STIMULATING INNOVATIVE RESEARCH IN ANAEMIA

RoFAR
Foundation for Anemia Research

RoFAR innovative research in anaemia

ROFAR – A MISSION TO STIMULATE INNOVATION IN ANAEMIA RESEARCH

The Roche Foundation for Anaemia Research (RoFAR) is an independent, charitable, non-profit organisation dedicated to encouraging innovative research that will open new avenues of exploration in the areas of anaemia, its mechanisms and outcomes in both anaemia related to kidney disease and cancer.

The RoFAR was established by F. Hoffmann-La Roche (Roche) in 2004 and funded by the company with an initial sum of 16 million CHF.

Roche is one of the world's leading research-focused healthcare groups in the fields of pharmaceuticals and diagnostics. As a supplier of innovative products and services for the early detection, prevention, diagnosis and treatment of disease, the Group contributes on a broad range of fronts to improving people's health and quality of life. RoFAR is a key example of the company's long-standing commitment to honour its responsibility to aid the advancement of science.

The Foundation is legally independent from Roche and is guided solely by a board of eight Trustees and an independently appointed Scientific Advisory Board whose role is to review all applications and make recommendations on their calibre and content to the Trustees. The development of the Foundation strategy and the framework within which scientific decisions are made is the responsibility of the Trustees.

Two cycles of grant applications for substantial awards of up to 200,000 CHF each are reviewed and granted each year. In addition, a special grant was announced in the spring of 2006 of up to 2,400,000 CHF that will be made to a single research group or institution to pursue a research topic of outstanding importance.

Since the conception of RoFAR in 2004, 4,107,440 CHF Swiss Francs ($USD 3.3 million) has been awarded to 20 research projects. These projects, now ongoing, are exploring key scientific questions in nephrology and diabetology, hematology, oncology and cardiology. This short booklet gives a flavour of the scope of these projects which have all met the exacting standards for scientific excellence, feasibility and originality set out by the Board of Trustees and Scientific Advisory Board.

Grant applications are encouraged from established members of academic staff at universities, dialysis centres and research institutes, with research projects relating to areas such as anaemia of chronic disease, anaemia related to congestive heart failure and stroke, erythropoietin or erythropoietin-like substances as protective drugs for various target organs, and the biology of anaemia and its outcomes.

For more information on how and when to apply for a RoFAR grant, please visit the website (www.rofar.org).

The first 20 RoFAR research projects

THE FIRST 20 ROFAR RESEARCH PROJECTS

NEPHROLOGY AND DIABETOLOGY

molecular

mechanisms

novel

erythropoietin

IS INFLAMMATION AN IMPORTANT FACTOR IN THE ANAEMIA OF CHRONIC RENAL FAILURE?

Dr. Edward Debnam Royal Free & University College London, UK

Anaemia is a feature of renal failure and it is, in part, due to insufficient production of the hormone erythropoietin (EPO), the action of which is to stimulate the bone marrow to produce mature red blood cells. However, an increased production of red blood cells also requires the absorption of adequate amounts of dietary iron (since iron is a vital component of red blood cells). It is a common clinical observation that many renal failure patients receiving EPO require intravenous iron supplementation, despite the fact that they have adequate dietary iron. This strongly suggests an abnormality of iron uptake by the intestine. Recent studies show that another hormone, hepcidin, reduces iron absorption from the gut. Interestingly, hepcidin secretion is increased during infection (also a common finding in renal failure) and this raises the possibility that raised hepcidin levels in renal failure interfere with gut iron uptake, i.e. high blood levels of hepcidin may override the effect of administered EPO in renal failure and limit the supply of dietary iron for red blood cell production. Hence the need for intravenous iron treatment. However, this procedure has risks of iron overload and tissue injury. To date, there is no information available on the relationship between hepcidin secretion, EPO secretion and infection in renal failure. If an association is found between infection and hepcidin expression in renal failure, the process by which hepcidin influences iron transport may prove to be an important target for therapy of this form of anaemia.

