EFFECT OF METHISOPRINOL ADMINISTERED IN OVO ON THE HISTOLOGICAL STRUCTURE OF THE BURSA OF FABRICIUS IN TURKEYS

BARTŁOMIEJ TYKAŁOWSKI, TOMASZ STENZEL, BOGDAN LEWCZUK¹, MARCIN ANDRZEJEWSKI, AND ANDRZEJ KONCICKI

Department of Poultry Diseases, ¹Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn, Poland bartekmozg@wp.pl

Received for publication April 30, 2009

Abstract

The study was aimed at determining the effect of a synthetic immunomodulator – methisoprinol – administered in ovo on the histological structure of the bursa of Fabricius in turkeys. The experiments were conducted on three groups of BUT 9 turkeys hatched from eggs to which, on day 26 of incubation, methisoprinol was administered in a dose of 5 mg (group I) or 20 mg per egg (group II). The poults hatched from the eggs treated with a physiological solution of NaCl in a dose of 0.1 ml per egg served as a control (group III). Turkeys were decapitated at the age of 5 d, and the bursas of Fabricius were collected and prepared for histological studies. The study demonstrated that the bursa of Fabricius in the turkeys hatched from eggs administered with 5 mg of methisoprinol, as compared to the organ in the control poults, differed in a greater number of lymphatic nodules, with a tangible division into the cortical and medullary parts. In turn, the bursa of Fabricius in the turkeys hatched from the eggs administered with methisoprinol at a dose of 20 mg/egg, was characterised by a thick wall with a prominent muscular coat and poorly developed folds with no lymphatic nodules. The morphometric examination of the folds showed a statistically lower number of lymphatic nodules in the bursas of Fabricius collected from the poults hatched from eggs to which methisoprinol was administered at a dose of 20 mg/egg, as compared to that recorded in the organs of turkeys hatched from the eggs treated with methisoprinol at a dose of 5 mg/egg and to that of the control group. Thus, the study demonstrated that methisoprinol used at a dose of 5 mg/egg might be applied as a preparation effectively modulating the immune processes in turkeys.

Key words: turkeys, methisoprinol, in ovo administration, bursa of Fabricius, morphological structure.
The study was aimed at determining the effect of a synthetic, low-toxic immunomodulator – methisoprinol – administered in ovo on day 26 of incubation on the histological structure of the bursa of Fabricius in turkeys.

Material and Methods

The study was conducted on three groups of BUT 9 type turkeys (35 birds in each group) hatched from eggs to which, on day 26 of incubation, methisoprinol (inosin-[(1-dimethyloamino-2-propanol)-4-acetamidobenzoate]) by VetAgro (Lublin, Poland) was applied at a dose of 5 mg/egg (group I) or 20 mg/egg (group II). The control group (III) consisted of poults from eggs to which a physiological solution of NaCl was applied at a dose of 0.1 ml per egg. The turkeys were reared following standard technology. At the age of 5 d, the birds were decapitated. The bursas of Fabricius were immediately collected from five turkeys randomly selected from each group, and fixed in 4% formalin. The 7 µm-paraffin sections were stained with haematoxylin and eosin and Mallor’s method. The images were acquired with a digital Nikon DS-5Mc camera (Nikon Co, Japan) connected to an Olympus BX21 light microscope (Olympus Life and Material Science Europa, Germany) and subjected to morphometric analysis with the use of AxioVision 4.6 software (Carl Zeiss, Germany). The data were elaborated statistically using one-way ANOVA followed by a Duncan test as a post-hoc procedure.

Results

The bursa of Fabricius in the turkeys hatched from the eggs treated with a physiological solution of NaCl (group III - control) was built of a mucous membrane covered by a stratified or simple cylindrical epithelium, and muscular and external coats (Fig. 1). The mucosa created long folds (Fig. 1). In the folds, the connective tissue formed centrally-running trabeculae, from which septa branched off and surrounded the lymphatic nodules. In most of the folds, there were two rows of nodules (Fig. 1), but in some of them there were up to four rows of nodules (Fig. 2). A typical feature was the presence of two kinds of nodules: those with a homogenous internal structure and those with a distinct division into medullary and cortical parts (Fig. 3). In the first case, the lymphocytes were intensively stained and were closely adhering one to another (Fig. 4); in the second case, the medulla consisted of loosely-arranged, brightly-stained cells (Fig. 5). Single eosinophilic and neutrophilic granulocytes were observed in some of the nodules (Fig. 6).

As compared to the control birds, the bursa of Fabricius in the turkeys hatched from eggs to which methisoprinol was administered at a dose of 5 mg/egg (group I) differed by the predominance of nodules, with a distinct division into the cortex and medulla (Fig. 7). The medullary part was limited by a continuous layer of epithelium-like cells (Fig. 8). A commonly observed feature was the presence of neck-like connections between the medullary part of the nodule and the epithelium covering the folds (Fig. 9). Numerous post-capillary venules were observed in some of the nodules. Moreover, many nodules contained a moderate number of eosinophils.

