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JSCS–4423 *Original scientific paper*

Strain differences in the toxicity of the vitamin K antagonist warfarin in rats

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*Abstrac*t: Warfarin (3-(α-acetonylbenzyl)-4-hydroxy coumarin) is a vitamin K (VK) antagonist that inhibits vitamin K-dependent (VKD) processes, such as blood coagulation. It also exerts an influence on some non-VKD-related activities. In this study, the effect of sub-acute (30-day) oral warfarin (2 and 1 mg L^{-1}) intake on hematological parameters was examined in two rat strains, Albino Oxford (AO) and Dark Agouti (DA), that differ in their sensitivity to certain chemicals. Greater susceptibility to the anticoagulant effect of 2 mg L^{-1} of warfarin was observed in AO rats and was associated with an increase in the relevant hematological parameters in this strain. Although both strains responded to 2 mg L^{-1} of warfarin with quantitative changes in the peripheral blood leukocytes, differential bone marrow and lung responses were observed. Strain-related differences in the pro-inflammatory activity of peripheral blood granulocytes and in mononuclear cell IFN-γ production were observed. Recognition of differences in quantitative and qualitative effects of oral warfarin on processes other than hemostasis might be of relevance for those humans who are on warfarin therapy.

Keywords: warfarin; rats; anticoagulant effect; hematology; peripheral blood leukocytes.

INTRODUCTION

Warfarin (3-(α-acetonylbenzyl)-4-hydroxy coumarin) and other coumarin analogs are antagonists of vitamin K that inhibit the vitamin K-dependent (VKD) step in the synthesis of several factors required for normal blood coagulation.[1]

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Interference with the synthesis of biologically active coagulation factors results in increases in the clotting time up to the point where no clotting occurs. Anticoagulants of the 4-hydroxy-coumarin type are used in prophylactic medicine to prevent thromboembolic diseases and as pesticides for rodents (anticoagulant rodenticides).[2,3] Warfarin inhibits vitamin K epoxide reductase (VKOR), giving rise to depletion of hydroquinone, a co-factor for the γ-glutamyl carboxylase, an enzyme that mediates carboxylation of glutamyl (Gla) residues on precursors of several proteins involved in coagulation, including II (FII, prothrombin), VII (FVII), IX (FIX), and X (FX) factors.[2] Inhibition of VKOR by warfarin also affects the catalytic rate of other VKD proteins, such as proteins required for the regulation of bone growth and calcification (bone Gla protein, BGP/osteocalcin and matrix Gla protein, MGP) as well as those involved in the growth of vascular smooth muscle cells and mesangial cells.[4–7] Moreover, warfarin exerts influence on some non-VKD-related activities, including anti-tumor and immunomodulating activities.[8,9] As regards the immune system, both stimulatory and suppressive effects of warfarin (and other coumarin congeners) were observed.[10–15] Some clinical complications of warfarin therapy and adverse reactions associated with inflammatory cells attendance in affected tissues implies the pro-inflammatory potential of this chemical.[16–21]

It was previously demonstrated that acute (intraperitoneal or epicutaneous route) or sub-acute oral administration of warfarin at doses resulting in anticoagulation exert systemic pro-inflammatory effects in rats and suggested that peripheral blood neutrophils are the target of this agent.[22–25] The impact of sub-acute oral warfarin intake on other hematological parameters besides peripheral blood leukocyte numbers and mononuclear cell activity was explored in this study in order to see whether there were broader effects of warfarin at the systemic level. Bearing in mind the well-known differences in the response of humans to anticoagulation by warfarin,[26] these effects were examined comparatively in two rat strains known for their differences in immune responses to chemical insult, *i.e.*, Albino Oxford (AO) and Dark Agouti (DA) rats.[27,28]

