2015 Scientific Conference

Aspirin in the 21st Century

Common mechanisms of disease and their modulation by Aspirin

Friday 28th August 2015

Caledonian Club
9 Halkin Street
Belgravia
London

9.00am - 5.00pm

Increasing the knowledge & understanding of Aspirin

Welcome

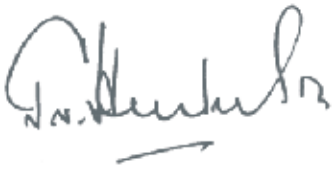
The International Aspirin Foundation welcomes you to our Scientific Conference.

The Foundation began in 1974 to increase the knowledge and understanding of the use of aspirin in medicine. We are delighted to welcome leading scientists, working with aspirin, to share their work and data within their specific areas of knowledge.

With the guidance of a Scientific Advisory Board, consisting of International key opinion leaders, The Foundation can encourage continued research into aspirin's use in medicine, via the Science Award Programme. In our fortieth year (2014) we instituted 2 Scientific Awards based on contributions to the advances in the use of aspirin in medicine, and we are delighted to welcome our inaugural winner of the Junior Investigator Award, Dr Hongmei Nan, to this Scientific Conference.

It is prudent to mention a learned and extremely knowledgeable Professor from Cardiff, Peter Elwood. Peter has provided wise, constructive and practical scientific advice since the beginning. In 2014 he formally took on the role of Chair of the Scientific Advisory Board and we are indebted to him for his support. The Board has worked hard in developing the Scientific Programme for this meeting. This meeting is vital as it provides a platform where professional colleagues can discuss and debate future uses of aspirin in an open manner. The purpose of the discussion session is to develop views for the benefits of using aspirin within primary prevention; secondary prevention and specific cancers.

We trust the setting of our Scientific Meeting will inspire you and we look forward to seeing you all again the in future.



Nick Henderson BSc HonFRCVS FCIPR
President

Pippa Hutchison MSc
Executive Director

From the Chairman of the Aspirin Scientific Advisory Board

Peter Elwood
Cardiff University, Cardiff, UK

"I had the privilege of being the first to report a randomised trial of aspirin in the reduction of vascular disease mortality, and 40 years later I was invited to chair the Aspirin Advisory Board. What an honour!

Those 40 years have been full of action and discovery. Granted the discovery has been almost entirely on the part of others, but what a thrill to have been involved in the discussions!

Aspirin has come a long way since 1974, and not just with reference to vascular disease, but also, thanks to inspired work by Rothwell and a very few others, in the prevention of cancer. I played no part in this (other than talking about it!) but it has been my privilege to conduct the first systematic review and meta-analysis of reports on aspirin in the treatment of cancer (about to be published).

Botanists know far more about salicylates than any medic, and way back in the 1970s Stan Pierpoint, a botanist, commented to me that from his knowledge of naturally occurring salicylates in plants, he could have predicted many of the effects of known aspirin in human subjects, and many more! His prediction still stands and one wonders what new benefit(s) will be found for the humble pill.

The Board, and before it the Aspirin Foundation, and before that The European Aspirin Foundation owes its existence to Nick Henderson, and many of us owe a substantial part of our careers, and the enjoyment and satisfaction therein, to him for his constant support and encouragement on all things aspirin. Bless you Nick! "

From the Chairman of the Aspirin Scientific Conference

Peter Rothwell
Oxford University, Oxford, UK

"The International Aspirin Foundation has organised many influential scientific meetings since it was founded by Nick Henderson over 40 years ago. The 2015 Scientific Conference, organised by Pippa Hutchison, the new Executive Director, reflects the Foundations continuing aim of increasing awareness of research on aspirin by stimulating the distribution and exchange of information and discussion.

We are extremely fortunate to have a truly world-leading group of speakers who will review some of the most interesting areas of current research and practice. I am honoured to chair the meeting and also to follow Peter Elwood as the next Chairman of the International Scientific Advisory Board.

Professor Elwood has made numerous important scientific contributions to our understanding of the effects of aspirin over five decades, including the first ever large randomised trials of aspirin in secondary prevention of myocardial infarction. His wisdom and enthusiasm have been a constant inspiration to those of us who have followed him in the study of aspirin."

Programme

Chairman: Peter Rothwell

Session One

9.00am - 11.00am

Mechanism of Action - Chair person Andy Chan

Mechanism of action of aspirin – Karsten Schror – Berlin

Aspirin triggered lipid mediators and their impact in resolution of inflammation – Joan Claria – Spain

Mechanisms in bleeding – Angel Lanas – Spain

Platelets versus extra-platelet components of aspirin pharmacodynamics – Paola Patrignani – Italy

Session Two

11.30am - 1.00pm

Aspirin in cancer - Chair person Ruth Langley

Primary prevention review – Andy Chan – USA

Effects of aspirin on colorectal cancer – Farhat Din – UK

Opportunities for using aspirin to treat cancer – Ruth Langley & Peter Elwood

Session Three

2.00pm - 4.00pm

Other aspirin-sensitive mechanisms of disease
Chair person Mike Gaziano

Hughes Syndrome, Aspirin and the Spectrum of Pregnancy – Graham Hughes – UK

Platelet activation and inhibition in diabetes mellitus – Carlo Patrono – Italy

Aspirin in HIV – Andrew Freedman – UK

Aspirin in treatment of acute pain – Ron Eccles UK

Session Four

4.00pm - 5.00pm

Looking at the future - Chair person Peter Rothwell

The Future - Group Discussion – Chairman/Scientific Advisory Board/ Speakers/Guests

Speakers

Andrew T. Chan MD, MPH

Associate Professor of Medicine, Harvard Medical School, USA.
Email: ACHAN@mgh.harvard.edu

Dr. Andrew T. Chan is an Associate Professor of Medicine at Harvard Medical School and an attending gastroenterologist at Massachusetts General Hospital (MGH) with a clinical specialty in familial gastrointestinal cancer syndromes. He is Chief of the Clinical and Translational Epidemiology Unit and the Program Director for gastroenterology training at MGH. His research focus is in clinical and translational epidemiology of colorectal cancer, with a focus on chemoprevention with aspirin.

For further details please see
http://www.massgeneral.org/gastroenterology/doctors/doctor.aspx?ID=17158

Joan Clària PhD

Department of Biochemistry and Molecular Genetics Hospital Clínic and Department of Physiological Sciences I, University of Barcelona, Barcelona, Spain.
Email: jclaria@clinic.ub.es

Joan Clària is currently a Senior Consultant at the Clinical Laboratory Service: Biochemistry and Molecular Genetics of the Hospital Clínic of Barcelona. He is also an Associate Professor at the Department of Physiological Sciences I at the School of Medicine of the University of Barcelona. He was trained as a specialist in Clinical Biochemistry and initiated his scientific career at the Liver Unit of the Hospital Clínic of Barcelona. From 1993 to 1996 he performed his post-doctoral studies as a Fulbright scholar at the Brigham and Women's Hospital and Harvard Medical School (Boston, MA) with Professor Charles N. Serhan, a leading scientist in the resolution of inflammation. He has also been a Visiting Scientist at the University of North Carolina (Chapel Hill, NC) (2001) and The Jackson Laboratory (Bar Harbor, ME) (2007) and a Visiting Professor at the Harvard Institutes of Medicine (Brigham and Women's Hospital/Harvard Medical School) (Boston, MA) (2010-2011). His laboratory is mainly interested in the study of lipid mediators implicated in the resolution of inflammation, with a special emphasis on the role of specialized pro-resolving mediators in obesity-associated liver complications.

Speakers

Farhat Din BSc MBChB MD FRCS

Cancer Research UK Clinician Scientist and Honorary, Consultant Colorectal Surgeon, Colorectal Unit, Western General Hospital, Edinburgh.
Email: Farhat.Din@hgu.mrc.ac.uk

Farhat Din completed her medical degree at St Andrews and Manchester University prior to returning to Edinburgh to undertake surgical training. She completed her MD thesis in examining the molecular mechanisms underlying NSAID- mediated prevention of colorectal cancer. She was awarded a Cancer Research UK Clinician Scientist fellowship in 2010. Currently she integrates her clinical practice as a consultant colorectal surgeon with her translational research programme investigating the effects of aspirin and other chemopreventive agents on mTOR signalling in colorectal cancer.

Colorectal cancer is a major cause of cancer death worldwide necessitating new strategies of disease control. Understanding the cellular signalling that governs growth of cancer cells holds the key to developing agents which interfere with cancer proliferation. The mTOR signalling pathway plays a pivotal role in controlling cell survival and regulation of cell metabolism and energy balance. Dysregulation of the mTOR signalling axis is implicated in the development of several cancers including colorectal cancer, supporting mTOR signalling as a promising target for chemoprevention. Our research focuses on colorectal cancer prevention by investigating the molecular mechanisms of the anti-tumour effects of chemopreventive agents such as aspirin on mTOR signalling to identify druggable targets in a pathway medicine approach.

http://www.nutshell-videos.ed.ac.uk/farhat-din-bowel-cancer-prevention/

Professor Ronald Eccles BSc, PhD, DSc

Director of Common Cold Centre and Healthcare Clinical Trials, Cardiff University, Wales.
Email: Eccles@cardiff.ac.uk

Professor Eccles is Director of the Common Cold Centre at Cardiff University, Wales UK. He is an expert on the symptoms of common cold and flu and medicines used to treat colds and flu. He has spent his career at Cardiff University and he established the Common Cold Centre in Cardiff in 1988, a Clinical Research Organisation that conducts scientific and clinical research on treatments for cough, colds and flu. His recent research has studied the effects of chilling on the onset of common cold symptoms and the effects of placebo treatments. He has special interests in studying nasal congestion and cough, and has published many scientific papers on these topics He is the author of some 250 publications and has contributed to many medical textbooks.

Dr Andrew Freedman MA, MB, BChir, MD, FRCP

Reader and Honorary Consultant, Infectious Diseases, Cardiff University and University Hospital of Wales. Email: Freedman@cardiff.ac.uk

Reader and Honorary Consultant in Infectious Diseases at Cardiff University and University Hospital of Wales. He qualified in Medicine from Cambridge University & St Thomas' Hospital in 1980, and subsequently trained in Infectious Diseases at St George's Hospital, London and Harvard Medical School, USA, before coming to Cardiff in 1994. His research interests include the effects of HIV on bone marrow and thymic function. He is a trustee of the British HIV Association and chair of its Audit & Standards sub-committee, and a previous member of the DH Expert Advisory Group on AIDS.

Speakers

Professor Graham R V Hughes MD FRCP

Head of The London Lupus Centre, London Bridge Hospital, London.
Email: Graham.Hughes@HCAConsultant.co.uk

Professor Graham Hughes trained at The London Hospital. In 1969-70, he spent 2 years with Professor Charles Christian in New York working on the introduction of anti-DNA assays in lupus.

In 1971 he set up Europe's first dedicated lupus clinic.

In 1983 he described the antiphospholipid syndrome (Hughes Syndrome).

Professor Hughes has published widely on lupus and connective tissue diseases, and has published 21 books. He is Founder and Editor of the international journal 'LUPUS', now in its 23rd year.

His honours include the ILAR International Prize for Rheumatology Research, and Docteur Honoris Causa in the Universities of Marseille and Barcelona.

Professor Hughes is currently Head of The London Lupus Centre, London Bridge Hospital.

For more details please see www.thelondonlupuscentre.com

Angel Lanas MD, D. Sc

Professor of Medicine, Chairman. Digestive Disease Service,
University Hospital, Zaragoza, Spain.
Email: angel.lanas@gmail.com

Angel Lanas is Professor of Medicine of the University of Zaragoza, Vice-Dean for Research Affairs at the Medical School of Medicine University of Zaragoza, Chairman of the Digestive Diseases Service at the University Hospital "Lozano Blesa" of Zaragoza, Spain and Scientific Director of the Aragón Health Research Institute.

Graduating from the University of Zaragoza, Spain in 1984, he completed his postgraduate training in internal medicine-gastroenterology at the same University. From November 1989 to December 1991 he was visiting professor to the Department of Gastroenterology at the University of Alabama in Birmingham, Alabama, USA where he worked with Professor Basil Hirschowitz. Professor Lanas joined the faculty at University of Zaragoza in 1992.

Dr. Lanas is actively involved in both clinical and basic investigation relating to acid peptic disorders, particularly NSAIDs, aspirin and gastrointestinal toxicity, as well as mechanisms of chemoprevention of colon cancer and Barrett's esophagus. Professor Lanas has published numerous articles pertaining to gastrointestinal damage, NSAIDs and inflammation in the most prestigious Medical Journals including the New England Journal of Medicine, The Lancet, Gastroenterology, Gut, The American Journal of Gastroenterology and American Journal of Medicine among others. He has also been invited as Visiting Professor to different American and European Universities including The Free University at Amsterdam, The North Carolina University at Chapel Hill, and the Huntsman Cancer Institute at the University of Utah at Salt Lake City and to numerous International Congresses and Symposiums.

For further details please see http://www.aspirin-foundation.com/speakers/angel-lanas/

Speakers

Dr Ruth Langley PhD FRCP

Oncologist/Senior Scientist, MRC Clinical Trials Unit at UCL,
Institute of Clinical Trials & Methodology, London.
Email: ruth.langley@ucl.ac.uk

Dr Langley is based at the UK Medical Research Council Clinical Trials Unit at University College London where she jointly leads the Cancer Group; and has a clinical consultant post at the Brighton and Sussex University Hospital.

She has a particular interest in gastro-oesophageal malignancy and has co-ordinated a series of trials and associated translational studies, and has led the investigation of the use of transdermal oestrogen in the treatment of prostate cancer.

Dr Langley is the Chief Investigator of an international trial (Add-Aspirin) designed to assess the effect of aspirin as an additional adjuvant agent in several common solid tumours.

Paola Patrignani, PhD

Professor of Pharmacology, Department of Neuroscience, Imaging and Clinical Sciences, Section of Cardiovascular and Pharmacological Sciences, "G. d'Annunzio" University, Chieti, Italy. E-mail: ppatrignani@unich.it

Paola Patrignani graduated from the Faculty of Biological Sciences, "La Sapienza" University of Rome (Italy). Then followed doctoral training in Pharmacology, at Catholic University of Rome, Italy. She worked for 2 years as Postdoctoral Research Fellow at Le Centre Hospitalier of Laval University (Quebec, Canada) and at the Department of Pharmacology of Merck Frosst (Kirkland, Quebec, Canada). Her academic career continued at "G. d' Annunzio" University, School of Medicine, Chieti, Italy, where she is currently Professor of Pharmacology and Head of the Research Unit of Pharmacology and Pharmacodynamics of eicosanoids at the Center of Excellence on Aging (CeSI). In 2009 she was Guest professor of Pharmacology at Goethe-Universität Frankfurt am Main (Germany).

Her scientific activity is documented by 150 peer-reviewed publications in international journals ranked in the Journal Citation Reports, and 21 chapters in national-international books. Her cumulative citation index is 6797: the h index is 42.

For further information please see http://www.aspirin-foundation.com/speakers/paola-patrignani/

Speakers

Carlo Patrono MD

Adjunct Professor of Pharmacology, Catholic University School of Medicine, Rome, Italy.
Email: cpatrono@unich.it

Carlo Patrono, MD, is Adjunct Professor of Pharmacology at the Catholic University School of Medicine in Rome, Italy and at the Perelman School of Medicine of the University of Pennsylvania in Philadelphia, USA. Dr. Patrono trained as a Postdoctoral Fellow in New York with the late Solomon Berson and Nobel Laureate Rosalyn Yalow.

Prof. Patrono's main research interest is in the study of platelet activation and inhibition in atherothrombosis and colorectal cancer. His research has characterized the human pharmacology of aspirin as an inhibitor of platelet COX-1 and provided the basis for the development of low-dose aspirin as an antithrombotic agent. During the past decade Prof. Patrono contributed to characterizing the human pharmacology of COX-2 inhibitors and evaluating their cardiovascular effects in different clinical settings.

Prof. Patrono has published over 200 research articles with an h index of 86.

For further details please see http://www.aspirin-foundation.com/speakers/carlo-patrono/

Prof. Dr. Karsten Schrör

Institute for Pharmacology and Clinical Pharmacology, Heinrich-Heine-University, Düsseldorf, Germany.
Email: schroer.frechen@uni-duesseldorf.de

Karsten Schrör is Professor and Chairman of the Department of Pharmacology and Clinical Pharmacology at the Heinrich-Heine-University in Düsseldorf, Germany. He obtained his M.D. at Martin-Luther-University Halle-Wittenberg (Germany) and carried out postdoctoral studies at the Universities of Halle, Mainz, Köln, the Jefferson Medical College, Philadelphia (USA) and the Wellcome Research Laboratories in Beckenham (UK). He held guest professorships at the Medical University of South Carolina at Charleston (USA) and the Department of Internal Medicine, Division of Hematology, University of Texas Medical Center, Houston (USA). Karsten Schrör is member of the German National Academy of Science (Leopoldina) and President of the German Society for Pharmacology.

