Morphometric Alterations, Steatosis, Fibrosis and Active Caspase-3 Detection in Carbamate Bendiocarb Treated Rabbit Liver

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ABSTRACT: Increasing use of pesticides all over the world makes it necessary to reveal the toxic risk in populations of nontargeted organisms. Bendiocarb is one of the 12 insecticides recommended by the World Health Organization for use in malaria control in Africa, and is used against a variety of insects. The liver has an important role in its process of detoxication and excretion. In our experiment 56 adult rabbits of breed HY+, 28 males and 28 females were used. Animals were divided into groups (control, days 10, 20, 30 of bendiocarb administration). The presence of many binucleated hepatocytes, the highest number of liver cells and their decreased size at 10 day after bendiocarb administration was observed as an evidence of the hepatic regeneration. After the long-term treatment pronounced changes were presented such as vacuolization and dilatation of hepatocytes, dilatation of sinusoids between hepatocytes, and focal infiltration of inflammatory cells. Numerous cells with caspase-3 activity were present throughout the organ, most commonly around the portal tract and close to the central vein. Short and long-term bendiocarb treatment showed the central vein thickened rim with increased deposition of collagen, spreading of collagen fibers into the perisinusoidal, and pericellular space surrounding the central veins,

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and septal fibrosis extended from the portal tract. Subsequently, presence of the lipid vacuoles both in the liver parenchyma and inner of the hepatocytes were observed. These results suggest that bendiocarb treatment leads to increased cell death, liver perisinusoidal fibrosis, and steatosis, especially during the long-term administration. Environ Toxicol 30: 212–222, 2015.
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INTRODUCTION

Pesticides are the significant contaminants of environment that are used in agriculture and households. Their widely use cause the question of toxicity pesticides to nontarget organisms, their persistence, accumulation, and combined effect with other chemicals. The worldwide annual consumption of pesticides is about 2 million tons of which 24% is consumed in the United States alone, 45% in Europe, and 25% in rest of the world (Uggini et al., 2010). In the European Union the wide using of pesticides are mainly organophosphates, carbamates, and pyrethroids. Further, over 25% of fruits, vegetables, and cereals grown in the European Union contain detectable residues of at least two pesticides while their total quantity, which Europe's population is exposed to is unknown (Bjørling-Poulsen et al., 2008). In 2003, over 800 pesticide substances were used in European agriculture, but only 380 of these are still authorized in March 2011 (Nougadere et al., 2011). At present, bendiocarb (2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate) is not approved for using in the European Union, mainly through Directive 91/414/EEC and Regulation (EC) No 1107/2009. Although, it is one of 12 insecticides recommended by the World Health Organization for use in malaria control in Africa (Sadasivaiah et al., 2007). The widespread pyrethroid resistance is becoming a major problem faced by several National Malaria Control Programmes throughout Africa. Field trials with the carbamates propoxur and bendiocarb for indoor residual spraying treatment have been very effective against pyrethroid resistant malaria vectors. Over the past few years, there was an increasing interest in testing these insecticides for public health purposes as alternatives to pyrethroids (Akogbeto et al., 2010). Bendiocarb is a broad spectrum pesticide belonging to the *N*-methylcarbamate group (Pacioni and Veglia, 2007). Using of bendiocarb can dramatically reduce the risk of insect-borne diseases that are transmitted by arthropod vectors such as mosquitoes (malaria, dengue fever, yellow fever, encephalitis, filariasis, West Nile fever and chikungunya), ticks (e.g., Lyme disease), and sandflies (leishmaniasis; Nauen, 2007). Bendiocarb inhibits acetylcholinesterase and elicits cholinergic hyperstimulation, however causes only reversible inhibition of acetylcholinesterase for a few minutes to a day (Kamel and Hoppin, 2004). This lability tends to limit the duration of carbamate poisonings, accounts for the greater span between symptom-producing and lethal doses, and it frequently invalidates the measurement of blood cholinesterase activity as a diagnostic index of poisoning (Reigart and Roberts, 1999). Further, carbamate insecticides inhibit cellular metabolism including energy, protein, and nucleic acid metabolism, thereby, causing cell regression, and death (Amanullah and Hari, 2011). Symptoms of bendiocarb poisoning include weakness, blurred vision, headache, nausea, abdominal cramps, chest discomfort, miosis, sweating, muscle tremors and incoordination, decreased pulse, low blood pressure, heart irregularities, giddiness, confusion, slurred speech, and loss of reflexes. Respiratory failure resulting from inhibition of the central respiratory drive and bronchospasms coupled with depolarizing blockade at neuromuscular junctions-diaphragm and intercostals (Krieger, 1991; Almasiova et al., 2012). Many studies proved that bendiocarb increases incidence of lymphoreticular tumours, such as lymphosarcomas, reticulosarcomas, lymphoid, and myeloid leukaemia (Dorko et al., 2011). Bendiocarb is degraded in the liver, which has a major role in the biotransformation and excretion of carbamate pesticides from the body. Reactions of detoxication take place in hepatocytes by the enzymatic system situated in the endoplasmic reticulum (Schenck and Kolb, 1990), and bendiocarb is excreted as a sulphate and β-glucuronide conjugates of the phenol derivatives (National Pesticide Information Center, 2002). In a study with rats, 90% of an oral bendiocarb dose was excreted in the urine, 1–3% in expired air, and 3–8% in the feces. Excretion was complete within 24 hours (Challis and Adcock, 1981).

