Changes of interleukin-10 mRNA expression are predictive for 9-day survival of pigs in an emergency preservation and resuscitation model

Wolfgang Sipos1 Catharina Duvigneau1 Wolfgang Weihs2 Fritz Sterz2 Alexandra Graf2 Romana Hartl1 Keywan Bayegan2 Andreas Janata2 Danica Krizanac2 Wilhelm Behringer2

1. University of Veterinary Medicine Vienna, Vienna, Austria; 2. Medical University of Vienna, Vienna, Austria

Introduction
Outcome after prolonged normovolemic cardiac arrest is poor. The technique of evaluating the neurologic state and the overall performance category in pigs resuscitated after cardiac arrest has already been optimized (1). Emergency preservation and resuscitation (EPR), which is the induction of hypothermia during the arrest, followed by hypothermic stasis during transport to resuscitation with cardiopulmonary bypass (CPB), might be a new concept for patients with prolonged cardiac arrest. We have shown previously that induction of deep cerebral hypothermia via ice cold saline aortic flush during cardiac arrest just before resuscitation improved neurologic outcome in pigs (2). To avoid fluid overload, we further developed a cardiopulmonary bypass cooling system (CPBCS) for rapid induction of hypothermia (3). We hypothesized that changes in cytokine expression during EPR could be predictive of resuscitability and 9-day survival.

Materials and Methods
21 Large White sows (31-38 kg BM) were subjected to 15 min of untreated ventricular fibrillation, followed by cooling with a CP-BCS for 1, 3, or 5 min achieving brain temperatures (Tbr) of 30.4 ± 1.6°C, 24.2 ± 4.6°C, and 18.8 ± 4.0°C, respectively. After 40 min of rewarming with CPB, pigs were defibrillated and kept at Tbr of 34.5°C for 20 hrs, survival was for 9 days. Plasma samples were analysed for interleukin (IL)-6, tumor necrosis factor-a (TNF-a), and IL-10 levels by ELISA. Total RNA out of PBMCs was analyzed by real-time PCR for IL-1, IL-2, IL-4, IL-10, TNF-a, interferon-γ, iNOS, and heme oxygenase-1 (HO-1) gene expressions.

Results
11 out of 21 pigs survived to day 9. In the plasma of all animals, a remarkable increase in proinflammatory cytokine levels could be observed following cardiac arrest and resuscitation. However, no significant differences in cytokine expressions could be found as a consequence of different intra-arrest hypothermia levels. On the other hand, IL-10 was identified as prognostic marker with decreasing mRNA levels during cardiac arrest in 9-day surviving animals but increasing ones in the non-survivors.

Discussion
The systemic changes in the postresuscitation syndrome are mainly characterized by a proinflammatory state similar to the systemic inflammatory response syndrome (SIRS) and thus termed a sepsis-like syndrome (4). Interestingly, SIRS is not only characterized by elevated inflammatory cytokine levels including IL-1, IL-6, and TNF-a, but also may be the consequence of a more profound dysregulation of the immune system, which is inferred from elevated IL-10 titers (5). Our study confirmed that pigs upregulate markers of systemic inflammation following cardiac arrest, intra-arrest hypothermia, and resuscitation, thus resembling the postresuscitation syndrome. The slight increase of IL-10 mRNA in non-survivors may account for a change in the functional properties of white blood cells. These findings hopefully will lead to improved intervention methods for the benefit of survival rate after restoration of spontaneous circulation following cardiac arrest.

References

This study was supported by a grant from the “Bürgermeisterfonds der Stadt Wien”, Project No: AP07075BGM.
Chemotherapy induces partial remission of mast cell leukemia in a minipig

Wolfgang Sipos¹ Johannes Hirschberger² Wolfram Breuer² Isabella Zenker² Sabine Elicker³
1. University of Veterinary Medicine Vienna, Vienna, Austria; 2. Ludwig-Maximilians University Munich, Munich, Germany; 3. Veterinary Practice Entenfellner, Stössing, Austria

Introduction
Mast cell tumors are classified as cutaneous or systemic. Cutaneous mast cell tumors represent a comparably frequent malignancy in dogs and cats, but only single reports about cutaneous mast cell tumors in pigs and only one referring to a case of porcine mast cell leukemia (1) can be found in the literature. In the following, a chemotherapy in a porcine leukemia patient is presented for the first time.

