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Effect of albendazole therapy on susceptible and resistant *Haemonchus contortus* larvae in Mongolian gerbils (*Meriones unguiculatus*) and distribution of inflammatory cells in the stomach wall

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Summary

The effect of albendazole therapy on the reduction of drug-susceptible and drug-resistant strains of *Haemonchus contortus* larvae on day 10 post infection (p.i.), distribution and the relative numbers of innate immunity cells – eosinophils/neutrophils and mast cells in the stomach wall of immunosupressed Mongolian gerbils on days 4/1, 7/4, 10/7 and 14/11 post infection/post therapy (p.i./p.t.) were investigated in the present study. The efficacy of albendazole was significantly lower on benzimidazole (BZ) resistant larvae (L3 and L4 stages) (58.92 %) than the efficacy on susceptible strain of larvae (94.15 %). *H. contortus* infection elicited strong inflammation in mucosal and submucosal layers of the stomach, where mucosal mast cells (MMC) were in the highest numbers in the *lamina propria mucosae* on day 7/4 p.i./p.t. Reduction of larval numbers following treatment resulted in a gradual decrease of MMC and connective tissue mast cells (CTMC). The lower counts of CTMC in the *submucosa* were seen in gerbils infected with BZ-susceptible strain during the whole period post therapy. In case of infection with BZ-resistant strain, peroxidase containig cells (eosinophils) peaked on day 7/4 p.i./p.t., whereas infection with BZ-susceptible strain elicited massive accumulation of these cells on day 4/1 p.i./p.t., particularly in the *submucosa*. No marked differences in eosinophils localisation were observed between both groups after the therapy. Goblet cells were found only in the proximal parts of *glandulae gastricae* close to the mucosal surface and no differences in the distribution in the stomach wall of both groups of animals were observed. After therapy the higher larval counts in case of BZ-resistant strain were in the correlation with the lower decline of CTMC and eosinophils, but MMC numbers were not significantly different between both treated groups. Present data indicate that in early stage post infection, the distribution of individual innate immunity cells might be directly affected by the larvae, and that the genetic and consequently biological differences related to the resistance to benzimidazoles probably had the impact on the interactions of larvae with the different immune cells in their niche.

Keywords: *Haemonchus contortus;* Mongolian gerbil; albendazole; mast cell; eosinophil; goblet cell

Introduction

Haemonchus contortus is one of the most pathogenic nematode of small ruminants causing a great economic losses in many countries worldwide. Benzimidazole anthelmintics are frequently used drugs to control disease caused by this blood-feeding nematode, however, this is hampered by the development of resistance to these drugs. Investigations were initially conducted using a gerbils model (*Meriones unquiculatus*) of *H. contortus* infection (Conder *et al*., 1990; 1991) for the study of host parasite interactions because of the histological similarity of the abomasum of sheep and the stomach wall of Mongolian gerbil (*Meriones unquiculatus)* (Conder *et al.*, 1992).
Efficacy of BZ carbamates with the broad spectrum of activity to parasites (oxfendazole, albendazole, tiabendazole), paraherquamide, levamisole (Ostlind *et al*., 2006; Conder *et al.*, 1990; Conder *et al*., 1991), verapamile, CL 347,099, moxidectin and ivermectin (Molento & Prichard, 1999; Ostlind *et al*., 2006) was evaluated in *H. contortus* experimental infection of this rodent laboratory model. González *et al*. (2004) and Johnson *et al*. (2004) used the gerbils to evaluate *in vitro* active compounds. Mongolian gerbil has also been recognized as a suitable to evaluate the efficacy of several natural substances with anthelmintic properties - tannins (Rojas *et al*., 2006), artemisine (Squires *et al*., 2011), orange oil emulsion (Squires *et al*., 2010) against the *H. contortus* infection. P-aminophenethyl-m-trifluoromethylphenyl piperazine (PAPP) has also found highly effective against co-infection of *H.*

contortus, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in Mongolian gerbil (White *et al*., 2007). The eosinophilia and mastocytosis are characteristic in mammals infected with gastrointestinal nematodes (Miller, 1984). Mechanical damage of the gastrointestinal tract by *H. contortus* larvae results in the chemotaxis of peroxidase containg cells (Px cells) comprising eosinophils (Eos), and probably also neutrophils (Neus) and basophils. Eos are the first cells of innate immunity which are suppose to kill parasites by means of produced free radicals. Eosinophil peroxidase is a haem containing protein, which shares 68 % amino acid identity with neutrophil myeloperoxidase as well as with other peroxidase enzymes and it is toxic for parasites. While the controversy regarding the possible protective role of the Eos in parasitic infections continues, the hypothesis that Eos and their products play a role in generating inflammatory changes during parasitic infections, likely to create an unfavourable environment for intestinal parasites, remains an attractive one (Dale *et al*., 1994).

Mast cells (MCs) and Eos may play an important role in immune responses that are associated also with the expulsion of gastrointestinal nematode. An increase in the numbers of peripheral blood and tissue Eos is a major hallmark of parasitic infections. Infestations of intestine-dwelling parasites in laboratory animals provoke a marked T lymphocyte dependent eosinophilia in the *mucosa*, which appears to be temporally associated with expulsion of the adult parasites. Only the Eo cells contain enough active peroxidase (Px) containing enzyme to interfere with the regular immunoperoxidase stainings in the sections of the rat stomach and the intestine. In uninfected rodents, the majority of Px cells are located in the *tunica propria* around the bottom of the gastric glands throughout the intact rodent stomach and scattered cells can be found in the *tunica propria* between the *gastric glands*, in the *submucosa*, and in the connective tissue spaces of the *muscularis externa* (Hunyady *et al*., 1996).

