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Intraepithelial but not lamina propria lymphocytes in the porcine gut are affected by dexamethasone treatment

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Abstract

It is well established that glucocorticoids are key regulators of the immune system and act as immunosuppressive agents in high concentrations. In the pig, effects on the gut immune system and trafficking of lymphocytes between tissues and blood plasma were not investigated so far.

Twelve pigs of 70 kg were fed 0.4 mg portions of dexamethasone (Dexa) twice daily for 9 days or remained untreated (controls) and were sacrificed for tissue collection at the end of Dexa treatment. Another six pigs with jugular vein catheters were left untreated for 7 days (control period) and then received Dexa for 9 days. Blood was drawn twice during the control period and at days 3, 6 and 9 of the Dexa period for characterization of peripheral blood leukocytes. Cells were obtained from thymus, mesenteric lymph nodes, jejunal mucosa and Peyer's patches. Lymphoid cells from gut tissue were isolated from two fractions: the EDTA-fraction, containing the intraepithelial lymphocytes (IEL), and the Collagenase-fraction, containing the lamina propria lymphocytes (LPL). In all samples, cell counts and phenotypic characterization of cells by flow cytometry (FCM) were performed.

In thymus, Dexa led to a more than 90% reduction of the absolute cell number, which was mainly found in the $CD4^+CD8^+$ subpopulation. Dexa effects on lymphocytes from mesenteric lymph nodes were less severe (50%) and led mainly to a decrease (71%) of B-lymphocytes.

The number of lymphocytes in the EDTA-fraction (IEL) of the jejunal mucosa decreased significantly by 56% in the Dexa-treated animals compared to the controls, whereas the number of lymphocytes in the Collagenase-fraction (LPL) decreased only moderately. In the Peyer's patches, a decreasing tendency in the number of lymphocytes in the EDTA-fraction was observed which, however, was not significant.

In blood, monocytes and granulocytes were significantly increased in an order of 60%.

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The data show that supraphysiological amounts of Dexa remarkably reduce cell numbers in thymus and also in the intraepithelial compartment of the jejunal mucosa and ileal Peyer's patches. In blood, a notable homeostasis was observed for several leukocyte populations whereas both monocytes and granulocytes increased.

Keywords: Swine; Dexamethasone; Intraepithelial lymphocytes; Lamina propria lymphocytes

1. Introduction

Glucocorticoids represent the main catabolic hormones, which counteract anabolic effects of the GH-IGF-1 axis and thus represent an important element for the regulation of cell turnover and tissue homeostasis. They are well known to regulate many individual phenomena such as gluconeogenesis in the liver, redistribution of glucoplastic amino acids, mineral homeostasis and cell differentiation. In addition, they are modulators of the immune system (Ashwell et al., 2000). The latter effect has gained considerable interest due to the pharmacological application of glucocorticoids in high doses as antiphlogistic and immunosuppressive agents, whereas low, physiological concentrations rather have an immunostimulatory function (Vermes and Beishuizen, 2001). The exact dose-dependencies between stimulatory and inhibitory effects were not exactly defined so far.

The immunomodulatory functions of glucocorticoids can be attributed to a combination of several effects. Thus, high levels of glucocorticoids lead to the involution of lymphatic tissues, including the gut associated lymphatic tissue (GALT). The concomitant decreased formation of lymphocytes is partly explained by inhibition of precursor cell proliferation (Pasquale et al., 1989), partly by an induction of apoptosis (Murosaki et al., 1997; Van Houten and Gasic, 1996; Van Houten et al., 1997; Brunner et al., 2001). In addition, lymphocyte and thymocyte formation is reduced due to decreased protein and nucleic acid synthesis (Cutroneo, 2002; Dumont and Robert, 1976; Munck et al., 1984). Inhibition of the nuclear transcription factor kappa B (NF-κB) decreased the formation of cytokines by glucocorticoids (Munck et al., 1984). In consequence, an impaired dialog between different types of lymphocytes adds to the overall immunosuppressive effect. The anti-inflammatory function of glucocorticoids in high doses is further explained by inhibition of vascularisation of inflamed tissues and the decreased permeability of capillaries for immigrating leukocytes (Perretti and Ahluwalia, 2000).

Effects of glucocorticoids on redistribution of lymphocytes contribute to immunosuppression: lymphocytes migrate from the intravascular compartment to the spleen, lymph nodes, thoracic duct and bone marrow (Chow et al., 1999). Similar observations were reported for monocytes whereas granulocytes leave the bone marrow and enter the blood. Specific distribution mechanisms to the gut compartment, so far, are less clear characterized.

Similar to other species, the gut associated lymphoid tissue (GALT) of the pig contains the largest accumulation of immunological cells in the body. It is organized in the Peyer's patches (PP), which are found in the duodenum and the ileum as well. They contain M-cells, which transport luminal antigens to macrophages, which again present the antigens to T-cells and immature B-cells (Köhne et al., 1996; Rothkötter et al., 1999b). In addition, lymphocytes are diffusely distributed along the whole intestinal tract and are located either in the lamina propria (LPL) or within the epithelium (IEL). Apart from their immunological function, it is likely that they have important effects to maintain the epithelial integrity and thus the barrier function of the gut. Until now, interest on the porcine GALT was focussed on the phenotypic characterization of cell types (Rothkötter et al., 1994; Haverson et al., 1999) and the trafficking of lymphocyte subpopulations (Rothkötter et al., 1995, 1999a,b), but the effects of glucocorticoids on the GALT of pigs were not investigated.

Therefore, in this study, the synthetic glucocorticoid dexamethason (Dexa) was applied for 9 days and the effects on leukocyte distribution in blood and primary and secondary lymphatic tissues were determined.