Case Report
This report describes a chemotherapeutic approach of treating mast cell leukemia in a three-year old, male-castrated Göttingen miniature pig weighing 27 kg. The animal had been submitted to the clinic due to a mildly depressed behaviour lasting for a month and a mild anemia. Hematologic analysis (Cell-Dyn 3500®; Abbott) including a modified Wright stain led to the diagnosis of a mast cell leukemia. Three days after the diagnosis of mast cell leukemia, chemotherapy was started based on a protocol applied in dogs suffering from cutaneous mast cell tumors (2). Induction therapy comprised weekly IV infusions of 2 mg/m² vinblastine sulfate for four weeks. Concomitantly, 2 mg/kg prednisolone were given daily per os. IV infusions were performed under general anesthesia achieved with IM application of 20 mg/kg ketamine and 2 mg/kg azaperone. Combined chemotherapy led to a reduction of total leukocyte count from 30.6 G/l to 25.3 G/l and a reduction of mast cell count from 18.05 G/l to 10.57 G/l. Animal’s behaviour became undisturbed again and no adverse side effects could be observed. For the next two weeks, no vinblastine was given. During this time, mast cell number increased to 35.34 G/l. As a consequence, weekly vinblastine applications were restarted and prednisolone was replaced by oral 50 mg cyclophosphamide boli supplemented by oral 20 mg furosemide boli, both given every second day. Furosemide administration aimed at achieving an accelerated clearance of cyclophosphamide metabolites and at abrogating the establishment of a hemorrhagic cystitis. Again, no adverse side effects could be recorded, but mast cell number continued to increase and peaked at 55.59 G/l one month thereafter. The multiagent protocol was applied for 6 weeks. Then vinblastine was given in a two-week interval. After 17 treatment rounds corresponding to an overall treatment period of half a year, overall behaviour became depressed again and feed consumption was decreased. At that point, total white blood cell number had increased to 83.7 G/l with a mast cell count of 33.48 G/l. At the same time, red blood cell count had dropped markedly. Therefore, the owner decided to stop treatment and to euthanize the animal.

At necropsy a reduced body condition, an anemic state, a splenomegaly, and gastric ulcers were evident. Histologically, an infiltration of inner organs as well as bone marrow by neoplastic cells, which stained multifocally positive for CD117 and more diffusely positive for chloroacetate esterase, could be found.

Discussion
Minipigs, which are kept as pets, are increasingly cared for at a higher medical standard as conventional pigs (3). Unfortunately, there is a lack of suitable treatment regimens of systemic mast cell tumors. Therefore, humans suffering from mast cell leukemia are treated mainly by corticosteroids and antihistamines. Prognosis for human mast cell leukemia is poor. Also in dogs systemic mast cell tumors are bad responders to chemotherapeutic interventions (4). In the presented case, the initial response to a vinblastine-prednisolone combination protocol was quite satisfying with mast cell number decreasing during the first four weeks of chemotherapy. Due to the following relapse another cytotoxic agent, cyclophosphamide, was included, but was unsuccessful in averting tumor progression.

References
**Improved neurologic deficit score for pigs in a cardiac arrest model**

Katharina Haas1 Michael Holzer2 Alexandra Graf2 Fritz Sterz2 Wolfgang Weihs2 Andreas Janata2 Wilhelm Behringer2 Wolfgang Sipos1

1. University of Veterinary Medicine Vienna, Vienna, Austria; 2. Medical University of Vienna, Vienna, Austria

**Introduction**

Cardiac arrest is unique in that when the heart stops, many otherwise healthy organs suffer injury, particularly the brain, the myocardium, and the endothelium. Therefore the goal of resuscitation is to restore victims to full health, including good neurological outcome. Resuscitation research studies the pathophysiology of acute terminal states and clinical death. Acute and short-term experiments in animals help to better understand mechanisms of dying and to evaluate the short-time effect of new resuscitation protocols. To evaluate long-term benefit of potential new treatments on neurologic recovery in resuscitation research, clinically realistic long-term outcome studies including evaluation of neurologic and histologic brain damage in animals high on the phylogenetic scale are necessary.