Except of the inflammatory cells, intestinal goblet cell hyperplasia and increased secretion of mucus by goblet cells can also help to eliminate parasites from alimentary tract (Miller, 1987). Mucins secreted by small intestinal goblet cells are considered to play an important role in the expulsion of *Nippostrongylus brasiliensis* (Tsubokawa *et al*., 2012).

MCs are inflammatory cells that belong to the host's non-specific immune system. MCs and Eos are often the first cells to attack the parasite upon direct contact with them. MCs release substances such as histamine, serotonin, heparin and proteoglycans. Enzymes released by MCs can trigger production of the inflammatory mediators, such as prostaglandins, leucotriens, bradykinins, creating typical symptoms of inflammation. Granular proteoglycans can bind with metachromatic dyes (Toluidine Blue, Alcian Blue/Safranin), thus MCs are visible under light microscope. According to their distribution and function, two types of MCs are recognized, those from connective tissue (CTMC) and a distinct set of MMC. The main protease of CTMC is tryptase that plays a key role in allergic reactions. Chymase, the main enzyme of MMC participates in collagen- 4 remodelling and degrading vasoactive intestinal peptide (Dale *et al*., 1994). It was demonstrated that sheep with increased numbers of degranulated MCs had fewer nematodes (Stear *et al*., 1995). The role of inflammatory cells may vary in the different host-parasite systems (Meeusen and Balic, 2000). Distribution of MCs during infection with BZ-resistant and BZ-susceptible *H. contortus* larvae in the stomach wall of Mongolian gerbils were described Königová *et al*. (2008).

In the present study we investigated the effect of albendazole treatment on the infection with of BZ-resistant and BZ-susceptible larvae of *H. contortus*, impact of treatment on the distribution of inflammatory cells - MCs (MMC, CTMC), Px cells (Eos/Neus) and goblet cells in the stomach wall of Mongolian gerbils by histochemical methods.

Material and methods

Animals and infection

In total 83 immunosuppressed Mongolian gerbils (*Meriones unquiculatus*) weighing 60 – 65 g were used in this study. Animals were provided the commercial rodent chow and water *ad libitum*. Experimental animals were suppressed by hydrocortisone intramuscularly with 6 mg of drug hydrocortison (5 % solution) (ICN, Czech Republic) seven days prior to inoculation with L3 nematode larvae and everyday during infection. Gerbils were inoculated with 1000 *H. contortus* L3 BZ-susceptible or BZ-resistant strain *per os*.

The *H. contortus* ISE strain used in this study was an anthelmintic-susceptible inbred strain (Roos *et al*., 2004), which was isolated from the field before BZ anthelmintics were on the market. Third stage larvae of this ISE strain were kindly provided by Dr. Frank Jackson, Moredun Research Institute, Edinburgh, UK. Larvae of the resistant strain (strain Courtion, Novartis AG, Switzerland), were obtained by cultivation from faecal samples (Hubert and Kerboeuf, 1984), which was carried out with the aim of standardizing *in vitro* tests for the detection of BZ resistance (Samson-Himmelstjerna *et al*., 2009). Three non-suppressed and uninfected gerbils served as controls for the histological observations.

Experimental design

The experiment was divided into two parts:

In the first part of experiment, the efficacy of albendazole (ABZ) against developing *H. contortus* larvae third larval stage (L3) and fourth larval stage (L4) was evaluated in Mongolian gerbils. Laboratory animals were divided into four groups. Group 1 and 2 were infected per os with 1000 L3 of BZ-resistant strain of *H. contortus* per animal. Groups 3 and 4 were infected with a BZ-susceptible strain of *H. contortus* at a dose of 1000 L3/per animal. Subsequently Group 1 and 3 were treated on day 3 p.i. with ABZ (Sigma Aldrich, Germany) with a dose of 10 mg/kg body

Table 1. Percentage of efficacy (PE) of albendazole and mean larvae counts ± SD of BZ-susceptible strain (SS) and BZ-resistant strain (RS) of *H. contortus* in experimentally infected gerbils on day 10/7 p.i./ p.t.

H. contortus	Day p. i./p.t.	Number of larvae in control group ± SD	Number of larvae in treated group ± SD	PE (%)
SS	10/7	174.00 ± 16.80	86.00 ± 10.20	94.15
RS	10/7	141.50 ± 12.30	98.50 ± 12.30	58.92

n = 20 (number of gerbils per group)

weight (b.w.). Gerbils were killed and examined on day 4/1, 7/4, 10/7 and 14/11 p.i./p.t. in the experiment for parasitological examination. Larvae were harvested from the stomachs of gerbils according to the technique of Conder *et al*. (1990). The stomachs were removed and opened longitudinally and incubated in 14 ml of distilled water at 37 °C for 5 hours. Following incubation, 1 ml of 4 % paraformaldehyde solution was added to each vial and processed for larval counting. The number of larvae *H. contortus* was determined microscopically in each animal/group.