He has over 500 original publications, reviews and several books on physiological and pharmacological aspects of myocardial infarction, pharmacology of platelet function, blood coagulation, prostaglandins, cellular effects of coagulation factors (thrombin, Xa) and signal transduction in vascular smooth muscle cells.

For further details please see http://www.aspirin-foundation.com/speakers/karsten-schror/

Session One

Mechanism of Action
Chair person Andy Chan

Mechanism of action of aspirin
Karsten Schror – Berlin

Aspirin triggered lipid mediators and their impact in resolution of inflammation
Joan Claria - Spain

Mechanisms in bleeding
Angel Lanas – Spain

Platelets versus extra-platelet components of aspirin pharmacodynamics
Paola Patrignani - Italy

Mechanism of action of aspirin

Karsten Schrör. M.D.

Professor of Pharmacology, em. Direktor, Institut für Pharmakologie und Klinische Pharmakologie,
Heinrich-Heine-Universität Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany. e-mail: schroer.frechen@uni-duesseldorf.de

Synopsis

Aspirin bears two active moieties within one and the same molecule: A reactive acetyl group and salicylate. Most if not all pharmacodynamic actions of aspirin are due to target structure acetylation. This includes thrombosis prevention, prevention of colorectal cancer and formation of "aspirin-triggered lipoxin" (ATL). These actions are seen at antiplatelet doses, i.e. maximum plasma levels of < 20 µM of unhydrolized aspirin. Mechanistically best known is inhibition of COX-1 in platelets with subsequent inhibition of thromboxane -dependent autocrine and paracrine platelet functions. This also includes inhibition of release of the platelet-storage products, including sphingosine-1-phosphate (S1P), an inflammatory mediator. Acetylation of COX-2 by aspirin allows for generation of 15-(R)HETE and, subsequently, ATL, another antiinflammatory mediator. Acetylation of eNOS by aspirin enhances NO production and improves endothelial oxygen defense. Many more acetylation targets have been identified are currently studied intensively, predominantly in connection with the anticancer effects of the compound.

Introduction – fresh insights into the pharmacology of aspirin

When aspirin was introduced into the clinics at the beginning of last century, it was generally believed that the compound itself was only the prodrug of its active metabolite salicylate and, needs first to become hydrolized in order to release the active salicylate moiety from the inactive "precursor". Consequently, it was assumed that the salicylate moiety fully accounts for all of the pharmacological actions of aspirin [1]. This view has changed fundamentally after detection of inhibition of prostaglandin biosynthesis by aspirin by Sir John Vane [2] and, shortly later, the detection of acetylation of the platelet cyclooxygenase (COX) as the molecular target of aspirin´s antiplatelet effect [3]. It is now clear that apparently all of the clinically relevant aspirin actions, i.e. antithrombotic and partially anti-inflammatory as well as anticancer effects in prevention of colorectal cancer (CRC) can be obtained at antiplatelet doses, i.e. doses between 75-325 mg/day and are mostly if not entirely due to target-specific acetylation. Most relevant is the acetylation of serine530 in the platelet COX-1, resulting in reduced generation of PG-endoperoxides and thromboxane A2 as well as acetylation of an epsilon-aminogroup of lysine residues in human endothelial cells and platelets at 1-30 µM concentrations [4]. Actually, more than 500 target proteins of aspirin induced acetylation have been identified by quantitative acid-cleavable activity-based protein profiling [5].

Another class of pharmacological effects of aspirin on lipid mediators results from the modulation rather than inhibition of the inducible form of cyclooxygenase, COX-2. A most challenging finding in this respect was the detection that acetylation of COX-2, in contrast to COX-1, results in the generation of a new product, 15-(R)-HETE. 15-(R)-HETE then can interact with lipoxygenase(s) of white cells to generate "aspirin-triggered lipoxin" (ATL) which like other lipoxins, is an anti-inflammatory mediator. The ATL-lipoxin axis also might stimulate NO-synthase in platelets and endothelial cells and upregulate hemeoxygenase-1 (HO-1), an antioxidative enzyme [6], [7].

This presentation reviews the actual data on pharmacokinetics and pharmacodynamics of aspirin. In particular focus as primary pharmacological candidate targets are the cyclooxygenases COX-1 and COX-2. The hypothesis is put forward that all of the effects of aspirin that are seen at commonly used antiplatelet doses – ca. 100-300 mg plain aspirin/day - are solely due to target protein acetylation without any evidence for direct involvement of the salicylate and acetate metabolites, respectively.

Inhibition of cyclooxygenase-1 (COX-1) by aspirin – the dose issue and pharmacokinetic aspects

The first pharmacological issue to be clarified before discussing specific pharmacodynaic effects of aspirin, is (i) which local (plasma) levels of aspirin and salicylate are required to inhibit COX-1 and COX-2 with the downstream mediators thromboxane, prostaglandins, lipoxins and nitric oxide and (ii) whether these concentrations can also be obtained at conventional aspirin doses in vivo. In the human, antiplatelet doses of aspirin result in peak acetylsalicylate levels of 1 - 3 µg/ml, i.e. about 6-20 µM [8]. Because of the longer half-life, concentrations of the salicylate metabolite in plasma are about 4 - 8fold higher. These levels are definitely below the millimolar range which is used in many in vitro studies (in protein-free media!), including studies in cancer cells. Salicylate concentrations, frequently above 2-5- mM, completely uncouple oxidative phosphorylation with numerous follow-up effects, most important non-specific

kinase inhibition [9]. Thus, it is likely that all biologically relevant effects of aspirin on COX-1 and COX-2 at antiplatelet doses are largely if not entirely acetylation-mediated. In this context, it is interesting to note that enteric-coated aspirin preparations are less potent antiplatelet drugs and inhibitors of thromboxane formation than a plain tablet at the same single oral dose [10]. Delayed absorption of the coated formulation will allow for longer contact times with aspirin esterases, eventually resulting in a higher proportion of the hydrolyzed acetyl group, i.e. inactive acetate. A weak though significantly higher aspirin hydrolytic activity has recently been shown in plasma of patients with coronary artery disease as well as a possible negative relation to inhibition of platelet function by aspirin [11].

Transacetylation of molecular targets by aspirin is irreversible – the duration of action is determined by the turnover rate of the acetylated protein(s)

Transacetylation by aspirin is nonselective and becomes preferentially detectable in platelets because of their insufficient protein synthesis. Irreversible COX-1 inhibition as the pharmacological mode of action of aspirin then results in prevention of platelet-COX-1-driven thromboxane formation, the only significant COX-product of platelets and the most important, aspirin-sensitive lipid mediator in the cardiovascular system. The antiplatelet action of aspirin does not require metabolic conversion but becomes rather reduced by high aspirin esterase activity in plasma [11].The plasma half-life of unmetabolized aspirin, amounting to 20-30 min, is an important determinant for its acetylation potential, i.e. to escape inactivation to acetate by hydrolysis, since the acetylation reaction results in irreversible covalent binding. The duration of action of aspirin is, therefore, determined by the biological half-life of the acetylated target, i.e. a few hours for endothelial cells, a few days for platelets, but about 20 days for albumin. This long protein survival will probably allow for cumulative acetylation with repeated dosing but, with the exception of platelets, has not been systematically studied yet.

Negative interactions between aspirin and nonopioid analgesic might abolish the antiplatelet effect of aspirin – even during continuous aspirin intake

Acetylation by aspirin of the platelet COX-1 has to block its enzymatic activity by > 95% in terms of thromboxane A2 forming capacity in order to become effective. The reason is the non-linearity of the concentration-response curve [12], resembling an all-or-none type of response. A remarkable finding are negative interactions of aspirin with some but not all nonopioid analgesics, most notably ibuprofen and dipyrone, with salicylate binding inside the COX-1 channel [13, 14]. Interestingly, these negative interactions, i.e. prevention of the antiplatelet effect of aspirin, can also be seen during continuous aspirin administration. The possible molecular explanation is the three orders of magnitude higher affinity of NSAIDs and dipyrone as opposed to aspirin to the hydrophobic bindings sites in the COX-1 channel [15].

Platelet-derived thromboxane A2 as the major lipid mediator targeted by aspirin with autocrine and paracrine actions

Pharmacological and clinical data data suggest that platelet COX-1 and subsequent platelet-derived thromboxane A2 formation is the primary target lipid mediator of aspirin. Inhibition of thromboxane formation will eliminate any further autocrine (platelet activation and recruitment) and paracrine actions of thromboxane on cells in the neighbourhood. This also includes multiple inflammatory actions exerted by non-lipid mediators [16]. In addition, there is also a role for inhibition of platelet-mediated recruitment of inflammatory white cells and activation of aspirin-sensitive soluble inflammatory mediators, such as C-reactive protein (CRP). It is not entirely clear, whether this interaction is a direct, TXA2 mediated effect or indirectly mediated via the platelet-stimulatory action of TXA2 [17]. In any case, the clinical significance of modification of these complex interactions between platelet-derived mediators and/or thromboxane and other cells, i.e. so-called "heterotypic" platelet functions [18] , though currently incompletely understood, might be considerable. For example, reduced plasma levels of CRP by aspirin were found to correlate with its cardioprotective effect in the Physians´Health study [19].

Release of sphingosine-1 phosphate (S1P) – a platelet-derived proinflammatory, COX-2 inducing and angiogenic mediator can be blocked by aspirin

Another interesting platelet-derived product is sphingosine-1-phosphate (S1P), a lipid mediator of the ceramide class. At the cellular level, S1P stimulates COX-2 expression, PGE-synthesis and angiogenesis and inhibits apoptosis. S1P has been shown to be crucially involved in inflammation and cancer development [20, 21]. In the circulation, S1P is stored in large quantities in platelets and is released upon platelet stimulation in a strictly thromboxane-, i.e. aspirin-sensitive, manner [22, 23]. S1P stimulates cell (monocyte) and endothelial cell migration and several other proinflammatory cell functions. S1P, most likely platelet-derived; is released during arterial thrombus formation, enhances expression of thrombin receptors such as PAR-1 and PAR-4 in human monocytes [24]. This in turn will sensitize monocytes at sites of injury by a thromboxane-dependent mechanisms which could be blocked by aspirin at antiplatelet doses. Moreover, we have recently shown that iv. aspirin not only reduces thrombin formation in patients with acute coronary syndroms but also the thrombin-induced platelet secretion of S1P in these patients [25].

Of particular interest is S1P as a putative link between platelets and tumorigenesis in colorectal carcinoma. We are working on the hypothesis that S1P might be (one of) the

platelet-derived factors that stimulate COX-2 expression in colonic epithelial cells and might also contribute to tumor growth, angiogenesis and metastasis (Rauch & Schrör, unpubl). Enhanced, aspirin sensitive levels of thromboxane have recently been described in patients with colorectal neoplasias[26].

Aspirin, COX-2 and "aspirin-triggered lipoxin"(ATL)

Lipoxins operate during self-limited acute inflammatory responses that enable the return to homeostasis by resolution of the inflammatory reaction[27]. Sweveral studies have described a relationship to the anti-inflammatory actions of aspirin. Acetylation of COX-2 by aspirin only partially reduces COX-2-dependent prostaglandin production but mainly changes the steric structure of the enzyme and its functionality towards a 15-lipoxygenase. This enzyme generates a new product, 15-(R)-HETE at at least 10fold higher amounts than the COX-metabolite PGE2 [28, 29]. 15-(R)-HETE, is the precursor of 15-epi-lipoxin A4 or "aspirin-triggered lipoxin" (ATL), resulting from synergistic interaction of acetylated COX-2 (15-(R)-HETE) with lipoxygenases from white cells [30]. Interestingly, ATL not only contributes to the anti-inflammatory actions of aspirin but might also be involved in endothelial protection by stimulation of NO-formation. Aspirin at antiplatelet doses of 75 mg/day in man has been shown to inhibit leukocyte accumulation at a local inflammatory site (skin blister). This involves stimulation of ATL-production as well as local upregulation of lipoxin receptors [31]. Thus, low-dose aspirin is able to interact with the lipoxin system and this possibly involves both COX-1 (inhibition of thromboxane) and COX-2 (generation of 15-(R)-HETE). These actions differ qualitatively from those of traditional NSAIDs and selective COX-2 inhibitors: Both classes of compounds only competitively, i.e. reversibly, inhibit COX-2 and COX-1 activities.

Aspirin, eNOS, heme oxygenase-1 and oxidative stress

Finally, aspirin has been shown to protect from low-grade inflammation-related oxidative stress via enhanced endothelial NO-synthase activity (eNOS) and subsequent NO-production [32]. Mechanistically, this is possibly due to posttranslational lysine acetylation in endothelial cells and platelets [4]. The required concentrations of aspirin are in the low µmolar range, in the in vitro study of Taubert and colleagues between 0.01 and 1 µM, the EC50 beeing 50 nM [32]. Interestingly, the aspirin analog 2-(acetoxy-phenyl) hept-2-ynyl sulfide (APHS) a 60fold more potent and 100 fold more selective COX-2 inhibitor than inhibitor of COX- 1[33], was found to be at least as potent as aspirin. NO stimulates the expression of downstream enzymes, among them hemoxygenase-1 (HO-1), thus improving oxygen defense and suggesting a connection between antithrombotic and anti-inflammatory actions of aspirin [7, 34, 35]. Recently, two randomized trials have demonstrated that aspirin (81 – 1,300 mg/day) significantly increased hemoxygenase-1 (HO-1) activity by about 50% and at the same time reduced assymetrical dimethyl arginine, an inhibitor of NO-synthase, by 30%. Both changes were highly significant and independent of the aspirin doses, suggesting HO-1 as another downstream target of aspirin [36, 37].

Outlook

The complex interactions of aspirin with other mediator systems and target structures are not fully understood yet. Specifically thromboxane, the key, aspirin-sensitive lipid mediator, has found renewed attention as aspirin-sensitive signaling molecule for autocrine and paracrine platelet functions. In addition to its established use in secondary prevention of myocardial infarction and several forms of ischemic stroke, inhibition of platelet function by aspirin has recently been found also to be proved to effective in prevention of venous thromboembolism (VTE) and, perhaps, as a chemopreventive of colorectal cancer (CRC). All of these actions are seen at antiplatelet doses of the compound. It is, therefore, likely that most if not all of these pharmacological actions are due to target structure acetylation whereas the salicylate component as well as the inactive acetate moiety are ineffective at these doses. Numerous new acetylation targets have been identified recently and their biological significance is still to be evaluated. Since the acetylation potential depends critically on the intact acetylsalicylic acid structure, it is of great pharmacological interest to increase the systemic bioavailability of non-hydrolized aspirin. A new micronized formulation has recently been introduced into the market [38]. Peak plasma levels of uncleaved acetylsalicylic acid are reached twice as fast and are three times higher than with plain conventional aspirin.

Acknowledgements:

This work was supported in part by the Forschungsgruppe Herz-Kreislauf, e.V. (Monheim)

Conflict of interest statement

The author is member of advisory boards of Bayer Healthcare and Daiichi/Sankyo-Lilly and also received speaker´s honoraria from these companies.

References

1. Dreser, H., Pharmakologisches über Aspirin (Acetylsalizylsäure). Pflügers Arch Physiol, 1899. 76: p. 306-318.
2. Vane, J.R., Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol, 1971. 231(25): p. 232-5.
3. Roth, G.J., N. Stanford, and P.W. Majerus, Acetylation of prostaglandin synthase by aspirin. Proc Natl Acad Sci U S A, 1975. 72(8): p. 3073-6.
4. Jung, S.B., et al., Histone deacetylase 3 antagonizes aspirin-stimulated endothelial nitric oxide production by reversing aspirin-induced lysine acetylation of endothelial nitric oxide synthase. Circ Res, 2010. 107(7): p. 877-87.