In order to increase using of bendiocarb against vectors of malaria, thus it is important to examine every potential aspect of its toxicology. The lack of information at all levels may be one of the most important causative factors of chemical intoxication in developing countries (Forget, 1991). At present, several studies of bendiocarb effect with rabbit as an animal model have been published. We can continue with results, particularly to organs which have a key role to biotransformation and excretion of bendiocarb. The aim of our study was to observe the morphometric alterations, fibrotic changes as well as the programmed cell death of the rabbit liver tissue treated with bendiocarb, mostly in the area of the central vein and portal tract.

MATERIAL AND METHODS

In our experiment were used 56 adult rabbits (age = 84 days) of breed HY+, 28 males and 28 females, and with average weight 2500 g from accredited animal farm (Nitra, Slovakia). Animals were kept in cages (two 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 groups (control, days 10, 20, 30 of administration), and each group comprised 14 animals. Rabbits in all experimental groups received bendiocarb (96% Bendiocarb, Bayer, Germany) *per os* in a dose 5 mg/kg per day (National Pesticide Information Center, 2002) and after day 11 in a same dose per 48 hours, with respect to the acute oral toxicity of bendiocarb to rabbit, 35–40 mg/kg of the body weight (Petrovova et al., 2011). Animals in control group were not treated and they were killed at 30 day from the beginning of the experimental work. Experimental animals were killed by thiopental (Thiopental Valeant 1 g, ICN, Czech Republic; 100 mg/kg of body weight) intravenous administration at days 10, 20, and 30 after bendiocarb treatment. Animal weight was recorded at first and last day of bendiocarb treatment, and calculation of the weight gain was made as the difference between rabbit body weight in experimental group and control group at days 10, 20, and 30 of the bendiocarb treatment. But the rabbit body weight of experimental animals was unchanged in relation to control during the whole experiment. The experimental work on rabbits was performed with approval of the Ethic Committee of the University of Veterinary Medicine and Pharmacy in Kosice (No. 2647/07–221/5) and State Veterinary and Food Institute in Bratislava (No. 1827/09–221/3) followed Slovakian protocols for ethical standards for the use of laboratory animals.

Histological Analysis

Liver samples from all animals were dissected immediately after the animals were killed and fixed in 10% neutral formalin. After the dehydration in 70–100% ethanol series they were paraffin embedded. Paraffin sections (7 μm thick) were stained for routine histological study using haematoxylin and eosin (H&E) for histological evaluation under light microscope (FL-800).

Morphometric Analysis

The morphometric model for liver characterization consisted of many stereological variables. All morphometric variables were obtained using a light microscope with two objectives 20x and 40x, and photo-equipment (Olympus Provis AX) to prepare images for morphometric analysis. More than 300 microscopic fields randomly selected were examined.

A 16-points square lattice was used to quantitative evaluation of hepatocytes (the total number of hepatocytes). The grid was overlaid onto each microscopic field (87296 μm^2) displayed on the monitor (Vertemati et al., 2012). From each animal of the control and experimental groups, we selected randomly of 5–6 fields of the liver parenchyma which were averaged. Subsequently, average for the group was measured. The morphometry was used to assess the diameter of the central vein and size of the liver cell in H&E stained sections of the liver tissue. Structures of interest were analyzed under 40× magnification, and the results of each sample were averaged and evaluated. The morphometric analysis of the vessel along the selected analysis path is based on the quantification of the cross-sectional areas and diameters for each cross-sectional section. For each in-plane direction, the maximum, distance between two boundary points parallel to that direction is determined. The minimum, average, and maximum vessel diameters are then computed as the minimum, average, and maximum values, respectively, of all of these distances (Boskamp et al., 2004). For the evaluation of the liver cell size were used of 120 measurements as well as 165 measurements for the diameter of the central vein. We used to display of the microscopic fields the software (Adobe Photoshop version 7.0), and for the measurement the Image Acquisition Software getIT (Olympus Soft Imaging Solution GmbH, Germany).

Oil red O Staining

Oil red staining was used for visualization of neutral lipids on frozen sections. Samples of the liver were fixed in 4% paraformaldehyde and sectioned on a cryomicrotome (Leica CM 1850) for 5–10 μm thick. Liver sections were rinsed in distilled water and 100% propylene glycol for 5 min, and stained by Oil Red (0,5% Oil red in 100% propylene glycol; Sigma-Aldrich, Germany) the preheated solution at 60°C, 8–10 min. Sections were differentiated in 85% propylene glycol and rinsed twice in distilled water. Then the sections were stained with hematoxylin for 30 seconds, differentiated and rinsed in distilled water, and mounted in Kaiser Glycerine-gelatine (Merck Millipore, Germany).