In the second part of experiment, distribution and dynamics of inflammatory cells (connective tissue mast cells – CTMC, mucosal mast cells – MMC), eosinophils (Eos) / neutrophils (Neus) and goblet cells in the stomach walls from gerbils were evaluated on day 4/1, 7/4, 10/7 and 14/11 p.i./p.t. in control and treated animals infected with either BZ-susceptible or BZ-resistant *H. contortus* strains. The stomach of each animal was divided into two pieces, one of which was fixed for 24h in 4 % paraformaldehyde and dissolved in phosphate-buffered saline (pH 7.4), and the second sample in the Carnoy's solution for 3 hours at room temperature. After fixation, tissues were dehydrated, cleared in xylene and embedded in paraffin wax. Tissue sections (5 – 7 μm thick) fixed in Carnoy's solution were stained with 0.1 % Alcian Blue (pH 0.3) for the identification of MMC as recognized by the presence of light-blue granules (Enerback, 1966). Paraformaldehyde-fixed sections were stained with Toluidine Blue solution (pH 0.5) in order to visualise connective tissue mast cells, granules of which are seen in pink colour (Enerback, 1981) and goblet cells, which were stained with Alcian Blue/Safranin solution. Px cells were identified as Eos and Neus and were visualized after the method of Hunyady *et al*. (1996) and only the intensity of accumulation and distribution were evaluated in the microscope. Counting of the MCs was performed under the light microscope using an eye-piece graticule (eye-piece x 10, objective x 60, covering area of 0.076 mm^2). The counts were made systematically from the *lamina muscularis mucosae* to the mucosal surface on at least 30 graticule fields and the mean number of MCs per mm^2 tissue was calculated separately for the *lamina propria mucosae* and the *submucosa* in each group (Hrčková *et al*., 2006).

Statistical analyses

Efficacy of ABZ treatment against BZ-susceptible and BZ-resistant larvae of *H. contortus* was calculated as percent larval reductation:

Percentage of efficacy (PE) = 100 x C – T/C, where C is a arithmetic mean number of larvae *H. contrortus* in an untreated control group of gerbils and T is the arithmetic mean number of larvae in a treated group inoculated with BZ-susceptible or BZ-resistant strain of *H. contortus*. Homogenity of data distribution was analysed by an F-test for small samples (n = 5). Differences between larval numbers and the numbers of MCs were evaluated using Kruskal-Wallis ANOVA test and Mann-Whitney U test with the P-level of significance indicated in the Tables. Analyses were performed using Statistica 6.0 (Stat Soft, Tulsa, USA) statistical package.

Table 2. Mean numbers of connective tissue mast cells (CTMC) in the *submucosa* in gerbils infected with BZ-susceptible and BZ-resistant *H. contortus* strains and post treatment with albendazole (ABZ) (10 mg/kg b.w.)

		CTMC (mean number/mm^2 tissue ± SD)			
		Days p.i./p.t.			
H. contortus	Group	4/1	7/4	10/7	14/11
SS	C	10.15 ± 6.88	10.85 ± 4.94	10.20 ± 2.74[a]	0.25 ± 0.35[b]
	ABZ	3.15 ± 2.38[b]	2.70 ± 1.38[b]	2.65 ± 0.76[ab]	0.30 ± 0.43[b]
RS	C	12.60 ± 2.73	7.35 ± 2.13	6.35 ± 3.80	4.75 ± 1.57[b]
	ABZ	10.45 ± 2.39[b]	6.60 ± 1.15[b]	5.95 ± 1.56[b]	3.65 ± 1.33[b]

n = 20 (number of data in each group), C- control group of experimental animal, ABZ- treated group of experimental animal with albendazole, SS- BZ-susceptible *H. contortus* strain, RS- BZ- resistant *H. contortus* strain

[a](P<0.05) Significant difference on certain day (p.i./p.t.) between untreated (control) and ABZ treated group after infection with BZ-susceptible/resistant *H. contortus* strain

[b](P<0.05) Significant difference on certain day (p.i./p.t.) between infections with BZ-susceptible and BZ-resistant *H. contortus* strain

Results

Efficacy of albendazole

The mean larvae (L3 and L4) count and efficacy of ABZ is shown in Table 1. The anthelmintic activity was evaluated as percentage of efficacy (%). Data presented in Table 1 show that ABZ was highly effective on BZ-susceptible larvae as evaluated on day 10/7 p.i./p.t., but not against *H. contortus* resistant larvae. The reduction of 94.15 % of the BZ-susceptible larvae was observed on day 10/7 p.i./p.t. However, the drug was only 58.92 % effective on BZ-resistant larvae, and 98.50 ± 12.30 survived in the stomach. In comparison to untreated group this was not significantly different. In our study we confirmed that this strain possesses genetically based resistance to ABZ and the therapy failed to eliminate significant portion of larvae.

Distribution of inflammatory and goblet cells in the stomach wall of infected and treated gerbils

Parasitic infection elicited the most intense inflammatory reactions in the *lamina propria mucosae* (LPM) during infection and post therapy. Multifocal infiltrates consisted mainly of Eos, Neus sensitive to Px-staining, but MCs and in the lesser extent macrophages and lymphocytes were also present (Fig. 1a). In the *lamina muscularis mucosae* (LMM) these inflammatory cells were observed mostly near the small venules and in the *submucosa* (SM), inflammatory cells were scattered through this layer. CTMC were seen through the whole layer of stomach wall and LMM in the glandular part of the stomach and also in LPM. The higher numbers of CMTC were recorded in the inflammatory foci in LPM in the stomach of gerbils infected with BZ-susceptible strain up to day 10 p.i. (Fig. 1b) in comparison to group infected with BZ-resistant strain (Fig. 1c). Following therapy, the mean numbers of CTMC were little changed in gerbils with BZ-resistant strain (Fig. 1d, day 4/1 p.i./p.t.) however decline was more intense in gerbils infected with the BZ-susceptible strain. CTMC were rarely seen outside the inflammatory foci. No detecable differences were observed in the localisation of MMC and CTMC in the stomach of gerbils infected with both BZ-susceptible and BZ-resistant *H. contortus* larvae.