5. Wang, Mapping sites of aspirin-induced acetylations in live cells by quantitative acid-cleavable activity-based protein profiling (QA-ABPP). Scientific Reports, 2015. 5.
6. Paul-Clark, M.J., et al., 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. J Exp Med, 2004. 200(1): p. 69-78.
7. Nascimento-Silva, V., et al., Novel lipid mediator aspirin-triggered lipoxin A4 induces heme oxygenase-1 in endothelial cells. Am J Physiol Cell Physiol, 2005. 289(3): p. C557-63.
8. Nagelschmitz, J., Blunk,M., Krätschmar, J. et al, Pharmacokinetics and pharmacodynamics of acetylsalicylic acid after intravenous and oral administration to healthy volunteers. Clin Pharmacol:Adv Applic, 2013. 5: p. 1-9.
9. Frantz, B. and E.A. O'Neill, The effect of sodium salicylate and aspirin on NF-kappa B. Science, 1995. 270(5244): p. 2017-9.
10. Grosser, T., et al., Drug resistance and pseudoresistance: an unintended consequence of enteric coating aspirin. Circulation, 2013. 127(3): p. 377-85.
11. Zhou, G., et al., Aspirin hydrolysis in plasma is a variable function of butyrylcholinesterase and platelet-activating factor acetylhydrolase 1b2 (PAFAH1b2). J Biol Chem, 2013. 288(17): p. 11940-8.
12. Reilly, I.A. and G.A. FitzGerald, Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet inhibitory drugs. Blood, 1987. 69(1): p. 180-6.
13. Catella-Lawson, F., et al., Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Engl J Med, 2001. 345(25): p. 1809-17.
14. Saxena, A., et al., Drug/drug interaction of common NSAIDs with antiplatelet effect of aspirin in human platelets. Eur J Pharmacol, 2013. 721(1-3): p. 215-24.
15. Hohlfeld, T., Schrör,K., Inhibition of antiplatelet effects of aspirin by non-opioid analgesics. Clinical Pharmacology & Therapeutics, 2015. 97(2): p. 131-4.
16. Weyrich, A.S., S. Lindemann, and G.A. Zimmerman, The evolving role of platelets in inflammation. J Thromb Haemost, 2003. 1(9): p. 1897-905.
17. Muhlestein, J.B., Effect of antiplatelet therapy on inflammatory markers in atherothrombotic patients. Thromb Haemost, 2010. 103(1): p. 71-82.
18. Passacquale, G. and A. Ferro, Current concepts of platelet activation: possibilities for therapeutic modulation of heterotypic vs. homotypic aggregation. Br J Clin Pharmacol, 2011. 72(4): p. 604-18.
19. Ridker, P.M., et al., Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med, 1997. 336(14): p. 973-9.
20. Liang, J., et al., Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. Cancer Cell, 2013. 23(1): p. 107-20.
21. Spiegel, S. and S. Milstien, The outs and the ins of sphingosine-1-phosphate in immunity. Nat Rev Immunol, 2011. 11(6): p. 403-15.
22. Ulrych, T., et al., Release of sphingosine-1-phosphate from human platelets is dependent on thromboxane formation. J Thromb Haemost, 2011. 9(4): p. 790-8.
23. Böhm, A., et al., Release of sphingosine-1-phosphate from platelets requires thromboxane synthesis and thromboxane receptor activation. Circulation, 2009. 120 S1080(S1080): p. Abstract 5299.
24. Mahajan-Thakur, S., et al., Sphingosine-1-phosphate induces thrombin receptor PAR-4 expression to enhance cell migration and COX-2 formation in human monocytes. J Leukoc Biol, 2014. 96(4): p. 611-8.
25. Polzin, A., et al., Aspirin inhibits release of platelet-derived sphingosine-1-phosphate in acute myocardial infarction. Int J Cardiol, 2013. 170(2): p. e23-4.
26. Li, H., Liu, K., Boardman, L.A. et al, Circulating prostaglandin biosynthesis in colorectal cancer and potential clinical significance. EBioMedicine, 2014.
27. Spite, M. and C.N. Serhan, Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. Circ Res, 2010. 107(10): p. 1170-84.
28. Mancini, J.A., et al., Mutation of serine-516 in human prostaglandin G/H synthase-2 to methionine or aspirin acetylation of this residue stimulates 15-R-HETE synthesis. FEBS Lett, 1994. 342(1): p. 33-7.
29. Lecomte, M., et al., Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. J Biol Chem, 1994. 269(18): p. 13207-15.
30. Claria, J. and C.N. Serhan, Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci U S A, 1995. 92(21): p. 9475-9.
31. Morris, T., et al., Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol, 2009. 183(3): p. 2089-96.
32. Taubert, D., et al., Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. Br J Pharmacol, 2004. 143(1): p. 159-65.
33. Kalgutkar, A.S., et al., Aspirin-like molecules that covalently inactivate cyclooxygenase-2. Science, 1998. 280(5367): p. 1268-70.
34. Grosser, N., et al., Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. Biochem Biophys Res Commun, 2003. 308(4): p. 956-60.
35. Grosser, N. and H. Schröder, Aspirin protects endothelial cells from oxidant damage via the nitric oxide-cGMP pathway. Arterioscler Thromb Vasc Biol, 2003. 23(8): p. 1345-51.
36. Hennekens, C.H., et al., A randomized trial of aspirin at clinically relevant doses and nitric oxide formation in humans. J Cardiovasc Pharmacol Ther, 2010. 15(4): p. 344-8.
37. Hetzel, S., et al., Aspirin increases nitric oxide formation in chronic stable coronary disease. J Cardiovasc Pharmacol Ther, 2013. 18(3): p. 217-21.
38. Voelker, M. and M. Hammer, Dissolution and pharmacokinetics of a novel micronized aspirin formulation. Inflammopharmacology, 2012. 20(4): p. 225-31.

Aspirin triggered lipid mediators and their impact in resolution of inflammation

Joan Clària, PhD, Senior Consultant and Associate Professor

Department of Biochemistry and Molecular Genetics Hospital Clínic and Department of Physiological Sciences I, University of Barcelona, Barcelona, Spain. Email: jclaria@clinic.ub.es

Synopsis

Aspirin (ASA) is the NSAID most widely used and the standard against which all new anti-inflammatory agents are compared. In addition to relieve inflammation, pain and fever, ASA is also useful in preventing myocardial infarction and sporadic colon cancer. Although most of the pharmacological properties of ASA are related to its ability to inhibit the enzyme cyclooxygenase (COX), leading to the blockage of the biosynthesis of the eicosanoids prostaglandins, the complete mechanism of action is still a subject of interest and debate. In this lecture, the generation of a novel series of eicosanoids named ASA triggered lipoxins (ATL) produced when COX-2 is acetylated by aspirin, will be discussed. Similarly, the formation of ASA-triggered resolvins from omega-3-PUFAs will be presented. The most important aspect of these novel mediators is that they exert anti-inflammatory and pro-resolution properties and therefore may effectively mediate, at least in part, the beneficial actions of aspirin.

Lecture Notes

Aspirin (acetylsalicylic acid, ASA) is the non-steroidal anti-inflammatory drug (NSAID) most widely employed and the standard against which all new anti-inflammatory agents are compared. Because of its long history of use and availability without prescription and its low cost and safety, ASA is the drug of choice for relieving inflammation and mild to moderate pain and fever. In addition to the well-known anti-inflammatory, analgesic and antipyretic properties, ASA also inhibits platelet aggregation and therefore is useful in preventing myocardial infarction and stroke (1). Numerous epidemiological studies have also shown that the long-term use of low doses of ASA represents a potentially viable option in the prevention of sporadic colon cancer (2). Although most of the pharmacological properties of ASA are related to its ability to acetylate cyclooxygenase (COX), leading to the irreversible inhibition of the biosynthesis of the eicosanoids prostaglandins (PG) and thromboxane (TX) (3), the complete mechanism of action underlying the pleiotropic effects of ASA is still a subject of interest and debate. At high doses, for instance, there are properties of ASA that are independent of COX and PG inhibition. In this regard, ASA is able to either activate the heat shock transcriptional factor and the p38 mitogen-activated protein kinase or to inhibit the mitogen-activated protein kinases p44Erk1 and p42Erk2 and the activity of transcriptional factors such as nuclear factor-kB and activator protein 1 (4-6).

In line with the principle that not all the beneficial effects associated with ASA consumption can be ascribed to the inhibition of PG and TX biosynthesis, the laboratory of Professor Serhan at Harvard University has provided solid evidence that ASA triggers the generation of a novel series of eicosanoids named ASA triggered lipoxins (ATL) (7-9). The acetylation capacity of ASA is a critical aspect in the ATL biosynthetic pathway and this property is not shared but any other NSAID. Indeed, this biosynthetic pathway triggered by ASA is initiated by acetylation of the inducible COX isoform (COX-2) which switches the enzyme´s catalytic activity from a PG synthase to a 15-lipoxygenase (15-LOX) (7). Thus, PG biosynthesis by ASA-acetylated COX-2 is inhibited and arachidonic acid is transformed to 15R-hydroxyeicosatetraenoic acid (15R-HETE). The further conversion of 15R-HETE by a 5-LOX present in immune cells to 15-epi-LXA4 (ATL) is the result of a process called transcellular biosynthesis. This process involves cell-cell interaction and processing of a metabolic intermediate generated by one cell (donor cell) by a vicinal cell (acceptor cell) for the production of an active eicosanoid that neither cell alone can generate (10).

The most relevant biological action of these ASA triggered eicosanoids (i.e. ATLs) is that they work as putative endogenous "breaking signals" for leukocyte recruitment and therefore play a key role in the resolution of inflammation (9). In recent years, resolution of inflammation has been described an active process under the control of a number of autacoids, including lipid mediators derived from omega-6-PUFAs, such as LXs as well as from omega-3-PUFAs, including resolvins, protectins and maresins. ATL are 15-epimers of LXs, which have a unique spectrum of bioactions indicative of anti-inflammatory and pro-resolution properties. For example these eicosanoids

work as "stop-signals" for inflammation and inhibit chemotaxis, selectin- and integrin-mediated adhesion to and transmigration across endothelial monolayers in response to LTB4 and formyl-methionyl-leucyl-phenylalanine, TNFa-stimulated superoxide generation and degranulation and interleukin-1 release by neutrophils (9). In vivo, LX stable analogs inhibit LTB4-induced leukocyte rolling and adherence and neutrophil margination and extravasation (9). LX analogs inhibit TNFa-stimulated leukocyte trafficking and chemokine secretion in murine air pouches and when applied topically to mouse ears dramatically inhibit leukocyte infiltration and vascular permeability (9). Also, ATL analogs protect mice from renal ischemia-reperfusion injury and glomerulonephritis (9). In an animal model of periodontal disease, LX and ATL analogs attenuate gingivitis and leukocyte recruitment (9). Intravenous delivery of LXs and ATL inhibit acute dermal inflammation and neutrophil infiltration of skin microabcesses and lungs in LTB4 receptor transgenic mice (9). In a murine model of asthma, LX and ATL stable analogs attenuate airway hyperreactivity and inflammation and accelerate resolution of pulmonary edema (9). Administration of a metabolically stable LXA4 analog in a mouse model of chronic airway inflammation and infection associated with cystic fibrosis suppresses neutrophilic inflammation, decreases pulmonary bacterial burden and attenuates disease severity (9). Finally, a randomized clinical trial in healthy subjects demonstrated that low-dose aspirin (81 mg daily), a dose used for long-term antithrombotic prophylaxis, initiates production of anti-inflammatory ATL opposite to the inhibition of the pro-thrombotic TX (11). Overall, LXs and ATL are anti-inflammatory and pro-resolution eicosanoids that work efficiently in reducing the signs and symptoms of inflammation in a wide range of disease models. Consequently, ATL biosynthesis during aspirin treatment may effectively mediate, at least in part, the beneficial actions of aspirin.

More recently, aspirin has been shown to trigger the conversion of omega-3-PUFA (i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to another group of anti-inflammatory and pro-resolution lipid mediators termed ASA-triggered resolvins and ASA-triggered-protectins (12,13). Similar to that described for the biosynthesis of ATL, endothelial cells expressing COX-2 acetylated by aspirin transform DHA into 17R-HDHA which is further converted by 5-LOX into the corresponding 17R-RvD1, 17R-RvD2 and other 17R-D resolvins, which are collectively known as aspirin-triggered (AT)-resolvins (14,15). ASA-triggered protectin D1 (AT-PD1) is biosynthesized in a similar process. Finally,biosynthesis of resolvins of the E-series from EPA is initiated with the formation of 18R-hydroperoxy-EPE (18R-HEPE) by endothelial cells expressing aspirin-acetylated COX-2 (13). 18R-HEPE is transformed by transcellular biosynthesis in neighboring 5-LOX-containing leukocytes into RvE1 (5S,12R,18R-trihydroxy-EPA) via a 5S,6-epoxide intermediate (13). Collectively, these omega-3-derived lipid mediators also exert anti-inflammatory and proresolution actions both in vitro and in vivo and contribute in the understanding of the observed preventive actions of aspirin and dietary omega-3PUFA.

References.

Patrono, C. (1996) Aspirin as an antiplatelet drug. N. Engl. J. Med. 330, 1287-1294.

Thun, M. J., Namboodiri, M. M., and Heath C. W. (1991) Aspirin use and reduced risk of fatal colon cancer. N. Engl. J. Med. 325, 1593-1596.

Vane, J. R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for the aspirin-like drugs. Nature 231, 232-235.

Jurivich, D. A., Sistonen, L., Kroes, R. A., and Morimoto, R. I. (1992) Effect of sodium salicylate on the human heat shock response. Science 255, 1243-1245.

Pillinger, M. H., Capodici, C., Rosenthal, P., Kheterpal, N., Hanft, S., Philips, M. R., and Weissmann, G. (1998) Modes of action of aspirin-like drugs: salicylates inhibit Erk activation and integrin-dependent neutrophil adhesion. Proc. Natl. Acad. Sci. USA 95, 14540-14545.

Koop, E., and Ghosh, S. (1994) Inhibition of NF-kB by sodium salicylate and aspirin. Science 265, 956-959.

Clària, J., and Serhan, C. N. (1995) Aspirin triggers previously unrecognized bioactive eicosanoids by human endothelial cell-leukocyte interaction. Proc. Natl. Acad. Sci. USA. 92, 9475-9479.

Clària J, Lee MH, Serhan CN (1996) Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. Mol Med 2:583-96.

Serhan CN. (2005) Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. Prostaglandins Leukot Essent Fatty Acids73, 141-62

Marcus AJ. (1986) Transcellular metabolism of eicosanoids. Prog Hemost Thromb 8:127-42

Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN (2004) Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci U S A 101:15178-83.

Serhan CN, Chiang N (2008) Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. Br J Pharmacol 153, Suppl 1:S200-15

Serhan C.N., Clish C.B., Brannon J., Colgan S.P., Chiang N., Gronert K (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J. Exp. Med. 192, 1197-1204.

Serhan C.N., Petasis N.A. (2011) Resolvins and protectins in inflammation resolution. Chem. Rev. 111, 5922-5943.

Serhan C.N. (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510, 92-101.

Mechanisms in bleeding

Angel Lanas, M.D.; D.Sc.

Affiliation: Service of Digestive Diseases. University Hospital. University of Zaragoza. IIS Aragón. CIBERehd. Zaragoza. Spain.
Email: alanas@unizar.es

Synopsis

Low-dose aspirin, alone or combined with other antiplatelets, is increasingly prescribed for cardiovascular prevention and probably in the near future for cancer prevention. These should be evaluated together with the bleeding risks, especially gastrointestinal and intracranial bleeding, although this one is very unfrequent. Low-dose aspirin is associated with upper and lower gastrointestinal injury, although lower gastrointestinal effects are poorly characterized. The mechanisms of bleeding are essentially linked to its antiplatelet effects and its interference with the coagulation process initiated by platelets. However there are other intrinsic and external factors that may people more prone to bleed. The gastrointestinal risk differs among antiplatelets drugs users. The most important risk factors are history of peptic ulcer, dose, older age and concomitant use of NSAIDs or dual antiplatelet therapy. The presence of previous lesions able to bleed when taking aspirin is another factor that must be considered. This risk can minimized and effective gastrointestinal prevention strategies are available and should be use in at-risk patients taking low dose aspirin. Proton pump inhibitors seem to be the best gastroprotective agents, whereas the benefits of H. pylori eradication are still unclear.

Lecture Notes

Cardiovascular (CV) diseases are important causes of morbidity and mortality. Low-dose aspirin (LDA) (75- 325mg daily) alone or combined with other antiplatelet drugs, is increasingly prescribed for either primary or secondary CV prevention. Cancer prevention may be another indication in the near future. LDA has been shown to be effective in preventing around one-fifth of vascular complications in patients with previous myocardial infarction, stroke or transient cerebral ischemia. Inducing bleeding in patients who need this drug may offset some of the beneficial effects of LDA. Bleeding events are for the most part mild (e.g. skin bruising, nose bleed, trauma, etc..) but they may be severe as those being intracranial and some gastrointestinal which are associated with morbidity and mortality (1,2).

Any bleed

In a recent meta-analysis (3) on 35 low-dose ASA-alone clinical trials involving 87,581 individuals with 338,735 person-years of follow-up evaluation we found a significantly increased risk of any type of bleeding using low-dose ASA compared with controls (Peto OR, 1.54; 95% CI, 1.36–1.74). Assuming similar baseline risks, the NNH value showed that 71 individuals had to be treated to harm a patient with any bleed. The Incidence Rate Difference was 8.1 (95% CI, 4.0–12.2) per 1000 person-years. We found an association between dose of ASA and increased risks of bleeding and also an increased risk for haemorrhagic strokes (Peto OR, 1.67; 95% CI, 1.12–2.48; P). Fatal bleeds were not associated significantly with low-dose ASA use (Peto OR, 1.22; 95% CI, 0.78–1.89) This was also the case with fatal GI bleeds (Peto OR, 0.94; 95% CI, 0.47–1.87; P = .87) and fatal haemorrhagic strokes (Peto OR, 1.42; 95% CI, 0.84–2.41). On the contrary LDA alone decreased the risk for all-cause mortality (relative risk, 0.93, 95% confidence interval [CI], 0.87-0.99), largely because of effects in secondary prevention populations. Also, based on the limited available data, it is uncertain whether aspirin therapy increases the risk of subdural hematoma. The incidence of subdural hematoma during aspirin therapy is low but varies widely depending upon the age of the patient population (4). In another recent meta-analysis the risk of ischemic stroke was comparable between clopidogrel and aspirin monotherapy (ischemic stroke: RR, 0.93; 95% CI, 0.83–1.05; P=0.24) and interestingly, the estimated number of patients needed to harm with clopidogrel and aspirin with respect to one additional major intracranial bleeding event would be 7606 (95% CI= -infinity to -1218 and 1793 to +infinity) (5)

Mechanisms of bleeding

The mechanisms whereby aspirin (or other NSAIDs) might promote or induce bleeding in only a small proportion of users are still not well known. Several potential mechanisms at the gastrointestinal (GI) level have been proposed and include: a failure of the gastroduodenal mucosa to adapt to NSAID/aspirin damage; a change in the mucosal balance between injurious/protective factors in susceptible people leading to increased severity of the ulcer diathesis and complications (6). Nevertheless, the question here is whether LDA is able to affect the gastroduodenal mucosa by inhibiting mucosal COX-1, the mosty important enzyme involved in the gastroduodenal mucosa defense.

