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INFORMATION

Hokkaido University confered the degree of Doctor of Philosophy on June 30, 2011 to 1 recipient.
The title of theses and other information are as follows:

Study on the effects of acute exposure to deoxynivalenol on the liver and immune system of pigs

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Deoxynivalenol (DON) is one of the major mycotoxic contaminants of grains. After absorption from the digestive tract, DON is partly detoxified in the liver and excreted. Although pigs are the animal most sensitive to DON, the effects of acute exposure to DON on the liver and immune system of pigs have not previously been established.

In chapter I, the cytotoxicity of DON to porcine hepatocytes was examined. Cell death of the primary cultured hepatocytes was observed in DON 100, 10, 1 and 0.1 μg/ml groups at 24 hours (hr) in a dose-dependent manner. The dead hepatocytes showed characteristic morphological changes of apoptosis with positive staining of nuclei by the TdT-mediated dUTP-biotin nick end-labeling (TUNEL) method. Increased caspase-3 activity was seen in DON 100, 10 and 1 μg/ml groups. Albumin secretion into the medium was significantly reduced in DON 100, 10, 1, 0.1 and 0.01 μg/ml groups. These results indicate that DON induced apoptosis through the caspase-3 activation pathway and caused functional disorder in porcine hepatocytes, which were considered the manifestations of the hepatotoxicity of DON.

In chapter II, six 1-month-old piglets were intravenously injected with DON to confirm the hepatotoxicity and immunotoxicity *in vivo* with special attention to apoptotic changes. Histopathological examination of the DON-injected pigs revealed systemic apoptosis of lymphocytes in lymphoid tissues and hepatocytes. Apoptosis of lymphocytes and hepatocytes was confirmed by the TUNEL method and immunohistochemical staining against single-stranded DNA and cleaved caspase-3. The numbers of TUNEL-positive cells in the thymus and Peyer's patches of the ileum were increased at 24 hr post-injection (PI) compared to 6 hr PI, but the peak in the liver was at 6 hr PI. These results show the apoptosis of hepatocytes suggesting the hepatotoxic potential of DON, in addition to an immunotoxicity in lymphoid organs with extensive apoptosis of lymphocytes induced by acute exposure to DON in pigs.

In chapter III, the baseline levels of expression of proinflammatory cytokine genes in lymphoid organs of pigs were established, and the expressions were determined in DON-injected piglets. Elevated expression of the interleukin (IL)-1β gene at 6 hr PI and a decrease of IL-18 expression at 24 hr PI were observed in the spleen in DON-injected pigs. IL-1β and IL-6 expressions increased significantly at 6 hr PI in the thymus, but tumor necrosis factor (TNF)-α decreased at 6 hr PI in the mesenteric lymph nodes. These results demonstrated the immunotoxic effects on the modulation of proinflammatory cytokine

genes in lymphoid organs induced by acute exposure to DON in pigs.

In chapter IV, we examined the leukocyte count, blood concentrations of several cytokines and acute-phase proteins and the chemiluminescence of neutrophils from peripheral blood to elucidate the effects of acute exposure to DON on inflammatory responses in miniature pigs. The number of leukocytes was transiently increased at 3, 6 and 12 hr PI due to the increased number of neutrophils. The chemiluminescence value of neutrophils was significantly elevated at 24 hr PI. Significant increases of IL-8 and TNF-α at 3 hr PI and IL-6 at 6 hr PI were detected in the serum, and the concentrations of haptoglobin and serum amyloid A were significantly increased at 24 hr PI. These results suggested that acute exposure to DON induced a temporary recruitment of neutrophils in the peripheral blood by IL-8 and subsequent activation of the bactericidal function, and a transient increase of proinflammatory cytokines and acute-phase proteins, indicating the immunomodulatory effects of DON in pigs.

These findings will contribute to a more comprehensive understanding of the toxicity of DON as well as pathophysiological elucidation of acute poisoning by DON in humans and animals.

The original papers of this thesis appeared in *Vet. Immunol. Immunopathol*., **90**: 203–207 (2002), *Toxicology*, **204**: 241–249 (2004), *J. Vet. Sci*., **11**: 107–113 (2010) and *J. Vet. Med. Sci*., **73**: 665–671 (2011).