

Publishable executive summary - 2006

Work performed and results achieved so far

After the second year, with a few exceptions, the partners report excellent progress, and for almost all work packages papers have been published. Here follows examples of work and results obtained, for each WP.

WP2. Regulation of the enzyme activity of lipoxygenases is complex. An arachidonic acid analogue was found not only to the catalytic domain of 15-LOX, but also to the N-terminal β -sandwich domain. This sheds light on a long suspected regulatory second fatty acid binding site, in lipoxygenases. Coactosin-like protein (CLP) binds to 5-LOX, effects of CLP on 5-LOX activity were elucidated. CLP can serve as a scaffold for 5-lipoxygenase activity, upregulating the formation of leukotrienes. The findings establish CLP as a novel factor relevant for 5-LOX activity. 3D crystals of rat LTC₄S have been obtained which diffract to about 6-8 Å.

WP3. Studies on the regulation of expression of 5-LOX and of COX-2 were performed. For 5-LOX, TGF β was found to confer both primary and secondary effects in Mono Mac 6 cells. Of considerable interest, Smad3/4 responsive elements in the far 3'-part of the gene (exon 10, introns J and M) were characterized. For COX-2, it was characterized how IL-1 β upregulates COX-2 expression in colon carcinoma cells. In CaCo-2 cells, transcription factor NF- κ B and p38 MAPK were important for the IL-1 β effect.

WP4. Several results on the roles of eicosanoids in inflammation and immunity were presented:

- On a role for LXA₄ in cytoskeleton rearrangement, of importance for macrophage phagocytosis of apoptotic cells.
- On the mechanism for upregulation of IL-2 gene transcription, involving NF- κ B-inducing kinase.
- On the regulated expression of the BLT1 in HUVEC, by proinflammatory factors including LTB₄ itself.
- On insulin resistance as a determinant of platelet activation in obese women.
- On a possible link between reduced endothelial COX-2 and NO-synthase expression and the increased risk of cardiovascular diseases affecting diabetic patient.
- On the sphingolipid analogue FTY720, which activates the SP1-P receptor, upregulating the Smad signalling cascade in renal mesangial cells.

WP5. Formation of NO in the GI-tract was further studied. Probiotic bacteria elicit a number of beneficial effects in the gut, lactobacilli can utilise nitrate and nitrite to generate nitric oxide, a gas with immunomodulating and antibacterial properties. Also, salivary nitrite is suggested to enhance the antimicrobial properties of gastric juice by conversion to nitric oxide (NO) and other reactive nitrogen intermediates in the stomach.

WP6. The research in this WP is focussed on the roles of enzymes and receptors of the cyclooxygenase (COX) pathways in vascular physiology and cardiovascular disease. Data were presented:

- On the influence of plasma protein on the potencies of inhibitors of COX-1 and -2.

- On the stronger inhibition by nonsteroid anti-inflammatory drugs of cyclooxygenase-1 in endothelial cells than platelets offers an explanation for increased risk of thrombotic events.
- On de novo synthesis of COX-1 counteracts the suppression of platelet thromboxane biosynthesis by aspirin.
- On reduced thromboxane biosynthesis in carriers of toll-like receptor 4 polymorphisms *in vivo*.
- On the suppression of RAGE as a basis of simvastatin-dependent plaque stabilization in type 2 diabetes.

WP7. The research in this WP is focussed on the roles of enzymes and receptors of the lipoxygenase (LOX) pathways in vascular physiology and cardiovascular disease. Data were presented:

- On the expression of 5-LOX and LTA₄ hydrolase in human atherosclerotic lesions correlates with symptoms of plaque instability.
- On the orphan receptor GPR17 identified as a new dual uracil nucleotide/cysLT receptor.
- On 15-LOX-mediated modification of high-density lipoproteins impairs SR-BI- and ABCA1-dependent cholesterol efflux from macrophages.

WP8. The research in this WP is focussed on the roles of NO and NO-synthases in vascular physiology and cardiovascular disease. Data were presented:

- On pharmacological characterization of 2NTX-99, a potential antiatherothrombotic agent with antithromboxane and nitric oxide donor activity in platelet and vascular preparations.
- On the effects of dietary nitrate on blood pressure in healthy volunteers.
- On nitric oxide down-regulating the expression of the catalytic NADPH oxidase subunit Nox1 in rat renal mesangial cells.

WP9. The research in this WP is focussed on the roles of eicosanoids and NO in diseases of the central nervous system. Data were presented:

- On the role of phospholipid hydroperoxide glutathione peroxidase isoforms in murine embryogenesis.
- On activation of cerebral endothelium, required for mononuclear cell recruitment in a novel *in vitro* model of brain inflammation.
- On the effect of arachidonic acid reacylation on leukotriene biosynthesis in human neutrophils stimulated with granulocyte-macrophage colony-stimulating factor and formyl-methionyl-leucyl-phenylalanine.
- On calcium-dependent expression of TNF α in neural cells is mediated by the calcineurin/NFAT pathway.