Caspase-3 Activity

The liver samples were dissected out and processed by the histological approach. Samples of the liver were fixed in 4% paraformaldehyde, sectioned on a cryomicrotome (Leica CM 1850) for 5 μm thickness, and stained immunohistochemically for observation of caspase activity, mainly close to the central vein and portal tract. The programmed cell death in the liver was observed by means of primary murine monoclonal antibody IgG 1–Caspase-3/CPP32 (BD Biosciences Pharmingen, California, USA) and secondary antibody conjugated with Rhodamine Red dye (Jackson ImmunoResearch Laboratories, Baltimore, USA). An apoptic signal such as granzyme B of cytotoxic T-cells or ICE-like proteases induces the intracellular cleavage of Caspase-3 from the inactive proform (32 kDa) to the active form, which consists of the p17 subunit. To visualize the nuclei in the liver, the respective sections were stained with Hoechst 33258 dye (Sigma-Aldrich, St. Louis, USA). The Rhodamine Red-conjugated antibody was red while the Hoechst

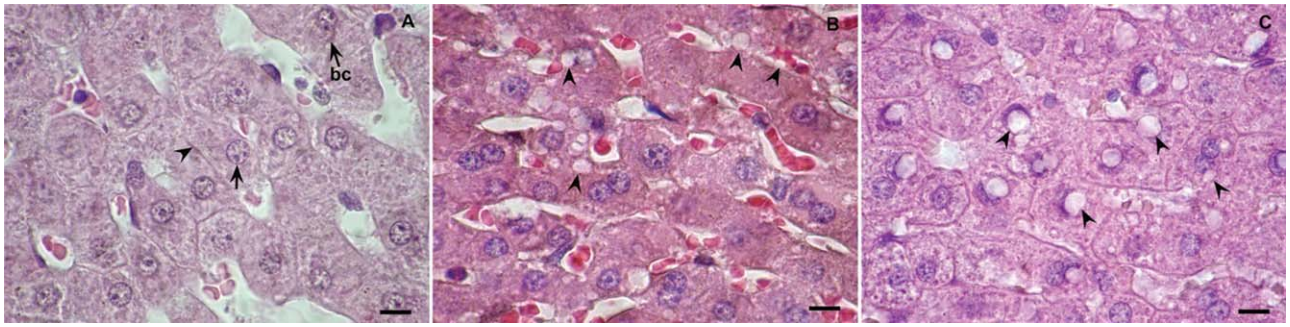

Fig. 1. Representative alterations in the bendiocarb treated rabbit liver. A: Moderately dilatation of the blood sinusoids (arrow) between the hepatocytes was observed in each treated group. Mostly at 10 days the binucleated cells (headarrows) were observed as well as the number of hepatocytes was increased in comparison with the other treated groups. B: Dilatation of hepatocytes was observed at 20 and 30 days. C: Affected lobules of the treated groups contained the focal infiltration of inflammatory cells (arrow), and the number of vacuoles (headarrows) in hepatocytes was observed at 10 and 30 days of bendiocarb administration, except at 20 day. Stained H&E, magnification 40×, Scale bars: 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

33258 stain was blue, and replacement fibrosis was green (WGA-wheat germ agglutinin staining). Image acquisition was performed on a Leica SPE confocal microscope using an oil immersed objective with 40× magnification. We used the viewing field of size 100 μm^3 for the counting the liver cells with active caspase-3.

Fibrosis

Picro-sirius red staining was used for visualization of collagen fibers and staging of hepatic fibrosis. Deparaffinized liver sections were washed in water, and stained in Picro-sirius red (0.1% Picro-sirius red in saturated aqueous picric acid; Sigma-Aldrich) for 1 hour at room temperature. Sections were rinsed twice in 0.5% acetic acid and stained in Hoechst (2 μl/200 ml 0.1% Triton/H_2O). Subsequently, histological sections were rinsed in distilled water, dehydrated in absolute ethanol and xylene, and mounted in Depex (VWR International GmbH, Austria). Microscopic evaluation of fibrosis was analyzed using an Olympus BX46 microscope equipped with a camera DP70 (Olympus, Hamburg, Germany) and objective with 20x magnification. Five central veins and areas of portal tracts in each animal were studied, for a total of 260 central veins and portal tracts.

Statistics

The data were analyzed and expressed as means ± SD (standard deviation of the mean), the minimum, average, and maximum values. Statistical analyses were performed using the statistics guide (GraphPad Prism version 5.0 software). Because the data are not normally distributed nonparametric Mann–Whitney test was used to compare between the data of the control and those of treatments. The p-value; $p \leq 0.05$ was the level of statistical significance.

RESULTS

Liver Histology

Physiological structure of the liver parenchyma is presented in the untreated samples. The polyedric liver cells are arranged into the trabecules and blood sinusoids between of them. Large, euchromatic nuclei were located in the centre of the hepatocytes, and few binucleated liver cells were observed as a physiological feature. The affected lobules of the treated groups contained the focal infiltration of lymphocytes and granulocytes. Dilatation of blood sinusoids between the hepatocytes was observed in each treated group, but dilatation of hepatocytes was observed at 20 and 30 days (Fig. 1). Mostly at 10 days the binucleated cells were observed as well as the number of hepatocytes was increased in comparison with the other treated groups (Table I). The number of vacuoles in hepatocytes was observed at 10 and 30 days of bendiocarb administration, except at 20 day. But the highest number of vacuoles was observed at 30 days, and they changed the shape of the nuclei to semilunar shape with pushing them away in the hepatocytes (Fig. 2). To further clarify whether lipid accumulation was indeed induced in the liver, the liver tissues were stained with Oil Red staining. The Oil Red staining clearly revealed moderate lipid accumulation in hepatocytes of bendiocarb-treated animals at 30 days. This result illustrates that the long-term bendiocarb administration tested in this study could lead to the liver steatosis (Fig. 3).