MOLECULAR MECHANISMS UNDERLYING THE HYPOXIC INDUCTION OF ERYTHROPOIETIN BY HIF-2

Dr. Christina Warnecke, University Erlangen-Nürnberg, Germany

The hormone erythropoietin (EPO), which is predominantly produced in the kidneys, is the primary regulator of red blood cell production. Hypoxia, i.e. insufficient oxygen supply as encountered at high altitudes or after blood loss, leads to a compensatory increase of EPO production due to an increase in EPO gene expression, which is mediated by hypoxia-inducible transcription factors. In disease states that impair kidney function such as diabetes, chronic renal disease or heart failure the production of erythropoietin in the kidney fails to meet the needs of the body and patients suffer from anaemia, which deteriorates their general condition and often aggravates the primary disease. Treatment with human recombinant EPO is highly effective and so far the therapy of choice for erythropoietin-deficiency anaemias. An alternative and very attractive approach, because of the high costs of recombinant EPO, could be the stimulation of endogenous EPO production. In 2001 a family of transcription factors inducible by hypoxia (hypoxia-inducible factor, HIF) was identified and their regulation has since then been actively investigated. Interestingly, although HIF was primarily identified as a regulator of EPO expression, it turned out that it also induces many other genes that serve to protect against oxygen deficiency. Although this underscores the importance of HIF, this lack of specificity makes it more difficult to use as a molecular target to stimulate EPO generation. Only recently, we and others demonstrated that one specific isoform of HIF, called HIF-2, is responsible for the regulation of erythropoietin, but not that of most other HIF-dependent genes. By studying the molecular basis of this specificity in EPO-producing cells lines we attempt to increase our understanding of the normal regulation of red cell production, as well as its impairment in different diseases and hope to identify novel targets for medical intervention.

HAEMATOLOGY

regulation

investigation

expression

signalling

REGULATION OF GENE EXPRESSION DURING ERYTHROPOIESIS

Dr. Diane Gilligan, Puget Sound Blood Centre, Seattle, USA

The goal of this project is to understand the regulation of appropriate gene expression during red blood cell production. Anaemia, the lack of sufficient numbers of red blood cells, is a common disorder that affects patients with many different illnesses. Specifically, it is a side effect of chemotherapy for cancer and it is a secondary sign of kidney failure. Much has been learned about the production of red blood cells since the discovery of erythropoietin, a hormone produced by the kidney that stimulates hematopoietic stem cells to produce red blood cells. At a molecular level, the binding of erythropoietin to its receptor signals the precursor cells to increase expression of genes that are important to red blood cells and to decrease expression of genes that are not important to red blood cells. This signaling pathway is not yet well understood and our experiments are designed to gain more information about the molecular steps that are required to increase production of red blood cells. We have been studying a family of genes, the adducins, that are expressed in all cells, but were first discovered as components of red blood cells. We have demonstrated lineage specific expression of two of the adducin genes. The beta adducin gene is highly expressed in red blood cell, but is not expressed in platelets, while the gamma adducin gene shows the opposite characteristics, being highly expressed in platelets, but not expressed in red blood cells. We will study the adducin gene family as a model system for lineage specific gene expression, with particular significance for red blood cell production. We may identify novel factors that are important for red blood cell gene expression and these results may lead to novel therapies for patients with anaemia.

INVESTIGATION OF THE ROLE OF JUNE-1 IN ERYTHROPOIESIS

Professor Alexander Maxwell, Queen's University Belfast, Belfast, UK

Erythropoietin (EPO) stimulates bone marrow cells to differentiate into mature erythrocytes. Recombinant human erythropoietin therapy is widely used to treat anaemias but there is relatively limited knowledge of how EPO regulates genes in cells and tissues. Investigation of EPO-regulated genes should improve our understanding of how EPO influences erythropoiesis and the function of non-haematopoietic organs which express EPO receptors, such as the brain and heart. We investigated transcriptional events occurring downstream of EPO binding to its receptor. We have now identified a novel gene, JUNE-1, encoding 5 exons expressing a 1.2 kb transcript translated into a 44 kDa protein. Its highly conserved DNA sequence has both nuclear targeting and plant homeodomain motifs, thought to mediate protein-protein or protein-DNA interactions. This suggests that JUNE-1 is involved in chromatin remodeling or transcriptional regulation. RT-PCR confirmed JUNE-1 expression in a variety of tissues and tumour cell lines, implicating JUNE-1 in processes beyond erythropoiesis. Functional studies of JUNE-1 are proposed using an in vitro model of erythropoiesis (murine erythroleukaemia (MEL) cell system), where proerythroblasts are cultured and differentiate into mature haemoglobin producing cells. We plan to localize JUNE-1 protein by immunohistochemistry. The consequences of overexpression and underexpression (knockdown via RNA interference) of JUNE-1 protein on erythroid cell proliferation and differentiation will be assessed. MEL cells will be examined for changes in phenotype, such as cell growth rate, apoptosis, and differentiation capacity. Since JUNE-1 may function as a transcription factor, the genes downstream of JUNE-1 will be identified in the MEL cell model using mouse gene microarrays. To identify proteins that interact with JUNE-1 mammalian expression vectors will be used to produce JUNE-1 fused to GST, and the protein then immobilized on a GST column. Cellular extracts will be loaded on the columns, and interacting proteins eluted and identified by SDS-PAGE and mass spectrometry. JUNE-1 specific antibodies will also be used to identify JUNE-1 interacting proteins via co-immunoprecipitations from the cell lysates. The proposed research should enable the functional characterization of JUNE-1, a novel EPO-regulated gene.