Several clinical studies have shown that gastric mucosal COX-1 inhibition is very susceptible to be inhibited with ASA even a very low dose (< 100 mg/day) and associated with mucosal damage (7,8). Enteric-coated aspirin

released in the small bowel has also been associated with small bowel mucosal damage and increase of mucosal permeability (8). However, inhibition of platelet and platelet dependent clotting mechanisms has been pointed out as the precipitating bleeding, particularly in the presence of previous lesions.

The acetylation of platelet COX-1 at serine-529 is the direct mechanism of action of low-dose aspirin. Patrignani et al. (9) in a phase I, single-arm, open-label study of EC aspirin (100 m/day) administered to 24 healthy subjects, over a 24 h-period on day 1 and 7, % platelet acetylated COX-1 (AceCOX-1) with traditional pharmacokinetic and pharmacodynamics parameters. Acetylation of platelet COX-1 was measurable before detection of aspirin levels in the systemic circulation and increased in a cumulative fashion upon repeated dosing. After the last dose of EC-aspirin, %AceCOX-1, serum TXB2 and CEPI-CT values were maximally and persistently modified throughout 24 h; they averaged 76 ± 2%, 99.0 ± 0.4% and 271 ± 5 s, respectively. EC-aspirin caused 75% reduction in urinary 11-dehydro-TXB2 excretion. After chronic dosing with aspirin, the pharmacokinetics of acetylsalicylic acid was completely dissociated from pharmacodynamics. In this way, it has been shown that, when tested within six hours of hospital admission, most aspirin related acute GI bleeders had an abnormal increase in skin bleeding time, which reversed to normal within several days.

To look for risk factors or mechanisms whereby aspirin may promote gastrointestinal bleeding 61 patients with previous aspirin related upper gastrointestinal bleeding and 61 matched controls were given 375 mg of aspirin and sequential skin bleeding time and blood aspirin levels were measured. Additional studies included platelet lumiaggregation, von Willebrand factor, Factor VIII, and coagulation studies. Baseline skin bleeding time was similar in bleeders and controls, but bleeders had a more prolonged skin bleeding time after aspirin use. Hyper-response was more frequent in bleeders (30% v 9.3%; $p < 0.01$) and was associated with more than one previous separate bleeding event and a lower packed cell volume during the preceding bleeding episode. No differences were found in other factors studied. Logistic regression analysis identified prolonged skin bleeding time after aspirin use as an independent factor contributing to aspirin related gastrointestinal bleeding (RR = 5.4; 95% CI: 1.8 to 17.1). The study concluded that 30% of patients with a history of aspirin related gastrointestinal bleeding had an exaggerated prolongation of skin bleeding time in response to aspirin, which may be a risk factor for bleeding. This intrinsic defect was not related to any known platelet defect or to subclinical von Willebrand disease or different aspirin metabolism or to subclinical von Willebrand disease or different aspirin metabolism (10).

A most recent study evaluated the impact of well known clinical and environmental risks factors for GI bleeding together with candidate gene polymorphisms on GIB susceptibility (11). This study was carried out as a case-control study comprising 644 consecutive patients hospitalized in a network of 8 Spanish hospitals due to non variceal GI bleeding, and 644 non-hospital sex- and aged- matched controls. Genomic DNA from patients and controls was typed for a panel of 89 polymorphisms in genes related to GI mucosal inflammation, coagulation pathways, arachidonic acid metabolism, and drug-metabolizing enzymes by using the Illumina Platform. Analysis of genotyping data was performed by the bioinformatic tool SNPator. Relative risks (RRs) of independent factors associated with either upper GIB (UGIB) or lower GIB (LIGB) are shown in the table shown below. Subgroup analysis by drug intake showed that patients treated with NSAID/ASA use carrying the PLAT (plasminogen activator, tissue) rs2020918TT geno- type had a higher risk of GI bleeding (RR:2.06, 95%CI:1.06-4) whereas carriers of the IL1B (Interleukin-1 beta) rs1143634C, and PTGER2 (prostaglandin-E receptor 2) rs708494TT variants showed a significant decrease (RR:0.34, 95%CI:0.12-0.91, and RR:0.67, 95%CI:0.43-1.06, respectively). Moreover, the COX1 (cyclooxigenase 1) rs883485TT variant was associated with overall GI bleeding risk in patients treated with PPIs (OR:2.01, 95%CI:1.17-3.46) whereas the PTGER2 rs708494TT genotype was associated with a lower risk (RR:0.63, 95%CI:0.43-0.92). The authors concluded the implication of genetic susceptibility on GI bleeding risk and reveal potential new targets for prevention and further investigation in this field.

Table 1: Enviromental and genetic risk factor for GIB

Rist Factor	Controls (n=644) n {%)	Upper GIB (n = 360) n (%)	RR (95% CI)	Lower GIB (n = 255) n (%)	RR (95% CI)
Hx of PUD	51 (7.9)	69 (19.2)	2.3 (1.4-3.6)	28 (11.0)	1.4 (0.8-2.4)
H. pylori infection	198 (51.7)	209 (59.4)	1.4 (1.0-1.9)	-	-
NSAID use	98 (15.2)	105 (29.2)	2.3 (1.6-3.4)	68 (26.7)	1.9 (1.3-2.8)
ASA use	88 (13.7)	60 (16.7)	1.5 (1.0-2.4)	70 (27.5)	2.1 (1.4-3.2)
PPI use	202 (31.4)	85 (23.6)	0.7 (0.5-1-0)	140 (54.9)	2.0 (1.4-2.8)
Anticoag. use	43 (6.7)	39 (10.8)	2.3 (1.3-4.3)	48 (18.8)	3.7 (2.3-6.1)
PLAT rs2020918TT (plasminogen activator, tissue)	70 (10.9)	52 (14.4)	1.3 (0.8-2.0)	50 (19.6)	1.9 (1.2-2.9)
COX1 rs883485TT	540 (83.9)	318 (88.3)	1.5 (0-9-2.4)	228 (89.4)	2.0 (1.2-3.4)
F2 (prothrom.) rs1799963GG	606 (93.2)	349 (96.9)	2.8 (1.1-7.4)	241 (94.5)	1.1 (0.5-2.5)
Adjusted by sex, age, hospital, smoking					

Gastrointestinal bleeding

LDA induces a wide spectrum of GI adverse event in the upper GI tract, ranging from symptoms without lesions to severe complications like peptic ulcer bleeding, and even death (12,13). Endoscopic-controlled studies have shown that approximately 60% of patients taking LDA have upper GI erosions. The incidence of ulcers seems to be lower (14,15). A study of 187 patient taking LDA without gastro-protectant drugs showed an ulcer prevalence of 11% (95% CI 6.3-15.1%) and an ulcer incidence, in 113 patients followed for 3 months, of 7% (95% CI 2.4-11.8 %). If we assumed a linear rate of ulcer development, the annual ulcer incidence could reach a high rate of 28%. Only 20% of patients had dyspeptic symptoms, which was not significantly different from patients without ulcer (12). The antrum and particularly the pre-pyloric area are the most frequent locations. All doses of LDA used in one study (13) (10mg, 81 mg and 325 mg) for 3 months, significantly reduced gastric mucosal prostaglandin concentration and significantly induced gastric mucosal injury. However, only aspirin at 81 mg and 325mg/day dose regimens reduced duodenal prostaglandin levels and only the 325 mg dose induced duodenal injury. These findings could explain aspirin's predominant gastric toxicity (7). However, the clinical significance of these endoscopic findings is unclear, since the incidence of GI complications is much lower. It is estimated that LDA use is associated with a 2-4-fold increase in symptomatic or complicated ulcers (3,6,12,14). The estimated average excess risk is five cases per 1,000 aspirin users per year (16). Observational studies have reported even higher risk estimates of upper GI bleeding (17-20). Death is the worst outcome of GI complication, but mortality data related to LDA are scarce and as commented above the risk-benefit balance is in favour in LDA when used in the secondary prevention of CV disease.

The association of LDA use with upper GI damage is well documented, however data on their effects on the lower GI tract is less clear. A systematic review found a small increase of fecal blood loss (0.5-1.5 ml per day) in LDA users. This amount increases up to 10 mL/day in some patients who take high dose aspirin (> 325mg/day) (21). One study in healthy volunteers showed that even enteric-coated LDA was associated with asymptomatic damage in 50% of volunteers, and some of them developed ulcers in their small bowel (22). However, it might be possible that these small bowel lesions could explain why some LDA users develop bleeding of "unknown" source, iron deficiency anaemia. A study in Health Professionals concluded that LDA increases significantly the risk of diverticular bleeding (23). A recent Japanese prospective study evaluated the effects of various drugs in diverticular disease. The drugs that significantly were associated with diverticular bleeding were some NSAIDs, LDA, clopidogrel and cilostazol. In another recent case-control study (24) that used data collected from consecutive patients hospitalized for gastrointestinal bleeding the use of anticoagulants, low-dose aspirin, and other drugs (non-aspirin-APA, 82.3% thienopiridines) was associated with upper and lower gastrointestinal bleeding; the risk was 2-fold higher for anticoagulants (RR, 4.2; 95% CI, 2.9-6.2) than for low-dose aspirin (RR, 2.1; 95% CI, 1.4-3.3) or other non-aspirin-APA drugs (RR, 2.0; 95% CI, 1.6-2.6).

The risk of development upper GI complications differs among LDA users. Risk factors are less well known than those for NSAIDs users, but in general, it is believed that risk factors for NSAIDs and LDA are the similar.

Several risk factors for GI bleeding in the setting of antiplatelet therapy have been reported: 1) history of peptic ulcer disease, 2) older age, 3) concomitant use of NSAIDs or other antiplatelet agents, 4) concomitant use of anticoagulants, 5) severe comorbidity, 6) higher aspirin doses, 7) H. pylori infection. Also, other potential risk factors have been mentioned; concurrent use of corticoids, male gender, smoking and alcohol use, and high body mass. The relative risk of GI bleeding increases with the number of adverse risk factors present in any patient.

Aspirin–induced GI complications seems to be dose related in the range of 30-1300 mg daily. But, this is based in great measure on indirect comparison of different trials and on a limited number of direct comparisons of different LDA doses (20,25-27). This dose-response relationship is though to reflect at least two COX-1 dependent components; 1) dose dependent inhibition of COX-1 in the GI mucosa and 2) dose independent inhibition of COX-1 in platelets. Because of that, the antithrombotic effect of aspirin can be dissociated, at least in part, from its GI effect. If we focus on the different doses of LDA, the results of several observational studies were inconsistent in demonstrating a lower GI risk with "low" LDA. (20, 25-28). Despite of this, current recommendation is to use the lowest possible aspirin dose (≤ 100 mg/ day) for the prevention of CV event, since within this dose range (75-100 mg/day) the aspirin effect on CV event prevention is not affected whereas the GI risk could be diminished.

Aspirin duration. The risk of GI complications with aspirin seems to be higher in the first month of treatment. With longer durations, the risk decreases but then remains constant over time (25-30). This phenomenon has been explained as the consequence of gastric adaptation to LDA (31) or as consequence of a fall in the proportion of susceptible individuals due to treatment withdrawal following GI intolerance, GI complication or other side effects.

Aspirin preparation. The use of enteric–coated or buffered preparations do not reduce the risk of GI complications (27). The reason for this is explained on the understanding that the main effect of aspirin on the gastric mucosa depends on the systemic effects rather than in the local "topical" effects of these compounds. (6).

Prevention of GI bleeding induced by aspirin

In order to minimize the upper GI damage induced by antiplatelets there are several strategies, which include: 1) reducing modifiable risk factors (including eradication of H. pylori infection); use the most appropriate aspirin dose and 2) use of gastroprotective agents

Reducing modifiable risk factors

Guidelines strongly suggest avoiding this combination if at all possible. In addition concomitant use of LDA with ibuprofen and perhaps naproxen should be avoided because these NSAIDs interfere with the antiplatelet effect of LDA. This is due to competition between both drugs for a common docking site within the COX-1 channel. If these combinations were used, LDA should be taken first and with time enough before dosing with ibuprofen or naproxen still interaction is possible. (32,33). Due to the long period of drug release with enteric-coated compounds, the chances of interaction between drug doses is very high.

In LDA users without history of peptic ulcer, the role of H. pylori eradication is controversial (34). Data are very scant. A small RCT, that included 32 patients, evaluated the role of eradication previous to begin long term LDA treatment. Eradication seemed to have a protective effect at 4 months follow-up (35). Several studies have evaluated the role of eradication in preventing recurrence of peptic ulcer (secondary prevention) in LDA users. Chan and colleagues performed a RCT that compared long term PPI treatment with H. pylori eradication as prevention strategies in patient H. pylori positive who had history of upper GI bleeding. The rebleeding rate was similar in both groups at 6 months of follow up, although the small sample size of the study left the question open. (36). The largest long-term prospective cohort study has been published recently and evaluated eradication as secondary prevention strategy (37). The incidence of upper GI bleeding were not significantly different between the H. pylori eradicated cohort (1.09; 95% CI 0.61-1.98) and the cohort of patients without history of peptic ulcer (0.67; 95%CI 0.42-1.06). Therefore, although eradication seems to be beneficial in LDA users as secondary prevention strategy, subanalysis of the H. pylori-related peptic ulcer bleeding cohort shows that despite of undergoing eradication, in the presence of other risk factors, these patients have higher risk of ulcer bleeding than the non- peptic ulcer history cohort. In summary, eradication may be an effective strategy to reduce risk of ulcer bleeding in patients taking LDA, but more evidence is necessary. Recommendation not to use maintenance PPI treatment in LDA users with history of ulcer bleeding may be yet too risky.

Gastroprotective agents

Three types of drugs have been used: misoprostol, proton pump inhibitors (PPI) and H2 receptor antagonists.

An endoscopic study showed that misoprostol, synthetic prostaglandin E1 significantly lowered the incidence of erosions in healthy volunteers taking LDA (38). Moreover, misoprostol seems to be superior to placebo for preventing recurrence of gastric ulcers among patients with prior peptic ulcer who are taking LDA and other NSAID (39). Side effects with misoprostol use when compared to other effective drugs have probably prevented the widespread use of this approach. No studies in the prevention g GI bleeding are available.

PPIs are potent inhibitors of gastric acid secretion. Several studies have explored the impact of PPI on reducing endoscopic damage and the risk of GI complications in users of LDA. PPI have been shown to prevent gastro duodenal ulcers associated with aspirin damage (40,41). The LAVANDER study (42) concluded that esomeprazole 20 mg over 48 weeks prevented the recurrence of peptic ulcers. Ulcer free rates were consistently lower in placebo group through week 48. The PLANETARIUM study, evaluated the efficacy and dose-response relationship (10 mg, 5 mg and active control) and safety of rabeprazole for peptic ulcer recurrence over 24 weeks in Japanese patients on LDA treatment. The cumulative recurrence rate of peptic ulcers were 1.4% and 2.8% in rabeprazole groups (5mg and 10mg, respectively), significantly lower that in the active control group (21.7%). In rabeprazole groups, there were not bleeding ulcers. (43). Lai et al. performed a RCT that compared lansoprazole (30 mg/ day) with placebo in LDA users with history of peptic ulcer and who had already received H. pylori eradication therapy (40). Patients on lansoprazole had significantly less recurrence of ulcer complications than those treated with placebo (1.6% vs 14.8%). Combined treatment (H. pylori eradication plus PPI) seems the most adequate therapy for these patients.

Aspirin interruption in GI bleeding events

The discontinuation of platelet inhibition in patients who develop an acute GI bleeding may have fatal consequences. There are two different scenarios: patients treated with LDA for CV prevention who develop upper GI bleeding and hospitalized patients who have just undergone a stent placement and develop a acute GI bleeding. Based on the current evidence, and in agreement with expert consensus report (15, 43), the best approach in patients with active ulcer bleeding should be treated endoscopically followed by high-dose PPI therapy. If endoscopy shows peptic ulcer with low-risk stigmata, LDA should not be withdrawn. However, if endoscopy shows high risk stigmata, LDA could be stooped and reintroduced early, preferably within 3 days after the last dose. If LDA was indicated for the primary prevention, a re-evaluation of the actual indication of LDA treatment should be performed and if considered appropriate reintroduced after ulcer healing or earlier. If the GI bleeding event occurs soon after the placement of coronary stent, the risk of

thrombosis is very high. In this setting, early endoscopy followed by high dose of PPI is the best option. A close collaboration with the cardiologist is necessary in order to balance the risks and benefits of maintaining one or the two antiplatelet agents.