Significantly higher number of the liver cells was observed at 10 day ($26.34 \pm 6.46/100\ \mu m^3$) and 30 day ($20.94 \pm 2.14/100\ \mu m^3$) of bendiocarb treatment in comparison with the control group ($19.55 \pm 1.95/100\ \mu m^3$). The number of hepatocytes of treatment group at 20 day was unchanged to control (Table I). Changes of the size of hepatocytes were observed at 10 and 20 day of the pesticide

TABLE I. Morphometric values of hepatocytes and vena centralis in the rabbit liver

	Day	*x*	SD[a]	Minimum	Maximum
Number of liver cells (100 μm^3)	Control	19.55	1.95	17.35	24.22
	10	26.34[b]	6.46	16.69	43.69
	20	20.67	2.61	14.57	24.55
	30	20.94[b]	2.14	17.18	25.20
Size of liver cells (μm)	Control	15.50	2.12	11.50	20.30
	10	14.20[b]	2.59	9.00	19.33
	20	16.50[b]	2.29	10.66	20.33
	30	15.50	3.50	9.83	23.50
Diameter of *v. centralis* (μm)	Control	118.23	22.36	58.34	158.78
	10	113.90	25.17	62.15	147.95
	20	118.71	33.21	63.19	155.87
	30	121.08	35.74	67.93	160.46

[a]Standard deviation.
[b]Statistical difference from the control; $p \leq 0.05$.

treatment. The size of hepatocytes was significantly decreased at 10 day (14.20 ±2.59 μm). But the hepatocyte size was increased at 20 day (16.50 ± 2.29 μm) against the size of hepatocytes in the control group (15.50 ± 2.12 μm; Table I). The diameter of central veins ranged from 58.3 to 160.5 μm, and the mean diameter was lower at 10 day (113.90 ± 25.17 μm) compared with control (118.23 ± 22.36 μm; Table I). Thus, they are representative central veins as a general term (Danko et al., 2011), also called centrolobular veins or terminal hepatic venules (Mak et al., 2012).

Caspase Activity

Almost no positive cells were detected in the untreated samples. After 10 days of bendiocarb treatment, numerous immune-reactive cells were present throughout the organ, most commonly around the portal tract (PT). Positive cells were still abundant at 20 days close to the central vein, with a decline at 30 days at which point vacuoles were observed in some cells together with replacement fibrosis.

After application of bendiocarb at 10 day, we observed that in the viewing field of size 100 μm^3 was the higher number of liver cells with caspases activity around the PT (13%). At this day only 7% of liver cells showed the caspase activity near to the central vein in comparison with the control. After application of bendiocarb at 20 day in the same viewing field of size the highest rate (14%) of caspase activated liver cells was observed close to the central vein in comparison to 7% of rate around the PT. At 30 day the number of positive cells was decreased, mainly around the PT (3%) in order to the space around the central vein (12%; Fig. 4).

Fibrosis

We observed the histological features of central veins with and without extension of collagen fibers into the perivenous parenchyma, perisinusoidal, and pericellular space, particularly at 10 and 20 days. The vein wall thickness ranged from 4 to 27 μm. Data of the vein wall thickness at 10 (14.9 ± 4.1μm) and 30 days (16.8 ± 4.38 μm) are higher than control value (12.6 ± 4.91 μm). At 20 day of bendiocarb treatment the central veins show thinner rim with decreased deposition of collagen fibers (9.9 ± 1.65 μm). As shown in Fig. 5, the vein wall thickness is increased at 10 and 30 days. Although Figure 6 shows that increase is without significance.

The control samples show a thin-walled central vein with fine fibrous extension by Sirius red staining of collagens. Fibrous extension was not detected in association with the wall thickness of the central vein. Portal tract shows increased collagen staining in the matrix. Further, at 10 and

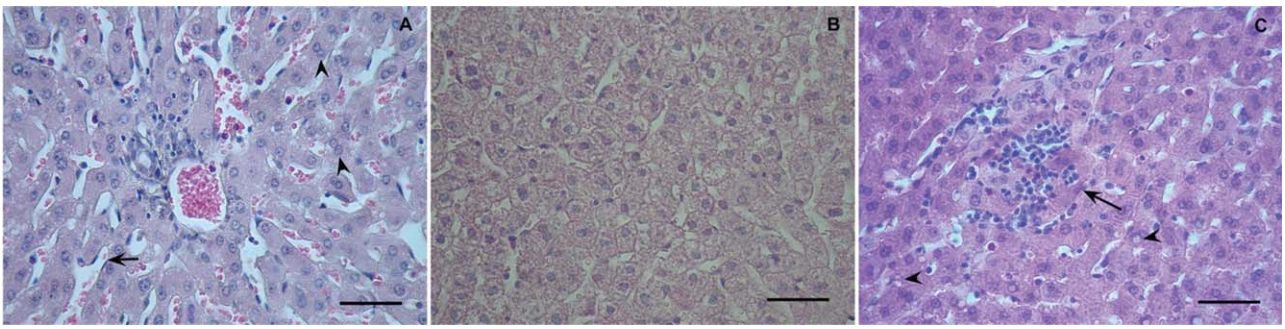

Fig. 2. The present of vacuoles in hepatocytes and liver parenchyma was observed at 10 and 30 days of bendiocarb treatment. A: Large, euchromatic nuclei (arrow) were located in the centre of the hepatocytes (headarrow), and few binucleated liver cells (bc) were observed as a physiological feature in the control. B: The number of vacuoles (headarrows) in hepatocytes and liver parenchyma was observed at 10 day of bendiocarb administration. C: The highest number of vacuoles was observed at 30 days. These vacuoles (headarrows) changed the shape of the nuclei to semilunar shape with pushing them away in the hepatocytes. Stained H&E, magnification 100×, Scale bars = 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

