REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 63 (2006)

Pharmacotherapeutic Potential of the P2 Receptors of Extracellular Nucleotides

Jean-Marie Boeynaems, Bernard Robaye and Didier Communi

Institute of Interdisciplinary Research, School of Medicine, Université Libre de Bruxelles, 1070 Brussels and 6041 Gosselies, Belgium

The release of nucleotides in extracellular fluids can result from cell necrosis, exocytosis of secretory granules, vesicular trafficking and efflux through membrane channels. Once in the extracellular fluids, nucleotides are degraded by ubiquitous ectonucleotidases that belong to two major families: the E-NTPDases (ecto-nucleoside triphosphate diphosphohydrolase) and the E-NPPases (ecto-nucleotide pyrophosphophatase/phosphodiesterase). The biological actions of extracellular nucleotides are mediated by two classes of membrane receptors. The P2X receptors are ion channels encoded by seven different genes. The P2Y receptors are coupled to G proteins and eight subtypes have been identified. Whereas P2X receptors are receptors of ATP, the different P2Y receptors are activated by distinct nucleotides, diphosphates or triphosphates, purines or pyrimidines, some of them being conjugated to sugars. The study of knockout mice has demonstrated that the P2X receptors play important roles in the neurogenic control of smooth muscle contraction, in pain and visceral perception and in macrophage functions. The phenotype of P2Y null mice is so far more restricted. Inhibition of platelet aggregation in response to ADP, increased bleeding time and resistance to thromboembolism are observed in both $P2Y_1^{-/-}$ and $P2Y_{12}^{-/-}$ mice. The only medicinal products currently on the market which target P2 receptors are the thienopyridines ticlopidine and clopidogrel, that produce a covalent inhibition of the $P2Y_{12}$ receptor via an active metabolite. UTP stimulates the secretion of chloride and water by various epithelial cells via the $P2Y_2$ receptor in the airways and the $P2Y_4$ receptor in the intestine. UTP derivatives with an extended half-life are currently in clinical trial for two indications related to their mucus and surface hydration properties : dry eye syndrome and cystic fibrosis. Nucleotides can also act as danger signals modulating the immune response. Their release may be a consequence of pathogen invasion or of an excessive inflammatory response. Accordingly they exert both pro- and antiinflammatory actions. In particular ATP acting via the $P2Y_{11}$ receptor induces a semi-maturation state of human dendritic cells that could be associated with immune tolerance. Indeed ATP stimulates the release of thrombospondin-1, that might activate latent TGF-β on the surface of regulatory T cells, and potentiates the activation of indoleamine 2,3-dioxygenase, an enzyme that exerts immunosuppressive effects on T cells via tryptophan depletion. These recent results suggest that $P2Y_{11}$ agonists might be used as tolerogenic adjuvants in therapeutic vaccination.

REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 64 (2006)

The Role of S1P Pathway in Cardiovascular and Inflammatory Processes

Giuseppe Cirino

Dipartimento di Farmacologia Sperimentale, Via Domenico Montesano 49, 80131 Napoli, Italy

Sphingosine-1-phosphate (S1P) is derived from sphingosine the backbone of most sphingolipids and it is now emerging as a vital lipid mediator. Sphingosine was named in 1884 after the Greek mythological creature, the Sphinx, because of its enigmatic nature. S1P was originally considered to be formed merely as an intermediate in the detoxification of sphingosine, by its phosphorylation and subsequent degradation, but since the discovery that S1P regulates cell growth and suppresses programmed cell death, there has been an explosion of important physiological and pathophysiological processes that are reported to be regulated by S1P in higher organisms.

Administration of exogenous SIP in vivo causes an oedema that is fast in onset and accompanied by a large eosinophil infiltrate. Similarly i.p. administration of S1P rapidly recruits eosinophils into the peritoneal cavity. The oedema formation is accompanied by a selective increase in rat paw tissue of CCR3. Purified human eosinophils constitutively express higher mRNA levels $S1P_1$ and to a lower extent $S1P_2$ and $S1P_3$. Challenging of eosinophils in vitro with S1P causes chemotaxis coupled with a strong upregulation of CCR3 and RANTES message. These data show that in inflammatory events, where S1P is released, eosinophils are involved leading to an immune response that is most likely the Th2 type.