References

1. Casado-Arroyo R et al. Role of ASA in the primary and secondary prevention of cardiovascular events. Best Pract Res Clin Gastroenterol. 2012;26(2):113–23.
2. US Preventive Services Task Force. Aspirin for the prevention of cardiovascular disease: US Preventive Services Task Force rec- ommendation statement. Ann Intern Med. 2009;150:396–404.
3. Lanas A, et al. Low doses of acetylsalicylic acid increase risk of gastrointestinal bleeding in a meta-analysis. Clin Gastroenterol Hepatol. 2011 Sep;9(9):762-768
4. Connolly BJ et al. Aspirin therapy and risk of subdural hematoma: meta-analysis of randomized clinical trials. J Stroke Cerebrovasc Dis. 2013 May;22(4):444-8
5. Gouya G, et al. .Antiplatelet treatment for prevention of cerebrovascular events in patients with vascular diseases: a systematic review and meta-analysis. Stroke. 2014 Feb;45(2):492-503.
6. Sostres C, et al. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol 2010; 24:121-32.
7. Cryer B, Feldman M. Effects of very low dose daily, long-term aspirin therapy on gastric, duodenal, and rectal prostaglandin levels and on mucosal injury in healthy humans.Gastroenterology. 1999;117:17-25.
8. Smecuol E, et al.. Low-dose aspirin affects the small bowel mucosa: results of a pilot study with a multidimensional assessment. Clin Gastroenterol Hepatol. 2009 May;7(5):524-9.
9. Patrignani P, et al. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. J Thromb Haemost. 2014 ;12:1320-30.
10. Lanas AI, et al. Aspirin related gastrointestinal bleeders have an exaggerated bleeding time response due to aspirin use. Gut. 1996 Nov;39(5):654-60.
11. Garcia-Gonzalez MA, et al. Gastroenterology 2013;144, Issue 5, S-141
12. Yeomans ND, et al. Prevalence and incidence of gastroduodenal ulcers during treatment with vascular protective doses of aspirin. Aliment Pharmacol Ther. 2005; 1;22(9):795-801.
13. Cayla G, et al. Prevalence and clinical impact of Upper Gastrointestinal Symptoms in subjects treated with low dose aspirin: the UGLA survey. Int J Cardiol. 2012; 156(1):69-75.
14. Weil J, et al. Prophylactic aspirin and risk of peptic ulcer bleeding. BMJ 1995;310:827–30.
15. Bhatt DL, et al. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol 2008;52:1502–17.
16. Hernández-Díaz S, García-Rodríguez LA. Cardioprotective aspirin users and their excess risk of upper gastrointestinal complications. BMC Med 2006 ; 4:22
17. Chan FK, et al. Eradication of Helicobacter pylori and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. Lancet 2002 ; 359(9300):9–13.
18. Moore A, et al. Evidence for endoscopic ulcers as meaningful surrogate endpoint for clinically significant upper gastrointestinal harm. Clin Gastroenterol Hepatol 2009; 7:1156–63.
19. García Rodríguez LA, et al. Association between aspirin and upper gastrointestinal complications: systematic review of epidemiologic studies. Br J Clin Pharmacol. 2001; 52(5):563-71.
20. Lanas A, et al. Risk of upper gastrointestinal ulcer bleeding associated with selective cyclo-oxygenase-2 inhibitors, traditional non-aspirin non-steroidal anti-inflammatory drugs, aspirin and combinations. Gut. 2006 ;55(12):1731-8.
21. Moore RA, et al. Faecal blood loss with aspirin, nonsteroidal anti-inflammatory drugs and cyclo-oxygenase-2 selective inhibitors: systematic review of randomized trials using autologous chromium-labelled erythrocytes. Arthritis Res Ther. 2008;10(1):R7
22. Smecuol E, et al. Low-dose aspirin affects the small bowel mucosa: results of a pilot study with a multidimensional assess- ment. Clin Gastroenterol Hepatol. 2009;2009(7):524–9.
23. Strate LL, et al. Use of aspirin or nonsteroidal anti-inflammatory drugs increases risk for diverticulitis and diverticular bleeding. Gastroenterology. 2011;140(5):1427–33.
24. Lanas A, et al. Risk of Upper and Lower Gastrointestinal Bleeding in Patients Taking Non-steroidal Anti-inflammatory Drugs, Antiplatelet Agents, or Anticoagulants. Clin Gastroenterol Hepatol 2015; 13(5):906-12.
25. Cea Soriano L, Rodriguez LA. Risk of upper gastrointestinal bleeding in a cohort of new users of low-dose asa forsecondary prevention of cardiovascular outcomes. Front Pharmacol 2010;1:126.
26. Serrano P, et al. Risk of upper gastrointestinal bleeding in patients taking low-dose aspirin for the prevention of cardiovascular diseases. Aliment Pharmacol Ther 2002;16:1945–53.
27. de Abajo FJ, Garcia Rodriguez LA. Risk of upper gastrointestinal bleeding and perforation associated with low-dose aspirin as plain and enteric-coated formulations. BMC Clin Pharmacol 2001;1:1.
28. McQuaid KR, Laine L. Systematic review and meta-analysis of adverse events of low-dose aspirin and clopidogrel in randomized controlled trials. Am J Med 2006;119:624–38.

29. Lanas A, et al. Nitrovasodilators, low-dose aspirin, other nonsteroidal antiinflammatory drugs, and the risk of upper gastrointestinal bleeding. N Engl J Med 2000;343:834–9.
30. Baigent C, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373:1849.
31. Graham DY, et al.. Gastric adaptation. Studies in humans during continuous aspirin administration.. Gastroenterology 1988;95:327
32. Capone ML, et al. Pharmaco- dynamic interaction of naproxen with low-dose aspirin in healthy subjects. J Am Coll Cardiol. 2005;45:1295–301.
33. Li X, et al. T. Differential impairment of aspirin-dependent platelet cyclooxygenase acetylation by nonsteroidal antiinflammatory drugs. Proc Natl Acad Sci U S A. 2014;111(47):16830-5.
34. Sostres C, et al. Peptic Ulcer Bleeding Risk. The Role of Helicobacter Pylori Infection in NSAID/Low-Dose Aspirin Users. Am J Gastroenterol. 2015;110(5):684-9
35. Giral A, et al. Gastroenterol Hepatol. 2004 ;19(7):773-7
36. Chan FK, et al. Preventing recurrent upper gastrointestinal bleeding in patients with Helicobacter pylori infection who are taking low-dose aspirin or naproxen. N Engl J Med. 2001;344(13):967-73
37. Chan FK et al.. Effects of Helicobacter pylori infection on long-term risk of peptic ulcer bleeding in low-dose aspirin users. Gastroenterology. 2013;144(3):528-35.
38. Yeomans N, et al. Efficacy of esomeprazole (20 mg once daily) for reducing the risk of gastroduodenal ulcers associated with continuous use of low-dose aspirin. Am J Gastroenterol 2008 Oct;103(10):2465–73.
39. Scheiman JM, et al. Prevention of peptic ulcers with esomeprazole in patients at risk of ulcer development treated with low-dose acetylsalicylic acid: a randomized, controlled trial (OBERON). Heart 2011 May;97(10):797–802.
40. Lai KC, et al. Lansoprazole for the prevention of recurrences of ulcer complications from long-term low-dose aspirin use. N Engl J Med 2002 Jun 27;346(26):2033–8.
41. Sugano K, et al. Multinational, double-blind, randomised, placebo-controlled, prospective study of esomeprazole in the prevention of recurrent peptic ulcer in low-dose acetylsalicylic acid users: the LAVENDER study. Gut. 2014 ;63(7):1061-8.
42. Iwakiri R, et al. Randomised clinical trial: prevention of recurrence of peptic ulcers by rabeprazole in patients taking low-dose aspirin. Aliment Pharmacol Ther. 2014 Oct;40(7):780-95.
43. Barkun AN, et al. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. Ann Intern Med. 2010 ;152(2):101-13

Platelets versus extra-platelet components of aspirin pharmacodynamics

Paola Patrignani, PhD, Professor of Pharmacology

Department of Neuroscience, Imaging and Clinical Sciences, Section of Cardiovascular and Pharmacological Sciences, "G. d'Annunzio" University, Chieti, Italy. E-mail: ppatrignani@unich.it

Synopsis

Indirect evidences suggest that the antiplatelet effect of aspirin plays an important role both in the prevention of atherothrombosis and cancer. In addition to their contribution to tumor metastasis, platelets may play a role in the early phases of tumorigenesis. Acetylation of cyclooxygenase (COX)-1 at serine-529 is the direct mechanism of action of low-dose aspirin as an antiplatelet agent. To address whether low-dose aspirin(enteric coated aspirin 100 mg daily administered to individuals undergoing CRC screening) preferentially targets platelet COX-1 versus extraplatelet sources of COX-1, e.g. colorectal mucosa, we used a novel assay which quantifies the extent of acetylation of COX-1. The results show a preferential impact of low-dose aspirin towards platelet COX-1. A lower extent of colorectal COX-1 acetylation was detected and this effect was associated with changes of rectal mucosa phenotype. In conclusion, the concurrent inhibitory effect of low-dose aspirin on COX-1 in platelets

Lecture Notes

Indirect clinical and experimental evidences suggest that mechanisms involved in the development of atherothrombosis are shared by colorectal cancer (CRC) (1,2). Both clinical conditions are associated with unremitting inflammation and enhanced platelet activation as a consequence of tissue damage/dysfunction. Moreover, the results of clinical studies have shown that the chronic administration of aspirin, even at the low-doses (75-100 mg daily) recommended for the prevention of cardiovascular disease, is associated with a reduction of cancer incidence and mortality, in particular colorectal cancer (CRC) (3-5). The data of the clinical pharmacology of low-dose aspirin show that the drug acts by causing a preferential inhibition of platelet cyclooxygenase(COX)-1 activity versus extraplatelet cellular sources of COX-isozymes (6,7). Thus, it has been proposed that similarly to its efficacy in preventing cardiovascular disease, the antiplatelet effect of the drug may play a central role in cancer chemoprevention. This hypothesis is convincingly supported by the efficacy for cancer prevention of a slow-release matrix formulation of 75 mg dose of aspirin used in the Thrombosis Prevention Study (TPT) (8).

In fact, this form of aspirin has been shown to affect platelet COX-1 in the presystemic compartment while leaving unaltered an index of systemic vascular COX-2 activity, such as the urinary levels of the enzymatic metabolite of PGI2, 2,3-dinor-6-keto-PGF1a(9). TPT contributed perhaps the largest amount of data of all the trials surveyed by Rothwell et al for cancer prevention (4.5). However, the trial has the limitation that lacked a conventional formulation of the same dose of aspirin as a comparator.

At the earliest phases of colorectal tumorigenesis, platelets may be activated as a consequence of intestinal epithelial damage/dysfunction induced by life-style and environment factors. This is a physiological mechanism to repair the damage. However, if the platelet response is unconstrained, it may contribute to the development of chronic inflammation through the persistent activation of stromal fibroblasts and immune cells. In the context of persistent platelet-stromal cell activation, intestinal epithelial cells may undergo epithelial-mesenchymal transition (EMT) which is a reversible dedifferentiation process that converts epithelial cells into cells with mesenchymal features (10). Activation of EMT participates in the generation of tumor-initiating cells in vivo (11) but also triggers tumor cell invasion and metastasis to distant organs (10).

Platelet-stromal cell cross-talk may contribute to the development of the vicious circle that leads to a chronic inflammatory response. Several lines of experimental evidence suggest a key role played by inflammatory signaling in the development of EMT and generation of tumor-initiating cells in vivo. Moreover, epithelial cells acquire phenotypic changes, including COX-2 overexpression, which enhances their tumorigenic potential (12). Intestinal epithelial cells expressing COX-2 attach to ECM components (e.g., laminin) with greater avidity than cells that do not express this enzyme, express lower levels of TGF-bII

receptor and E-cadherin, resist to apoptosis and express high levels of the anti-apoptotic protein BCL-2. Recently, it has been shown a functional role for COX-2 in the regulation of epidermal growth factor receptor (EGFR) (13), a transmembrane receptor tyrosine kinase of the ErbB family implicated in the etiology of CRC.

Numerous evidences sustain a key role of COX-2 overexpression in the development and progression of cancer, in particular CRC (14,15). Among them, it is noteworthy the efficacy of selective COX-2 inhibitors (named coxibs) to decrease the risk of colorectal adenoma recurrence (16-20). However, the use of coxibs as chemopreventive agents seems to be inappropriate due to the interference with cardiovascular homeostasis by the coincident inhibition of vascular COX-2-dependent PGI2 (21).

A three-step model has been proposed to explain the potential contribution of blood platelets in colorectal carcinogenesis (22,23): (1) activated platelets may promote early events of tumorigenesis through the release of several factors [i.e., thromboxane (TX)A2, ADP, growth and angiogenic factors and inflammatory cytokines]; (2) they trigger an inflammatory response in stromal cells leading to COX-2 induction and the release of soluble mediators, i.e. prostanoids and proteins, such as cytokines, growth and proangiogenic factors; (3) these events may participate in epithelial cell transformation into an adenomatous lesion in part as a consequence of COX-2 overexpression and EMT induction.

Another mechanism which might take part in the early phases of colorectal tumorigenesis involves an altered biosynthesis of prostaglandin(PG)E2 in intestinal epithelial cells via the COX-1 pathway. COX-1 is constitutively expressed in the gastrointestinal tract and plays a role to maintain a basal rate of prostanoid biosynthesis, mainly PGE2, which are involved in protecting the gastrointestinal mucosa by stimulating the synthesis and secretion of mucus and bicarbonate, inhibiting gastric acid secretion, increasing mucosal blood flow and promoting epithelial proliferation (24). The cellular levels of PGE2 are under the control of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), in particular the Type I PGDH which uses nicotinamide adenine dinucleotide (NAD+) as a cofactor (25). This enzyme catalyses the first step in the conversion of PGE2 into its significantly less biologically active metabolite 15-keto-PGE2. 15-PGDH is a colorectal tumour suppressor and it is downregulated in colorectal carcinomas and even in very small adenomas from patients with familial adenomatous polyposis (FAP) (26). It has been proposed that 15-PGDH downregulation is a very early event in intestinal tumorigenesis occurring even before COX-2 induction (27). Thus, enhanced PGE2 might be produced early in colorectal neoplasia through the activity of COX-1 and PGDH downregulation.

In addition to the role of activated platelets in the early phases of intestinal tumorigenesis, platelets may contribute to tumor metastasis through different mechanisms(28): (i) the formation of platelet aggregates surrounding tumor cells which support tumor cell survival and protection from immune elimination; (ii) the increase of the adhesion of tumor cells to the endothelium thus leading to tumor cell arrest and extravasation; (iii) tumor vascularization; (iv) the induction of a malignant phenotype in epithelial cancer cells via the phenomenon of EMT (29,30). Thus, the antiplatelet effect of aspirin is plausibly involved in the findings of Rothwell et al. [31, 32] showing that aspirin prevention of distant metastasis could account for the early reduction of mortality of different types of cancer in trials of daily aspirin versus control.

In order to clarify the mechanisms of action of aspirin in the prevention of initial stages of intestinal tumorigenesis, we have performed a clinical study in individuals undergoing CRC screening. The primary objective was to compare the effects of low-dose enteric coated aspirin (100 mg daily for a week) on platelets versus extraplatelet cellular targets using a direct biomarker of drug action, i.e. the acetylation of COX-1 at serine-529. To provide definitive evidence of the impact of low-dose aspirin on the platelet versus extra-platelet components of aspirin pharmacodynamics we used a novel assay of aspirin action which assesses the extent of acetylation of COX-1 at serine-529 (33); this is the specific target of aspirin when administered at therapeutic doses (34). In fact, the cyclooxygenase activity of acetylated of COX-1 is irreversibly inhibited and the protein is unable to produce prostanoids. We used a novel strategy for the absolute quantification (termed AQUA) of proteins and their modification states which consists in the use of peptides with incorporated stable isotopes as ideal internal standards to mimic native peptides formed by proteolysis (35). Such AQUA internal standard peptides are then used to precisely and quantitatively measure the levels of proteins and post-translationally modified proteins after proteolysis by using a selected reaction monitoring analysis in a tandem mass spectrometer (MS).

We compared this direct biomarker of aspirin action with indirect biomarkers, including prostanoid biosynthesis ex vivo and in vivo. Finally, the impact of chronic aspirin treatment on molecular pathways implicated in the etiology of CRC, including mTOR signaling, EGFR and COX-2 expression and the tumor suppressor pathway mediated by 15-PGDH has been investigated in rectal tissue.