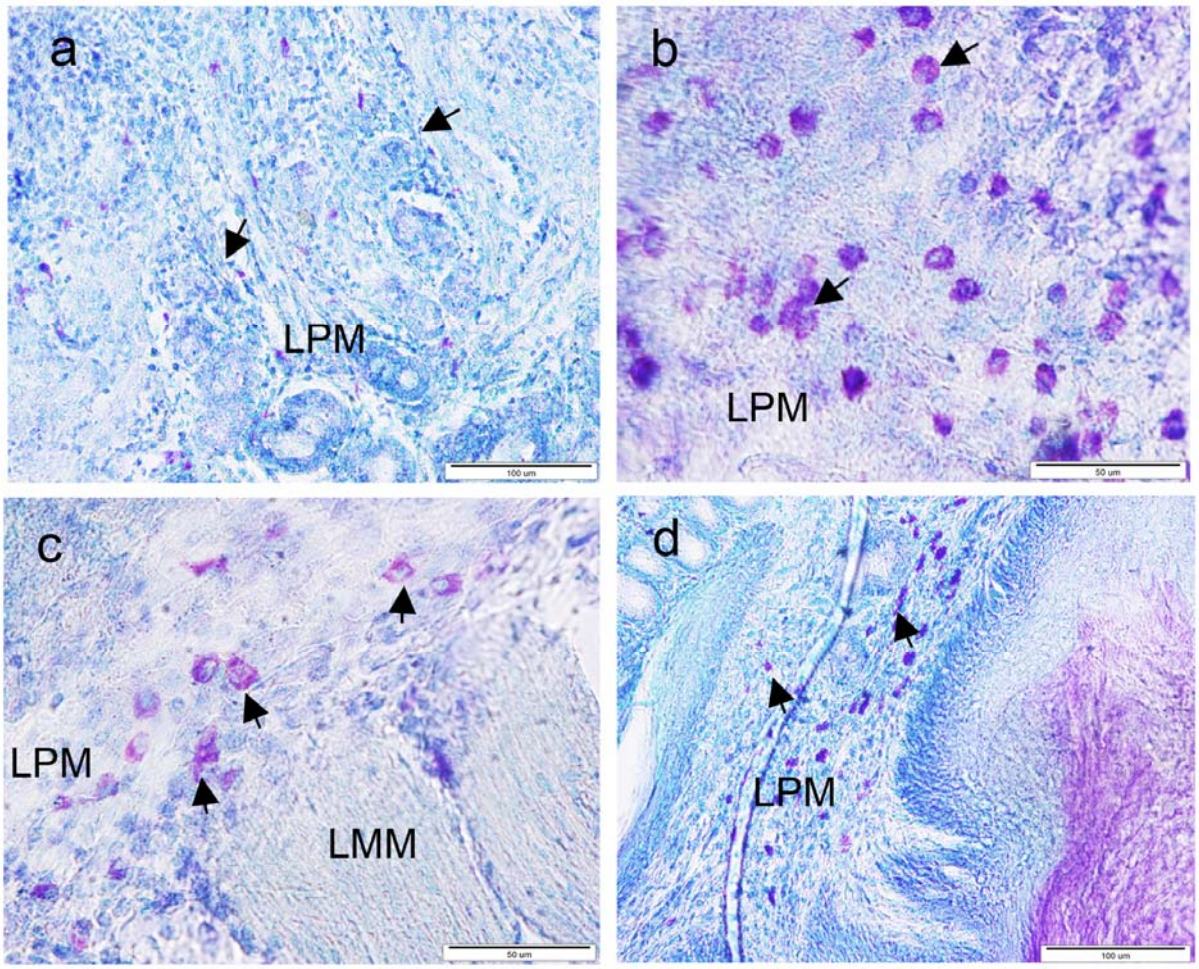

Fig. 1. **a** – Multifocal infiltrates (arrows) in the *lamina propria mucosae* (LPM) in the stomach of infected and treated gerbil; **b** – The inflammatory foci with CTMC (arrows) in the *lamina propria mucosae* (LPM) in the stomach of untreated gerbil infected with BZ-susceptible *H. contortus* larvae on day 10 p.i.; **c** – CTMC in the *lamina propria mucosae* (LPM) (arrows) in the stomach of untreated gerbil infected with BZ-resistant *H. contortus* strain on day 10 p.i. (LMM – *lamina muscularis mucosae*); **d** – CTMC (arrows) in the *lamina propria mucosae* (LPM) in the stomach of treated gerbil infected with BZ-resistant *H. contortus* strain on day 4/1 p.i./p.t.