INNOVATIVE DRUG DESIGN USING RNA APTAMERS FOR VARIOUS ANAEMIAS.

Dr. Jun-ichi Nishimura, Duke University Medical Centre, USA

Aptamers are small RNA molecules that bind to target proteins similar to antibodies. Through a selection process, termed SELEX, aptamers have been identified against a wide range of therapeutic targets. Dr. Sullenger has established the SELEX system, and Dr. Nishimura has recently successfully identified RNA aptamers for treatment of paroxysmal nocturnal haemoglobinuria, a rare type haemolytic anaemia. Our overall goal is to develop aptamers to treat various anaemias using the SELEX system. In this proposal, we exclusively focus on the development of aptamers that inhibit red cell adhesion to prevent or treat vaso-occlusion in sickle cell disease (SCD). Vaso-occlusive crises are the major clinical feature of SCD, and the adhesion of sickle erythrocytes (SS-RBC) to vascular endothelium is important to the generation of vaso-occlusion. SS-RBC express many adhesion molecules, such as Lutheran protein (B-CAM) and LW. Adhesive SS-RBC may bind to endothelial cell P-selectin, integrin V3, as well as extracellular matrix proteins, such as laminin. All of these molecules and their ligands are potential targets for reagents to prevent or treat the vaso-occlusive crises of SCD, and we focus 3 molecules, including B-CAM, P-selectin, and integrin V3. Since RNA aptamers for P-selectin have already been identified, we have synthesized one of these known P-selectin-binding aptamers. We have also begun to identify RNA aptamers that bind specifically to B-CAM and integrin V3. Once these aptamers are optimized, we will advance to flow chamber assays, to test their ability to inhibit adhesion of SS-RBC to vascular endothelium. Selected high-affinity and high-inhibitory aptamers will be further modified in preparation for *in vivo* studies. The development of combinatorial blocking aptamers against these 3 adhesion molecules represents a novel potential therapeutic option for patients with SCD.

THE ROLE OF SMAD4-DEPENDENT SIGNALING IN ANAEMIA

Professor Radek Skoda, University Hospital Basel, Basel, Switzerland

We observed that mice lacking the signaling protein SMAD4 developed a severe anaemia. SMAD4 is an essential mediator for signals generated by the transforming growth factor beta (TGF) family of proteins. This signaling pathway is important for proper embryonic development and regulates differentiation and growth in many adult tissues. However, an association of the TGF signaling pathway with the appearance of anaemia in adults has not been described to date. We used an inducible system to delete the gene for SMAD4 in adult mice and observed anaemia 3-4 weeks after induction. Blood cells lacking SMAD4 developed normally when transplanted into healthy recipients, but anaemia developed when normal blood cells were transplanted into SMAD4 deficient recipients. Thus, SMAD4 is required to provide the environment for normal red blood cell homeostasis. Our preliminary results suggest that loss of SMAD4 causes alterations in the iron metabolism that could interfere with red blood cell formation. Iron is essential for the formation of haemoglobin and iron deficiency is the most common cause of anaemia. More subtle changes in the availability of iron are thought to play a role in anaemia associated with chronic inflammatory diseases and cancer. We propose to elucidate the mechanism of how anaemia develops in these mice and to define a novel role for TGF signaling in the regulation of red blood cell formation.

ROLES OF SOX6 IN ERYTHROPOIESIS

Dr Véronique Lefebvre, Cleveland Clinic Foundation, Cleveland OH, USA

This project is designed to help us better understand how the gene called Sox6 controls formation and function of red blood cells in the mouse under normal and anaemia conditions. Sox6 codes for a protein (a transcription factor) that helps specific types of cells activate the genes that they need to fulfil their specialized functions. We found recently that red cells are among these cell types. Both the overall production and the quality of red cells are affected in mouse foetuses and pups that lack Sox6.