The results of this study show a preferential impact of low-dose aspirin towards platelet COX-1. However a lower extent of colorectal COX-1 acetylation was detected and this effect was associated with changes of rectal mucosa phenotype.

In conclusion, the use of a novel and direct assay of drug action has allowed to shed light into aspirin pharmacodynamics in preventing colorectal cancer. Our results suggest that the concurrent inhibitory effect of low-dose aspirin on COX-1 in platelets and colorectal epithelium may impact the development of CRC at early stages.

References

Patrono C, Patrignani P, García Rodríguez LA. Cyclooxygenase-selectiveinhibition of prostanoid formation: transducing biochemical selectivity intoclinical read-outs. J Clin Invest 2001; 108(1): 7-13. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol 2012; 9(5): 259-67.

Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol 2012; 9(5): 259-67.

Flossmann E, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet 2007; 369(9573): 1603-13.

Rothwell PM, Wilson M, Elwin CE, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality, 20-year follow-up of five randomised trials. Lancet 2010; 376(9754): 1741–1750.

Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011; 377(9759): 31-41.

Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest 1982; 69(6): 1366-72.

FitzGerald GA, Brash AR, Oates JA, Pedersen AK. Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthase in man. J Clin Invest 1983; 72(4): 1336-43.

Meade TW, The Medical Research Council's General Practice Research Framework. Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischaemic heart disease in men at increased risk. Lancet 1998; 351(9098): 233-41.

Clarke RJ, Mayo G, Price P, FitzGeral GA. Suppression of thromboxane A2 but not of systemic prostacyclin by controlled-release aspirin. N Engl J Med 1991; 325(16): 1137–1141.

KalluriR , Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009; 119(6): 1420-8.

Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Göktuna SI, Ziegler PK, Canli O, Heijmans J, Huels DJ, Moreaux G, Rupec RA, Gerhard M, Schmid R, Barker N, Clevers H, Lang R, Neumann J, Kirchner T, Taketo MM, van den Brink GR, Sansom OJ, Arkan MC, Greten FR. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties Cell. 2013 Jan 17;152(1-2):25-38.

Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell. 1995;83(3):493-501.

Li H, Zhu F, Boardman LA, et al. Aspirin prevents colorectal cancer by normalizing EGFR expression. EBioMedicine 2015. http://dx.doi.org/10.1016/j.ebiom.2015.03.019.

Dixon DA, Blanco FF, Bruno A, Patrignani P. Mechanistic aspects of COX-2 expression in colorectal neoplasia. Recent Results Cancer Res 2013; 191: 7-37.

Prescott SM. Is cyclooxygenase-2 the alpha and the omega in cancer? J Clin Invest 2000; 105(11): 1511-3.

Arber N, Eagle CJ, Spicak J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med 2006; 355(9): 885–95.

Bertagnolli MM, Eagle CJ, ZauberAG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med 2006; 355(9): 873–84.

Baron JA, Sandler RS, Bresalier RS, et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology 2006; 131(6): 1674–82.

Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000; 342(26): 1946-52.

García Rodríguez LA, Cea-Soriano L, Tacconelli S, Patrignani P. Coxibs: pharmacology, toxicity and efficacy in cancer clinical trials. Recent Results Cancer Res 2013; 191: 67-93.

Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. J Clin Invest 2006; 116(1): 4-15.

Guillem-Llobat P, Dovizio M, Alberti S, Bruno A, Patrignani P. Platelets, cyclooxygenases, and colon cancer. Semin Oncol 2014; 41(3): 385-96.

Dovizio M, Alberti S, Guillem-Llobat P, Patrignani P. Role of platelets in inflammation and cancer: novel therapeutic strategies. Basic Clin Pharmacol Toxicol 2014; 114(1): 118-27.

Patrono C, Patrignani P, García Rodríguez LA. Cyclooxygenase-selectiveinhibition of prostanoid formation: transducing biochemical selectivity intoclinical read-outs. J Clin Invest 2001; 108(1): 7-13.

Ensor CM, Tai HH. 15-hydroxyprostaglandin dehydrogenase. J Lipid Mediat Cell Signal 1995; 12(2-3): 313-319.

Myung SJ, Rerko RM, Yan M, et al. 15-Hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis. Proc Natl Acad Sci USA 2006; 103(32): 12098–12102.

Smartt HJ, Greenhough A, Ordóñez-Morán P, et al. β-catenin represses expression of the tumour suppressor 15-prostaglandin dehydrogenase in the normal intestinal epithelium and colorectal tumour cells. Gut 2012; 61(9): 1306-14.

Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. Nat Rev Cancer 2011; 11(2): 123–134.

Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 2011; 20(5): 576–590.

Dovizio M, Maier TJ, Alberti S, et al. Pharmacological inhibition of platelet-tumor cell cross-talk prevents platelet-induced overexpression of cyclooxygenase-2 in HT29 human colon carcinoma cells. Mol Pharmacol 2013; 84(1): 25–40.

Rothwell PM, Price JF, Fowkes FG, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet 2012; 379(9826): 1602–1612.

Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on riskof cancer metastasis: a study of incident cancers during randomised controlled trials. Lancet 2012; 379(9826): 1591–160.

Patrignani P, Tacconelli S, Piazuelo E, Di Francesco L, Dovizio M, Sostres C, Marcantoni E, Guillem-Llobat P, Del Boccio P, Zucchelli M, Patrono C, Lanas A. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. J Thromb Haemost. 2014;12(8):1320-30.

Patrono C, Baigent C, Hirsh J, Roth G. Antiplatelet drugs: American college of chest physicians evidence-based clinical practice guidelines (8th Edition). Chest 2008; 133(Suppl 6): 199S-233S.

Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. Proc Natl Acad Sci USA 2003; 100: 6940–5.

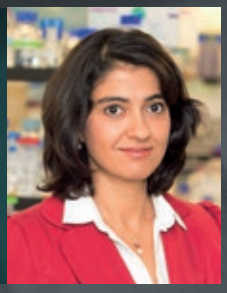
2015 Scientific Conference
Aspirin in the 21st Century - Common mechanisms of disease and their modulation by Aspirin

Session Two

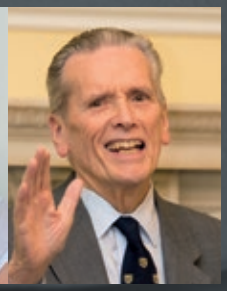
Aspirin in cancer
Chair person Ruth Langley

Primary prevention review
Andy Chan – USA

Effects of aspirin on colorectal cancer
Farhat Din – UK



Opportunities for using aspirin to treat cancer
Ruth Langley & Peter Elwood



A Review of Aspirin's Role in the Primary Prevention of Cancer

Andrew T. Chan, MD, MPH

Associate Professor of Medicine, Harvard Medical School, USA. Email: ACHAN@mgh.harvard.edu

Synopsis

Remarkably consistent experimental and epidemiologic evidence demonstrates that aspirin is associated with a lower risk of colorectal cancer. Five placebo-controlled randomized controlled trials (RCTs) among individuals with a history of colorectal neoplasia showed that aspirin reduced the risk of recurrent adenomas, the precursors of the vast majority of cancers. Additionally, data from long-term follow-up of a RCT of aspirin among individuals with the Lynch hereditary colorectal cancer syndrome and a RCT of aspirin among women for primary prevention of cardiovascular disease and cancer demonstrated a lower risk of colorectal cancer associated with randomized aspirin treatment. Moreover, recent results from secondary analyses of RCTs of aspirin for cardiovascular prevention has shown that aspirin's effects may extend to benefits for cancers beyond the colon, suggesting a case for a broader role for aspirin in cancer prevention. In this presentation, we will review the evidence supporting a role for aspirin in the primary prevention of cancer, with a focus on colorectal cancer.

Effects of aspirin on energy and metabolism signalling in colorectal cancer

Farhat VN Din BSc MBChB MD FRCS

Institute of Genetics and Molecular Medicine, University of Edinburgh, UK. Email: Farhat.Din@igmm.ed.ac.uk

Synopsis

Colorectal cancer is eminently preventable, and yet it is the 2nd most common cause of cancer death in the UK (15,700 deaths annually, 55% 5-year survival) and worldwide incidence is 1.24 million (~610,000 deaths). There is substantial rationale for developing approaches aimed at defining and modifying colorectal cancer risk. Chemoprevention is a promising research avenue, aiming to reverse or prevent cancer initiation and/or impede progression from premalignant to invasive disease[1].

Compelling epidemiological and randomised trial data demonstrate that aspirin has striking chemopreventive properties against colorectal cancer. Aspirin substantially reduces colorectal cancer incidence and mortality (RR ~ 0.6)[2,3]. We have shown protection even with low dose aspirin (75mg) in average risk subjects[4]. Moreover, aspirin may also improve survival from colorectal cancer[5]. Understanding the mechanisms underlying these beneficial effects would enable more effective, and safer, chemopreventive approaches. It would also shed new light on cellular and molecular mechanisms underlying colorectal carcinogenesis.

Lecture Notes

Colorectal cancer is a complex disorder, with both environmental and genetic factors influencing disease causation. Environmental exposure accounts for 50-60% of the variance in risk. Several colorectal cancer risk factors result in energy metabolism imbalance including type 2 diabetes mellitus, obesity, physical inactivity and metabolic syndrome. Although the colorectal cancer genetic landscape is complex; somatic driver mutations converge on a few key pathways governing cellular fate and metabolism[6].

Physical activity is associated with 30% lower colorectal cancer risk, whilst obesity increases risk by ~10% overall (30-70% in men)[7,8]. Given this background, it appears that energy-metabolism imbalance may initiate and promote colorectal cancer[9].

The mTOR pathway is pivotal role in controlling cell survival, regulation of metabolism and energy homeostasis[9]. mTOR integrates stimuli from growth factors, nutrients and several signalling pathways which converge at the TSC1/2 complex[10]. Growth factors, via Akt and ERK, induce phosphorylation of TSC2 and increase mTOR activity[11]. In contrast, TSC2 phosphorylation by AMP kinase (AMPK) leads to mTOR inhibition. Hence, both environmental and genetic risks converge on aberrant signalling pathways which alters colorectal crypt metabolism and growth. Crucially metabolic activity determines cell fate and mTOR may act as a rheostat for stem cell maintenance [10].

In colorectal tumorigenesis mTOR is progressively dysregulated from early aberrant crypt foci to adenoma to carcinoma, suggesting pathway dependency driven by metabolic requirements[11, 12]. Obesity and physical inactivity increase colorectal cancer risk emphasising metabolic derangement as a key link between obesity and cancer.

Aspirin may prevent colorectal cancer through targeting several pathways during disease initiation and progression from adenoma to carcinoma. Given intratumoral heterogeneity and redundancy in signalling pathways, there is unlikely to be a single cancer prevention molecular target. Drugs which modulate multiple targets increase efficacy by circumventing negative feedback signalling[8]. Aspirin influences several pathways leading to colorectal cancer cell death but the effects on complex signalling relationships are not fully elucidated. mTOR dysregulation is implicated in colorectal carcinogenesis, supporting mTOR signalling as a promising target. Determining the mechanism of action is critical to developing safer agents. We have shown that the anti-tumour activity of aspirin may be attributed to aspirin's potent inhibition of mTOR signalling and activation of AMPK in colorectal cancer[13]. Our current work examines the effect of aspirin-induced mTOR inhibition on translation regulation in colorectal cancer. We show that aspirin not only inhibits initiation of protein translation but may also play a key role in inhibiting translation elongation. Our data present novel insights into the molecular effects of aspirin on protein translation in colorectal cancer.

References:

1. M. B. Sporn, Cancer Res 36, 2699 (1976).
2. M. J. Thun et al., N Engl J Med 325, 1593 (1991).
3. P. M. Rothwell et al., The Lancet 376, 1741 (2010).
4. F. V. Din et al., Gut 59, 1670 (2010).
5. A. T. Chan et al., JAMA 302, 649 (2009).
6. B. Vogelstein et al., Science 339, 1546 (2013).
7. R. M. Speck et al., in Physical Activity, Dietary Calorie Restriction and Cancer, A. McTiernan, Ed. (Springer, 2011), vol. 3.
8. M. Bardou et al., Gut 62, 933 (2013).
9. S. D. Hursting, Cancer Treat Res 159, 21 (2014).
10. R. C. Russell et al., Development 138, 3343 (2011).
11. N. Alqurashi et al., Human Pathology 44, 2089 (2013).
12. Y. J. Zhang et al., Ann Surg Oncol 16, 2617 (2009).
13. F. V. Din et al., Gastroenterology 142, 1504 (2012).

Opportunities for using aspirin to treat cancer

Ruth Langley & Peter Elwood

This talk will focus on the evidence supporting the use of aspirin as a potential treatment for cancer and opportunities to incorporate aspirin into current treatment paradigms. In particular we will review the epidemiological evidence demonstrating beneficial effects of aspirin use after a cancer diagnosis in preventing metastases and prolonging survival. We will also present the evidence relating to specific tumour mutations as potential biomarkers of aspirin response, and discuss the challenges relating to the perceived and actual bleeding rates on aspirin. Finally we will present a number of planned or ongoing clinical trials aimed at establishing a role for aspirin in the treatment of cancer.

Some key relevant papers:

Langley RE, Burdett S, Tierney JF, Cafferty F, Parmar MK, Venning G. Aspirin and cancer: has aspirin been overlooked as an adjuvant therapy? Br J Cancer. 2011; 105(8): 1107-13. Gasic GJ, Gasic TB, Murphy S. Anti-metastatic effect of aspirin. Lancet. 1972; 2(7783): 932-3. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials.

Lancet. 2010; 376(9754): 1741-50.

Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials.

Lancet. 2011; 377(9759): 31-41.

Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, Tognoni G, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet. 2012; 379(9826): 1602-12.

McCowan C, Munro AJ, Donnan PT, Steele RJ. Use of aspirin post-diagnosis in a cohort of patients with colorectal cancer and its association with all-cause and colorectal cancer specific mortality. Eur J Cancer. 2013; 49(5): 1049-57.

Bastiaannet E, Sampieri K, Dekkers OM, de Craen AJ, van Herk-Sukel MP, Lemmens V, et al.

Use of aspirin postdiagnosis improves survival for colon cancer patients. Br J Cancer. 2012; 106(9): 1564-70.

Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA. 2009; 302(6): 649-58.

Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin Use, Tumor PIK3CA Mutation, and Colorectal-Cancer Survival. New England Journal of Medicine. 2012; 367(17): 1596-606.

Domingo E, Church DN, Sieber O, Ramamoorthy R, Yanagisawa Y, Johnstone E, et al.

Evaluation of PIK3CA Mutation As a Predictor of Benefit From Nonsteroidal Anti-Inflammatory Drug Therapy in Colorectal Cancer. J Clin Oncol. 2013; 31(34): 4297-305.

Tran B, Jorissen RN, Desai J, Day F, Lipton LR, Wong H-L, et al. Aspirin use and survival outcomes in patients (pts) with PIK3CA mutant colorectal cancer (CRC). ASCO Meeting Abstracts. 2013; 31(15_suppl): 3598.

Kothari N, Yeatman TJ, Fisher K, Schell MJ, Kim RD. Association of aspirin use with improved 5-year survival in colorectal cancer patients with PIK3CA mutation. ASCO Meeting Abstracts. 2013; 31(15_suppl): 3644.

Holmes MD, Chen WY, Schnitt SJ, Collins L, Colditz GA, Hankinson SE, et al. COX-2 expression predicts worse breast cancer prognosis and does not modify the association with aspirin. Breast Cancer Research and Treatment. 2011; 130(2): 657-62.

Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, Tognoni G, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet. 2012.

Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. Lancet. 2012.

Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. Ann Intern Med. 2013; 159(2): 77-85.

Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet. 2012; 378(9809): 2081-7.

Holmes MD, Chen WY, Li L, Hertzmark E, Spiegelman D, Hankinson SE. Aspirin intake and survival after breast cancer. J Clin Oncol. 2010; 28(9): 1467-72.

Bastiaannet E, Sampieri K, Dekkers OM, de Craen AJ, van Herk-Sukel MP, Lemmens V, et al.

Use of Aspirin postdiagnosis improves survival for colon cancer patients. Br J Cancer. 2012.

Liu J-F, Jamieson GG, Wu T-C, Zhu G-J, Drew PA. A Preliminary Study on the Postoperative Survival of Patients Given Aspirin After Resection for Squamous Cell Carcinoma of the Esophagus or Adenocarcinoma of the Cardia. Annals of Surgical Oncology. 2009; 16(5): 1397-402.

Langley RE Clinical evidence for the use of aspirin in the treatment of cancer Ecancermedicalscience.2013

Session Three

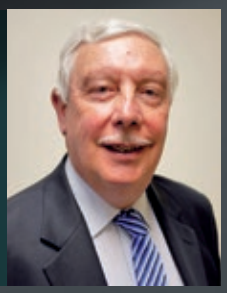
Other aspirin-sensitive mechanisms of disease
Chair person Mike Gaziano

Hughes Syndrome, Aspirin and the Spectrum of Pregnancy
Graham Hughes – UK

Platelet activation and inhibition in diabetes mellitus
Carlo Patrono - Italy

Aspirin in HIV
Andrew Freedman – UK

Aspirin in treatment of acute pain
Ron Eccles UK



Hughes Syndrome, Aspirin and the Spectrum of Pregnancy

Graham Hughes

Head of The London Lupus Centre, London Bridge Hospital, London.
Email: Graham.Hughes@HCAConsultant.co.uk

Synopsis

The antiphospholipid syndrome (APS) is an auto-immune prothrombotic syndrome, with a tendency to both venous and (critically) arterial thrombosis. It is also a cause of miscarriage, now being recognised as the commonest, treatable cause of recurrent pregnancy loss.