Recently, our group published a novel and reliable score for evaluation of neurologic outcome in pigs after cardiac arrest.1 The neurologic deficit score (NDS) consists of four equally weighted sections: mental status, breathing pattern, cranial nerve reflexes, and motor and peripheral sensory functions. Aim of this study was to re-evaluate and optimize the neurologic examination procedure in order to make it even more observer-independent by elucidating and then topping off the critical points of the NDS.

**Materials and Methods**

16 female Large White pigs (32 kg in the mean) were put into ventricular fibrillation cardiac arrest of 15 min duration followed by cooling to achieve different degrees of preservative hypothermia for 40 min. After resuscitation (ROSC: restoration of spontaneous circulation), controlled ventilation was for 24 h, and intensive care for 3 days. Neurologic evaluation was done independently by two observers, blinded to the experiments, at 72 h and 9 days post ROSC. The critical points of the NDS as published by Sipos et al. (2008), namely the evaluation of the mental state and, interestingly, the breathing pattern, were optimized.

**Results**

A significant difference in the NDS score between hypothermia groups was found (f-test p=0.0127). No significant difference in the NDS score was found between the two observers. The ICC (intra-class correlation coefficient) determined using analysis of variance components for the NDS score was 20.7 % indicating that 20.7 % of the total variability can be explained by the variability between the pigs. The largest contribution to the total variability came from the variability between time points (intervisit SD=68.22). Furthermore, a large contribution came from the variability between hypothermia groups (inter-treatment SD=47.15). The large variation over days and treatments also explained the small ICC. Practically no contribution to the total variability came from the variability between the observers (intrarater SD <0.001), which indicates a good repeatability of the score between observers.

**Discussion**

The evaluation of a pig’s mental state is very tricky under special situations, as for sure is the immediate post-no-flow time, as pigs are, by their nature, psychically very autonomous animals. However, the optimized NDS showed to be even more examiner-independent than the already published one, as also the mental state as well as the breathing pattern were now judged the same by both investigators in the majority of cases. At the same time, also the published overall performance category (OPC) score (1) was optimized (data not shown). The OPC score gave nearly 100 % identical results by both investigators and may be even more appropriate than the NDS in specific clinical settings.

**References:**

Ovariectomy and calcium shortage have no obvious effect on bone metabolism in adult sows

Wolfgang Sipos1 Martina Rauner2 Peter Pietschmann3
1. University of Veterinary Medicine Vienna, Vienna, Austria; 2. Carl Gustav Carus Universitätsklinikum Dresden, Dresden, Germany; 3. Medical University of Vienna, Vienna, Austria

Introduction
For a long time, osteoporosis was ascribed nearly exclusively to an increased activity of osteoclasts, but now it has been recognized, that also depressed osteoblastogenesis contributes to osteoporosis. Recently, osteoimmunology has emerged as a central area of interest as peripheral blood cells, cytokines, and other soluble immunomodulators exert their impact on a series of osteopathologies including osteoporosis (1). Thus, osteoporosis has been recognized to involve more than simply a skeletal system with decreased bone mineral density. Consequently, new therapeutic strategies need to be developed. To date, the majority of basic osteologic experiments has been performed using rodents, which have a lot of advantages in terms of laboratory management, but also large animal models might be beneficial for osteoporosis research due to biomechanical issues.

Multiparous sows – in contrast to nulliparous sows – being fed a standard diet containing 1.5 % calcium exhibited significantly increased plasma PTH and calcitriol levels at 8 months post ovariectomy and alkaline phosphatase levels at 12 months post ovariectomy. No significant changes concerning bone chemistry and histomorphometry could be observed (2). On the other hand, there have already been successful efforts to establish an osteoporosis minipig model (3). Ovariectomy in 4 months old minipigs resulted in a 6 % decrease in bone mineral density (BMD), 15 % in bone volume, and 13 % in trabecular number, and an increase of 15 % in trabecular separation, whereas ovariectomy in combination with a mild nutritive calcium shortage (0.75 % Ca2+) led to a 10 % reduction in vertebral BMD and significant increases in final erosion depth and vertebral marrow star volume. These data implicate, that an ovariectomy alone is sufficient to induce an osteoporotic phenotype in (mini)pigs, which can be even more pronounced by restricting calcium supply.