Using cellular and molecular approaches, we have found that Sox6 helps red cell precursors proliferate, develop quickly and undergo correct terminal maturation. Sox6 is thereby required for optimal function and long-term survival of red cells in the blood stream. Sox6 is made in red cell precursors in response to erythropoietin and thus contributes to mediate the effect of this essential hormone in boosting red cell formation. Our first aim is to continue our studies in the mouse to determine whether Sox6 is also important in red cell formation in childhood and adulthood under normal conditions and to recover quickly from anaemia. Our second aim is to use cellular and molecular assays to identify the specific genes that Sox6 activates and that have major roles in red cell formation. We will particularly ask whether Sox6 helps red blood cells assemble the specialized protein network (cytoskeleton) that critically helps them mature and acquire their specific shape, and thereby fulfil their functions and survive in the circulation. We anticipate that this study will greatly increase our molecular understanding of red cell formation and the roles of Sox6 in this process and in anaemia, and will thereby suggest genetic causes for some types of anaemia diseases and new treatments for various forms of anaemia diseases.

ERYTHROPOIETIN OR ERYTHROCYTE TRANSFUSION FOR ANAEMIA?

Prof Jürg Schifferli (principal applicant) Dr Christoph Hess (co-applicant), University Hospital Basel, Switzerland

Over the years evidence has accumulated suggesting that blood transfusions may be immunosuppressive, favour infections and diminish the survival of patients with severe disease. The mechanisms responsible for this immunosuppression are not well understood. Red blood cells release small vesicles during storage. These vesicles bud off from the cell surface of red blood cells. They are found in every red blood cell bag, and apparently do no immediate harm when transfused. The aim of the present project is to test the hypothesis that these small vesicles released by red blood cells are immunosuppressive. This hypothesis is based on the similarities between the structure of vesicles released by red blood cells and white blood cells (polymorphonuclear leucocytes); the latter have been shown to have immunosuppressive properties. To test our hypothesis, we will perform experiments in the laboratory using vesicles released by human and mouse red blood cells, to see whether they inhibit inflammation and immunity in cell culture models and in mice. The next steps would be to see whether such mice are prone to infections. An immunosuppressive activity of red blood cell vesicles would mean that transfusion should be avoided whenever possible, and particularly in patients who are already immunosuppressed. The logical consequences would be to explore further the possibility to replace red blood cell transfusion by erythropoietin treatment, particularly in chronic diseases.

ONCOLOGY

tumour

response

analysis

resistance

IN VITRO ANALYSIS OF TUMOUR RESPONSE TO RADIATION IN OXIC AND HYPOXIC CONDITIONS

Dr Marco Merlano, Division of Medical Oncology, S. Croce General Hospital, Cuneo, Italy

Despite therapeutic improvements and efforts to develop more efficacious therapies, the majority of Head and Neck Squamous Cell Carcinoma (HNSCC) patients face a poor prognosis. Therefore, the primary goal of current treatment is improvement of quality of life (QOL) and prolongation of survival. Anaemia frequently occurs in HNSCCs and has been associated with decreased QOL, impaired treatment outcomes and shortened survival. Furthermore, anaemia is a causative factor of tumour hypoxia, which compromises the efficacy of radiotherapy. Thus, correction of anaemia seems to have a beneficial effect on QOL and outcome. The human recombinant erythropoietin (rHuEPO) has proven efficacy and safety in correcting anaemia in numerous clinical studies and over a decade of clinical practice. The relationship between anaemia, hypoxia, transfusion and treatment outcome is complex and there certainly remains a lot of room for discussion about the role of hypoxia for tumour development and radiation response. Nevertheless, understanding the biological mechanisms is important in order to have the ability to reverse radioresistance, improve QOL, cancer control and clinical efficacy of radiation in anaemic patients. The purpose of the present study is to establish an experimental model and to provide experimental evidence to examine the relationship between hypoxia, EPO/EPOR and EGFR transcription/expression and their effects on the cellular response to radiation; we will investigate different cellular responses after radiation under oxic and hypoxic conditions and compare these findings to what happens when the cells cultured in hypoxia are reported to oxic conditions and then irradiated. The aim is to better define the biological and molecular bases for the *in vitro* response to hypoxia and to explore its effects on radiotherapy, in order to increase knowledge on the mechanisms underlying the negative effects of anaemia on radiotherapy outcome. The identification of the causes of treatment failure may facilitate the development of treatment strategies to improve efficacy and reduce toxicity.