Recent studies have indicated the importance of S1P in the cardiovascular system, particularly its essential role in vascular development and a potential role in cardiovascular diseases. Cellular levels of S1P are low and tightly regulated by sphingosine kinase (SPK). Moreover S1P is released by platelets during platelet activation and can be detected in significant amounts in the serum. S1P was found to act as an extracellular mediator by binding to specific members of the EDG-family of G-protein coupled receptors, now termed $S1P_{1-5}$ receptors and as an intracellular messenger. Interestingly, its effects depend on its source e.g. extracellular vs intracellular. Particularly, its role in cardiovascular system has been ascribed to its receptor-mediated effects. The majority of the studies, until now, explored the extracellular pathway ($S1P/S1P_1$) through which S1P regulates eNOS.

Recently we have investigated on the potential intracellular second messenger role of S1P in eNOS activation in aortic vessel and on the contribution of this alternate pathway in the control of vascular tone. We have determined by negative ESI LC-MS that aortic rings incubated with Ach produce detectable amount of S1P indicating that the sphingolipid pathway contributes to the ach-induced vasorelaxation. The contribution of this pathway to signalling appears to be important in vessel function since the blockade of S1P generation, by using specific inhibitors of sphingolipid pathway such as a known inhibitor of sphingosine kinaase (DTD), reduces by about 50% the vasorelaxant effect of Ach. The eNOS/hsp90 co-immunoprecipitation experiments strongly confirm this hypothesis suggesting that sphingosine kinase is a prerequisite for eNOS activation. The eNOS/hsp90 co-immunoprecipitation experiments consistently showed increased association of hsp90 with eNOS following exposure of cells to BK or calcium ionophore, A-23187. Interestingly, in sharp contrast to A23187, BK effects were significantly inhibited by pre-treatment of the cells with the SPK inhibitor DTD. These data suggest an involvement of calcium as a trigger for the eNOS/hsp90 coupling. Surprising exogenous S1P induced the co-immunoprecipitation of hsp90 with eNOS too, coupling abrogated by pre-treatment with DTD. In addition DTD abrogated S1P-induced vasorelaxation of rat aortic rings, suggesting that S1P promotes, via $S1P_1$ activation, its own intracellular synthesis through SPK. Thus the intracellular pool of S1P enhances hsp90/eNOS interaction through intracellular calcium and in turn increases NO production.

Thus an interplay exists among SPK, S1P, hsp90, and eNOS where intracellularly generated S1P promotes the coupling of eNOS to hsp90 and this contributes to the modulation of vascular tone as demonstrated by our functional and molecular studies.

REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 65 (2006)

Regulatory Issues of Pharmacogenetics: A National and a European Perspective

Gottfried Kreutz, Dr.-med., Dipl.-Chem., Director and Professor

International Medicinal Product Matters, Federal Ministry for Health, D - 53121 Bonn, Germany

Pharmacogenetic information has gained increasing recognition in medicine in recent years.

The importance in the context of development of medicinal products has been appreciated by many stakeholders involved in this process.

The professional public is discussing *personalized medicine* as a new approach to therapy with medicinal products. Hopes are nourished that when increased specificity of treatment by exposing those subpopulations to a treatment only which have optimal benefit treatment failure or incorrect dosing frequency may be reduced. Additionally and not less importantly identifying subpopulations prone to increased adverse reaction frequency due to pharmacogenetically based susceptibility may less frequently or less severely be followed by adverse effect experience or become excluded from exposure before avoidable risks are taken.

Many pharmaceutical companies have started to regularly study Pharmacogenetic issues at an early stage of development. They are increasingly genotyping and phenotyping subjects or patients exposed in all phases within a development programme. Additionally they are asking for advice from regulatory authorities before a new step in the development of a medicinal product is undertaken and they are increasingly interested in discussing pharmacogenetic information before a next step in the development is approached or an application for performing a specific study or an application for approval is launched.

Regulatory authorities have increasingly been involved by transforming pharmacogenetic data into recommendations for the application of a medicinal product as provided with the Standard of Product Characteristic as a regulatory document. They have also been involved by applicants and asked to provide early advice and define regulatory standards which normally have to be fulfilled.

