ALTERATIONS IN THE RABBIT LYMPHOID TISSUES AND SELECTED PARAMETERS OF HOMEOSTASIS AFTER BENDIOCARB ADMINISTRATION

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ABSTRACT

The carbamate compounds are a class of cholinesterase inhibiting pesticides, and bendiocarb is the most widely used carbamate insecticide used to control disease vectors (mosquitoes, flies, household and agricultural pests). In this study histological structure of rabbit lymphoid tissue and selected parameters of homeostasis after bendiocarb administration were studied. Bendiocarb was perorally administered for 60 days (D60). The structure of intestinal wall of experimental animals revealed any significant changes. In the small intestine a slight increase in the height of enterocytes and diameter of crypts was detected indicating increased cellular activity. More detailed analysis of Peyer’s patches in experimental groups after administration of bendiocarb revealed a relative decrease in areas rich in lymphocytes. Observations of the lymph node showed that on D10 and D20 there was a significant increase in relative volume of medulla at the expense of the relative volume of the cortex and a decrease in the number of lymphocytes. However, we recorded an increase in diameter of lymphocytes. Bendiocarb addition caused imbalance in internal milieu of rabbits. Main changes were observed in serum creatinine content and activity of serum enzymes (creatinine and GGT on D30, AST on D10 and D30, and GLUD on D10) describing possible failure of liver and/or kidney caused by bendiocarb. Decrease of some hematological parameters (hematocrit, mean corpuscular haemoglobin concentration, platelet count) in all experimental groups suggests possible significant defection in haemoplastic system.

Key Words: Bendiocarb, Lymphoid tissue, Blood biochemistry, Haematology, Rabbit

INTRODUCTION

Pesticides are a group of chemicals with high biological activity that are worldwide introduced into the environment and expose large populations of living organisms. Several pesticides are carcinogenic, other may cause reproductive toxicity or have endocrine disrupting properties. Bendiocarb (2,2–dimethyl–1,3–benzodioxol–4–yl–methylcarbamate) is a carbamate broad–spectrum insecticide used to control disease vectors such as mosquitoes and flies, as well as household and agricultural pests. The immune system is one of the essential integrating systems in the organisms producing and maintaining optimum conditions for functioning of other physiological functions, sustaining homoeostasis and supporting adaptation to external influences. Exposure to pesticides can cause a number of effects on the immune system, varying from a slight modulation of immune functions to the development of clinical immune diseases. An alteration of the normal immune function may have two types of consequence: the first one is a reduction of the immune activity, which can evolve into immune deficit and increased susceptibility to infectious diseases and neoplasm. The second one is an enhancement of...
the normal immune response, which can evolve into allergy and autoimmunity. Regarding carcinogenicity, genotoxicity and mutagenicity, pesticides pose high risk particularly to dividing cells and thus also active cells of the lymphoid system.

The aim of the study was to describe and quantify structural and functional changes in the structure of the rabbit small intestine, mucosal lymphoid tissue (Payer’s patches, lymphocytes in lamina propria), and a lymph node as well as selected parameters of homeostasis after peroral administration of bendiocarbamate.