Materials and Methods
32 Large White sows aged 33.5 months in the mean were included and distributed equally to four groups:
1. sham-ovariectomy, 0.75 % Ca2+
2. sham-ovariectomy, 0.3 % Ca2+
3. ovariectomy, 0.75 % Ca2+
4. ovariectomy, 0.3 % Ca2+

Blood samples were collected by venipuncture every 2 months. The experiment was terminated after 10 months. Serum levels of following bone metabolism markers were determined by commercially available ELISA kits: receptor activator of nuclear factor-kB ligand, osteoprotegerin, calcitriol, PTH, bone specific alkaline phosphatase, osteocalcin, CICP, pyridinoline, and crosslaps.

Results
Analyses of indicated marker kinetics revealed no meaningful trends in general or differences between the four groups and thus gave no evidence of any ovariectomy- or calcium shortage-induced change in bone metabolism.

Discussion
Interestingly, ovariectomy and calcium shortage alone as well as a combination of these two did not lead to any effect on bone metabolism as delineated from the analysed marker kinetics. This is quite surprising, as other authors (2) found increasing PTH, calcitriol, and BAP plasma levels in sows of the same age group as the ones we investigated, although those were only ovariectomized but fed a standard diet. On the other hand, most investigators analysing the effects of ovariectomy and/or calcium shortage used minipigs or growing pigs, thus making a comparison with the data obtained from our study difficult.

References

This study was supported by a grant from the Austrian Wissenschaftsfonds, Project No: P20337-B13.
Cytokine modulation of yeast derivatives investigated on a lipopolysaccharid-activated monocyte-macrophage cell line

Anja Ganner; Christian Stoiber; Manuela Pelz-Studlar; Simone Schaumberger; Gerd Schatzmayr

Biomin Research Center, Tulln, Austria

Introduction
Cytokines are signaling molecules which play an important role in cellular communication. Modulation of cytokine secretion may offer novel approaches in the prevention and treatment of a variety of diseases in human and veterinary medicine. TNF-alpha, IL-1, IL-6 are important cytokines which are secreted by immune cells such as macrophages after endotoxin (LPS) exposure and are involved in endotoxic shock which can cause severe economic losses in swine production. Therapeutical approaches in veterinary field aim to minimize or to prevent inflammatory parameters triggered by endotoxins. Counteraction of pro-inflammatory cytokines with feed additives such as yeast derivatives might be an interesting approach.

Yeast derived beta-glucan fractions are described in the literature to interact with cytokines. Aim of the present study was to investigate the effect of different yeast derivatives such as beta-glucans, whole yeast, yeast extract and yeast nucleotides on nitric oxygen (NO) production, pro-and anti-inflammatory cytokine levels after LPS stimulation of the murine monocyte-macrophage cell line J774A.1.

Materials and Methods
Monocyte-macrophage cell line:
J774A.1 is a BALB/c mouse derived monocyte-macrophage cell line isolated from a tumour. Cells were cultured in tissue culture flasks at 37°C in DMEM complete growth medium supplemented with 10% fetal bovine serum. Subcultures were prepared by scraping adherent cells 1 to 2 times per week and re-seeded 1:4 into fresh media.

Cell culture assay:
J774A.1 cell number and viability were assessed by trypan blue dye exclusion on a Neubauer hemacytometer. For the experiment, cells were seeded at a concentration of 106 cells/well in a 24-well tissue culture plate. Cells were incubated for 48 hours with LPS of E. coli 0127:B8 and the yeast derivative, which was either yeast derived beta-glucan, yeast nucleotide, yeast extract or whole yeast (0.1%, 0.02%, 0.01% solutions in phosphate buffered saline). Produced nitric oxygen was determined after 48 hours of incubation in the supernatant of the cultures using a Griess reagent which provides a colorimetric reaction. Pro-inflammatory cytokines IL-1, IL-6, TNF-alpha and the anti-inflammatory cytokine IL-10 were detected with ELISA assay (R&D Systems).

