than in rat skeletal muscle deserves further molecular studies to determine which isoforms of CPT I are expressed in the piglet skeletal muscle.

Communication no. 18

Isoprostanes (8-epi PGF2a), products of lipid peroxidation, as oxidant stress markers. C. Feillet-Coudray, A. Mazur, E. Rock, Y. Rayssiguier (Laboratoire des maladies métaboliques et micronutriments, Inra, Theix, 63122 Saint-Genès-Champanelle, France)

Free radicals have been implicated in the pathophysiology of a wide variety of diseases including cancer, atherosclerosis, neurodegenerative disorders and even the normal ageing process. Measurement of lipid peroxidation is often employed to evaluate oxidative stress, and more precisely, oxidation products of polyunsaturated fatty acids (TBARS, conjugated diene). These assays, however, suffer from inherent problems related to specificity and sensitivity. Thus, there is a need to provide more reliable markers of oxidative stress in vivo. Recently, isoprostanes have been proposed and seem to be particularly valuable markers. Isoprostanes are produced by the free-radical peroxidation of arachidonic acid. Morrow et al. (Morrow et al., Proc. Natl. Acad. Sci. USA 87 (1990) 9383–9387) were the first to quantify free isoprostanes in plasma and urine by GC/MS, and to demonstrate the increased levels of lipid peroxidation in animal models. Measurement of isoprostanes allowed the detection of oxidative stress in animal models and provided evidence for a role of oxidative stress in human disease. In animals, administration of CCl4 to normal rat and diquat to selenium-deficient rats, combined with vitamin E/selenium and iron overload caused increased levels of isoprostanes. In humans, increased levels of isoprostanes were observed in relation to age, chronic cigarette smoking, diabetes and hypercholesterolemia; administration of vitamin E decreased these levels (Morrow et al., Biochim. Biophys. Acta 1345 (1997) 121–135).

We carried out a large number of nutritional and metabolic disorder studies in animal models, using GC/MS methods (which are difficult and expensive) and recently developed immunoassay methods (which are less time consuming and less expensive). Deficiencies in antioxidant micronutriments (zinc, copper), ageing, alcohol intake and diabetes (STZ) were investigated in rats. Increased urinary levels of isoprostanes were observed in Cu-deficient animals on a prooxidant diet (fructose). Membrane alterations in Cu-deficiency which are related to haemolytic anaemia, may explain this observation. No modification in the levels of isoprostane was observed in other studies. In humans, trials are under way to assess isoprostane levels in degenerative disorders associated with ageing.

The discovery of isoprostanes has opened up new areas of investigation regarding the occurrence of free radical damage in human physiopathology and may provide a valuable approach to evaluate the efficiency of antioxidant micronutriments.

SESSION 3:

TISSUE GROWTH

Communication no. 19

Apoptosis in myogenic cells: effect of oxidative stress and flavonoid antioxidants. A. Orzechowski[a], J. Skierski[b], W. Zimowska[b], B. Balasinska[b], T. Motyl[b], B. Lukomska[c] ([a] Department of Animal Physiology, Warsaw Agricultural University, Nowoursynowska 166, 02-785 Warsaw, Poland; [b] Flow Cytometry Laboratory, Drug Institute, [c] Surgery Research and Transplantation Department, Medical Research Center Institute, Warsaw, Poland)

Muscle differentiation is initiated by a Rb (retinoblastoma) gene product (Okuyama

et al., FEBS Lett. 397 (1996) 219–224) that possesses an antiapoptotic activity (Wang et al., Canc. Res. 57 (1997) 351–354). The upstream regulator of Rb expression is the Bcl-3 protein that may further protect cells from apoptosis owing to its similarity to the Bcl-2 family (Shiio et al., Oncogene 12 (1996) 1837–1845). The antiapoptotic properties of Bcl-2 proteins are in turn explained by their ability to protect cells against oxygen free radicals (OFR) (Hockenberry et al., Cell 75 (1993) 241–251). It is assumed that susceptibility of myogenic cells to programmed cell death (PCD) should fluctuate with the consecutive stages of muscle tissue development and might be influenced by cellular redox status. Extensive formation of OFR was induced by either of the selected oxidants (H_2O_2 (10 mmol·L^{-1}), 2,2'azobis (2amidinopropane) dihydrochloride-AAPH (1 mmol·L^{-1}), $CuSO_4$ (1 mmol·L^{-1})) in L6 rat skeletal myoblasts. Muscle growth impairment was also achieved by intragastric administration of dexamethasone disodium phosphate (DEX, 2 mg·kg b.w.$^{-1}$·day^{-1}) for 5 days to male Sprague-Dawley rats (6 weeks old). Quercetin aglycon was applied as an antioxidant for in vitro studies (100 mmol·L^{-1}), whereas quercetin-3-rutinoside (2 mg·day^{-1}·animal^{-1}) pre-treatment was chosen as antioxidant in rats. The extent of apoptosis in cultured growing L6 myoblasts was calculated using flow cytometry with combined labelling by fluorochrome (DAPI + SF and HO33342 + PI) that enables detection of cell cycle phases, early- and late-apoptosis, and necrosis. Bcl-2 expression was determined by flow cytometry in cells labelled with FITC-conjugated anti-Bcl-2 monoclonal antibodies. Post mortem cross sections of soleus muscle were subject to simultaneous TUNEL assay and Hoechst 33342 labelling in order to calculate the number of apoptotic nuclei of muscle fibres and satellite cells. A higher incidence of apoptosis was induced by oxidative stress: H_2O_2 (6.22 %), AAPH (19.8 %), $CuSO_4$ (7.8 %), with (3.58 %) in control. Diminished expression of Bcl-2 protein in myoblasts exposed to oxidants was positively correlated to the rate of apoptosis ($P < 0.05$). When quercetin aglycon was present with oxidants, Bcl-2 expression was not altered, but the rate of apoptosis was still accelerated. Dexamethasone caused oxidative insult confirmed by the decline of tissue glutathione ($P < 0.05$) and lower superoxide dismutase activity ($P < 0.05$). Apoptosis in merely singular muscle satellite cells and nuclei present within muscle fibres were detected after DEX loading. Rutin pre-treatment did not affect this process. The results indicate that myogenic cells were prone to PCD but were less vulnerable after differentiation (muscle fibres). This process was not affected by quercetine.

Communication no. 20

Indispensability of polyamines for survival of HC11 mouse mammary epithelial cells. T. Ploszaj[a], T. Motyl[a], W. Zimowska[a], J. Skierski[b] ([a] Department of Animal Physiology, Veterinary Faculty, Warsaw Agricultural University, Nowoursynowska 166, 02-787 Warsaw, Poland; [b] Flow Cytometry Laboratory, Drug Institute, Warsaw, Poland)

Polyamines are organic polycations indispensable for mammary cell proliferation, differentiation and secretory activity. The high biosynthesis rate and high level of polyamines in milk at the onset of lactation coincide in time with the highest rate of mammary gland development and high demands of neonates for these compounds. Ornithine decarboxylase (ODC) – a key enzyme in the polyamine pathway is involved in the signal transduction pathway of hormones and growth factors that regulate mammary gland remodelling and milk secretion (e.g. prolactin, EGF, IGF-I, TGF-β_1). In the present study the effect of α-difluoromethylornithine (DFMO) (an irreversible inhibitor of ODC and exogenous putrescine