MATERIAL AND METHODS

Experiment

In the experiment 36 adult rabbits (Oryctolagus cuniculus; age=54 days) of breed HY+ (female=18, male=18) with average weight of 1250 g from accredited animal farm (Nitra, Slovakia) were used. Animals were kept in cages (2 per a cage) at standard conditions (temperature 15–21°C, 12 hour light period and relative humidity of 45%) and fed with granular feed mixture (O–10 NORM TYP, Slovakia). Drinking water was available for all animals ad libitum. Animals were divided into four groups (control, D (day) 3, D10, D20, D30, D60 of administration). Rabbits in all experimental groups (n=6) received bendiocarb (96% Bendiocarb, Bayer) perorally in a dose 5 mg/kg per day and after day 13 in a same dose per 48 hours. Whereas a treatment dose was strong (in some animals we observed diarrhoea, dehydration and alopecia), it was reduced after D13. Animals were killed by ether after D3, D10, D15, D20, D25, D30 and D60 of administration. Rabbits in all experimental groups (n=6) received bendiocarb (96% Bendiocarb, Bayer) perorally in a dose 5 mg/kg per day and after day 13 in a same dose per 48 hours. Whereas a treatment dose was strong (in some animals we observed diarrhoea, dehydration and alopecia), it was reduced after D13. Animals were killed by ether after D3, D10, D15, D20, D25, D30 and D60 of administration. The lymph node was evaluated on D3, D10 and D20 after bendiocarb administration. The blood analysis was realized on D3, D10 and D20 after bendiocarb administration. The blood analysis was realized on D3, D10 and D20 after bendiocarb administration. The blood analysis was realized on D3, D10 and D20 after bendiocarb administration. The blood analysis was realized on D3, D10 and D20 after bendiocarb administration. This study was carried under the institutional ethical authority of decision No. 2647/07–221/5.

Histology

The samples were dissected out for microscopic analysis of small intestine (Payer’s patches) and lymph node (lymphonodi jejunales), subsequently were processed by a standard way for histological examination. Samples were fixed in 10% formalin for a week. After fixation the samples were dehydrated in a graded series of ethanol (70, 80, 90, 100%), saturated in benzene, benzene–paraffin and embedded into paraffin blocks. Blocks of samples were sectioned after 2 weeks on a microtome (Reichter, Austria) into 7 µm thick sections and stained with haematoxylin and eosin.

Samples were evaluated qualitatively and quantitatively by means of an optical microscope with photo–equipment (Olympus Provis AX). The structure of the small intestine and lymph nodes was evaluated directly using software for quantitative image analysis Image ProPlus. In the small intestine was evaluated altitude of enterocytes, diameter of intestinal crypts (µm) and number of cells in lamina propria mucosae (per 1000 µm²). We also evaluated quantitatively the relative volume of cortex (%) and medulla (%) in the lymph node with the number of lymphocytes (per 1000 µm²) and lymphocyte diameter (µm) in the lymph node. Results of morphometric structure of small intestine and lymph node in individual groups were compared statistically employing standard Student’s t–test. Values of P p<0.05 were considered significant.

Homeostasis

Blood samples from vena auricularis from rabbits were taken from all animals by macromethod. For biochemical analyses blood samples were centrifuged for 30 min at 3000xg and blood serum was obtained. Selected metabolites, electrolytes and enzymes in serum were determined using Ecoline kits and automatic analyzer Microlab 300 (Merck®, Germany), spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc, USA) and microprocessor–controlled analyzer EasyLite (Medica, Bedford, USA) according to manufacturer conditions. In blood selected haematological parameters were measured using haematology analyzer Abacus junior VET (Diatron®, Austria). To calculate basic statistic characteristics, determine significance of differences, and compare the results the analysis of variance, one-way ANOVA test and Duncan’s test were performed at p level less than 0.05. The SAS and Sigma Plot 11.0 (Jandel, Corte Madera, USA) statistical software were used. P
RESULTS AND DISCUSSION

Histology – small intestine

Evaluation of structural changes in the small intestine involved height of enterocytes, diameter of intestinal crypts and total number of cells in *lamina propria mucosae* per 1000 µm². Quantitative evaluation of the observed structures showed that the height of enterocytes in the control group reached 26.44±3.64 µm. In all experimental groups a slight increase in this parameter was detected. A significant increase was detected on D20 and D30 after administration of bendiocarb (28.88±5.03 and 30.00±4.65 µm). The diameter of intestinal crypts in the control group was 26.02±5.74 µm and significant increase to the level of 31.11–37.33 µm was observed on D10, D20 and D30 (Fig. 1). On D3, D20 and D30 following administration of bendiocarb, the number of cells in *lamina propria mucosae* decreased significantly (14.12±4.10–16.72±5.33/ per 1000 µm²) compared to the control (19.62±4.45/per 1000µm²).