Results
Yeasts extract and yeast nucleotides inhibited LPS induced NO-production between 20 and 50%; beta-glucan fraction up to 80% according to the used concentration. Whole yeast did not affect LPS induced NO-production. IL-6 was inhibited between 20 and 40% by beta-glucan, by yeast nucleotide around 20%. Whole yeast did not inhibit IL-6 on a notable level. IL-1 was inhibited to 70% by a yeast nucleotide product, between 40 and 100% by beta-glucan and to 50% by whole yeast. TNF-alpha was inhibited around 40% by nucleotides, beta-glucans and also by whole yeast. All yeast derivatives tested induced IL-10 production.

Discussion
The present results revalue that yeast derivatives inhibited LPS induced pro-inflammatory cytokines and elevate anti-inflammatory IL-10 levels. However, the mechanism behind, the mode of action is not yet understood. A fully chemical characterization concerning mannan-and glucan content and nucleotide profile might be illuminating.

In conclusion, yeast derivatives which interfered in this in vitro study with IL-1, IL-6, TNF-alpha and IL-10 might be an interesting approach in the control and prevention of endotoxic shock in swine production and this might impact positively on animal health. However, it is too early to estimate to what extent these expectations are going to be fulfilled in vivo. In any case they justify further multidisciplinary research in this field.

References
Olson et al. (1996); Infection and Immunity 64(9): 3548-3554
Bedirli et al. (2007); Shock 27(4): 397-401
**Introduction**

Isoimmune thrombocytopenic purpura (ITP), firstly described by Stomorken et al. in 1963, is a sporadic disease affecting newborn piglets, mainly from secondiparous or multiparous sows. Large White and Landrace are the most affected breeds. ITP is an immune-mediated disease subsequent to colostral transfer antiplatelet (PLTs) antibodies produced by sow against the antigens originating from the boar semen (Saunders and Kinch, 1968). Multiple haemorrhages develop in piglets when platelets are lower than 30,000-50,000/μl, leading to death the whole litter or a part of it. The Authors describe a case of ITP occurred in a farrow to finish pig herd.

**Materials and Methods**

An hemorrhagic syndrome occurred in 28 litters of a farrow to finish pig herd between May 2008 and Jan 2009. Only one of the sows was primiparous. Sows had been inseminated during 3 days using mixed semen from different boars. During the first week of age seventeen percent of the piglets showed haemorrhagic diathesis, characterised by multiple haemorrhages, spontaneous petechiae and diffuse ecchymoses. The death rate ranged from 0% to 50%. Six piglets were submitted to necropsy, histological bacteriological, virological and toxicological examinations. Furthermore, 6 piglets with hemorrhagic syndrome and 3 coehtaneous healthy piglets from the same farrowing batch were submitted to complete blood cell count (CBC).

**Results**

Hemorrhagic lesions characterised by multiple haemorrhages, spontaneous petechiae and ecchymoses were detected in piglet skin, subcutaneous tissue, inner organs and mucous/seros membranes. The most affected sites were head, abdomen, groin and soles. Kidneys and lymph nodes presented disseminated hemmorhages and hemorrhagic suffusions were evident in mesocolon and epicardium. Histology confirmed the presence of multivisceral interstitial hemorrhages, extramedullary hematopoiesis and interstitial pneumonia. Bacteriological, virological and toxicological examinations provided negative outcomes. CBC results are presented in the following table.

**Table 1.**

<table>
<thead>
<tr>
<th></th>
<th>ITP (mean)</th>
<th>Healthy (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (Hb) gr %</td>
<td>5.5</td>
<td>9.3</td>
</tr>
<tr>
<td>Hematocrit (HCT) %</td>
<td>17.5</td>
<td>28.2</td>
</tr>
<tr>
<td>RBCs /μm3</td>
<td>2.525,000</td>
<td>4.390,000</td>
</tr>
<tr>
<td>PLTs /μm3</td>
<td>33,557</td>
<td>241,133</td>
</tr>
<tr>
<td>Leukocytes /μm3</td>
<td>6,750</td>
<td>4,833,3</td>
</tr>
<tr>
<td>MCV fL</td>
<td>69,9</td>
<td>64,3</td>
</tr>
<tr>
<td>MCHC gr %</td>
<td>32,2</td>
<td>33,1</td>
</tr>
<tr>
<td>MCH pgr</td>
<td>22,6</td>
<td>21,3</td>
</tr>
<tr>
<td>RDW %</td>
<td>22,1</td>
<td>19,2</td>
</tr>
<tr>
<td>NRBC/100 WBC</td>
<td>13,2</td>
<td>1,7</td>
</tr>
<tr>
<td>Reticulocytes %</td>
<td>12,2</td>
<td>2,35</td>
</tr>
<tr>
<td>Lymphocytes /μm3</td>
<td>2,358</td>
<td>1,692</td>
</tr>
<tr>
<td>Neutrophils /μm3</td>
<td>3,225</td>
<td>2,867</td>
</tr>
<tr>
<td>Eosinophils /μm3</td>
<td>113,05</td>
<td>120</td>
</tr>
</tbody>
</table>