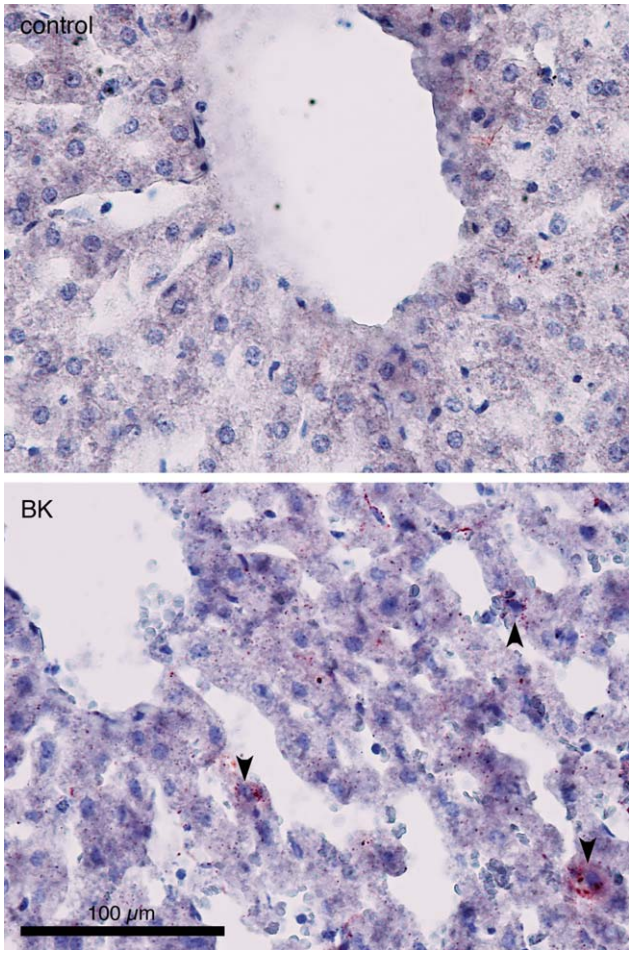

Fig. 3. Representative Oil Red staining of the rabbit liver tissue in control and experimental group after bendiocarb treatment at 30 days (BK; arrows). Oil Red staining, magnification 40×, Scale bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

30 days the fibrous septa of variable lengths are spreading from PT into the lobules and perisinusoidal space. The perisinusoidal fibrosis is focal, which is marked by increased collagen staining along the sinusoidal borders. Fine fibrous strands extend from PT for a short distance into the lobules at 20 day of bendiocarb treatment (Fig. 7).

DISCUSSION

It should be noted that many pesticides are transformed in the environment through physical, chemical, and biological processes that are intended to detoxify them, but often the transformation process forms products that are more toxic than the parent compound (Van Dyk and Pletschke, 2011). It is well known that there is comparatively a low risk of carbamates for mammals, which is based on their biodegradation (Sogorb and Vilanova, 2002). But on the contrary, our study shows affected lobules of the treated groups contained the focal infiltration of inflammatory cells and dilatation of sinusoids. The increased number of lipid vacuoles in hepatocytes after long-term bendiocarb treatment we observed with Oil Red O staining. Oil Red O staining is considered as a superior fat stain (therefore staining myelin), which has extremely good depth of color and yet leaves cellular structures intact (Cholewiak et al., 1968). In the cholesterol fatty rabbit liver, Nile red staining was comparable to that of Oil Red O, a commonly used dye for tissue cholesteryl esters and triacylglycerols. In contrast, Nile red staining of rabbit aortic atheroma revealed ubiquitous lipid deposits not observed with Oil Red O staining. These latter results suggest that Nile red can detect neutral lipid deposits, presumably unesterified cholesterol, not usually seen with Oil Red O or other traditional fat stains (Fowler and Greenspan, 1985). A causal link between chronic exposure to pesticides and their possible health effects is difficult to establish because consequences appear years after a generally intense exposure or after repeated low-intensity exposures over many years (Nougadere et al., 2011). Regeneration process was observed on 10 day after bendiocarb treatment. Histology is long known to be an important tool for determining the nature and extent of tissue damage (Kundu et al., 2011). Histological examination of the liver of pregnant rat females exposed to 400 ppm ditiocarbamate–propineb showed a dilatation of the wall of the central vein, as well as irregularity and degeneration in hepatocytes around the vein. Moreover, an increase in the number of vacuoles and hyalinization were observed in hepatocytes. Also the study showed a very clear dilatation of the sinusoids between the hepatocytes (Güven et al., 1999). Further, vacuolization of cell cytoplasm, infiltration of lymphocyte, and congestion in liver were evaluated by Cengiz et al. (2001) after Thiodan (33.7% endosulfan) treatment of mosquitofish, *Gambusia affinis*. Carbendazim caused changes in heterochromatin with pronounced sporadic cell death of individual cells around the central lobule area. Changes in heterochromatin caused by imazalil were similar as in the carbendazim treated group, and a sporadic cell death could be seen in areas close to central vein (Dikic et al., 2011). The rabbit liver appeared the most pronounced change on day 3 after bendiocarb administration (5 mg/kg of body weight per day) with massive accumulation of inflammatory cells within the portal spaces, and regenerative features, such as binucleated hepatocytes, were common (Holovska et al., 2011). We observed only weak septal fibrosis (septa formation without bridging fibrosis) around the portal tract, and perisinusoidal fibrosis close to the central vein of the rabbit liver with the Picro-Sirius Red staining. Previous studies of picro-dye reactions demonstrated wide variations in the binding of different dyes. Picro-Sirius Red was recommended because it colors all collagens intensely and is suitable for polarization microscopy (Puchtler et al., 1988). Sirius Red staining is presented as a

