Effect of the bendiocarb on the ultrastructure of rabbit skeletal muscle

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Abstract

Bendiocarb belongs to the group of carbamate insecticides that inhibit acetylcholinesterase. In agriculture, it is used to control a variety of insects, therefore it is important to examine every potential aspect of its toxicology. The aim of this study was to observe the effect of bendiocarb on the ultrastructure of the skeletal muscle in rabbits. Rabbits in all experimental groups received capsules of bendiocarb (96% Bendiocarb, Bayer, Germany) per os daily at a dose of 5 mg/kg body weight. Samples of skeletal muscles were collected on days 10 and 20. On day 10 of the experiment, muscle fibres were not affected consistently. The observed changes were moderate and focal. Electron microscopy revealed dilatation of sarcoplasmic reticulum, and myofilament disorganization. On day 20 of the experiment, the ultrastructural changes in muscle fibres were more intense and more frequent. The most important alteration was the disruption of the sarcomeres due to the lysis of both thick and thin myofilaments. However, in the unchanged regions of muscle fibres a prominent mitochondrial swelling was observed. Many mitochondria lacked cristae and thus appeared as large membrane-bound cytoplasmic vesicles. The results presented in this study indicate that bendiocarb affects the ultrastructure of skeletal muscles. The intensity of damage (dissolution of myofilaments and disruption of sarcomeres) was related to the duration of administration of bendiocarb.

Morphology, pesticide, toxicity, muscle fibre

Bendiocarb (2,2-dimethyl-1,3-benzodioxol-4-yl-N-methylcarbamate) is a broad spectrum insecticide belonging to the N-methyl carbamate group. It is one of the insecticides recommended by the World Health Organization for malaria control in Africa (Sadasivaiah et al. 2007). Similar to other carbamate insecticides, bendiocarb is a reversible inhibitor of acetylcholinesterase (AChE), an essential nervous system enzyme, which plays an important role in neurotransmission. Generally, carbamates are excreted rapidly and do not accumulate in mammalian tissues. In rabbits, 80–95% of radioactively labelled bendiocarb was eliminated in 24–48 h. However, it was found that rats fed 20 mg/kg of ^{14}C-bendiocarb in their diet for 10 days had detectable ^{14}C residues in the analysed tissues (fat, liver, kidney, muscle, and brain) 6 days after termination of dietary feeding (Bendiocarb 2002). Due to high fat solubility, carbamates easily penetrate through cell membranes and are quickly distributed throughout the body (Tos-Luty et al. 2001). The oral LD50 (median lethal dose) for bendiocarb has been previously observed between 35–40 mg/kg for rabbits, 34–156 mg/kg for rats and 3.1–16 mg/kg for birds, which are extremely sensitive to this substance (Hayes and Laws 1990).

If the use of bendiocarb against vectors of malaria is to be increased (Forget 1991), it is important to examine every potential aspect of its toxicology. As the effect of bendiocarb was investigated in several studies using rabbits as an animal model, we have continued with these observations in order to obtain its complete picture. The aim of this study was to describe ultrastructural changes in the rabbit skeletal muscles after administration of bendiocarb.

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Materials and Methods

In the experiment we used a total of 30 adult rabbits (54 days old) of the Hyla hybrid (*Oryctolagus cuniculus*), with a mean body weight of 2.5 ± 0.144 kg. The rabbits were kept in a well-ventilated environment and received standard diet (O-10 NORM TYP, Bonamix, Gemerská Panica, Slovak Republic) and water *ad libitum.* The animals were divided into three groups, ten animals in each (control, days 10 and 20 of administration). Rabbits in all experimental groups received capsules of bendiocarb (96% Bendiocarb, Bayer, Germany) *per os* daily at a dose of 5 mg/kg of body weight. This dose was selected based on the results of a previous study that demonstrated morphological changes induced by bendiocarb (Flesarova et al. 2007). The animals' reaction to bendiocarb was relatively strong, accompanied by dehydration, diarrhea, and alopecia. After day 11, experimental animals received the same dose of bendiocarb (5 mg/kg b.w. in capsules) every 48 h. Experimental and control animals were killed with thiopental (Thiopental Valeant 1 g, ICN, Czech Republic; 100 mg/kg of body weight) intravenously at days 10 and 20 after bendiocarb treatment. The experimental study on rabbits was performed with the approval of the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Kosice (No. 2647/07–221/5) and the State Veterinary and Food Institute in Bratislava (No. 1827/09–221/3) and followed Slovakia's protocols for ethical standards for the use of laboratory animals.