MECHANISMS FOR ERYTHROPOIETIN RESISTANCE IN TRANSFORMED AND NON-TRANSFORMED CELLS

Dr Peter Mertens, Medizinische Klinik II Universitätsklinikum Aachen, Germany

Recent studies indicate that erythropoietin (EPO) fulfills important functions not only in haematopoiesis, but also those related to cell survival of non-transformed and transformed cells under hypoxia. Our goal is to unravel causes of cellular EPO resistance with the focus being on an archetypal stress responsive protein, namely Y-box protein-1 (YB-1). This transcription factor is hypothesized to counteract EPO cytoprotective effects in non-transformed cells at several key levels, including EPO gene transcription, EPO signalling and target gene regulation. Our data indicates that YB-1 itself is regulated under hypoxia. After elucidating whether YB-1 has an immediate effect on the hypoxic response, e.g. by binding to specific DNA regulatory elements within the EPO gene, further results will deal with the effects of manipulated cellular YB-1 levels (up and down) on EPO signalling and cell survival. For tumour cells an increased nuclear YB-1 content has been described, which is associated with poor outcome due to metastasis formation. An underlying mechanism may be counteracted EPO regulation with the initiation of a hypoxia cell program. Such a response includes the upregulation of target genes relevant for angiogenesis and metastasis formation. Our approach is to test for the EPO response in dependency of YB-1 expression levels that will be manipulated by molecular biology tools. The in-depth understanding of EPO resistance for mesenchymal cells as well as tumour cells may provide the rationale for specific interventions. These may include targeting of YB-1 under both conditions, to sensitize cells to EPO and thereby increase the survival of mesenchymal cells under hypoxia, and to shut off the “angiogenesis program” of tumour cells responsible for metastasis formation in a wide range of tumours, like breast and lung cancer.

NEUROPROTECTIVE EFFECT OF ERYTHROPOIETIN ON CHEMO- AND RADIOTHERAPY-INDUCED TOXICITY

Dr. Carole Soussain, Oregon Health and Sciences University, Portland, USA

Combined radiotherapy and chemotherapy approaches have provided significant efficacy against brain tumours such as primary central nervous system lymphoma (PCNSL). A significant number of patients experience severe neurotoxicity with these therapeutic approaches, and it is a limiting factor in the management of patients with intracerebral cancer. Erythropoietin (EPO) is an important hormone for the development of foetal brain and homeostasis of the adult brain, and both EPO and EPO receptor (EPO-R) are expressed on astrocytes and neurons in adult brain. Exogenous EPO has demonstrated a protective effect on neuronal cell cultures after injuries such as nitric oxide or glutamate exposure, as well as in animal models of ischaemia, inflammation, seizures, and subarachnoid haemorrhage. In clinical trials, EPO has been safely and efficiently used to prevent neurological damage after acute stroke. These findings suggest that EPO may be neuroprotective against chemotherapy and radiotherapy-induced neurotoxicity. In this proposal, the potential therapeutic role of erythropoietin in reducing therapy-induced neurotoxicity will be tested in molecular and cellular studies in *in vitro,* and in *in vivo* animal models. We will assess the effect of EPO on tumour cell growth and chemotherapy toxicity *in vitro,* and determine the protection provided by EPO in an *in vitro* neuronal cell model. We hypothesize that EPO has no growth and survival activity on lymphomatous cells, but is neuroprotective against chemotherapy and radiotherapy toxicity in cultured neuronal cells. We will search for EPO-induced alteration of the blood-brain barrier permeability in normal rats and in rats with intracerebral tumours. We hypothesize that EPO does not prevent the entry of chemotherapy in the brain. Finally, we will determine if there is a neuroprotective effect of EPO in rats exposed to neurotoxic doses of chemotherapy and radiotherapy. We hypothesize that EPO chemoprotection can improve the management of treatment-related CNS toxicities.

CARDIOLOGY

epoetin beta

subcutaneously

administered

dose

EFFECT OF 5,000 IU EPOETIN BETA ONCE WEEKLY SUBCUTANEOUSLY (SC.) ADMINISTERED FOR THREE MONTHS IN PATIENTS SUBJECTED TO PERCUTANEOUS CORONARY INTERVENTION (PCI) DISPLAYING REDUCED LV-EJECTION FRACTION DUE TO REGIONAL LEFT VENTRICULAR WALL MOTION DEFECTS

Dr Martin Bergmann, Katheterlabor, Franz Volhard Klinik, Germany

Despite the increasing success of interventional methods to restore blood supply to ischaemic myocardium, the resultant functional improvement of myocardial contractility is often limited. Erythropoietin has been shown to protect cardiomyocytes from apoptosis, enhance the level of circulating progenitor cells and possibly contribute to restore functional myocardium in previously ischaemic areas and increase oxygen supply by increasing the haemoglobin levels. All these effects may enhance myocardial function after percutaneous coronary interventions (PCI). Therefore, the study will test the effect of a once weekly dose of epoetin beta applied subcutaneously beginning at the time of PCI on the recovery of regional wall motion defects at three months. Cardiac function will be precisely assessed by cardiac magnetic resonance imaging.