The Committee on Human Medicinal Products (CHMP) as the responsible body to provide professional advice to the European Medicines Agency (EMEA) and to the European Commission has set up a permanent working group, the Pharmacogenetic Working Party (PgWP), to help with these tasks. Experts from many European countries are members to this group.

Aspects of the organization of this work and some of its content will be presented.

REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 66 (2006)

Chemical Biology in Academia: Challenges and Opportunities

Joe D. Lewis Ph.D.

Chemical Biology Core Facility, EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Small molecules play essential roles in many areas of basic research and are often used to address important biological questions. The ability to identify and characterize small molecules that can be used as *biotools* is a highly interdisciplinary undertaking. To enable this at the EMBL, the Chemical Biology Core Facility was inaugurated at the beginning of 2004 with the aim of allowing research groups to identify and optimize small molecule *biotools* with which to dissect biological processes. The Facility provides the hardware and software infrastructure, compound libraries and expertise to guide research groups through developing assays for high through-put screening and characterization of active compounds. Further chemical optimization of hit compounds can be done through one of our chemistry partners.

The challenges and opportunities of pursuing this exciting area of research in an academic environment will be discussed. Cases studies that demonstrate the utility of the *biotool* approach in providing novel biological insights to basic cellular processes, from both cell based and biochemical screens, will shown. For example, structural insights derived by the Conti group at EMBL as to how the Aurora A kinase activity is controlled has demonstrated that there is an allosteric regulatory site which can be bound by TPX2. We have performed a screen to find inhibitors of Aurora A kinase and identified a small number of compounds that may target this allosteric site. The targeting of Aurora kinases through allosteric mechanism of action, in contrast to ATP competitive compounds, may provide an alternative mechanism for dissecting their role in the cell cycle.

REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 67 (2006)

Treatment of Duchenne Muscular Dystrophy by Pharmacological Means

Urs T. Ruegg, Francois-Xavier Boittin, Peggy Mittaud, Olivier M. Dorchies and Emmanuelle Roulet

Laboratory of Pharmacology, Geneva-Lausanne School of Pharmaceutical Sciences, University of Geneva, Quai Ernest Ansermet 30, CH-1211 Geneva 4, Switzerland

Duchenne muscular dystrophy (DMD) is caused by the lack of dystrophin, a cytoskeletal protein. DMD is an X-linked disease affecting 1/3500 male births. Mutations in the p21 region of the X chromosome, lead to deficiency of dystrophin, a 427 kDa protein located in the inner face of the plasma membrane. Dystrophin provides a link between the cytoskeleton and the extracellular matrix and its absence in dystrophic fibers leads to progressive weakness and muscle degeneration.

Therapies such as myoblast transfer, gene delivery/repair or muscle specific utrophin expression, appear promising, but are unlikely to become available in the near future. However, pharmacological approaches may provide a more promising short- and medium-term intervention strategy for treating DMD and other neuromuscular disorders. Thus, our main goal is to identify the key pathophysiological pathways active in DMD, and then target these pathways for therapeutic intervention.

We and others have found that muscle cells from DMD patients or from one of the animal models of DMD, the *mdx* mouse, display elevated cytosolic calcium concentrations ($[Ca^{2+}]_c$) when exposed to stress. Our results show that drugs correcting this $[Ca^{2+}]_c$ dysregulation in *mdx* myotubes or fibers are also effective in preventing muscle necrosis in *mdx* mice.

The ion channels participating in the enhanced Ca^{2+} entry and subsequent cytosolic Ca^{2+} overload, appear to be store-operated Ca^{2+} channels and their activity is greatly enhanced in dystrophic skeletal *Flexor Digitorum Brevis* (FDB) fibers isolated from *mdx*^{5cv} mice. We showed that this Ca^{2+} entry is under the control of Ca^{2+}-independent phospholipase A_2 and the exaggerated Ca^{2+} influx occurring in dystrophic fibers can be attenuated by inhibitors of this enzyme. This pathway therefore appears as an attractive target to reduce excessive Ca^{2+} influx and subsequent de-generation occurring in dystrophic fibers.

The *mdx*^{5Cv} mouse model was also used to investigate the effects of green tea extract, its major component (-)-epigallocatechin gallate, and pentoxifylline on dystrophic muscle quality and function. After 1 or 5 weeks of treatment, a delay in muscle necrosis and an increase in resistance to tetanic tensions were observed, reaching values close to those of normal mice. These results demonstrate that diet supple-mentation protected muscle against the first massive wave of necrosis, and stimulated muscle adaptation towards a stronger and more resistant phenotype.