The specimens of skeletal muscle (m. gastrocnemius) were cut into 1 mm^3 sections and immersion-fixed in 3% glutaraldehyde (Sigma-Aldrich, Bratislava, Slovak Republic) in 0.15 M cacodylate buffer (Sigma-Aldrich, Bratislava, Slovak Republic) pH 7.2–7.4 for 2 h at 4 °C. Specimens were postfixed for 1 h at 4 °C in 0.15 M cacodylate buffered 1% osmium tetroxide (Sigma-Aldrich, Bratislava, Slovak Republic), pH 7.2–7.4, washed in the same buffer before dehydration in acetone (Sigma-Aldrich, Bratislava, Slovak Republic), and embedded in Durcupan ACM (Fluka Chemie AG, Buchs, Switzerland). The Durcupan blocks were sectioned with glass knives attached to an ultramicrotome Tesla BS 490. The obtained ultrathin sections (90–120 nm) were mounted on copper grid, stained with 1% uranyl acetate (Sigma-Aldrich, Bratislava, Slovak Republic) for 30 min, followed by 0.3% lead citrate (Merck, Bratislava, Slovak Republic) for 15 min. The samples were evaluated under a transmission electron microscope Tesla BS 500.

Results

The ultrastructure of normal skeletal muscle is shown in (Plate II, Fig. 1). Myofibrils in longitudinal sections showed cross-striations formed by alternating segments of dark anisotropic (A) and light isotropic (I) bands. Each anisotropic band contained a centrally located Hensen disc (H band) with a dark transverse plate, the mesophragm. The isotropic bands were subdivided by dark thin Z lines demarcating regular segments, the sarcomeres. Mitochondria were situated between the myofibrils. The oval pale nuclei were located under the sarcolemma.

On day 10 of the experiment, muscle fibres were not affected consistently. The ultrastructure of many muscle fibres was compared with that of the control. These normal muscle fibres were situated adjacent to the injured ones. The changes observed within the fibres were moderate and limited to small areas. In these areas, spaces between myofibrils were enlarged due to dilated sarcoplasmic reticulum. Other focal changes were characterized by disorganisation of contractile myofilaments. In such regions the muscle fibres contained mitochondria of normal shape and size, but with barely recognizable cristae (Plate II, Fig. 2).

On day 20 of the experiment, the ultrastructural changes in muscle fibres occurred at higher frequency and higher intensity. In these regions, myofilaments of sarcomeres were completely interrupted. Lysis of both thick and thin myofilaments caused this sarcomeric disruption. Z lines in these regions were irregular, faint, or absent. Areas of disorganization of the myofilaments could be observed immediately next to normal areas of the myofibrils (Plate III, Fig. 3). However, these unchanged regions contained dilated mitochondria. The mitochondrial swelling was prominent. Many mitochondria lacked cristae and thus appeared as large membrane-bound cytoplasmic vesicles (Plate III, Fig. 4).

Discussion

Many studies indicated that the intensity of damage to muscle fibres depended on the density of motor end plates in the skeletal muscle (Patterson et al. 1987). The

accumulated acetylcholine causes unusual contraction of myofibrils in the respective section of the muscle fibre, which can eventually lead to its damage (Simons 1996). This was confirmed also by studies of other authors who observed necrotic changes in muscle fibres at the site of the motor end plate (Dettbarn 1984; Patterson et al. 1987). In parallel with inhibition of AChE, Ca^{2+} is released from the endoplasmic reticulum (Mense et al. 2003). The released Ca^{2+} might also be responsible for muscle degeneration. Increased intracellular level of Ca^{2+} could stimulate loss of muscle proteins (Sugden and Fuller 1991), and is associated with dissolution of myofilaments (Publicover et al. 1978). In our study, dissolution of myofilaments was observed on day 10 of the experiment. The intensity of damage was related to the duration of administration of bendiocarb. On day 20 of the experiment we were able to detect damage to the entire sarcomeres. Hyperstimulation as well as accumulation of Ca^{2+} ions in the sarcoplasm might play a role in the lysis of both thick and thin myofilaments and the loss of sarcomeric organization (Bright et al. 1991). It has been recognised that the high intracellular Ca^{2+} concentration causes damage to mitochondria (Choi and Rothman 1990; Milatovic et al. 2006). Our examinations showed that the observed muscle fibres displayed pronounced mitochondrial swelling with the injured cristae on day 20 of the experiment.