EXPERIMENTAL

Chemicals

Warfarin sodium (Serva Feinbiochemica, Heidelberg, Germany), dextran 500,000, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phorbol-12-myristate-13-acetate (PMA), hexadecyltrimethylammonium bromide (HTAB), *o*-dianisidine dihydrochloride and myeloperoxidase (MPO) (all from Sigma Chemical Co., St. Louis, MO, USA), nitroblue tetrazolium (NBT) (ICN Pharmaceutical, Costa Mesa, CA, USA), concanavalin-A (ConA) (Pharmacia, Uppsala, Sweden) were used in experiments. The culture medium RPMI-1640 (PAA laboratories, Austria) supplemented with 2 mM glutamine, 20 μg mL^{-1} gentamycin (Galenika a.d., Serbia) and 5 % (v/v) heat-deactivated fetal calf serum (PAA Laboratories, Austria), *i.e.*, complete medium, was used in the cell culture experiments.

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Animals and warfarin treatment

Twelve-week old, female DA (weighing 200–232 g) and AO rats (weighing 295–325 g), conventionally housed at the Institute for Biological Research "Siniša Stanković" (Belgrade, Serbia) were used in the experiments. The treatments were performed in adherence with the Guidelines of the Ethical Committee of the Institute. In the pilot experiments, rats were given 4 mg L^{-1} of warfarin solution in drinking water, which (at consumption volume of 20–30 mL of solution daily) corresponded to 0.367 (±0.047) mg of warfarin per kg daily. However, massive hemorrhage was observed in AO rats during the last week of warfarin intake, with subsequent death of more than 80 % of individuals, while less than 15 % of the DA rats died. No fatal outcome was observed in either rat strain at two (2 mg L^{-1}) and four times (1 mg L^{-1}) lower warfarin doses. Thus, the consequences of the intake of these two lower non-lethal warfarin doses were analyzed further. At least two independent experiments were conducted with four to six animals assigned to each treatment group. Rats were given warfarin solution in drinking water *ad libitum* for 30 days. Control rats were given drinking water only.

Prothrombin time

The prothrombin time (*PT*) was determined in samples of blood withdrawn from the abdominal artery diluted in citrate buffer (blood to citrate ratio 5:1) by a one-stage method using citrate plasma and Thromborel S reagents (Behring Diagnostics GmbH, Marburg, Germany) with Siemens BCS-XP Dade Behring equipment (Marburg, Germany).

Hematology

Complete blood tests analyses were conducted automatically using a Siemens ADVIA 120 flow cytometer (Terytown, N.Y., USA). In this way, the white blood cell count (*WBC*) and percentage of leukocytes, red blood cell count (*RBC*), hemoglobin concentration (*Hb*), hematocrit (*HCT*), mean corpuscle volume (*MCV*), platelet number (*PLT*) and mean platelet volume (*MPV*) were measured.

Bone marrow leukocyte counts

Total bone marrow counts were determined using an improved Neubauer hemocytometer following cell staining with Türck solution. Differential cell counts were determined by differentiating at least 1000 cells of bone marrow smears stained according to the May–Grünwald–Giemsa protocol.

Lung histology

Lungs were excised, cut and immediately fixed in 4 % formaldehyde (pH 6.9). After processing, the tissue was embedded in paraffin wax for sectioning at 5 μm. Hematoxylin and eosin (H&E)-stained histology slides were subsequently analyzed in a blinded manner by experienced pathologist using a Coolscope digital light microscope (Nikon Co., Tokyo, Japan).

Peripheral blood granulocyte and mononuclear cell isolation

Peripheral blood leukocytes were isolated from the heparinized blood by dextran sedimentation and centrifugation (700 *g*, 20 min at room temperature) on an OptiPrep (Nycomed AS, Norway) density gradient. Polymorphonuclear cells (granulocytes) were obtained from the pellet fraction, following the lysis of erythrocytes with the isotonic NH_4Cl solution. The mononuclear cells were harvested from the band at the interface of plasma and OptiPrep density medium.

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MTT assay for leukocyte viability

Granulocyte and mononuclear viability was determined in freshly isolated cells by a quantitative colorimetric assay for metabolic viability described for humans,[29] which is based on the reduction of the tetrazolium salt MTT to a colored end-product, formazan, by several mitochondrial dehydrogenases in viable cells. The formazan produced was dissolved in an acidified sodium dodecyl sulfate solution (10 % SDS–0.01 M HCl) and the optical density (*OD*) was measured using a microplate spectrophotometer (GRD, Rome, Italy) at 540 nm.