Supported by grants from the Swiss National Science Foundation, the Association Française contre les Myopathies, the Duchenne Parent Project, and the Swiss Foundation for Research on Muscle Diseases

REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 68 (2006)

Do we Need Pharmacoproteomic Markers in Addition to Pharmacogenomic Data for Cardiovascular Diseases?

Gérard Siest

INSERM U.525, Université Henri Poincaré Nancy 1, Nancy, France.
Email: Gerard.Siest@pharma.uhp-nancy.fr

Personalized medicine is based on a better knowledge on biological variability with an important part due to genetics. When we are trying to identify the genes and their products involved in differential responses to cardiovascular drugs we have to follow a strategy in five steps:

- Pharmacokinetic related genes and phenotypes
- Pharmacodynamic targets genes and products
- Cardiovascular diseases and risks dependent on specific or large metabolic cycles
- Physiological variations of the previous identified genes and proteins
- Environment influences on them

We will focus on one risk factor, i.e. the genes and proteins involved in hypertension because many processes are involved in eventually all phases of atherogenesis.

Personalized medicine in this case needs to know the genes and there expression in the metabolism of anti-hypertensive drugs, their transport, their target and the variations of the proteomic markers in healthy populations. But it appears that we still have to go in getting these points across the physician's education and across the general public to explain the promise of personalized medicine and pharmacogenomics-pharmacoproteomics to better medical care.

REFERENCES

1. Siest G., Jeannesson E., Berrahmoune H., Maumus S., Marteau J.B., Mohr S., Visvikis S.: Pharmacogenomics and drug response in cardiovascular disorders. *Pharmacogenomics 5*: 779-802 (2004)

2. Siest G., Marteau J.B., Maumus S., Berrahmoune H., Jeannesson E., Samara A., Batt A.M., Visvikis-Siest S.: Pharmacogenomics and cardiovascular drugs: Need for integrated biological system with phenotypes and proteomic markers. *Eur. J. Pharmacol. 527*: 1-22 (2005)

3. Marteau J.B., Gambier N., Jeannesson E., Batt A.M., Siest G., Visvikis-Siest S.: From the pharmacogenetics to the pharmacogenomics of antihypertensive drugs. *Pharmacogenomics* (submitted)

REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 69 (2006)

Cell Necrosis: A Futile, Unregulated Process, or a Novel Drug Development Target?

Csaba Szabó

Department of Human Physiology and Experimental Research, Semmelweis University, Budapest, Üllöi út 78/A, H-1082, Hungary
E-mail: szabocsaba@aol.com

Key words: Necrosis, apoptosis, free radicals, oxidants, cell death, stroke, myocardial infarction.

INTRODUCTION

In contrast to apoptosis, cell necrosis has received less attention, and was generally dismissed as an accidental and unregulated form of cell death. A key difference between the two forms of cell death is that during necrosis, the membrane integrity breaks down, cytosolic constituents are released into the extracellular space through the damaged plasma membrane, whereas during apoptosis cells shrink, their nuclei condense, resulting in their encapsulation into apoptotic bodies, followed by consumption by macrophages. Recent work challenges the dogma that necrosis is a futile or unregulated process, and identifies several pathways and pharmacological tools and drug development candidates which favorably affect this process. This talk will focus on the mechanisms and pathways of necrosis and will overview the pharmacological methods and drug development candidates, which are able to prevent it.