The bursa of Fabricius in the group of turkeys hatched from the eggs to which methisoprinol was administered at a dose of 20 mg/egg (group II) differed markedly from that of the groups I and III. The wall of the bursa was considerably thicker than in the birds of two other groups and contained a well-developed muscular coat. The folds were poorly developed, and in many of them no lymphatic nodules occurred (Fig. 10). Numerous, deep invaginations of the epithelium into the folds, sometimes leading to the formation of cysts, were observed (Fig. 10). The division into the medullary and cortical parts was not observed in the nodules situated in the folds. In turn, such a division was observed in the nodules located at the base of the folds (Fig. 11). Numerous eosinophilic granulocytes were found in the connective tissue stroma (Fig. 12). An obvious, negative relationship was noted between the number of nodules and granulocytes.

A morphometric examination demonstrated a statistically lower number of lymphatic nodules in the bursa of Fabricius in the turkeys from the group II than in birds from the groups I and III (Table 1).

Discussion

An effectively functioning immune system in birds is indispensable in assuring their capability of surviving in an environment characterised by an ever increasing risk from pathogens, often inducing a state of immunosuppression (7, 9, 17-19, 25). A typical trait of such pathogens is their replication in the organs of the immune system and the impairment of the production of precursor cells to B and T lymphocytes, as well as macrophags and granulocytes. Proliferating viruses additionally impair the metabolism of immune cells, evoke a blockade of cytokine receptors, suppress the synthesis of interferon, and block the expression of MHC on the surface of infected cells (14, 15, 26). This condition is often intensified by the action of non-infectious immunosuppressors linked to breeding conditions (3) and feeding (1, 4, 23), as well as with the use of some drugs (6). These factors affect, among other things, the bursa of Fabricius, whose major function is providing conditions for the maturation process and differentiation of B lymphocytes. Therefore, this organ plays a key role in humoral immunity.

In light of the above, in the present study an attempt was made to use the immunomodulation already in the course of embryogenesis, i.e. when a developing embryo is not yet affected by some potentially-immunosuppressive factors.
Fig. 1. Section through a wall of the bursa of Fabricius of a control turkey. A fold with two rows of nodules. HE, 90x.

Fig. 2. Section through a fold of the bursa of Fabricius of a control turkey. Presence of numerous nodules. HE, 90x.

Fig. 3. Diversified structure of lymphatic nodules in the bursa of Fabricius of a control turkey. Visible nodules with a homogenous structure (1) and nodules with a distinct division into medullary and cortical parts (2). HE, 180x.

Fig. 4. Lymphatic nodule with a homogenous structure in the bursa of Fabricius of a control turkey. HE, 360x.

Fig. 5. Lymphatic nodule with a distinct division into medullary and cortical parts in the bursa of Fabricius of a control turkey. HE, 360x.

Fig. 6. Eosinophilic granulocytes (arrow) in a lymphatic nodule of the bursa of Fabricius of a control turkey. HE, 570x.
Fig. 7. Numerous lymphatic nodules with a prominent division into cortex and medulla in the bursa of Fabricius of a turkey from the group I. HE, 180x.

Fig. 8. Epithelial cells (arrows) separating lymphocytes in the medullary and cortical parts of a nodule in the bursa of Fabricius of a turkey from the group I. HE, 360x.

Fig. 9. Lymphatic nodule forming connection with the fold’s surface in the bursa of Fabricius of a turkey from the group I. HE, 180x.

Fig. 10. Thick muscle coat in the bursa of Fabricius of a turkey from the group II. Lack of lymphatic nodules in folds. HE, 90x.

Fig. 11. Lymphatic nodule with a prominent cortex and medulla in the bursa of Fabricius of a turkey from the group II. HE, 360x.

Fig. 12. Numerous eosinophilic granulocytes in folds of the bursa of Fabricius of a turkey from the group II. A lack of lymphatic nodules. HE, 180x.
Previous investigations have shown that methisoprinol administered to turkey embryos on day 26 of incubation does not induce any disturbances in the hatching process, and has no negative effect on the health status of the reared turkey poults (28). Methisoprinol administered in ovo at a dose of 5 mg/egg and then, from day 8 till day 10 of poult rearing, applied in a feed mixture at a dose of 50 mg/kg was shown to enhance the post-vaccinal humoral immunity against NDV (10, 21). Earlier research also demonstrated that methisoprinol administered in ovo at a dose of 5 mg/egg stimulates the mainly non-specific mechanisms of the humoral immunity in the 5-old turkey poults hatched from those eggs, as was indicated by a higher percentage of subpopulations of CD3+ and CD4+ T lymphocytes in the peripheral blood and spleen (27). The available literature, however, lacks data on the impact of methisoprinol applied in ovo at the final stage of incubation on the histological structure of the bursa of Fabricius in birds hatched from such eggs.

The study presented demonstrates that the bursa of Fabricius in the turkeys hatched from eggs to which methisoprinol was administered in various doses correspond with the observations made by Rumińska-Groda (21) and Stenzel et al. (27). The current study confirms that methisoprinol administered in ovo at a dose of 5 mg/egg increases the number of lymphatic nodules characterised by a distinct division into the cortical and medullary parts. Hence, it should be concluded that this preparation applied in ovo at the above-mentioned dose effectively modulates the immune processes in turkeys and, therefore, may be applied in practice in the commercial breeding of these birds.

### References