OTHER AREAS

characterisation

family

chaperones

concept

CHARACTERISATION OF A NOVEL INTESTINAL HEME TRANSPORTER

Dr Andrew McKie, Life Sciences, Kings College London, UK

Iron is an essential nutrient required by the body to make the protein haemoglobin in red blood cells which is essential for delivering oxygen to working muscles and other cells. Too little iron in the diet (iron deficiency) leads to anaemia causing fatigue. On the other hand too much iron is toxic and can damage vital organs like the heart and liver. Red meat is a good source of iron because it contains a lot of heme iron which is efficiently absorbed by the small intestine. We have now identified the protein responsible for the absorption of heme from the diet. In this application we study the proteins involved. One aim will be to develop synthetic dietary supplements based on the structure of heme which would be suitable for treatment of iron deficiency anaemia in vegetarian communities.

CHARACTERIZATION OF A FAMILY OF PUTATIVE MAMMALIAN HEME CHAPERONES

Dr Chris Vulpe, Department of Nutritional Sciences and Toxicology, University of California, Berkeley, USA

Iron is an essential nutrient that is required for a wide range of biochemical reactions in the body. One of the important roles of iron is in heme which is used by a variety of proteins including haemoglobin, cytochromes (important for mitochondrial function) and P450s (involved in chemical detoxification). Despite the importance of heme and the recent progress in understanding iron metabolism in both yeast and mammals, the distribution of heme from the mitochondria to the organelles and proteins that require it has remained a central intractable enigma of mammalian metal metabolism. We have identified a family of eukaryotic proteins that very likely represent the long elusive heme chaperones which deliver heme to apo-proteins. We propose to characterize the mammalian members of this family of proteins and investigate the mechanisms of heme transport and delivery. Understanding heme metabolism is vital to the understanding of iron deficiency, the most common nutritional disorder in the world, and the resulting anaemia. This study will provide insight into the clinical consequences of anaemia including impaired psychomotor and cognitive development in children, increased morbidity in anaemic mothers and diminished work capacity in affected adults and possibly lead to therapeutic interventions.

ERYTHROPOIETIN REDUCES BRAIN, EYE AND LUNG DAMAGE IN VERY PRETERM INFANTS: PROOF OF CONCEPT STUDY

Professor Hans-Ulrich Bucher, University Hospital Zurich, Zurich, Switzerland

Erythropoietin (EPO) has been shown to be protective against hypoxic-ischaemic and inflammatory injuries in a broad range of tissues and organs besides promoting red cell formation. In particular protective effects on brain, retina and bowel have been shown in animal models and initial human studies. EPO has been used widely for several weeks in preterm infants to prevent anaemia and is well tolerated. No short and long-term adverse effects have been documented with EPO treatment in preterm infants. Because EPO has been shown to influence several mechanisms associated with these short-term and long-term complications of prematurity and furthermore has been shown to have a positive effect even post hoc, i.e. if given within a period of hours after an hypoxic-ischaemic insult, EPO may ameliorate the damage in very premature infants. Very preterm infants may suffer from a variety of short-term complications and long-term sequelae of premature birth. The most critical period is the first few days after birth. Inflammatory changes as a consequence of hypoxia-ischaemia or infection seem to have a major impact on short-term as well as permanent damage. To determine whether early administration of EPO alters the incidence and severity of complications typically associated with preterm birth in infants born between 24 and 27 gestational weeks, we want to investigate, in a clinical trial, prophylactic therapy with EPO.