DISCUSSION

The triggers of cell necrosis are multiple. Tissue ischemia and hypoxia deplete cellular energetic pools (energy starvation), followed by cellular ionic imbalance, mitochondrial alterations, and calcium overload. In cerebral ischemia, excitotoxins are also released and trigger cell death. Restoration of blood flow to a previously ischemic tissue induces additional damage (*reperfusion injury*), that is mediated, in part, by the extra- and intracellular formation of reactive oxygen and nitrogen species. The cellular effectors of necrosis involve oxygen- and nitrogen-derived free radical and oxidant species, including superoxide, hydroxyl radical and peroxynitrite. Sodium and water uptake and inhibition of Na/K ATP-ase is another key factor. Calcium overload and activation of proteases and nucleases and phospholipases are also important. Cellular calcium overload is a potent inducer of cell death, which involves the activation of proteases, lipases and other factors. Activation of the nuclear enzyme poly (ADP-ribose) polymerase (PARP) plays a distinct suicidal role in cell necrosis. Even though the processes associated with necrosis are complicated by multiple auto-amplifying cellular processes of injury, there are several key checkpoints of necrosis which are drug development targets. One approach is the neutralization of reactive oxygen and nitrogen species (e.g. with catalytic antioxidants). Pharmacological inhibition of PARP is another distinct opportunity to suppress cell necrosis of myocytes, neurons and other cell types: the first series of ultrapotent PARP inhibitors are already in clinical trials. A recent cell-based high throughput screen identified a series of molecules, called necrostatins, which inhibit apopto-necrotic cell death via a new caspase-independent pathway.

CONCLUSION

Necrosis can no longer be viewed as a futile or unregulated process. Instead, necrosis presents itself as *the other form* of regulated cell death, and offers multiple opportunities for pharmacological intervention. As necrosis is the principal mechanism of cell death in many acute severe diseases (e.g. myocardial infarction or stroke), the identification of new effectors of necrosis and the development of new anti-necrotic drugs is of major practical importance.

REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 70 (2006)

Acute Administration of Vitamin E Triggers Preconditioning *via* K_{ATP} Channels and cyclic-GMP without Inhibiting Lipid Peroxidation

Ioanna Andreadou[1,2], Efstathios K. Iliodromitis[2], Konstantinos Tsovolas[2], Catherine Gaitanaki[3], Anastasia Zoga[2], Ioanna-Katerina Aggeli[3], Isidoros Beis[3], Dimitrios Th. Kremastinos[2]

[1]Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Athens, Greece, [2]Second University Department of Cardiology, Medical School, Attikon General Hospital, University of Athens, Greece [3]Department of Animal & Human Physiology, School of Biology, University of Athens, Athens, Greece

Key words: Vitamin E, preconditioning, infarct size, antioxidant, mitochondrial K_{ATP} channels

INTRODUCTION

Vitamin E (VitE) is considered an antioxidant agent. Mitochondrial K_{ATP} channels (Mit K_{ATP}), cyclic-GMP (c-GMP) and free radicals are involved in the protective mechanism of preconditioning (PC), whereas some antioxidants abolish this benefit. The purpose of the present study was to evaluate the effect of VitE on infarct size, on PC and on oxidative status in vivo.

METHODS

Male rabbits were divided into seven groups and were subjected to 30min of myocardial ischemia (isc) and 3h reperfusion (rep) with the following interventions before: 1) Control (no intervention) 2) E_{150} group (i.v. VitE at a total dose of 150 mg kg^{-1} for 75 min, starting 40min before index isc and lasting on the 5th min of rep) 3) E_{300} group (i.v. VitE at a total dose of 300 mg kg^{-1} as previously described) 4) PC group (two cycles of 5min isc and 10min rep), 5) combined E_{150}-PC group and 6) combined E_{300}-PC group. A seventh group was treated as E_{150} group with the addition of the Mit K_{ATP} blocker 5-hydroxydecanoic acid (5-HD) at a dose of 10 mg kg^{-1}. At the end of the experiment the area at risk and the infarct zone were determined with the aid of fluorescent particles and triphenyl tetrazolium chloride staining. Respective to the first series groups were used in a second series of experiments; heart tissue samples were taken at the time of long ischemia for malondialdehyde (MDA) and conjugated dienes (CDs) assessment (as lipid peroxidation markers) and for c-GMP.

RESULTS

VitE, at both doses, reduces the infarct size (19.7±2.8% and 18.8±4.9%) vs 47.4±2.6% in control ($P<0.05$) and does not attenuate the protection from PC (10.2±3.1%, 12.4±2.2%, vs 13.5±3.3%). Combined VitE and 5-HD treatment abrogates this benefit (37.4±6.5%, $P<0.05$ vs E_{150} and NS vs control). VitE increases intracellular c-GMP and CDs equally well as PC does with no effect on MDA.

CONCLUSION

VitE decreases the infarct size via Mit K_{ATP} and c-GMP and preserves the benefit of ischemic PC without eliciting antioxidant properties.