Studies investigating myopathy caused by AChE inhibitors showed that the rise in intracellular calcium may result in the stimulation of numerous intracellular calcium dependent processes, e.g. the activation of nitric oxide synthase (NOS), which increased production of nitric oxide (NO) (Baggeta et al. 1993). The pathway through which NO induces cellular injury involves its reaction with superoxide anion and the formation of peroxynitrite which is known to mediate oxidative injury (Wang and Zweier 1996; Jeyarasasingam et al. 2000).

The toxic effect of bendiocarb involves not only AChE inhibition but also production of reactive oxygen species (Sobekova et al. 2009). Bendiocarb was shown to alter homeostasis (Capcarova et al. 2010; Mojzisova et al. 2012) and induce morphological changes in different organs such as the thymus, kidneys, and liver (Flesarova et al. 2007; Almášiová et al. 2014; Holovska et al. 2014). In agreement with previously published studies, it can be concluded that bendiocarb adversely affected the structure of rabbit tissues and organs.

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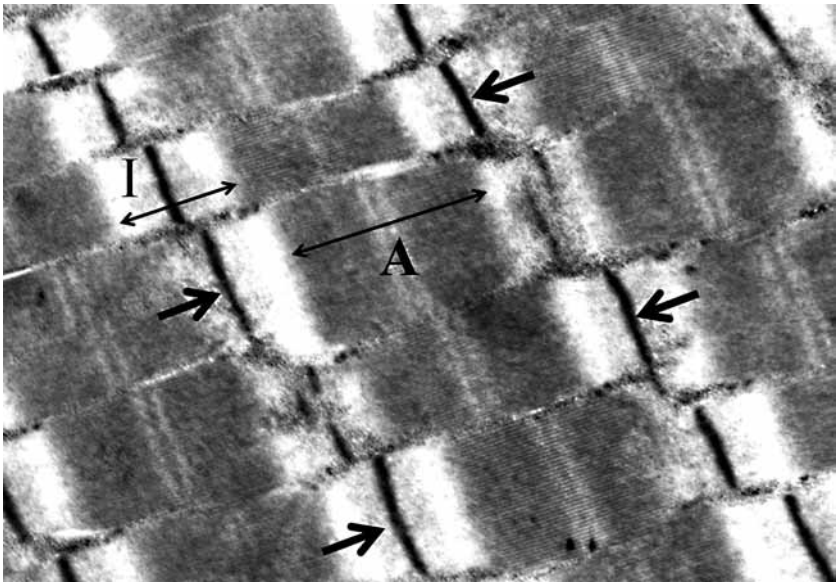

Fig. 1. Electron micrograph of the muscle fibre of the control group. Magnification × 22 700; A – anisotropic band, I – isotropic band, arrows – Z lines.