The syndrome, diagnosed by the presence of antiphospholipid antibodies (aPL), is becoming recognised worldwide as common, with cases of APS seen in every practice, presenting, for example, with migraine, memory loss, epilepsy, angina, recurrent miscarriage.

In obstetrics, aPL are associated not only with recurrent miscarriage (some women suffering a dozen or more miscarriages), but also with intra uterine growth retardation, pre-eclampsia and late pregnancy loss (stillbirth).

Treatment to date has largely been directed towards anticoagulation – aspirin, heparin and warfarin – with limited experience of the newer oral anticoagulants.

The initial studies of aspirin use in aPL positive women in pregnancy were striking, a previous pregnancy success rate of under 20% increasing to over 90%.

The combination of aspirin and low molecular weight heparin has become the chosen treatment for aPL-positive women with severe pregnancy histories and/or previous thrombosis. Current topics of discussion include whether to test all pregnancies, or all miscarriages or all cases of multiple pregnancy loss.

In summary, aspirin is playing a major role in the management of pregnancy loss. The introduction of more widespread aPL testing (simple inexpensive blood tests) will help direct treatment to more vulnerable subsets of patients.

References:

1. Hughes GRV.
   Hughes Syndrome/APS. 30 years on, what have we learnt? Opening talk at the 14th International Congress on Antiphospholipid Antibodies, Rio de Janeiro, October 2013.
   LUPUS 2014 23 : 1225-1226
2. Wong LF, Porter TF, de Jesus GR.
   Recurrent early pregnancy loss and antiphospholipid antibodies. Where do we stand?
   LUPUS 2014 23 : 1226-1228
3. Hughes GRV & Khamashta MA
   Hughes Syndrome : Highways & Byways
   Published by: Springer

Platelet Activation and Inhibition in Diabetes Mellitus

Carlo Patrono

Adjunct Professor of Pharmacology, Catholic University School of Medicine, Rome, Italy.
Email: cpatrono@unich.it

Synopsis

Both type 1 diabetes mellitus (T1DM) and T2DM are characterized by persistently enhanced TXA2 biosynthesis (1,2). When a coronary plaque undergoes fissuring or rupture, blood platelets are primed to over-react contributing to increased thrombotic risk in this setting. ESC/EASD guidelines recommend using the same antithrombotic strategy in DM as in patients without DM. However, there is a lack of dedicated studies in DM, and the results of sub-group analyses should be interpreted with caution.

The conventional once daily dosing regimen of aspirin and thienopyridines may be sub-optimal in at least a fraction of the T2DM population (3), and RCTs testing a personalized antiplatelet regimen (eg, bid) are warranted (4).

The residual cardiovascular risk in acute coronary syndromes, despite improved dual or triple antiplatelet regimens, is substantial (>10% at 1 yr in DM patients), and the mechanisms underlying these events deserve further investigation (5).

References

Davì G, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C. Thromboxane biosynthesis and platelet function in type-II diabetes mellitus. N Engl J Med, 322:1769-1774, 1990.

Davì G, Chiarelli F, Pomicio MP, Santilli F, Vigneri S, Falco A, Basili S, Ciabattoni G, Patrono C. Enhanced lipid peroxidation and platelet activation in the early phase of type 1 diabetes mellitus. Role of interleukin-6 and disease duration. Circulation, 107:3199-3203, 2003.

Rocca B, Santilli F, Pitocco D, Mucci L, Petrucci G, Vitacolonna E, Lattanzio S, Mattoscio D, Zaccardi F, Liani R, Vazzana N, Del Ponte A, Ferrante E, Martini F, Ghirlanda G, Davì G, Patrono C. The Recovery of Platelet Cyclooxygenase Activity Explains Interindividual Variability in Responsiveness to Low-Dose Aspirin in Patients With and Without Diabetes. J Thromb Haemost, 10:1220-30, 2012.

Patrono C. The multifaceted clinical read-outs of platelet inhibition by low-dose aspirin. J Am Coll Cardiol, 2015 (in press).

Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med, 357:2482-2494, 2007.

Aspirin in HIV

Andrew Freedman, MA, MB, B Chir, MD, FRCP,

Reader in Infectious Diseases/Honorary Consultant Physician, Cardiff University School of Medicine.
Email: Freedman@cardiff.ac.uk

Synopsis

The advent of highly active antiretroviral therapy (HAART) in the late 1990s had a dramatic effect in reducing mortality in patients with HIV and AIDS. However, patients on fully suppressive HAART have a residual increased risk of non-AIDS-defining morbidities, including cardiovascular, renal, hepatic and neurological disease, as well as malignancies. Evidence suggests that a state of heightened immune activation and inflammation may be a significant contributory factor. A short term, pilot study of low dose aspirin given for one week to patients with HIV on treatment and control subjects demonstrated significantly increased platelet activation at baseline in HIV patients. Aspirin led to a fall in platelet aggregation and cellular activation markers, including sCD14. Two large, randomized trials of aspirin in HIV patients on HAART are underway, with results from the first expected later this year.

In the meantime, studies indicate that, at present, aspirin is markedly under-prescribed in patients with HIV and cardiovascular risk factors.

Lecture notes

Prior to the advent of combination antiretroviral therapy in the late 1990s, the majority of patients with HIV infection progressed to advanced disease (AIDS) after an average of 8-10 years and this was universally fatal (1). The situation now is very different & the majority of patients, if not diagnosed too late, can expect a near normal life expectancy (2, 3). HIV is now a chronic treatable condition but, despite full viral suppression on therapy, there remains an increase in a variety of morbidities. These include malignancy as well as cardiovascular, renal, hepatic and neurological disease. These co-morbidities are likely to become more prevalent as the cohort of patients with HIV gets older.

Cardiovascular Disease

Studies have demonstrated increased rates of both myocardial infarction (4) and ischaemic stroke (5) in patients with HIV compared to age-matched controls. Venous thrombo-embolic disease appears also to be more frequent, esp in advanced HIV disease. The causes of vascular disease in HIV infection include direct effects of HIV, and antiretroviral drug toxicity, particularly abacavir (6), as well as traditional risk factors, such as smoking. The mechanisms by which HIV itself cause vascular disease remain incompletely understood, but there is evidence for vascular endothelial dysfunction, increased coagulation activity and platelet aggregation (7), as well as increases in serum lipids.

Chronic Immune Activation

There is a growing body of evidence that HIV infection is associated with heightened immune activation and a 'chronic inflammatory state' (8). Importantly, this is only partially reversed by fully suppressive antiretroviral therapy and it has been postulated that this persisting inflammation may lead to the various morbidities described above. A number of theories have been proposed to account for this, including direct effects of ongoing, low level viral replication, loss of normal immune regulation, co-infections such as CMV, and damage to the gut mucosa. It is believed that the latter leads to a compromised mucosal barrier that allows translocation of microbial products, particularly lipopolysaccharide (LPS), a component of Gram negative bacterial cell walls that binds CD14, triggering immune activation (8).

Two recent studies (9,10) have demonstrated that serum inflammatory markers, particularly soluble CD14 (sCD14), are independent predictors of both morbidity from non-AIDS events (myocardial infarction, stroke, malignancies and serious bacterial infections) and overall mortality. The authors of both these studies concluded that agents which can reduce this immune activation, given in conjunction with antiretroviral therapy, might be beneficial and should be evaluated in clinical trials.

Trials of Aspirin

The first pilot study of aspirin in patients with HIV was undertaken by Meaghan O'Brien and colleagues (11). 25 such patients, all on antiretroviral therapy, as well as 44 HIV negative controls, were given Aspirin, 81 mg daily, for one week. Measurements were performed at baseline and following treatment, and included platelet aggregation, markers of cellular activation and inflammatory markers. At baseline, platelet activation was significantly increased in the HIV patients compared to the controls, as were markers of T-cell activation (CD38 and HLA-DR) and monocytes (sCD14). After one week of aspirin, ADP and arachidonic acid induced platelet aggregation fell in all subjects and

there was also a fall in CD38, HLA-DR and sCD14 in the HIV patients. No change in CRP, IL-6 or D-dimer was observed. It was concluded that the study provided evidence that platelet activation may contribute to immune activation and inflammation in HIV and that low dose aspirin might have a possible therapeutic role in attenuating this.

To investigate this further, the same group have undertaken a prospective, double blind, randomized trial (ACTG 5331) (12) of aspirin 300mg vs 100mg vs placebo for 12 weeks with 40 HIV patients in each of the 3 arms. All patients were taking fully suppressive ART. The primary endpoint was plasma sCD14 concentration; secondary measures included other inflammatory markers, lymphocyte subsets, urinary thromboxane as well as endothelial function, assessed by brachial artery flow mediated dilatation. As of now, all patients have completed the studies but the samples are yet to be analysed and results are towards the end of this year (Meaghan O'Brien, personal communication).

A second NIH trial (13) is also underway in HIV elite controllers (the small subset of patients who maintain undetectable viral loads without needing ART) as well as patients on suppressive ART, randomized to receive either low dose aspirin (81mg) or atorvastatin (40mg) once daily for 9 months. Again the primary outcome measure will be soluble CD14 & other measures will include carotid artery MRI. Results of this study will not be available until 2017.

HIV-associated neurocognitive disorders (HAND)

HIV is known to spread to the central nervous system soon after initial infection. The virus infects brain macrophage and microglial cells. A proportion of patients with HIV develop varying degrees of cognitive impairment & in a few, this progresses to dementia. In the majority, however, neurocognitive impairment is mild and usually asymptomatic. HAND does not always improve with the initiation of antiretroviral therapy, and indeed can occasionally develop in patients already taking fully suppressive treatment. The pathophysiology of HAND is not fully understood but is believed to involve persistent microglial cell activation and plasma sCD14 levels have been reported to be higher in HIV patients with lower neurocognitive test scores (14).

To investigate the potential therapeutic effect of aspirin in HAND, Blanchard (15) used a HIV-1 transgenic (HIV-1 Tg) rat model. HIV-1 Tg rats develop neuropathology and exhibit behavioural changes as they age. They have significantly higher brain arachidonic acid (AA) -derived eicosanoid levels than wild-type rats. However, these levels fell to those of wild-type rats after 42 days of feeding them low dose aspirin in their drinking water. This suggests low dose aspirin may have a role in reducing the upregulated brain AA metabolism and potentially the progression of HAND.

Current usage of aspirin in HIV as 1° and 2° CHD prevention

Most guidelines for the treatment and care of patients with HIV emphasise the importance of assessing cardiovascular risk and addressing risk factors. Regular monitoring of weight, blood pressure, lipids and blood sugar are recommended, as are smoking cessation, dietary modification, exercise and statin therapy, when indicated. There is, however, very little mention of the use of aspirin. Two recently published studies highlight the fact that aspirin is relatively under-prescribed in this patient group, compared to the general population.

Burkholder and colleagues performed a cross-sectional study of nearly 2,000 patients attending the HIV clinic in Birmingham, Alabama in 2010 (16). They calculated that 400 of them qualified to receive aspirin as primary CVD prevention, according to the US Preventive Services Task Force guidelines (based on 10 year CHD risk in men and 10 year stroke risk in women). However, only 66 of these (17%) were prescribed aspirin; for those in the highest risk groups (> 10%), the figure was still only 22%.

More recently Suchindran and colleagues (17) investigated prophylactic aspirin usage in over 4,000 HIV patients and 36,00 HIV negative control subjects enrolled in the Partners HealthCare System in Boston, USA. The overall rate of aspirin usage was lower in the HIV infected patients than the uninfected controls – 12.4 % vs 15.3% ($p < 0.001$). A greater difference was observed in rates for those with high cardiovascular risk – 22.1% vs 42.4% ($p < 0.001$), but a much smaller, but still significant difference for those with prevalent CHD (51.6% vs 65.4%). They suggested that the lower rate of aspirin usage in the HIV patients, may be explained in part by higher rates of conditions that would contra-indicate aspirin, such as chronic liver disease. However, they also highlighted the absence of CHD prevention guidelines specific to the HIV population and the lack of clinical trial data on aspirin in this group.

References

1. Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus infection. N Engl J Med 1993; 328: 327-35.
2. Palella FJ Jr, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr. 2006 Sep;43(1):27-34.
3. May M, Gompels M, Delpech V, Porter K, Post F, Johnson M, Dunn D, Palfreeman A, Gilson R, Gazzard B, Hill T, Walsh J, Fisher M, Orkin C, Ainsworth J, Bansi L, Phillips A, Leen C, Nelson M, Anderson J, Sabin C. Impact of late diagnosis and treatment on life expectancy in people with HIV-1: UK Collaborative HIV Cohort (UK CHIC) Study. BMJ 2011 Oct 11; 343: d6016.

4. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007 Jul; 92(7): 2506-12.
5. Chow FC, Regan S, Feske S, Meigs JB, Grinspoon SK, Triant VA,. Comparison of ischemic stroke incidence in HIV-infected and non-HIOV-infected patients in a US health care system. J Acquir Immun Defic Syndr. 2012 Aug 1; 60(4): 351-8.
6. Friis-Møller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr WM, Reiss P, Thiébaut R, Morfeldt L, De Wit S, Pradier C, Calvo G, Law MG, Kirk O, Phillips AN, Lundgren JD; Data Collection on Adverse Events of Anti-HIV Drugs (DAD) Study Group. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med. 2003 Nov 20;349(21):1993-2003.
7. Gresele P, Falcinelli E, Sebastiano M, Baldelli F. Endothelial and platelet function alterations in HIV-infected patients. Thromb Res. 2012 Mar; 129(3): 301-8.
8. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006 Dec;12(12):1365-71.
9. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC; INSIGHT SMART Study Group. Plasma levls of soluble CD14 independently predict mortality in HIV infection. J Infect Dis. 2011 Mar 15;203(6):780-90.
10. Tenorio AR, Zheng Y, Bosch RJ, Krishnan S, Rodriguez B, Hunt PW, Plants J, Seth A, Wilson CC, Deeks SG, Lederman MM, Landay AL. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis. 2014 Oct 15;210(8):1248-59.
11. O'Brien M. Montenont E, Hu L, NardiMA, Valdes V, Merolla M, Gettenberg G, Cavanagh K, Aberg JA, Bhardwaj N, Berger JS. Aspirin attenuates platelet activation and immune activation in HIV-1-infected subjects on antiretroviral therapy: a pilot study. J Acquir Immune Defic Syndr. 2013Jul 1; 63(3): 280-8.
12. ACTG 5331: Modulation of immune activation by aspirin. https://clinicaltrials.gov/ct2/show/NCT02155985.
13. Elite controller and ART-treated HIV+ statin versus ASA treatment intervention study. https://clinicaltrials.gov/ct2/show/NCT02081638.
14. Plasma sCD14 is a biomarker associated with impaired neurocognitive test performance in attention and learning domains in HIV infection. Lyons, JL, Uno H, Ancuta P, Kamat A, Moore DJ, Singer EJ, Morgello S, Gabuzda, D. J Acquir Immune Defic Syndr. 2011 Aug 15;57(5):371-9.
15. Blanchard HC, Taha AY, Rapoport SI, Yuan ZX. Low-dose aspirin (acetylsalicylate) prevents increases in brain PGE2, 15-epi-lipoxin A4 and 8-isoprostane concentrations in 9 month-old HIV-1 transgenic rats, a model for HIV-1 associated neurocognitive disorders. Prostaglandins Leukot Essent Fatty Acids 2015;96:25-30.
16. Burkholder GA, Tanhane AR, Salinas JL, Mugavero MJ, Raper JL, Westfall AO, Saag MS, Willig JH. Underutilization of aspirin for primary prevention of cardiovascular disease among HIV-infected patients. Clin Infect Dis. 2012 Dec; 55(11): 1550-7.
17. Suchindran S, Regan S, Meigs JB, Grinspoon SK, Triant VA. Aspirin Use for Primary and Secondary Prevention in Human Immunodeficiency Virus (HIV)-Infected and HIV-Uninfected Patients. Open Forum Infect Dis, 2014; 1(3):ofu076.

Aspirin in treatment of acute pain

Professor Ron Eccles BSc, PhD, DSc

Director of Common Cold Centre and Healthcare Clinical Trials, Cardiff University, Wales. Email: Eccles@cardiff.ac.uk

Synopsis

This presentation will discuss the unusual origins of aspirin's analgesic activity from the role of salicylic acid as a plant hormone to the acetylation of salicyclic acid to form aspirin. The origins of aspirin as an analgesic go back millions of years to the evolution of plant defense mechanisms and the use of salicylic acid as a plant hormone for activation of plant defense mechanisms.

