

## **Publishable executive summary - 2005**

### **Background and project objectives**

The Eicosanoids and nitric oxide (NO) are signalling molecules in many physiological and pathological processes, including cardiovascular, cerebral and neoplastic disorders. Together, these diseases account for the vast majority of health problems and health care in Europe, with a major socio-economic impact. The partners in Eicosanox jointly carry out an ambitious project, aiming to increase the knowledge about these autacoids (eicosanoids and NO) with the goal to develop novel therapeutic strategies and medical treatments.

We perform molecular studies on key enzymes and receptors to elucidate biochemical properties, catalytic mechanisms and structure-function relationships. We address the functional genomics of the Eicosanoid and NO cascades to characterize gene expression profiles and regulation under normal and disease states, and identify novel potential drug targets. Genes and proteins are characterized using proteomics, structural genomics and model organisms. In parallel, the partners will conduct cell biological work on gene regulation, gene silencing, signalling systems, and cross-talk between pathways. This gives increased understanding regarding pathophysiological processes and disease mechanisms such as chronic inflammation, aberrant immune responses and angiogenesis. In turn, these insights are translated into investigations of diseases using animal models and clinical applications.

The basic research together with applied and clinical studies act in synergy to identify novel targets for pharmacological intervention and drug design for the treatment of patients afflicted with cardiovascular, cerebral and neoplastic disorders. We also create a long-lasting infrastructure for R&D activities, education, training, and exploitation. Results are disseminated to users at multiple levels, such as research organisations, funding agencies, patient associations, industry, insurance companies, and society in general.

In the work plan, the 15 work packages are structured in five clusters (A-E). The titles of these illustrate the **general project objectives**:

- A. MOLECULAR STUDIES OF THE EICOSANOID AND NO CASCADES
- B. ROLE OF EICOSANOIDS AND NO IN IMMUNOLOGY AND INFLAMMATION
- C. DISEASES OF THE CARDIOVASCULAR AND CENTRAL NERVOUS SYSTEMS
- D. CANCER AND ANGIOGENESIS
- E. INFRASTRUCTURE AND COMMUNICATION

Cluster A is focused on biochemistry and molecular biology, while clusters B, C, and D are more oriented to functional studies using cell and animal models, as well as studies on human subjects. Cluster E concerns the structure of the consortium, as well as internal and external communication.

In EICOSANOX, we have brought together 15 top European laboratories within Eicosanoid and NO research, one world leading Canadian group and two SMEs in a large multidisciplinary and highly competitive Consortium. The partners (contractors involved) are described in tabular format.

## **Work performed and results achieved so far**

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

WP1. As PGE<sub>2</sub> is involved in pain and fever, inhibitors of the enzyme mPGES-1 may become future anti-inflammatory drugs. New chemical entities have been developed and screened for inhibitory activity on the human mPGES-1 enzyme. Lead molecules have been identified and further optimised regarding drugability aspects. A Research Collaboration and Licensing Agreement between the EICOSANOX SME Biolipox and Boehringer Ingelheim was signed to further discover and develop human mPGES-1 inhibitors.

WP2. It was found that glycerides have a direct effect on the 5-LOX enzyme. As described previously for Ca<sup>2+</sup>, OAG renders 5-LOX activity resistant against inhibition by glutathione peroxidase activity. Intriguingly, a 5-LOX mutant lacking tryptophan residues (Trp-13, -75, and -102) important for the binding of the 5-LOX C2-like domain to phospholipids was not stimulated by OAG. Apparently, OAG directly stimulates 5-LOX by acting at a phospholipid binding site located within the C2-like domain.

WP3. COX-2 gene expression is regulated through both transcriptional and posttranscriptional mechanisms. It was found that Ca<sup>2+</sup>/Calcineurin/Nuclear Factor of Activated T Cells and AP-1 pathways play an essential role in the up-regulation of COX-2 expression in a variety of cell lines (T lymphocytes, endothelial cells, colon carcinoma cells). In macrophages, the induction of COX-2 and mPGES-1 involves the cooperation of LPS/MAPKs and GPCR/cAMP signalling pathways.

WP4. A novel type of drug, nitroaspirin, was evaluated in relation to cancer vaccination. The aim was to investigate the potential effects of NSAIDs and NO-NSAIDs, on tumor induced immune suppression and on the immune response elicited by anti-tumor DNA vaccination in a mouse transplantable tumour model. By interfering with the inhibitory enzymatic activities of myeloid cells, orally administered NO aspirin:

- normalised the immune status of tumour-bearing hosts restoring T lymphocyte function
- increased the number and function of tumor-antigen-specific T lymphocytes
- enhanced the preventive and therapeutic effectiveness of the antitumor immunity elicited by cancer vaccination.

WP5. For NOS-independent NO generation in the stomach, a role of salivary nitrite was described. Apparently, such NO generation in the human stomach is important for prevention of gastric ulcers. Thus, intragastric nitric oxide is abolished in intubated patients and restored by nitrite. Effects of nitrite-derived NO in the stomach were increased mucus thickness and mucosal blood flow.