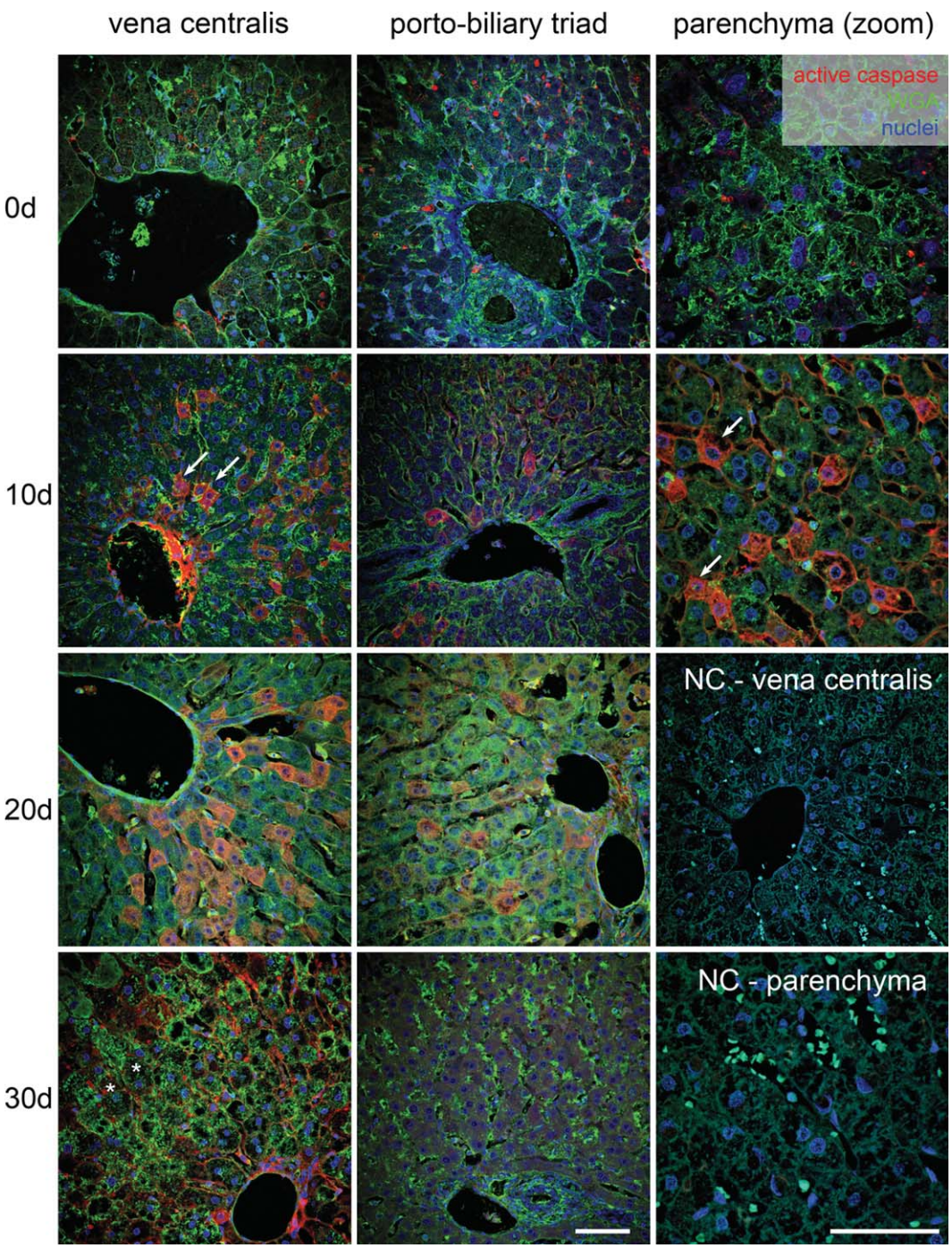

Fig. 4. Active caspase-3 is detected in bendiocarb treated rabbit liver. Almost no positive cells were detected in the untreated sample (0d). After 10 days of treatment, numerous immunoreactive cells (white arrows) were present throughout the organ, most commonly around the portal tract. Positive cells were still abundant at 20 days, with a decline at 30 days, at which point vacuoles were observed in some cells (asterisks) together with replacement fibrosis (wheat germ agglutinin staining; WGA, green). Negative control (NC) showed only dim background autofluorescence under identical exposure conditions. Stained immunohistochemically, magnification 40×, Scale bars = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

method for collagen determination, enabling quantitative morphometric measurements to be performed in locally defined tissue areas (Malkusch et al., 1995). By means of staining with Sirius Red F3BA in a saturated picric acid solution, the collagen contents of rat livers with varying degrees of fibrosis have been measured quantitatively in

