Toxicological Pathology

EXPRESSION OF IgA AND PIgR IN THE ILEUM OF GF MICE AFTER ADMINISTRATION OF *L. REUTERI* AND CHALLENGE WITH PCV-2

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Introduction: Porcine circovirus-2 (PCV-2) is a very stable virus replicating in mammalian cells that can persist in infected herds. *Lactobacillus reuteri*, a normal gastrointestinal inhabitant of man and animals, produce reuterin with antimicrobial activity. The aim of this study was to investigate the effect of *L. reuteri* on transcription of genes encoding immunoglobulin (Ig)A and the polymeric Ig receptor (pIgR) and to evaluate the amount of infected virus in faeces after challenge of germ-free mice with PCV-2.

Materials and Methods: Four-week-old GF mice ($n = 30$) were divided into three groups: PCV-2 (0.3 ml/ mouse per os on day 7), LPCV-2 (10^9 CFU/0.1 ml; from day 1 to 7 of the experiment + PCV-2) and controls. Samples of ileum were homogenized and total RNA was isolated. Primers for IgA and pIgR were used. The PCV-2 was quantified by RT-PCR. Amplification and detection of specific products was performed using the CFX 96 RT system (Bio-Rad, USA) with a predefined programme.

Results: Relative mRNA expression from the gene encoding IgA was upregulated in the LPCV group compared with control ($P < 0.001$) and PCV ($P < 0.01$) at both sampling points (5 and 14 days post infection; dpi). However, mRNA expression from the gene encoding pIgR was upregulated in LPCV compared with control ($P < 0.01$) and PCV ($P < 0.05$) groups only at 14 dpi. The mean amount of PCV2 in faeces was significantly lower from 3 dpi in the PCV-2 group compared with the LPCV-2 group.

Conclusions: A beneficial effect of *L. reuteri* was observed, with increased expression of IgA and pIgR genes in the ileum and a decreased amount of PCV-2 in the faeces.

HISTOPATHOLOGICAL EFFECTS OF PENTOXFYLLINE ON SKELETAL MUSCLE INJURY INDUCED BY ACUTE HINDLIMB ISCHAEMIA–REPERFUSION IN RATS

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Introduction: Skeletal muscle ischaemia–reperfusion injury can occur with diseases, trauma and during orthopaedic surgical procedures. Ischaemia–reperfusion injury is characterized by the production of oxygen-free radicals leading to disturbances in vasomotility and microvascular permeability. This study evaluated the effects of pentoxifylline, a methylxanthine derivative, on skeletal muscle injury after acute hindlimb ischaemia–reperfusion.

Materials and Methods: Twenty male Wistar rats were allocated randomly into two groups: ischaemia–reperfusion and ischaemia–reperfusion + pentoxifylline. All animals underwent 2 h of ischaemia by occlusion of the femoral artery followed by 24 h of reperfusion. Rats in the ischaemia–reperfusion + pentoxifylline group were given an intraperitoneal injection of pentoxifylline (40 mg/kg) immediately before reperfusion. After the reperfusion period, animals were killed and the left gastrocnemius muscle was harvested for histopathological analysis.

Results: In the ischaemia–reperfusion group, the changes were more intense with haemorrhage and necrotic muscle fibres, inflammatory cell (lymphocyte and neutrophil) infiltration and interstitial oedema.

Conclusions: The administration of pentoxifylline significantly decreased skeletal muscle injury induced by acute hindlimb ischaemia–reperfusion. Pentoxifylline improves microcirculation flow, reduces migration of neutrophils, reduces release of cytokines, increases production of prostacyclines and reduces release of reactive oxygen species.

DETECTION OF APOPTOTIC EVENTS, USING DIFFERENT METHODS, IN RENAL TISSUES AFTER ACUTE HAEMORRHAGE

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Introduction: Apoptosis plays an important role in cellular damage caused by acute renal hypoperfusion. The aim of this study was to detect apoptotic events in an animal model of acute haemorrhage, followed by volume replacement with different intravenous solutions.

Materials and Methods: Renal samples were collected from animals subjected to passive arterial bleeding and reperfusion with a crystalloid (RL) (group 1) and with a synthetic colloid (HES 130/0.4) (group 2). All procedures were carried out under personal and project licenses approved by the Ethical Committee of the national regulatory office. Immunohistochemistry was performed using cytochrome c antibody to detect mitochondrial activity and the in-situ TUNEL method was used to evaluate endonucleosomic cleavage of DNA by TdT. An immunofluorescence method, the M30 Cytodeath, was also used to detect early apoptotic events.

Results: In all groups, apoptosis was detected in the epithelial tubular cells of the proximal and distal convoluted tubules, in the loop of Henle and in the collecting tubules. However, the percentage of apoptotic cells and the intensity of the reaction was significantly higher in group 2.

Conclusions: Apoptosis plays an important role in cellular damage in renal tubules following acute haemorrhage and volume replacement. The type of solution used for volume replacement may influence the extent of renal damage resulting. In the present study, the use of RL was related to decreased cytosolic cytochrome c and a lower apoptotic index in the tubular renal cells.

CHROMIUM CHLORIDE CYTOTOXICITY ON BALB/C MOUSE EMBRYO FIBROBLASTS

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Introduction: Chromium is an element commonly occurring in nature in trivalent (Cr III) and hexavalent (Cr VI) forms. The reduction of Cr VI to Cr III results in formation of reactive intermediates, such as superoxide anion, singlet oxygen and hydroxyl radicals, as well as Cr V and Cr IV intermediates, which may interact with microfilaments, mitochondria, lysosomes and the nucleus. Inside the cells, chromium Cr (III) compounds can directly bind with DNA. The aim of this study was to examine the cytotoxic effect of chromium chloride on mouse fibroblasts.

Materials and Methods: The experiments were performed on the BALB/c 3T3 cell line. The cells were cultured in DMEM (2×10^5 cells/ml), supplemented with FBS and antibiotics. After 24 h of incubation the medium was exchanged for fresh medium supplemented with $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$ at final concentrations of 0 (control), 50, 100, 200, 300, 400, 500, 600, 700 and 800 μM. After 24 h the cell viability was measured by MTT reduction, LDH release and neutral red uptake. Apoptosis was determined by photometric enzyme immunoassay.

Results: Chromium chloride decreased cell viability (confirmed by all three cell viability tests). Moreover, mono- and oligonucleosomes increased in the cytoplasm of cells.

Conclusions: The suggested mechanism of chromium action in mouse embryo fibroblasts is that the chromium in cells interacts first with mitochondria, then with the cell membrane and finally with lysosomes. Chromium chloride also interacts with DNA of cells.