Peripheral blood granulocyte and mononuclear cell activity

Granulocyte activation was evaluated by a cytochemical nitroblue tetrazolium (NBT) reduction assay for the respiratory burst based on the capacity of granulocytes to reduce NBT *via* respiratory burst oxidase.[30] Briefly, NBT (10 μL, 5 mg mL^{-1}) was added to a granulocyte suspension (5×10^5 cells $well^{-1}$ of a 96-well plate, in 100 μL) and incubated for 30 min with 100 ng mL^{-1} PMA (stimulated NBT reduction) or solely in medium (spontaneous NBT reduction). The absorbance of the produced formazan was measured as described above.

The granulocyte myeloperoxidase (MPO) activity was assessed based on the oxidation of *o*-dianisidine dihydrochloride by cells.[31] To 966 μL of substrate solution (0.167 mg mL^{-1} *o*-dianisidine dihydrochloride and 0.0005 % H_2O_2 in 50 mM potassium phosphate buffer, pH 6.0), 33 μL of granulocyte lysate, obtained by repeated freeze–thaw, was added. The absorbance was read at 450 nm (at three-minute intervals up to ten minutes) against an MPO standard. The values are expressed as MPO units per 10^6 cells.

To examine interferon-γ (IFN-γ) production, mononuclear cells were cultured at 5×10^5 cells $well^{-1}$ in a 96-well plate for 48 h in culture medium solely (spontaneous production) or in the presence of 1 μg mL^{-1} of ConA (ConA-stimulated production). The concentration of IFN-γ produced was measured by enzyme-linked immunosorbent assays (ELISA) for rat IFN-γ (R&D systems, Minneapolis, USA). The cytokine titer was calculated using a standard curve constructed with known amounts of recombinant IFN-γ.

Data display and statistical analysis

The results were obtained from two independent experiments and are expressed as means ± standard deviation (*SD*). Statistical analysis was performed using Statistica 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA). The statistical significance was defined by the Mann–Whitney U test. Values of p less than 0.05 were considered significant.

RESULTS

Anticoagulant effects of warfarin

Rats were given 1 and 2 mg L^{-1} of warfarin in their drink water. Every individual consumed 20–30 mL of warfarin solution daily, in this way consuming 0.090±0.009 (0.078–0.107) and 0.191±0.016 (0.164–0.215) mg kg^{-1} day^{-1} at 1 and 2 mg L^{-1}, respectively. Prolongation of the mean prothrombin time was observed in the AO rats following consumption of 2 mg L^{-1} of warfarin only (Fig. 1). No changes in *PT* were observed at the lower (1 mg L^{-1}) warfarin dose.

Hematology

Thirty-day consumption of 2 mg L^{-1} of warfarin resulted in significantly lower total leukocyte counts and in a tendency (p = 0.07) of decreasing in the AO and DA rats, respectively (Table I). A decrease in lymphocyte with unchanged

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neutrophil concentrations was observed in the individuals of both strains. Besides leukocytes, several other hematological parameters were affected in AO rats, including an increase in red blood cell counts, hemoglobin concentration, hematocrit, mean corpuscle volume and platelet counts. No changes in these parameters were observed in DA rats, although the highly variable platelet counts resulted in a numerical increase at this warfarin dose. Consumption of 1 mg L^{-1} was without effect, except for an increase in HCT in AO rats.