**Discussion**

On the basis of laboratory results ITP was diagnosed. BCC showed a marked decrease in PLTs (absence of PLT aggregation), Hb, HCT and RBCs in affected animals. Moreover, a pattern of regenerative anemia and reticulocytosis was detected. For differential diagnosis, infectious and toxic diseases were ruled out. Combining all data a diagnosis of an immuno-mediated disease was suggested. The marked thrombocytopenia and the absence of direct or indirect signs of erythrocytolysis allowed to rule out the haemolytic disease of newborn pigs (Taylor 2008). From Jan 2009 the insemination practice was modified using the semen from one boar only for each sow. Consequently, one boar was associated with the occurrence of the diseases in offspring and it was culled.

**References**

Agents isolated in pericarditis cases from pigs in the nursery, growing and finishing in Brazil

Jose L. Santos; Lucas F. Santos; Daniel L. Santos; Hellen P. Pasqualon; Walter V. Guimaraes

Microvet, Vicosa, MG, Brazil

Introduction

Pericarditis in pigs can be caused by various pathogens, such as Haemophilus parasuis, Pasteurella multocida, A. pleuropneumoniae, A. suis, Streptococcus suis and Mycoplasma sp (Mores, 2007). Pericarditis is the inflammation of the parietal and visceral surfaces of the pericardial cavity. May occur independent of other lesions, but is often observed in association with a pleurisy. Among the various forms of pericarditis, fibrinous pericarditis is more commonly found. It’s characterized by the presence of a serous fluid and marked fibrin deposition on the surfaces of the pericardium and epicardium, leading to heart failure (Jones et al., 2000). This study aims to identify the mainly agents involved in fibrinous pericarditis in pigs in the nursery, growing and finishing.

Materials and Methods

We evaluated 129 cases of fibrinous pericarditis in animals with ages ranging from 20 to 106 days. Swabs were collected from pericarditis, then added 3 ml PBS and plated on blood sheep agar and blood sheep agar with staff nurse. The plates were incubated at 37° C for 24 to 48 hours. And were performed the morphology identification of the colonies and biochemical tests for confirmation of the agent. And serology for H. parasuis and S. suis were performed in Microvet laboratory.

Results

We obtained a result quite varied among the several agents. The mainly agent observed was the H. parasuis (Hps) 38/129, distributed as: non serotypable (14/129), serotype 5 (10/129), serotype 4 (4/129), serotype 1 (3/129), serotype 2 (3/129), serotype 12 (2/129) and serotype 14 (2/129). Other agents were observed such as: P. multocida type A (Past A) 20/129 and P. multocida type D (Past D) 12/129. S. suis 8/129, distributed as: serotype 2 (4/129), serotype 3 (2/129), serotype 7 (1/129) and serotype 8 (1/129). A. pleuropneumoniae (APP) 4/129, A. suis (3/129). Some co-infection were observed between APP and the Past A (1/129), Hps serotype 12 and the Past D (1/129), Hps non serotype and Past D (1/129) and S. suis serotype 2 and Past D (1/129). And 40 animals had were negative for bacterial isolation (Graphic 1).

Discussion

This study allows us to conclude that the onset of pericarditis is actually connected to various agents as described by Mores, in 2007. However, it is noteworthy that the H. parasuis has a great contribution to pericarditis. The serotype 5 is closely associated to conditions of Glasser’s disease in Brazil (Santos et al. 1998), and was found in most reported cases of pericarditis caused by this agent, like H. parasuis non serotyppable. Other agents such P. multocida A and D, can also has great importance in the development of fibrinous pericarditis.

References


Graphic 1: Agents involved in pericarditis from pigs in Brazil