THE IMPACT OF ERYTHROPOIETIN ON THE HYPOXIC VENTILATORY RESPONSE OF MOUSE AND MAN

Prof Max Gassmann Vetsuisse, University of Zurich, Switzerland

For decades, the blood hormone erythropoietin (EPO) has been thought to exert solely an erythropoietic function. Within the last few years, we and others discovered that EPO is expressed by neuronal cells too and that it has (neuro)protective effects such as protecting the brain from stroke or the retina from degeneration. However, a physiological role for brain-derived EPO has not been established so far. We very recently demonstrated that EPO directly influences the respiratory center via central (brainstem) and peripheral (carotid bodies) organs (Soliz et al., in press). This finding proves that EPO has a crucial role in the acclimatisation to reduced environmental oxygen. We propose to extend our studies as follows: a) considering that women and female mammals demonstrated a better capacity to adapt to hypoxia, we are interested to define whether gender-specific differences occur in the EPO-enhanced ventilatory response in hypoxia. b) We also plan to investigate whether EPO increases the carotid body sensitivity to oxygen changes in blood. c) As the data described above has been obtained in mice only, we plan to determine in a first step whether EPO influences ventilation in man, too. We expect to provide new mechanistical insights into the EPO-mediated ventilatory response to hypoxia that may translate into clinical application involved in ventilatory diseases such as Chronic Mountain Sickness and premature newborn apnoea.

EFFECTS OF SYSTEMIC ERYTHROPOIETIN THERAPY ON CEREBRAL AUTOREGULATION AND THE INCIDENCE OF DELAYED ISCHAEMIC DEFICITS IN PATIENTS WITH ANEURYSMAL SUBARACHNOID HAEMORRHAGE

Peter J Kirkpatrick, University of Cambridge, United Kingdom

Intracranial bleeding from a ruptured cerebral blood vessel (called a subarachnoid haemorrhage) affects 7000 patients each year in the UK and is a source of considerable death and disability, even in young adults. Recent observations indicate that these bleeds can cause narrowing of the brain vessel (vasospasm) leading to reduced blood flow and eventual stroke. In this study we wish to use erythropoietin, a widely used natural human hormone for treating anaemia, to reduce vasospasm and clinical deterioration from low blood flow. A man-made version has shown promise in improving outcome of general stroke patients, and beneficial effects can be seen within a few days of treatment. In this proposal we would like to treat subarachnoid haemorrhage patients with erythropoietin soon after they are admitted to hospital. We will compare these patients with those treated with a dummy drug (placebo). Safety will be scrutinised, and ultrasound used to examine aspects of cerebral blood flow known to influence patient outcome. We also aim to identify any evidence of reduced episodes of neurological worsening in patients given erythropoietin. Results from this study will help in the design of a larger trial needed to examine clinical outcome. Benefits identified may be helpful in other conditions associated with cerebral haemorrhage.

EFFECT OF ERYTHROPOIETIN ON BRAIN INJURY AND REGENERATION IN BACTERIAL MENINGITIS

Prof Stephen L. Leib, University of Berne, Switzerland

Bacterial meningitis is associated with a mortality rate of up to 30% and up to half of survivors suffer from permanent neurological sequelae including deafness and learning impairment. The dramatic mortality and morbidity rates have remained unchanged for several decades in spite of advances in antimicrobial and intensive care therapies. Injury caused by bacterial meningitis prominently affects the inner ear and two brain structures, namely the cortex and the hippocampus. In the cortex, the damage includes areas of cerebral infarction. A specific form of brain injury, namely apoptosis in the hippocampus has been observed in patients dying from bacterial meningitis. The affected brain structure is responsible for learning and memory functions. Erythropoietin has been shown to protect the brain from injury by stroke, a disease with similarities to bacterial meningitis. In addition to this protective effect, erythropoietin has been shown to increase brain repair mechanisms. We thus hypothesize that adjuvant erythropoietin exerts a beneficial effect in bacterial meningitis by the combined effect of preventing acute brain damage and increasing brain repair mechanisms. In the proposed project we plan to assess in experimental bacterial meningitis a) whether therapy with erythropoietin prevents acute brain damage; b) whether this beneficial effect is still evident when therapy with erythropoietin is delayed until symptomatic disease and started together with antibiotics at 18 hours after infection (reflecting the clinical situation); c) whether erythropoietin attenuates injury to the inner ear and thus prevents hearing loss, the most frequent sequel of bacterial meningitis; d) whether erythropoietin increases brain repair mechanisms (e.g. expansion of brain stem cells) in the late phase of the disease and; e) whether the combined effect of erythropoietin mediated protection and increased brain repair leads to improved outcome assessed by learning and hearing performance in long term survivors of bacterial meningitis.