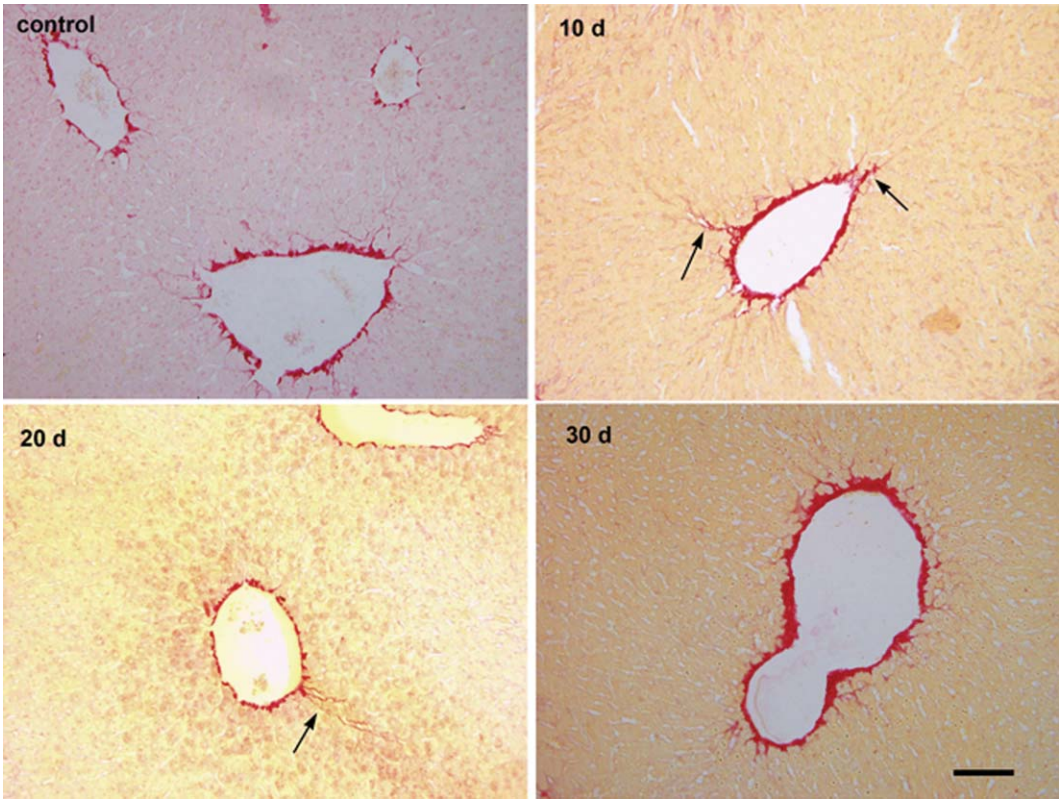

Fig. 5. Control samples show a thin-walled central vein with fine fibrous extension by Sirius red staining of collagens. At 10 and 30 days of bendiocarb treatment the wall of central vein increased in thickness. Furthermore, fibrous strands emerge from the central vein into the parenchyma and perivascular space, particularly at 10 and 20 days (arrows). Sirius Red staining, magnification 20×, Scale bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fixed and sectioned material. For analysis of collagen accumulation in rat liver, section-based methods seem to be useful and reliable, the extraction method giving the quickest results for large-scale screening, and the histophotometric method being more appropriate to take readings in selected areas (James et al., 1990). Miranda et al., (2008) studied the bioaccumulation of chlorinate pesticides and PCBs in the tropical freshwater fish *Hoplias malabaricus*, and they found the most important alterations in the liver were lesions such as fibrosis, large necrosis area, and leukocyte infiltration. The mammalian liver cells (WBF344) were the most sensitive to insecticide bendiocarb, with significant suppression of their proliferative activity (Pollakova et al., 2012). Atypical mitoses, cytologic alterations, cytomegaly, pigmentation, necrosis, and neoplastic nodules of the liver in rats were induced at 6 months after methyl carbamate treatment (0 or 400 mg/kg of body weight), 5 days per week (Chan et al., 1992). The study of the other rabbit organs (thymus, spleen, lymphoid tissue of the small intestine) detected changes in the structure, after an experimental long-term bendiocarb administration (Flesarova et al., 2007; Petrovova et al., 2010a, 2011). Bendiocarb addition caused imbalance in internal milieu of rabbits. Significant increase of creatinine content, increase of aspartate aminotrasferase (AST) and gamma glutamyl transferase (GGT), inform about possible failure of liver caused by bendiocarb after 25 days of administration (5 mg/kg b.w. per day; Capcarova et al., 2010). Animals have served as models of human response for decades

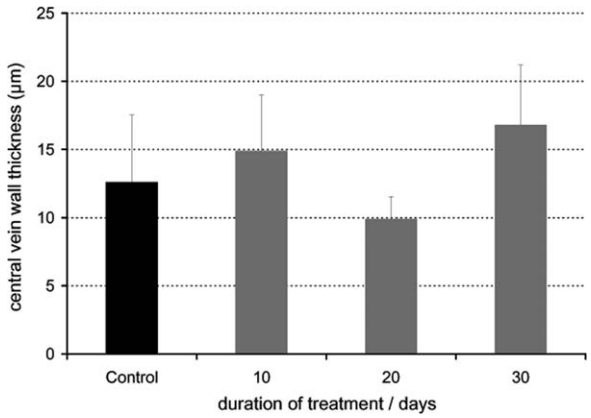

Fig. 6. The central vein wall thickness in bendiocarb-treated rabbit liver.