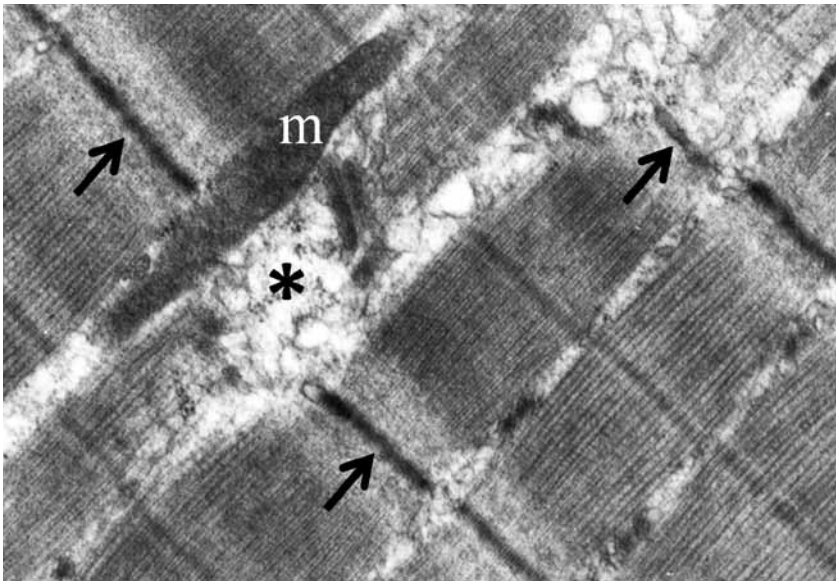

Fig. 2. Electron micrograph of the muscle fibre on day 10 of exposure to bendiocarb. Magnification × 28 700; Arrows – Z lines; m – mitochondrion; asterisk – disorganized myofilaments and dilated sarcoplasmic reticulum.

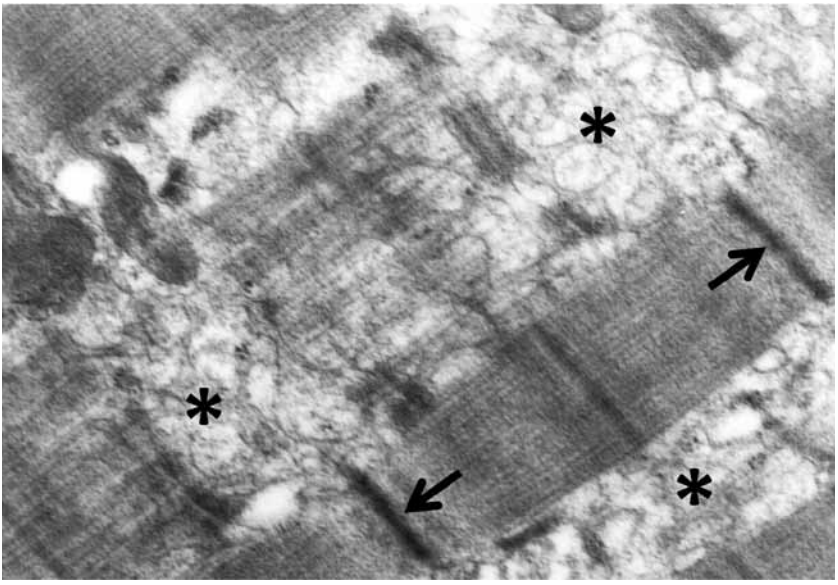

Fig. 3. Electron micrograph of the muscle fibre on day 20 of exposure to bendiocarb. Magnification × 32 000; Arrows – Z lines, asterisks – disorganized myofilaments and dilated sarcoplasmic reticulum.

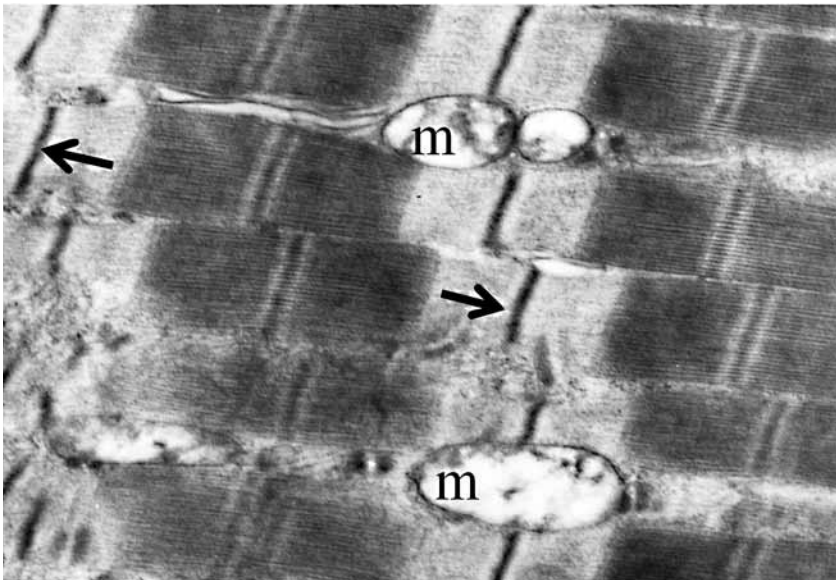

Fig. 4. Electron micrograph of the muscle fibre on day 20 of exposure to bendiocarb. Magnification × 32 500; Arrows – Z lines, m – mitochondrion