WP10. Inflammatory cells can either promote or inhibit tumor growth. It was presented that CD40, a key molecule for adaptive immune response, has a role in mammary carcinogenesis of BALB/NeuT transgenic tumor-prone mice. Activated platelets, which may interact with and activate ECs, are a possible source of CD40L, the anti-platelet drug clopidogrel inhibits platelet CD40L expression. Treatment of BALB/NeuT mice reduced tumor growth to a level similar to CD40-deficient mice. This points to a participation of CD40/CD40L in the angiogenic processes associated with mammary carcinogenesis. Also, it was presented that PMA-mediated induction of genes that are significantly associated with inflammation, tumour

growth and metastasis, such as COX-2 and VEGF, is inhibited by PPAR α ligands in the human colorectal carcinoma cell line SW620.

WP11. 15-LOX isoforms were analysed in normal and malignant ovarian tissue. A significantly elevated expression of 15-LOX-2 in primary ovarian cancer was found. In metastases expression was also augmented (20-fold). For 15-LOX-1 and 5-LOX mRNA no significant differences were observed. Also, a drastic upregulation (29-fold upregulation) was observed for mammaglobin-2 (MGB2).

WP12. Activation of NFAT transcription factors requires their dephosphorylation by the phosphatase calcineurin. NFATs contain two calcineurin binding sites: Results suggest a model in which the different calcineurin binding characteristics of the two sites enable different NFAT members to influence each others activities in cells where they are co-expressed. Also, the involvement of angiotensin converting enzyme (ACE) in angiogenesis was elucidated. It was presented that ACE inhibitors promote angiogenesis in coronary endothelium, by increasing the expression and production of FGF-2 via the stimulation of eNOS.

WP13. Measures have been taken to further promote sharing of key technologies within the Consortium. A list of the existing core facilities and technical platforms e.g. basic methods in functional genomics and proteomics, structure biology and structural genomics, animal models, facility for conduction of clinical phase-1 studies, technology for screening and identification of lead structures and research tools is under preparation. Protocols and research tools are being collected regularly and made available to the partners at the Eicosanox intranet. Group KI:b has provided a list of antibodies available, generated through a project collaboration with the Human Protein Atlas (<http://www.proteinatlas.org/index.php>), that can be used by the Consortium. The leader of WP13 is currently negotiating with a publisher to assemble the techniques in a book or dedicated issue of a scientific Journal.

WP14. The partners made an inventory of the educational needs and plan for the corresponding courses and workshops. Educational and training activities at different partner institutions were collected and have been made available at the Consortium level. We have assembled an operative Education and Training Committee and allocated postgraduate courses that cover scientific, technical and management skills. A summer school for graduate students has been held in Sandhamn, Stockholm, that covered experimental and clinical aspects of the arachidonic acid pathway. Information material, e.g. website, poster and brochure has been produced and maintained.

WP15. Our Project Office with Director, Project Managers and Project Administrator now operates to serve the needs of the Consortium. Tools used to facilitate transfer of information and knowledge among the consortium members, have been continuously updated. These include, an intranet site, which allows project members to exchange files, work with a common calendar, have a common archive for files, have discussions and exchange information. In addition, all partners have access to a web based conferencing system. Meetings of the Work Management Group are held regularly every second week using this software.

To further promote integration and collaboration between partners, the WMG decided to arrange Cluster meetings. Three such meetings were arranged; for cluster C in Chieti 060706-07, for cluster A in Stockholm 060827, and for cluster B in Madrid 061016. These meeting

were very appreciated among the participants and led to a number of new collaborative initiatives.

To get a fruitful collaboration within the project, the Consortium gathers at least once a year for discussions on scientific progress of the project as well as general issues. The annual meeting was held in Madrid in October 2006. The meeting of the Project Steering Committee (PSC) was held in direct conjunction with the annual meeting.

Expected end results and intentions for use and impact

Eicosanoids and NO are involved in a number of severe endemic diseases addressed in FP6, e.g. atherosclerosis, myocardial infarction, thrombosis, dementia and cancer. In addition, these inflammatory mediators (eicosanoids and NO) are involved in many other disorders. Examples are diseases of the respiratory system (asthma), autoimmune disorders, and the sequelae after severe trauma. Together, these conditions account for the vast majority of mortality and morbidity, in Europe as well as the rest of the world.

As inflammatory mediators, eicosanoids and NO also have beneficial effects in normal physiology. In normal life, we constantly defend ourselves against pathogens, and inflammation is part of normal mechanisms, for example preventing tumorigenesis. Consequently, eicosanoids and NO most probably are of relevance also for diseases characterized by defective defence mechanisms, such as HIV.

Thus, our project is aimed at increasing the knowledge about the mechanisms by which eicosanoids and NO trigger and maintain physiological and pathophysiological processes, in both health and disease. In addition, we aim to identify new drug targets, evaluate the therapeutic potential of recently developed lead structures, improve existing therapies and develop novel drugs and therapeutic strategies. Hence, our project has the potential to prolong the life expectancy and significantly improve the health, quality of life, and prosperity of the citizens of Europe.

Main elements of the plan for using and disseminating knowledge

During the second year of the Eicosanox project 63 articles, with an average impact factor of 6.9, have been published in scientific journals, and the results have been presented at several international conferences. To disseminate information to other scientists and to the public, the external website has been updated in a regular manner (<http://www.eicosanox.org/>).