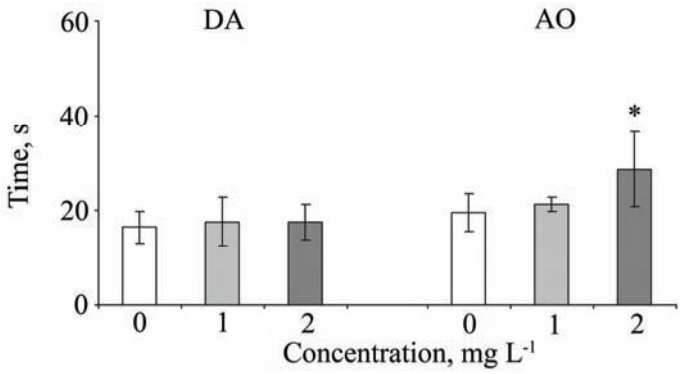

Fig. 1. Anticoagulant effect of warfarin intake in AO and DA rats expressed through the prothrombin time (*PT*). The results are expressed as mean values ± *SD* from two independent experiments with six animals per each animal group. Significance at * $p < 0.05$ *vs*. controls (0 mg L^{-1} of warfarin) of the respective strain.

Bone marrow leukocyte counts

Given the quantitative changes in the peripheral blood leukocytes at 2 mg L^{-1} of warfarin, an analysis of bone marrow leukocytes was conducted in rats that consumed this warfarin dose. Significant increase in number of immature neutrophils (metamyelocytes) and a tendency ($p = 0.060$) of an increase in the mature granulocyte pool were observed only in DA rats (Table II). No changes in the number of lymphocytes or monocytes were observed in either strain.

Lung histology

As lungs harbor an intravascular reservoir of leukocytes, predominantly neutrophils, called marginated pool, which constantly exchanges with the circulating cells,[31] histological analysis of lungs from rats that consumed 2 mg L^{-1} of warfarin was conducted next (Fig. 2). It revealed neutrophil attendance in AO rats (neutrophil lung infiltration noted in three out of four animals), as compared to DA rats, where neutrophils were observed in the lungs of one out of four individuals that had consumed warfarin.

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TABLE I. Hematological parameters following consumption of warfarin; abbreviations: *WBC*, white blood cell count; *NE* / %, percent of neutrophils; *LY* / %, percent of lymphocytes, *MO* / %, percent of monocytes; *EO* / %, percent of eosinophils; *BA* / %, percent of basophils; *RBC*, red blood cell count; *Hb*, hemoglobin concentration; *HCT*, hematocrit; *MCV*, mean corpuscular volume; *MHC*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin concentration; *PLT*, platelet count; *MPV*, mean platelet volume; data represent the mean values ±*SD* from two independent experiments with four to six animals per group

Parameter		DA warfarin dose, mg L^{-1}			AO warfarin dose, mg L^{-1}		
		0	1	2	0	1	2
WBC	/ 10^9 L^{-1}	9.91±1.70	7.88±2.02	7.32±1.07	5.88±2.06	4.13±1.88	3.22±0.68[a]
NE	/ 10^9 L^{-1}	4.61±0.56	3.07± 1.17	4.66±0.54	2.24±0.93	1.55±0.74	2.12±0.50
	/ %	45.05±5.15	38.20±4.76	64.03±3.61	37.13±5.85	37.54±4.58	65.61±7.05
LY	/ 10^9 L^{-1}	4.88±1.10	4.39±0.80	2.37±0.50[a]	3.35±0.76	2.36±1.14	0.91 ±0.25[b]
	/ %	48.82±4.67	56.33±4.04	32.17±3.19	57.83±4.37	56.80±4.76	28.57 ±7.46
MO	/ 10^9 L^{-1}	0.29±0.11	0.34±0.07	0.22±0.10	0.21±0.07	0.12±0.07	0.14±0.04
	/ %	2.84±0.79	4.33±0.58	2.83±1.17	3.75±1.50	3.20±2.17	4.23±0.95
EO	/10^9 L^{-1}	0.11±0.05	0.06±0.01	0.06±0.04	0.05±0.01	0.08±0.02	0.06±0.02
	/ %	1.16±0.44	0.77±0.21	0.73±0.51	0.93±0.19	2.08±0.92	1.73±0.72
BA	/ 10^9 L^{-1}	0.03±0.01	0.03±0.002	0.02±0.01[a]	0.02±0.01	0.02±0.01	0.01±0.01[a]
	/ %	0.36±0.15	0.37±0.06	0.23±0.15	0.38±0.10	0.36±0.17	0.20±0.12
RBC	/ 10^{12} L^{-1}	7.06±0.59	7.91±0.11	7.52±0.37	7.55±0.61	7.95±0.34	8.51±0.22[b]
Hb	/ g L^{-1}	124.60 ±10.43	127.00±1.73	136.50±8.53	139.50±11.45	138.80±4.09	160.71±7.02[a]
HCT	/ %	0.36±0.02	0.40±0.01	0.41±0.02	0.39±0.02	0.42±0.01[a]	0.46±0.02 [b]
MCV	/ fl	51.52±4.18	50.83±1.98	54.47±1.91	51.28±2.62	53.28±1.25	54.71±1.19[a]
MCH	/ pg	17.67±0.85	16.25±0.23	18.16±0.98	18.54±1.80	17.54±0.30	18.90±0.78
MCHC	/ g L^{-1}	343.58 ±38.77	320.26±9.10	334.12±10.00	361.25±38.54	327.27±4.11	347.21±8.99
PLT	/ 10^9 L^{-1}	87.60 ±145.40	694.33 ±19.01	759.67 ±112.61	620.50±61.07	712.80±70.96	724.71 ±55.50[a]
MPV		7.44±0.81	8.53±0.12	8.47±0.27	7.75±0.81	8.12±0.04	8.23±0.46

