
7 Summary

An isolated haemoperfused distal cow limb model for experimental studies on the pathogenesis of bovine laminitis

The actual knowledge on the pathomechanisms of bovine laminitis is still incomplete despite of progressively increasing research activities. Isolated perfused distal limbs are a promising model to study the biological mechanisms that lie behind the laminitis syndrome. For ethical and financial reasons experiments using this model are a preferable alternative to animal experiments. The aim of this work was to develop an isolated haemoperfused distal cow limb model basing on an already existing porcine limb model used already for studying the effects of pharmaceuticals.

The isolated limbs were obtained from routinely slaughtered cows and subsequently perfused in the laboratory of a local biotech company (Vitro-tec Entwicklungsgesellschaft mbH, Berlin). A standard perfusion apparatus enables isolated limb perfusion for up to 5 hours under close to physiological conditions. Perfusion pressure and flow were calculated and adjusted basing on values available for horses and pigs. The comparison of the different initial experiments showed that 190 ml/min is an optimal value for the flow of perfusate. The oxygen saturation was adjusted to 100 % and the pH preset between 7.35 and 7.45. Glucose was added as nutrient. The weight increase of the limb and the perfusion pressure served as vitality indicators. The threshold for an acceptable weight gain of the distal limb was 10 % and for the upper and lower values of perfusion pressure 150 mmHg and 30 mmHg respectively. In addition, systematic light- and electron microscopic examination of tissue samples from all segments of the claws was carried out to evaluate tissue vitality according to a list of standardised morphological criteria. A thermographic camera was used to detect "cold spots" as an indicator for ischemic areas. A complete electrolyte solution added with washed erythrocytes (haematocrit 8 – 10 %) turned out to be optimal for perfusion and was used as standard perfusate.

The model was challenged in a series of 23 experiments. Thereby the effects of candidate bioactive molecules relevant for the pathogenesis of laminitis on perfusion pressure and resulting tissue alterations were tested. The modern hypotheses on the pathogenesis of laminitis attribute a central role to disturbances in microcirculation and direct damage to the vascular endothelium in the initial phase of the development of laminitis. These disturbances and alterations are caused by toxins, vaso-active substances or metabolic by-products. Therefore, in the perfusion experiments of this work the effects of some of these substances most frequently discussed in relation to laminitis have been tested.

The claw tissue was perfused under oxygen deficiency and reduced flow rates. Furthermore the model was challenged by endotoxin, lactate and histamine to detect whether these substances produce changes in perfusion or structure of the vascular system or cause tissue alterations relevant for the development of laminitis.

It was possible to show that an oxygen deficiency between 15 – 30 % for 4 hours and agents like histamine and E. coli endotoxin provoke changes in perfusion parameters and alterations in tissue structure. The following changes were detected: An increase in pressure and organ resistance, alterations of vascular permeability, formation of oedema and cell damage. These alterations perfectly fit in the actual hypotheses on the pathogenesis of laminitis. The limited period of 5 hours however does not permit final conclusions whether this condition finally would lead to clinical laminitis.

The model introduced here is interesting to investigations on a variety of specific questions in Veterinary Medicine. It is attractive in particular for further research into equine and bovine laminitis. Furthermore it is also attractive for research in human medicine with possible applications in Pharmacology and Toxicology. In combination with modern analytical methods such as PCR techniques the reactions of cells to the challenging factors could be detected on the molecular level. The combination of the described novel ex vivo model with real-time PCR for quantitative analysis of effects on the cellular protein expression is recommended for future investigations using this model.