![Graph showing quantitative evaluation of intestinal structures and number of cells in lamina propria mucosae after bendiocarb administration](image)

**Fig. 1**: Quantitative evaluation of intestinal structures and number of cells in *lamina propria mucosae* after bendiocarb administration

[Legend: AE – Height of enterocytes (µm); DIC – Diameter of intestinal crypts (µm); NC – Number of cells in *lamina propria mucosae* (1000 µm²)]

In the Peyer’s patches of the rabbit small intestine the proportion of sites relatively abundant and poor in lymphocytes were evaluated. Diameter of the observed lymphocytes was studied in more detail. Measurements showed that the areas relatively poor in lymphocytes constituted 56.98–76.29% in all observed groups. The areas rich in lymphocytes reached 23.71–43.02%. Any significant differences were observed between experimental groups and the control. Detailed analysis confirmed that diameters of lymphocytes observed in Peyer’s patches in individual experimental groups ranged between 7.79 and 9.19 µm while in the control group reached 8.47 µm. Significant differences in comparison with the control were observed on D20 and D60 after administration of bendiocarb. (Fig. 2).

The structure of intestinal wall of experimental animals revealed any significant changes. In the small intestine a slight increase in the height of enterocytes and diameter of crypts was detected indicating increased cellular activity. More detailed analysis of Peyer’s patches in experimental groups after administration of bendiocarb revealed a relative decrease in areas rich in lymphocytes.

Histology – Lymph Node

Evaluation of the lymph node focused on changes in relative proportion of medulla and capsule and involved also more detailed morphometric analysis aimed at number and diameter of lymphocytes in the lymph node on days 3, 10 and 20 after administration of bendiocarb. The relative volume of cortex in the control lymph node was 22.12±4.26%. The relative volume of cortex...
decreased significantly in all experimental groups exposed to bendiocarb (Fig. 3). Significant differences were observed on D10 (14.7±5.08%) and D20 (14.64±3.85%). The relative volume of medulla in the lymph node of the control group formed in average 77.88%. Highly significant differences were observed in medulla on D10 and D20 compared to control. The number of lymphocytes in the bendiocarb–exposed lymph nodes in experimental groups ranged from 32.70 to 34.02 per 1000 µm². Number in experimental groups showed a decreasing tendency compared to the control but the differences were insignificant. The diameter of the observed lymphocytes in the control group reached 2.86±0.33 μm. The diameter of lymphocytes in experimental groups showed a slight increase. The differences compared to the control were significant on days 10 and 20 after administration of bendiocarb (Fig. 3).

![Graph](image1)

**Fig. 2:** Relatively poor and rich lymphocyte areas and diameter of lymphocytes in Payer’s patches after bendiocarb administration

[Legend: PLA – Poor lymphocyte areas (%); RLA – Rich lymphocyte areas (%); DL – Diameter of lymphocytes (µm)]

![Graph](image2)

**Fig. 3:** Relative volume of basic structure and number of lymphocytes in rabbit lymph node

[Legend: LC – Lymph node cortex (%); LM – Lymph node medulla (%); NL – number of lymphocytes (per 1000 µm²); DL – Diameter of lymphocytes (µm)]
Evaluation of the lymph node indicated a more marked effect of bendiocarb as a more pronounced increase in the relative volume of medulla with decreasing relative volume of cortex at a slight decrease in the number of lymphocytes was found. In the experimental groups diffuse lymphatic tissue is concentrated into the smaller follicles which form inner cortex. However, the diameter of lymphocytes showed a slight increase which suggested their maturation and activation.