[a]Significantly different from control animals of the respective strain (0 mg L^{-1}) at $p < 0.05$; [b]significantly different from control animals of the respective strain (0 mg L^{-1}) at $p < 0.01$

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TABLE II. Total and differential bone marrow leukocyte counts; data represent the mean values ±*SD* from two independent experiments with four to six animals per group

Cell number × 10^{-6} per femur	DA Warfarin dose, mg L^{-1}		AO Warfarin dose, mg L^{-1}	
Parameter	0	2	0	2
Total	72.4±10.7	74.9±6.6	72.7±7.1	70.7±1.2
Metamyelocyte	8.70±2.1	11.77±2.3[a]	6.48±0.9	6.36±0.4
Granulocyte	31.02±4.9	34.16±3.2	24.92±3.5	24.21±0.8
Lymphocyte	31.73±5.7	29.82±9.0	40.42±4.1	39.19±1.5
Monocyte	0.94±0.3	1.13±0.5	0.83±0.2	0.88±0.1

[a]Significantly different from control animals of the respective strain (0 mg L^{-1}) at $p < 0.05$

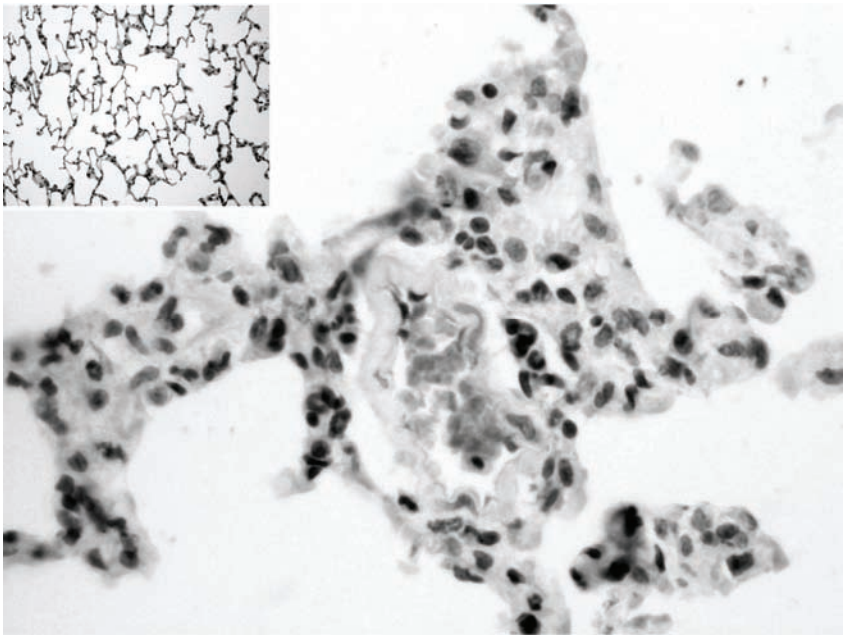

Fig. 2. Histology of lungs of an AO rat following oral intake of 2 mg L^{-1} of warfarin. Perivascular neutrophil attendance and interstitial edema. Inset: lung histology of a control animal (0 mg L^{-1} of warfarin).