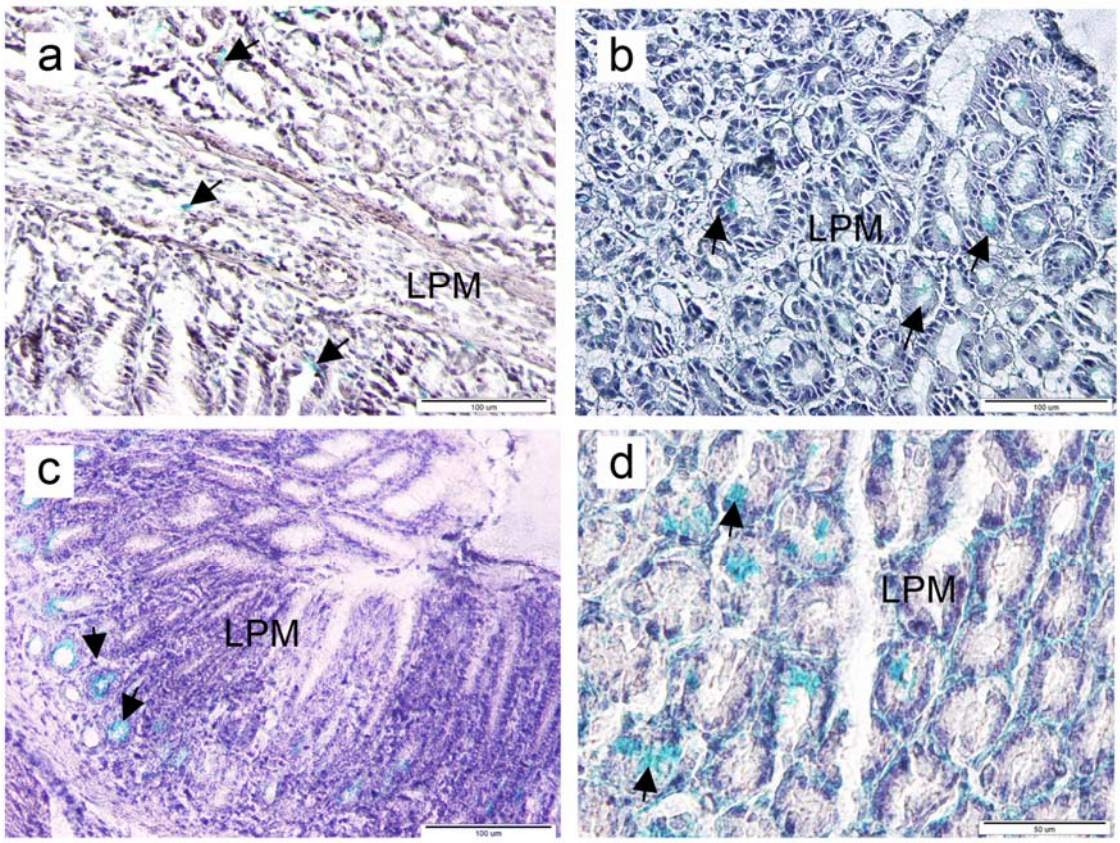

Fig. 2. **a** – MMC (arrows) in the *lamina propria mucosae* (LPM) in the gerbil stomach; **b** – MMC (arrows) in the *lamina propria mucosae* (LPM) in the stomach of treated gerbil infected with BZ-susceptible *H. contortus* strain on day 4/1 p.i./p.t.; **c** – Distribution of MMC (arrows) in the *glandulae gastricae* in the *lamina propria mucosae* (LPM) in the stomach of treated gerbil infected with BZ-resistant *H. contortus* strain on day 4/1 p.i./p.t.; **d** – Distribution of MMC (arrows) in the *lamina propria mucosae* (LPM) in the stomach of untreated gerbil infected with BZ-resistant *H. contortus* strain on day 7 p.i.

MMC were observed mainly in LPM, in the inflammatory lesions, which are results of larvae attachment and tissue damage (Fig. 2a). The occurrence of MMC was sporadic on day 4/1 p.i./p.t. and the cells were localized in the close proximity to proximal parts of the *glandulae gastricae*, during infection with both BZ-susceptible (Fig. 2b) and BZ-resistant *H. contortus* larvae, respectively (Fig. 2c). Interestingly, a higher density of MMC in LPM was re-

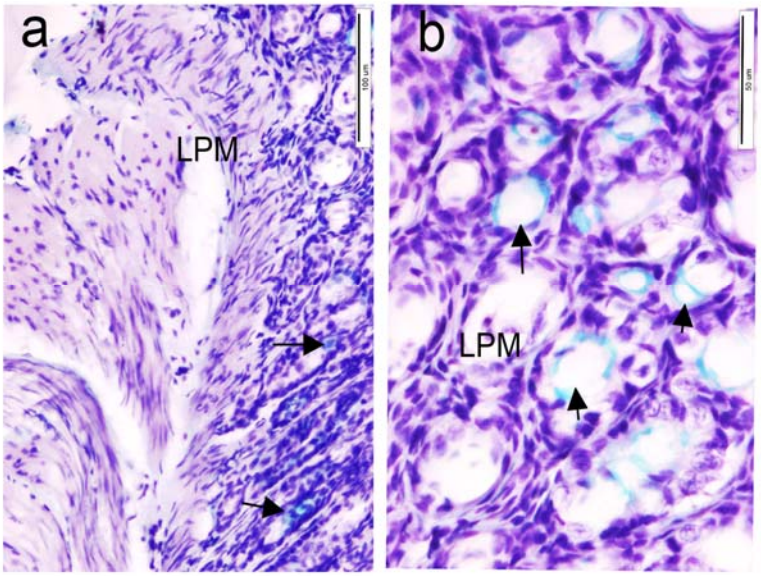

Fig. 3. **a** – Goblet cells (arrows) in the proximal part of *glandulae gastricae* in the *lamina propria mucosae* (LPM) in the gerbil stomach; **b** – Mucine in the goblet cells (arrows) in the *lamina propria mucosae* (LPM) in the gerbil stomach