**Homeostasis – Blood Analysis**

From all tested minerals in blood serum only in sodium content were observed significant differences in the group with lowest as well as the highest bendiocarb concentration. In the case of other minerals (calcium, phosphorus, magnesium, potassium, chlorides), values in control group did not significantly differ from those in experimental groups. On D10 the highest level of serum glucose was observed versus other groups. AST serum activity was increased in experimental groups in comparison with control group. The highest AST activity was observed in experimental group on D25. Also the highest GGT activity was found on D25 (P<0.05). The activity of GLUD decreased in all experimental groups in comparison with control group. Significant difference (P<0.05) was found between control and D25. Serum creatinine concentration was increasing with bendiocarb administration. Any significant differences were observed among the groups in levels of urea, total proteins, cholesterol, bilirubin, triglycerides, ALT and ALP (Fig. 4).

The tendencies of haematological parameters are presented in Fig. 5. WBC and RBC counts decreased insignificantly (P>0.05) below control group as a consequence to the increase in dose of bendiocarb administered to the rabbits. Consequently, haemoglobin content exhibited a decrease gradually with increasing dose of bendiocarb. Significant difference and D25 (P<0.05) was found between control group and D25. Bendiocarb also induced decrease of MCH and MCHC. PLT count decreased gradually to D25 in comparison with in control group (P<0.05).

![Fig 4: Selected serum homeostasis parameters](image)

<table>
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<tr>
<th>Control</th>
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<td>Ca</td>
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<td>CREA (:10)</td>
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[Legend: Ca – calcium (mmol/l); P – phosphorus (mmol/l); Mg – magnesium; GLU – glucose (mmol/l); CHOL – cholesterol (mmol/l); AST – aspartate aminotransferase (µkat/l); GGT – gamma glutamyl transferase (µkat/l); CREA – creatinine (mmol/l)]
Pesticides possess properties that make them different from other chemicals mainly because they are introduced to environment. They are important for their stability in the environment, exposure of population and high biological activity. Though, toxic effect of the pesticides is specialized to specific species. They may endanger also human health, and both domestic and wildlife animals.

The annual application of synthetic pesticides to food crops in the EU exceeds 140,000 tones, an amount that corresponds to 280 grams per EU citizen per year. Acute toxic symptoms of carbamate poisoning, e.g. miosis, urination, diarrhea, diaphoresis, lacrimation, salivation, and excitation of the central nervous system, are generally caused by inhibition of the enzyme acetylcholinesterase, which leads to accumulation of acetylcholine. Studies on chronic exposure to carbamate insecticides and case reports of long-term exposure give equivocal results. Because of lack of human exposure data, the major source of information for studying potential health effects of chemicals on humans is generally obtained from animal dose response experiments. Animal data are often evaluated in two aspects via statistical analysis: qualitative testing and quantitative estimation. The acute oral LD$_{50}$ in rabbits ranges from 35-40 mg/kg b.w.

**CONCLUSION**

The study investigated the influence of bendiocarb on selected organs of the rabbit – lymph node, small intestine wall and Peyer’s patches that play an important role in local immunity of the intestine. The immune system reacts sensitively to a concentration of chemical substances that are not yet toxic for other systems of organism. An alteration of the normal immune function may have two types of consequence: the first one is a reduction of the immune activity, which can evolve into immune deficit and increased susceptibility to infectious diseases and neoplasm. The second one is an enhancement of the normal immune response, which can evolve into allergy and autoimmunity. Adult rabbits, administered bendiocarb perorally for 90 days at a dose 5 mg/kg b.w. exhibited a moderate toxic effect. In this study, based on long-term administration of bendiocarb, we observed an increased volume of cortex and decreased volume of thymus medulla. Moreover, morphometric analysis detected lower number of thymus cells as well as their smaller diameter in comparison with the control.

Results of interaction effects among pesticide mixtures on human peripheral blood lymphocytes indicate that cytotoxicity was induced at very low concentrations by mixtures compared to individual
pesticides.

With regard to the fact that environmental cleanness and negative influence of its pollution on animal and human health are presently a serious issue we stress the importance of evaluation of the effect of pesticides on live organisms and the need for additional information on their potential harmful effects.

REFERENCES