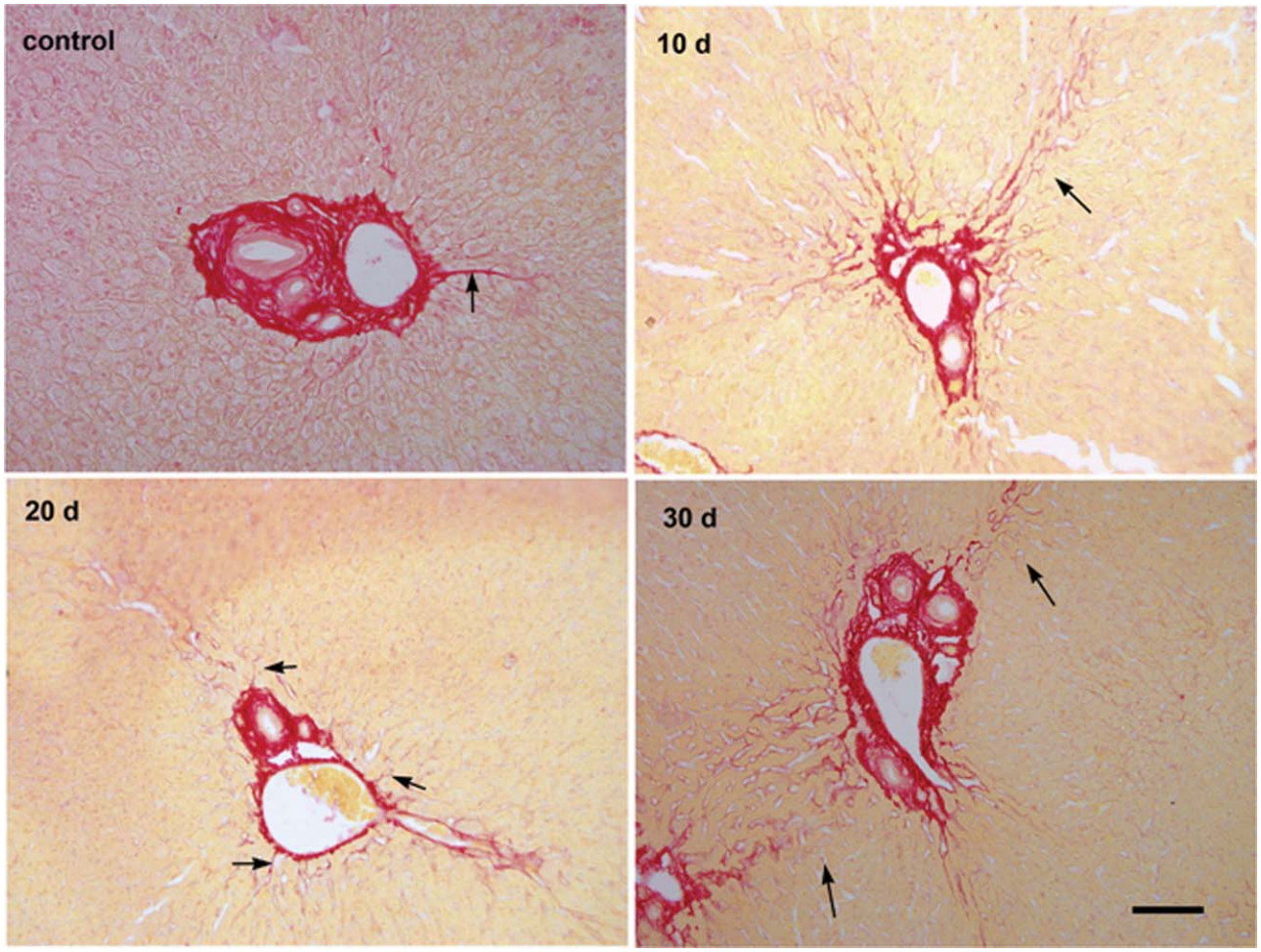

Fig. 7. Portal tract shows increased collagen staining in the matrix. Fibrous septa of variable lengths are spreading from portal tract into the lobules and perivascular space (arrow). Sirius Red staining, magnification 20×, Scale bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

because the biology of the test animals is, in general, similar to that of humans (Krewski et al., 2010). Better understanding of the patterns of exposure, the underlying variability within the human population, and the links between the animal toxicology data and human health effects will improve the evaluation of risks to human health posed by pesticides. Improving epidemiology studies and integrating this information with toxicology data will allow the human health risks of pesticide exposure to be more accurately judged by public health policy markers (Alavanja et al., 2004).

In view of the data of this study, it can be concluded that bendiocarb has moderately toxic effect on the rabbit liver tissue, regardless of gender. After the short-term bendiocarb treatment the regeneration behavior of the liver was observed in relation to binucleated hepatocytes, the higher number of liver cells and their decreased size. But long-term treatment causes vacuolization and dilatation of hepatocytes, dilatation of sinusoids between hepatocytes, and focal infiltration of inflammatory cells. And the presence of lipid vacuoles both in the liver parenchyma and inner of the hepatocytes could lead to the liver steatosis after prolonged exposure. Our results agree with other studies of bendiocarb both *in vitro* and *in vivo* toxicity (Capcarova et al., 2010; Petrovova et al., 2010b, 2011; Holovska et al., 2011; Almasiova et al., 2012; Pollakova et al., 2012). The case with poisoned animals in this study would not develop any systematic poisoning observable at the level of the whole human organism.

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