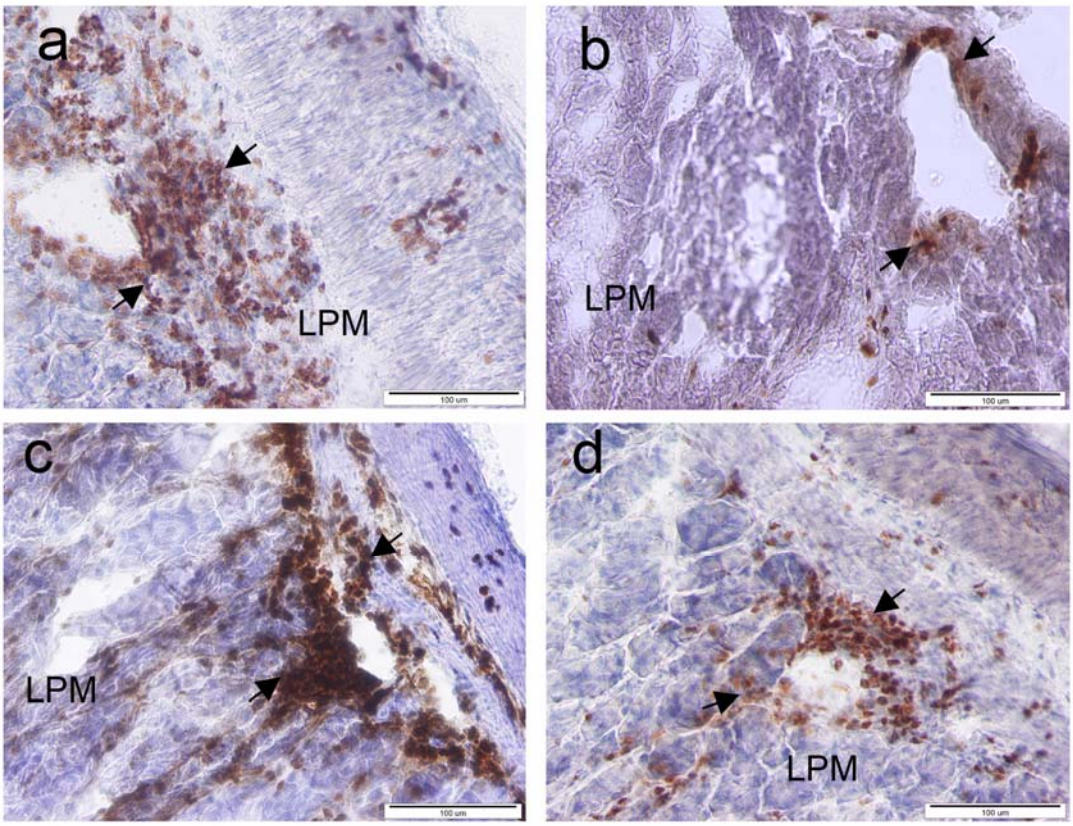

Fig. 4. **a** – Strong inflammation comprising mostly of Px cells (arrows) which were found in the *lamina propria mucosae* (LPM) in the stomach of treated gerbil during infection of BZ-susceptible *H. contortus* strain on day 4/1 p.i./p.t.; **b** – Px cells (arrows) in the *lamina propria mucosae* (LPM) in the stomach of treated gerbil infected with BZ-resistant *H. contortus* strain on day 4/1 p.i./p.t.; **c** – Distribution of Px cells (arrows) in the *lamina propria mucosae* (LMP) in the stomach of treated gerbil during infection BZ-resistant *H. contortus* strain on day 7/4 p.i./p.t.; **d** – Px cells (arrows) in the *lamina propria mucosae* (LPM) in the stomach of treated gerbil infected with BZ-susceptible *H. contortus* strain on day 7/4 p.i./p.t.

corded on day 7 p.i. near to *glandulae gastricae* in both untreated groups, what was followed by decline (Fig. 2d). Goblet cells were found only in the proximal parts of *glandulae gastricae* close to the mucosal surface of the stomach wall (Figs. 3a,b). However, the differences in cell counts after treatment of animals infected with BZ-susceptible and BZ-resistant strains of *H. contortus* were not significant. In addition, MMC were sporadically seen in sites free of inflammation.

Eos and Neus (Px cells) were present in several times higher numbers than MCs. The different pattern of distribution and kinetics of Px cells was found in LMM and LPM of the stomach wall from gerbils infected with both strains of the parasite. In group infected with BZ-susceptible strain, the largest foci of these cells were seen on day 4/1 p.i./p.t. (Fig. 4a) and mild in the second group (Fig. 4b). On day 7/4 p.i./p.t. cells accumulation peaked in gerbils infected with BZ-resistant strain (Fig. 4c) and a rapid decline was recorded in second group of gerbils (Fig. 4d). It is possible that higher eosinophilia in BZ-resistant strain after therapy persisted as the consequence of the higher numbers of BZ-resistant larvae. The gradual reduction of the numbers of all inflammatory cells was found, higher during the follow-up the therapy.

Numbers of inflammatory cells in the stomach wall of infected and treated gerbils

The numbers of CTMC in SM showed a decreasing trend following infection and therapy (Table 2). In comparison to the infection with BZ-susceptible strain significantly ($P < 0.05$) higher numbers of CTMC were recorded after infection with BZ-resistant strain and subsequently therapy. Statistical differences ($P < 0.05$) between control and ABZ treated group was recorded only in gerbils infected with BZ-susceptible strain on day 10/7 p.i./p.t. After the treatment of gerbils infected with BZ-resistant strain *H. contortus* in SM the significantly higher CTMC count ($P < 0.05$) was found from the beginning of the experiment until day 14/11 p.i./p.t.

The MMC counts in LPM and SM following infection with BZ-susceptible and BZ-resistant *H. contortus* larvae and ABZ therapy on the sections of the stomach of experimentally infected gerbils is shown in Table 3. Significant differences ($P < 0.01$) in MMC counts between LPM and SM were found mainly in gerbils infected with BZ-susceptible strain. Distribution of MMC was seen mostly in LPM. In comparison with treated groups of both infections with BZ-resistant and BZ-susceptible strain the numbers of MMCs were present in much higher values ($P < 0.05$, $P < 0.01$) in control groups. In LPM, MMC gradually increased

Table 3. Kinetic changes of mucosal mast cell numbers (MMC) in the *lamina propria mucosae* (LPM) and in the *submucosa* (SM) of the stomachs from gerbils infected with BZ-susceptible and BZ-resistant *H. contortus* strains