RECOMBINANT HUMAN ERYTHROPOIETIN: A NEW TREATMENT FOR FRIEDREICH`S ATAXIA

Dr Barbara Scheiber-Mojdehkar, Medical University of Vienna, Austria

Friedreich's ataxia (FRDA) is the most common of the inherited ataxias, affecting one in 50,000 people. FRDA is caused by a GAA-trinucleotide expansion in the frataxin gene, resulting in a reduced expression of frataxin, a small mitochondrial protein. The exact physiological function of frataxin is unknown, but it may be involved in mitochondrial iron homeostasis and/or assembly of iron-sulfur (FeS) proteins and heme synthesis. Clinically there is an intramitochondrial iron accumulation in heart, liver, nervous system and spleen of FRDA-patients, as well as a reduction of mitochondrial DNA, the FeS cluster-containing subunits of the mitochondrial electron transport chain (complex I-III) and of the enzyme aconitase. We found that additionally to its reported neuro- and cardioprotective properties recombinant human erythropoietin (rhuEPO) significantly increases frataxin expression in primary lymphocytes from FRDA-patients. Additionally rhuEPO can increase frataxin expression in many other cell types among them the most affected in FRDA such as neurons and cardiac cells. The potential therapeutic role of rhuEPO for the treatment of FRDA will be directly tested in an open-label, single-dose pilot study in FRDA-patients. For this study we will recruit 13 FRDA patients where 7 out of 13 have been tested for *in vitro* response of their lymphocytes to rhuEPO-treatment. We will test if the effects on frataxin expression seen *in vitro* can also be seen in patients. Therefore the safety and efficacy of rhuEPO for the treatment of FRDA will be tested in an open-label, single-dose pilot study.

ERYTHROPOIETIN NEUROPROTECTION IN RETINAL NEURODEGENERATION

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Programmed cell death (apoptosis) is the final common pathway of neural loss in the visual system in a variety of neurodegenerative diseases, some of which are primarily genetic, such as photoreceptor degenerations (retinitis pigmentosa, cone dystrophies and Stargardts macular dystrophy) and retinal ganglion cell (RGC) degenerations (inherited optic neuropathies and glaucoma). We have recently generated a novel model of RGC neurodegeneration, in which the genetic defect is in the murine gene OPA1, and leads to a primary retinal ganglion cell loss in postnatal life by a mechanism of apoptosis triggered by mitochondrial dysfunction. The human counterpart of this is the primary inherited optic neuropathy, autosomal dominant optic atrophy (ADOA), caused by mutation in the OPA1 gene. We have also established *in vitro* model systems using primary retinal ganglion cells, with OPA1 knockdown, in our laboratory. The identification of the growth and survival factor erythropoietin (EPO) receptor on tissues and cells other than red cell progenitor cells suggests that EPO may have biological roles and functions other than the stimulation of erythropoiesis. Central nervous system (CNS) EPO receptors are expressed on neurons, astrocytes, microglia and myelin sheaths and EPO is thought to have neuroprotective and neurotrophic effects on neuronal cells. The aim of this study is to explore the neuroprotective effect of EPO in neural retina in a model of primary RGC degeneration, using both a whole organism and cells in culture. We suggest that EPO, which is expressed in neural retina and acts at the mitochondrial membrane to protect nerve cells from apoptosis under adverse cellular conditions, can protect against RGC loss triggered by single gene defects in the neural retina. Our models will be used to investigate the effect of EPO administered systemically and locally on retinal ganglion cell loss and retinal degeneration. The end-point will be the assessment of retinal morphology, functional vision and cell survival.

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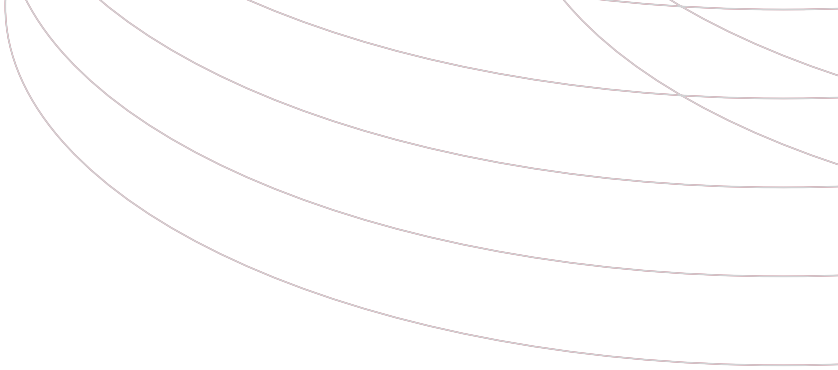

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