WP6. Immunocytochemistry of carotid plaques revealed infiltration of inflammatory cells, which was most evident in the shoulder of “symptomatic” plaques. “Symptomatic” plaques contained higher expression of COX-2, mPGES-1, MMP-2 and MMP-9 as compared to specimens from “asymptomatic” patients. TLR4 was expressed in carotid plaques and was localized at plaque shoulder in association with CD68 macrophages. Immunoreactivity for TLR4 was significantly higher in “symptomatic” than in “asymptomatic” plaques. Comparing the expression of TLR4 and COX-2 in plaques, an association between these two proteins was observed.

WP7. Expression of an atherosclerosis relevant gene (12/15-LOX) during foam cell formation was determined. An *in vitro* foam cell model, based on the uptake of acetylated LDL by murine macrophages was used. 12/15-LOX expression protected the cells from intracellular lipid deposition. This effect was related to an attenuated uptake of modified LDL as indicated by impaired expression of scavenger receptor A, and to accelerated intracellular lipid metabolism. The results indicate that the role of 12/15-LOX in atherogenesis may not be restricted to oxidative LDL modification. Expression of this lipid-peroxidizing enzyme may impact both, lipid uptake and intracellular lipid turnover.

WP8. Cellular proteins required for eNOS activity in endothelial cells, with possible relevance for type 2 diabetes, were described. It was demonstrated that protein kinase A (PKA) and not Akt is the kinase that phosphorylates and activates eNOS. The activation of PKA in endothelial cells exposed to fluid shear stress was found to be independent of an increase in cAMP, but dependent of the activation of the tyrosine phosphatase SHP2 and the presence of the Gab1 scaffolding protein. The role of Gab1 in the activation of eNOS is particularly interesting as levels of this protein are attenuated in type 2 diabetes.

WP9. In the brain of IL-4 overexpressing animals, the gene expression pattern of EICOSANOX relevant genes (LOX, COX, PG receptors, LT receptors, NO synthases etc.) was determined using microarray technology and RT-PCR. The most dramatic response was observed for the 12/15-LOX. These data indicate for the first time expression of 12/15-LOX in murine central nervous system and also suggest that induction of this enzyme by IL-4 is not restricted to *in vitro* cultures of monocytic cells but can also be observed *in vivo* in the CNS.

WP10. 15-deoxy $\Delta^{12,14}$ -PGJ<sub>2</sub> inhibits AP-1 activation, and this leads to an inhibition of VEGF and COX-2 expression in colon carcinoma cells. This cyclopentenone inhibits AP-1 activation at 2 levels; upstream the activation of the c-jun terminal kinase (JNK) and by preventing the binding of AP-1 transcription factor to the DNA of those promoters. Interestingly the redox sensitive mechanism does not involve binding of PGJ<sub>2</sub> to PPAR $\gamma$ .

WP11. For BL41 E95A B-lymphocytic cancer cells it was observed that 5-LOX protein expression decreases during induction of cell proliferation by cell culture splitting. This suggests that there is a link between cell proliferation and 5-LOX protein expression in B-cells. It was found that in these cells, 5-LOX is regulated by caspases, by means of specific limited proteolysis. Apparently, splitting of BL41 cells induces *de novo* synthesis of a protein involved in the activation of casp-6, which cleaves 5-LOX.

WP12. The expression pattern of the different EP (PGE<sub>2</sub> receptors) was analysed. Expression of EP1, EP2 and EP4 in HUVEC, and EP2, EP3 and EP4 in HeLa, were detected. Furthermore, there was an increase in VEGF mRNA in HUVEC treated with PGE<sub>2</sub>. Accordingly, PGE<sub>2</sub> induces VEGF promoter transcriptional activity in this cell type.

A number of *in vivo* angiogenesis assays were set up, which will be useful to validate the findings obtained by the *in vitro* experiments.

WP13. All partners have been carefully made aware about the need to share 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.

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 at Frankfurt that covered experimental and clinical aspects of the arachidonic acid pathway. Information material, e.g. website, poster and brochure has been produced.

WP15. A Project Office with Director, Project Managers and Project Administrator was set up at the start of the project. Tools have been purchased to facilitate transfer of information and knowledge among the consortium members, including 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. The Work Management Group has appointed three different committees from the Eicosanox Congress: 1) Science and Society Group, 2) Education and Training Committee, and 3) Ethical Review Panel.

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. Two meetings were held during the first year, the kick-off meeting at the start of the project and the first annual meeting, which was held in Stockholm in November 2005. 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 defense 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 first year of the Eicosanox project a number of results have been generated within the consortium. 71 articles have been published in scientific journals, and the results have been presented on several international conferences.

As also small and medium-sized enterprises (SMEs) are part of Eicosanox, productivity does not only mean scientific publications. An exploitable result of one of the SMEs (Biolipox) during 2005, was to go forward with the development of inhibitors for the human mPGES-1 enzyme.