Salicylic acid inhibits plant enzymes similar to the cyclo oxygenase enzymes found in animals, and this is the unusual and chance effect that makes aspirin such an effective analgesic. The acetylation of salicylic acid to form aspirin was done to increase tolerability, but again by chance, and not design, it produced an amazing medicine that is taken in many millions of doses every day.

Introduction

Aspirin is the most widely used medicine in the world, and billions of tablets are consumed each year to relieve common aches and pains. It has the advantage of being cheap, and freely available without prescription, and throughout its long history of use as a pain killer it has been established as a safe and effective treatment. What I find fascinating about aspirin is how plant products containing salicylic acid were first discovered to be effective in treating pain and inflammation and then how salicylic acid was acetylated by reaction with acetic acid to produce acetyl salicylic acid (ASA) or aspirin. Dr Felix Hoffman first synthesised pure and stable ASA in 1897 in an attempt to develop a salicylate that had fewer side effects than salicylic acid(1). But in acetylating the salicylic acid he produced a much more effective medicine, and it would take nearly a hundred years of research to discover how important the acetylation was in the mechanism of action of ASA as a medicine.

Salicylic acid and its plant origins

The story of the discovery of the beneficial effects of plant extracts containing salicylic acid goes back thousands of years. The Ebers papyrus which was written about 3,500 years ago recommended the use of dried leaves of Myrtle to treat rheumatic pains and similarly Hippocrates used tree barks containing salicylates to treat the pain of child birth and fever(2). The first tests or trial of salicylates was made in 1763 by a country parson, Edward Stone, who used Willow bark to treat fevers as he believed the treatment for diseases such as fevers was to be found in those areas where the disease was common, and fever was often seen in damp wet areas were Willow trees were commonly found growing(1). The latin name for Willow is Salix and this gives its name to the extract from Willow bark, salicin and salicylates. The name Aspirin is believed to originate from spiric acid which is another name for salicylic acid, and spiric acid was obtained from plants such as Meadowsweet (Filipendula ulmaria) of the genus Spirea. An extract of Meadowsweet was used by German chemists as a source of salicylic acid for their experiments on producing a less toxic pain killer, and this lead to the eventual discovery of Aspirin.

Plants are the world's best biochemists and organic chemists, and they produce a huge number of medicines that are useful to man; local anaesthetics such as cocaine, muscle relaxants such as curare, heart stimulants such as digitalis, pain killers such as morphine and codeine, and common cold remedies such as eucalyptus and menthol. Plants are not altruistic, and they do not waste metabolic energy synthesising biochemicals for human use – so what is the benefit to a plant in producing these biochemical? In most cases the biochemical are produced to deter predators from eating the plants. Many plant biochemical such as cocaine and morphine are toxic to the central nervous system of animals, and a caterpillar or larger animal eating a plant containing these toxic biochemical will become ill or die as a result. Menthol is a good example of a plant biochemical widely used by man in common cold remedies, chewing gum and mints, but which is toxic to plant predators such as snails and slugs(3). Menthol interacts with CNS neuronal cation channels for calcium and is toxic in high doses to slugs and snails, but in lower doses in man, acts on sensory neuronal calcium channels to give a cool refreshing sensation.

Salicylic acid is present in a wide range of plants and as described above is useful to man as an analgesic and antipyretic. Salicylic acid does not appear to act as a deterrent to plant predators, and the reason that it is produced by so many plant species is that it acts as a phytohormone or messenger, to regulate plant growth and development, and also to initiate plant defences against pathogens such as viruses(4). Plants that are bred without genes to synthesise salicylic acid are much more susceptible to viral infections(5).

The biochemistry of plants shares many fundamental biochemical pathways to those of animals, as plants and animals divided from a common ancestor that was already a sophisticated biochemical organism with all the basic

biochemical pathways present for metabolism and life. Plants produce prostaglandin like molecules such as jasmonic acid as local mediators, and the biosynthesis of jasmonic acid mirrors those of animal prostaglandin derivatives of arachadonic acid(6). Jasmonic acid has multiple functions and effects on plant physiology and therefore has a similar physiological mediator role to animal prostaglandins, but it is especially involved in plant defence against infection(7). Jasmonic acid is synthesised from linolenic acid by lipoxygenase enzymes (LOX) and these enzymes are inhibited by salicyclic acid that is produced by the plant as a phytohormone(6, 7).

Thus it seems that the benefit to man of aspirin as an analgesic is related to the role of salicylic acid in plants as a phytohormone and inhibitor of plant LOX, an enzyme closely related to animal cyclo oxygenase enzymes(COX), that synthesise animal prostaglandins involved in pain.

Mechanism of action of aspirin in acute pain

The mechanism of action of aspirin was clarified in 1971 when Vane and Smith showed that aspirin and indomethacin blocked the synthesis of prostaglandins through inhibition of cyclo oxygenase enzymes(COX)(8, 9). The history and implications of this discovery have been reviewed in detail by Vane and Botting in 2003(10). Prostaglandins are numerous and they have wide ranging functions in the body from control of reproduction and kidney function to secretion of mucus in the stomach but their influence on acute pain is mainly mediated by PGE2 which acts to sensitise pain nerve endings to the actions of bradykinin(10, 11). Aspirin has a very specific effect on COX as it acetylates the enzyme at a crucial structural point that is important for the activity of the enzyme. The location of the acetylation is now known in detail as one of the serine residues (Ser 530) located 70 amino acids from the C terminus of the enzyme(12). This acetylation is irreversible and it means the enzyme is permanently inhibited and new enzyme must be synthesised to restore COX activity. The irreversible inactivation of COX is important for long term effects in platelets as new enzyme cannot be synthesised in platelets, but in inflamed tissue new enzyme is continually being synthesised and the inactivated enzyme can be replaced.

The initial discovery of plant extracts such as Willow bark as analgesics for acute pain utilised the effects of salicylic acid and salicylates and not aspirin. Salicylates are effective reversible inhibitors of COX but not as potent as aspirin(13). Aspirin is rapidly metabolised to salicylates after ingestion and it has been debated that the major action of aspirin as an analgesic is to act as a pro-drug for salicylates, and that the major analgesic effect of aspirin is mediated by salicylates rather than aspirin(14). If salicylates are the major source of analgesia when aspirin is ingested then the analgesic effect is obviously not limited to acetylation of COX by aspirin, and other mechanisms are also likely to be involved(15, 16).

Efficacy of aspirin in treatment of acute pain

Aspirin has been used as an analgesic for over 100 years and it was the first freely available analgesic to be made widely available to the public because of its unquestioned efficacy and its lack of addictive properties. Because aspirin was universally accepted as an effective analgesic there was little need to demonstrate efficacy in clinical trials, and the hard scientific and clinical evidence for efficacy came late in the history of aspirin.

Aspirin is now widely used for treatment of acute pain in headache and migraine, common cold and flu, muscle aches and pains, menstrual pain, toothache and pain after tooth extraction, and minor aches and pains of arthritis(17). Randomised, placebo controlled clinical trials have been conducted in all these pain conditions and they now provide hard clinical evidence for the efficacy of aspirin as an analgesic.

In order to study the efficacy of analgesics it is necessary to have a patient pain model that is relatively predictable and standardised, and the pain associated with removal of impacted third molars has proven useful as a model for clinical trials because it is a relatively common dental procedure and the timing and intensity of the pain is predictable(18). Studies on dental pain have consistently demonstrated the efficacy of aspirin above placebo and also indicate that aspirin is a more useful analgesic than paracetamol in the control of postoperative pain after third molar surgery(18).

Aspirin is effective in treatment of both migraine and tension-type headache and it is recommended as first-line treatment for each of these disorders in various management guidelines(19, 20).

Pain associated with common cold such as sore throat pain is one of the most common minor pains because of the high incidence of common cold throughout the world. Aspirin has been shown to be effective in treating sore throat pain, muscle aches and headache associated with common cold(21), and also shown to be effective in treating fever associated with upper respiratory tract infection(22).

Patients with acute pain demand an analgesic that provides rapid relief of pain and studies on aspirin have shown that the formulation of the aspirin product has marked effects on its absorption and pharmacokinetics. Simple aspirin tablets have the slowest absorption into the blood stream and the speed of absorption can be greatly increased by administering aspirin as an effervescent formulation, and can be further speeded by buffering the aspirin(23, 24). The time to peak plasma concentration of aspirin varies between 33-83 minutes in tablet form, and recently the rate of absorption has been greatly accelerated by using micronized tablets with an effervescent nucleus, so that absorption time can be reduced to only 18 minutes(20).

Aspirin's journey from plant hormone to the world's most popular medicine

Salicylic acid was synthesised in plants millions of years before humans such as Edward Stone and Felix Hoffman investigated its efficacy as a treatment for acute pain. Plants, as said above, are far superior to man when it comes to developing biochemicals that have beneficial effects in man as medicines. In this case, salicyclic acid was synthesised in plants as a hormone messenger that could activate plant defence mechanisms against infection, and part of this response involved turning off or inhibiting another plant mechanism mediated by jasmonic acid which is also a plant hormone. Salicyclic acid inhibits the synthesis of jasmonic acid by inhibiting plant LOX enzymes that are similar to the COX enzymes in animals.

Over a thousand years of trial and error humans discovered plants that had beneficial effects on disease, and discovered the beneficial effects of plants containing salicyclic acid for the treatment of pain and inflammation. Clinical and scientific investigations like those of Edward Stone in 1763 confirmed the benefits of salicylic acid as a treatment for pain and fever, but it took another hundred years or more before salicyclic acid could be extracted in a pure form from plants and then acetylated by Felix Hoffman in 1897 to produce the less irritant aspirin. By acetylating salicylic acid Hoffman not only increased the tolerability of the medicine, but by chance, introduced the acetyl group that could now be irreversibly attached to the COX enzyme, causing permanent inhibition of the enzyme which is especially relevant for its actions in platelets which cannot synthesise new enzyme.

The aspirin story is not yet over and new developments and therapeutic opportunities for aspirin have been found in the treatment of cancer and Alzheimer's disease, as well as its role in treatment of cardiovascular diseases. Aspirin is a truly remarkable medicine and its future as probably the most commonly used and versatile medicine in the world is assured.

References

1. Vane JR, Botting RM, editors. Aspirin and other salicylates. London: Chapman & Hall; 1992.
2. Vane J, Botting R. The history of aspirin. Aspirin and other salicylates. 1992:3-16. In; Vane JR, Botting RM, editors. Aspirin and other salicylates. London: Chapman & Hall
3. Eccles R. Menthol and related cooling compound s. J Pharm Pharmacol. 1994;46:618-630.
4. Kumar D. Salicylic acid signaling in disease resistance. Plant Sci. 2014;228:127-134.
5. Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, et al. A Central Role of Salicylic-Acid in Plant-Disease Resistance. Science. 1994;266(5188):1247-1250.
6. Schultz JC. Shared signals and the potential for phylogenetic espionage between plants and animals. Integrative and Comparative Biology. 2002;42(3):454-462.
7. Pozo MJ, Van Loon LC, Pieterse CMJ. Jasmonates - Signals in plant-microbe interactions. Journal of Plant Growth Regulation. 2004;23(3):211-222.
8. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971;231(25):232-235.
9. Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nat New Biol. 1971;231(25):235-237.
10. Vane JR, Botting RM. The mechanism of action of aspirin. Thromb Res. 2003;110(5-6):255-258.
11. Claria J. Cyclooxygenase-2 biology. Curr Pharm Des. 2003;9(27):2177- 2190.
12. Roth GJ, Majerus PW. The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. J Clin Invest 1975;56(3):624-632.
13. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci U S A. 1993;90(24):11693-11697.
14. Higgs G, Salmon J. Is aspirin a pro-drug for salicylate? In: Vane J, Botting R, editors. Aspirin and other salicylates. London: Chapman Hall; 1992. p. 63 - 73.
15. Wu KK. Aspirin and salicylate - An old remedy with a new twist. Circulation. 2000;102(17):2022-2023.
16. Tegeder I, Pfeilschifter J, Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. FASEB J. 2001;15(12):2057-2072.
17. Gaciong Z. The real dimension of analgesic activity of aspirin. Thromb Res. 2003;110(5-6):361-364.
18. Seymour RA, Hawkesford JE, Sykes J, Stillings M, Hill CM. An investigation into the comparative efficacy of soluble aspirin and solid paracetamol in postoperative pain after third molar surgery. Br Dent J. 2003;194(3):153-157.
19. Lampl C, Voelker M, Steiner TJ. Aspirin is first-line treatment for migraine and episodic tension-type headache regardless of headache intensity. Headache. 2012;52(1):48-56.
20. Lecchi M, D'Alonzo L, Negro A, Martelletti P. Pharmacokinetics and safety of a new aspirin formulation for the acute treatment of primary headaches. Expert Opin Drug Metab Toxicol. 2014;10(10):1381-1395.
21. Eccles R, Loose I, Jawad M, Nyman L. Effects of acetylsalicylic acid on sore throat pain and other pain symptoms associated with acute upper respiratory tract infection. Pain Med. 2003;4(2):118-124.
22. Bachert C, Chuchalin AG, Eisebitt R, Netayzhenko VZ, Voelker M. Aspirin compared with acetaminophen in the treatment of fever and other symptoms of upper respiratory tract infection in adults: a multicenter, randomized, double-blind, double-dummy, placebo-controlled, parallel-group, single-dose, 6-hour dose-ranging study. Clin Ther. 2005;27(7):993-1003.
23. Leonards JR. The influence of solubility on the rate of gastrointestinal absorption of aspirin. Clin Pharmacol Ther. 1963;4:476-479.
24. Davison C. Salicylate metabolism in man. Ann N Y Acad Sci. 1971;179:249-268.

Notes

Notes

Notes

Notes

The International Aspirin Foundation Scientific Advisory Board 2014-2015

Dr Andrew T Chan MD MPH
Boston. USA

Professor J Michael Gaziano MD
Boston, USA

Professor Ronald Eccles
Cardiff, UK

Dr Ruth Langley PhD FRCP
London, UK

Professor Peter Elwood MD (Chair)
Cardiff, UK

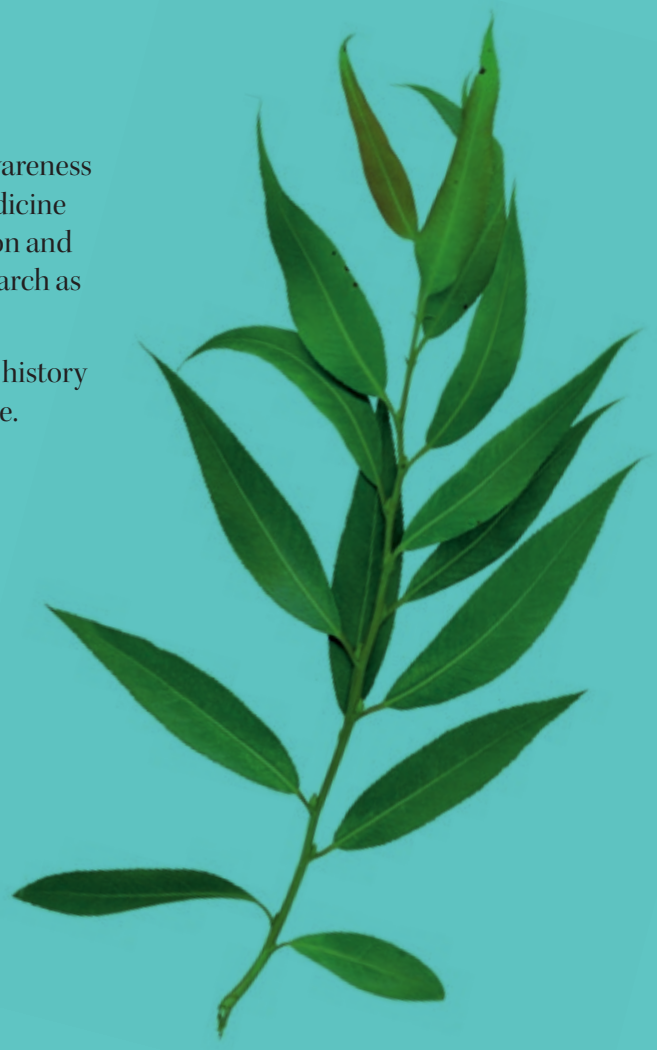
Professor Carlo Patrono MD
Rome, Italy

The International Aspirin Foundation aims to increase the knowledge and understanding of aspirin, probably the world's oldest and most widely used medicine.

The International Aspirin Foundation helps to increase awareness and medical research interest in this vitally important medicine by stimulating the distribution and exchange of information and discussion on all aspects of Aspirin, including current research as well as old and new therapeutic uses for it.

Aspirin is a versatile and trusted home remedy with a long history that also promises important new applications in medicine.

The Aspirin Foundation is guided by an independent International Scientific Advisory Board.



For further information please contact

International Aspirin Foundation
34 Bower Mount Road
Maidstone
Kent ME16 8AU

Tel: +44(0) 7764 616122
Fax: +44(0) 1436 840194
Email: aspirin@healthcom.eu.com

www.aspirin-foundation.com