H. contortus	Group	Number MMC (mean number/mm^2 tissue± SD)				
		Distribution	Day 4/1 p.i./p.a.	Day 7/4 p.i./p.a.	Day 10/7 p.i./p.a.	Day 14/11 p.i./p.a.
SS	C	LPM	16.20 ± 3.90^{a}	39.25 ± 9.70^{aabb}	17.25 ± 4.11^{aabbcc}	3.45 ± 1.82^{b}
		SM	7.30 ± 3.15 ^{a}	8.00 ± 3.69^{aa}	1.80 ± 2.45^{aa}	2.05 ± 1.50
	ABZ	LPM	9.10 ± 2.80 ^{a}	8.55 ± 2.60^{bb}	3.80 ± 2.82^{bb}	0.55 ± 0.51^{b}
		SM	3.82 ± 2.01 ^{a}	3.30 ± 2.34	2.25 ± 1.33	0.40 ± 0.68^{c}
RS	C	LPM	11.50 ± 2.90	32.55 ± 6.38^{aabb}	6.35 ± 2.11^{bcc}	5.45 ± 1.39^{b}
		SM	10.80 ± 3.75	9.60 ± 3.43 ^{aa}	3.00 ± 1.20	2.08 ± 1.25
	ABZ	LPM	9.30 ± 5.75	4.05 ± 2.74^{bb}	1.75 ± 1.29^{b}	0.55 ± 0.82^{b}
		SM	5.10 ± 2.30	4.20 ± 2.24	2.70 ± 1.89	2.05 ± 0.39^{c}

n = 20 (number of data in each group), C- control group of experimental animal, ABZ- treated group of experimental animal with albendazole, SS- BZ-susceptible *H. contortus* strain, RS- BZ-resistant *H. contortus* strain

^{a}(P<0.05), ^{aa}(P<0.01) Significant difference on certain day (p.i./p.t.) between LPM and SM after infection with BZ-susceptible/resistant *H. contortus* strain

^{b}(P<0.05), ^{bb}(P<0.01) Significant difference on certain day (p.i./p.t.) between untreated (control) and ABZ treated group after infection with BZ-susceptible/resistant *H. contortus* strain

^{c}(P<0.05), ^{cc}(P<0.01) Significant difference on certain day (p.i./p.t.) between infections with BZ-susceptible and BZ-resistant *H. contortus* strain

following the infection and peaked on day 7/4 p.i./p.t. In SM, infection with BZ-resistant larvae elicited slightly higher mastocytosis than did the infection with BZ-susceptible strain, which peaked on day 4/1 p.i./p.t. In comparison to BZ-susceptible strain significant lower MMC counts ($P < 0.05$) after ABZ therapy were observed in the gerbils infected with BZ-resistant strain on days 10/7 and 14/11 p.i./ p.t. in LPM and SM, respectively.

Discussion

An increasing number of parasite populations resistant to the most common anthelmintics such as BZ carbamates prompted the search of new classes of drugs by means of exploiting the parasite´s specific molecular mechanisms (Geary *et al*., 2004). However, anthelmintic resistance in nematodes is now highly occurred prevalent, and cases of multiresistance (i.e. resistance against BZ and ivermectine or levamisole) are appearing in many areas of the world (Gaba *et al*., 2010; Várady *et al*., 2011). As yet, new effective drugs against resistant strains of intestinal nematodes such as *H. contortus* and *T. colubriformis* are not available and the search for novel lead molecules is in the centre of attention of pharmacologists. To be able to assess mode of action of potential drugs, a better understanding of the physiology of drug-resistant and drug-susceptible nematodes and their pathological effects on the host is necessary.

Our data confirmed that i*n vivo* gerbil model is a suitable for the study of BZ-resistance of *H. contortus*. In our study low efficacy of ABZ on resistant strain and more than 95 % clearing of immature adult stage of BZ-susceptible strain were described. The same observation was reported by Conder *et al*. (1991). Rojas *at al*. (2006) did not find larvae of susceptible strain *H. contortus* in the stomach of Mongolian gerbils after ABZ treatment on day 9 p.i. Similarly, using levamisole resistant strain of *H. contortus* larvae in Mongolian gerbils, levamisole analogs showed similar rate of reduction (≥ 68.9 %) for a levamisole resistant strain of *H. contortus* and for susceptible parasites (≥ 93.5 %) (Conder e*t al*., 1990; 1991).

H. contortus is predominantly localized in the abomasum of small ruminants being the definitive hosts, where immune responses elicited by nematodes and regulating the intensity of infection was determinated (Balic *et al*., 2000). The abomasum of sheep is histologically identical with the stomach of gerbils, however, local immunological or pathophysiological responses to infections are probably different. In the course of parasitic infection, inflammation is triggered not only by excretory/secretory products of larvae (Knox & Jones, 1990) but also by mechanical damage and blood sucking activity of larvae. The first line of defense is manifested by migration and accumulation of Px cells like Eos, Neus and later also MCs. Distribution and kinetics of Px cells in the acute stage of inflammation in gerbils with *H. contortus* infection were studied for the first time in our experiment. These cells released mediators and produced reactive oxygen species (ROS) aimed to eliminate *H. contortus* larvae. We assume, that the massive accumulation of Eos and Neus is primarily the consequence of mechanical damage and it correlated with the numbers of larvae. Increased numbers of Px cells in the inflammatory foci were found early day p.i. in gerbils infected with BZ-susceptible strain but their accumulation peaked on day 7/4 p.i./p.t. in stomach wall in case of BZ-resistant strain. Although we were not able to count these cells, foci of Px cells seemed to be more abundant post therapy in gerbils infected with BZ-resistant larvae. In the intestinum of rabbits infected with *T. colubriformis,* no differences in the distribution and numbers of Eos were found (Mallet & Hoste, 1995). Decreased numbers of Eos at the beginning of parasitic infection and increased values of these cells in gerbils infected with BZ-resistant strain of the parasite on day 7/4 p.i./p.t., might be the consequence of higher larval numbers due to their resistance to ABZ. It

is possible, that the antioxidant defence system, mainly glutathion-S-tranferase (GST), could be up-regulated in BZ-resistant strain of nematodes.

Polymorphonuclear cells (Eos, Neus) release lysosomal enzymes following stimulation with mediator acetylcholine (Ach) (Zurier *et al*. 1974). Lysosomal enzymes are mediators of acute inflammation and Ach plays an important role in this process. Therefore it is possible that acetylcholine esterase (AChE) secreted by nematodes may interfere with the process by hydrolysing the AChE before it reaches its target cells, thus reducing inflammation in the immediate vicinity of nematodes (Lee, 1996). Expulsion of larvae BZ susceptible and resistant strains of *H. contortus* from experimental rodent model may be associated with differences in AChE release. AChE produced by nematodes *Haemonchus* and *Ostertagia* might reduce local ulceration by hydrolysing Ach, which stimulates gastric acid secretion (Lee, 1996). ACh is involved in the release of histamine through stimulation of receptors on the surface of histamine containing cells - MCs in the alimentary tract, also in the release of mucus from goblet cells (Lee, 1996). Upon antigenic stimuli, MCs undergo a degranulation process, release histamine and proteases and give rise to goblet cells (Balic *et al*. 2000).

It was found that thiabendazole resistant L3 stage of *H. contortus*, *Ostertagia circumcincta* and *T. colubriformis* contains significantly greater amounts of AChE than susceptible strains of these nematodes (Sutherland & Lee, 1993). In the study of Mallet and Hoste (1995), BZ-resistant strains of *T. colubriformis* larvae secreted this enzyme in higher concentration *in vitro* than BZ-susceptible larvae did. In contrast, no AChE was found to be secreted by *H. contortus* L3 larvae *in vitro*, but *in vivo* secretion by L3, L4 stage of larvae or adult parasites was not investigated and can not be ruled out (Mallet & Hoste, 1995).

Expulsion of nematode larvae may also be associated with the population of inflammatory cells, neurons, smooth muscle cells, intersticial cells, mucosal epithelial cells, MCs, endocrine cells and immunocytes (Winter e*t al*., 2012). MMC signalling to enteric neurons, activates in the enteric nervous system and alarm program involving alterations in gastrointestinal motility and secretion (Nassauw *et al*., 2007). Expulsion of *Strongyloides venezuelensis* from mice is closely associated with the generation of intestinal mastocytosis. The timing of parasite expulsion and the degree of intestinal mastocytosis were almost comparable regardless of the dose of infection (Khan *et al*., 1993). In some cestode infection, the presence of MCs is controversial, whereas in mice infected with *Hymenolepis nana* the expulsion of this parasite seemed to be IgE and MC mediated (Watanabe *et al*., 1994). On another side, in rats infected with *Hymenolepis diminuta* MMC have no direct role in expulsion of this cestode (Featherston *et al*., 1992).

In our study, differences in MMC distribution were seen in LPM and SM of experimental animals. CTMC were found in SM, where also MMC were scattered and both types of MCs can interact. A rapid decrease of the CTMC numbers in the stomach wall of gerbils infected with BZ-susceptible strain was observed after treatment. CTMC are located mainly in the connective tissue around the blood vesels and MMC are found in the intestinal *mucosa* (Winter *et al*., 2012). CTMC are involved in many pathophysiological processes including the modulation of fibrogenesis, which develops as a response to tissue injury (Sellge *et al*., 2004). CTMC peaked on day 7/4 and 10/7 p.i./p.t. in control group of gerbils infected with BZ-susceptible strain of *H. contortus* in SM, the only larval stage which can feed on blood and cause massive tissue injury. In rabbits infected with BZ-resistant and susceptible strains of *T. colubriformis* MC counts increased in the intestinum within the course of infection. At this later stage, the higher MC numbers present in rabbits with resistant infection correlated with the reduction in egg production but not with parasite establishment (Mallet & Hoste, 1995). Our experimantal results differ from data found in this report, what could be explained by using the gerbils as laboratory animal and the mature larvae.

The different proteoglycan content of both types of MCs within the intestinal *mucosa* suggests that they might serve different functions in maintaining the integrity of the intestinal *mucosa*. It is well known that both the number and composition of MCs change with immunopathological conditions. Mezey and Palkovits (1992) showed that immunocytes in the LPM of the alimentary tract posses muscarinic receptors and their play a role in certain inflammatory diseases. MC counts were also significanly higher in the duodenum in rabbit infected with the resistant strain *T. colubriformis* (Mallet & Hoste, 1995). Their results suggest that genetic variation in the nematode, such as anthelmintic resistance, is associated with variations in worm biology and physiology as well as differences in the inflammatory response of host.

Gerbils seem to be suitable models for evaluation effect of drugs and it is important to know differences in mucosal immunity after infection with BZ-susceptible and BZ-resistant nematode strains. Secondly, it is important to know mechanism by which infective larvae of nematodes affect immunity, while taking into account much higher secretion of crucial enzyme AChE by BZ-resistant strain of *H. contortus.*

This study confirmed that Mongolian gerbil is a suitable model to asses the efficacy of BZ anthelmintic. Our results showed low ABZ efficacy on BZ-resistant strain of *H. contortus.* In spite of the high number of MCs in the stomach wall of Mongolian gerbils we assume that they were not activated and histamin was not released. This probably accounted for suppressed expulsion of this strain of larvae and higher secretion of AChE also played the role. Our results suggest that expulsion of *H. contortus* larvae from the intestine after treatment does not depend on the intensity of mastocytosis and eosinophilia, but rather on the functional state of these cells.

In conclusion, present data revealed that BZ-resistant and BZ-susceptible strains of *H. contortus* can elicit a different local cellular immune response and that mutation in DNA of gene encoding tubulin may modulate functions of BZ-

resistant nematodes, which influence functional state of innate immunity cells.

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