Peripheral blood granulocyte viability and activity

To determine whether there were qualitative changes in the peripheral blood leukocytes, some of the basic aspects of activity (metabolically-based viability, activation/priming for respiratory burst and myeloperoxidase activity) were examined in neutrophils taken from animals that had consumed the higher warfarin dose (2 mg L^{-1}). As shown in Fig. 3, an increase in the MTT reduction by freshly isolated peripheral blood granulocytes of DA rats that had consumed warfarin was observed, while a similar capacity of reduction was observed in the control

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and warfarin-exposed AO rats (Fig. 3A). No difference in level of spontaneous NBT reduction could be observed between the control and rats that had consumed warfarin (Fig. 3B). Stimulation with PMA resulted in an increase in the NBT reduction by cells from the control and treated animals of both strains. Although PMA-stimulated NBT reduction capacity was more pronounced in AO compared to DA rats ($p < 0.05$), no difference was noted between control and the warfarin-treated AO rats, while the levels of reduction attained in DA rats that had consumed warfarin were higher than those from the controls.

Measurements of the intracellular MPO content in neutrophils revealed lower values in the control DA (*vs.* AO) rats, but it increased significantly after warfarin consumption in the DA rats (Fig. 3C), while it remained unchanged in the AO rats.

Peripheral blood mononuclear cell viability and activity

As a drop in the peripheral blood lymphocyte counts was observed in rats that had consumed 2 mg L^{-1} of warfarin, the viability of the blood mononuclear cells was next determined. No difference in MTT reduction was observed between the control and the warfarin-treated rats of either strain (Fig. 4A). To determine whether warfarin exerted an influence on the mononuclear cell activity, the production of IFN-γ was determined (Fig. 4B). While there was no effect on the spontaneous IFN-γ production by mononuclear cells from DA rats, consumption of warfarin resulted in an increase in the AO rats. Stimulation with ConA, however, resulted in significantly higher production of this cytokine in DA rats that had consumed warfarin, while it was similar in the control and warfarin-treated AO rats.

DISCUSSION

In this study, the effects of sub-acute (30-day) oral intake of warfarin on hematological parameters and on peripheral blood granulocyte and mononuclear cell activity were examined comparatively in two rat strains known to differ in their susceptibility to chemical insult. The lower susceptibility to the anticoagulant effect of warfarin, the increase in some of the hematological parameters (RBC and PLT counts, Hb concentration and HCT values) as well as the lack of qualitative effects (peripheral blood granulocyte) or different pattern of mononuclear cell activity in DA *vs.* AO rats demonstrated the differential responsiveness to warfarin treatment in the two rat strains. The differential susceptibility to hemorrhage might have accounted for differences in mortality at the highest warfarin dose, in line with data showing that impaired coagulation, along with the hemorrhage generally resulted in the death of rodents.[3] The increase in prothrombin time, which reflects the basic biological activity of warfarin, at a dose that resulted in no changes in this parameter in DA rats, also depicts the higher responsiveness of AO rats to warfarin anticoagulation. Strain-related differences in the metabolism of warfarin shown in laboratory rat strains[32,33] might be responsible for the differences observed in the prothrombin time in AO *vs.* DA rats.

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Differences in cytochrome P450 (CYP) enzymes as well as vitamin K epoxide reductase complex subunit 1 (VKORC1) might have accounted for these differences, as polymorphism in these enzymes affects the anticoagulant action of warfarin in humans.[34]

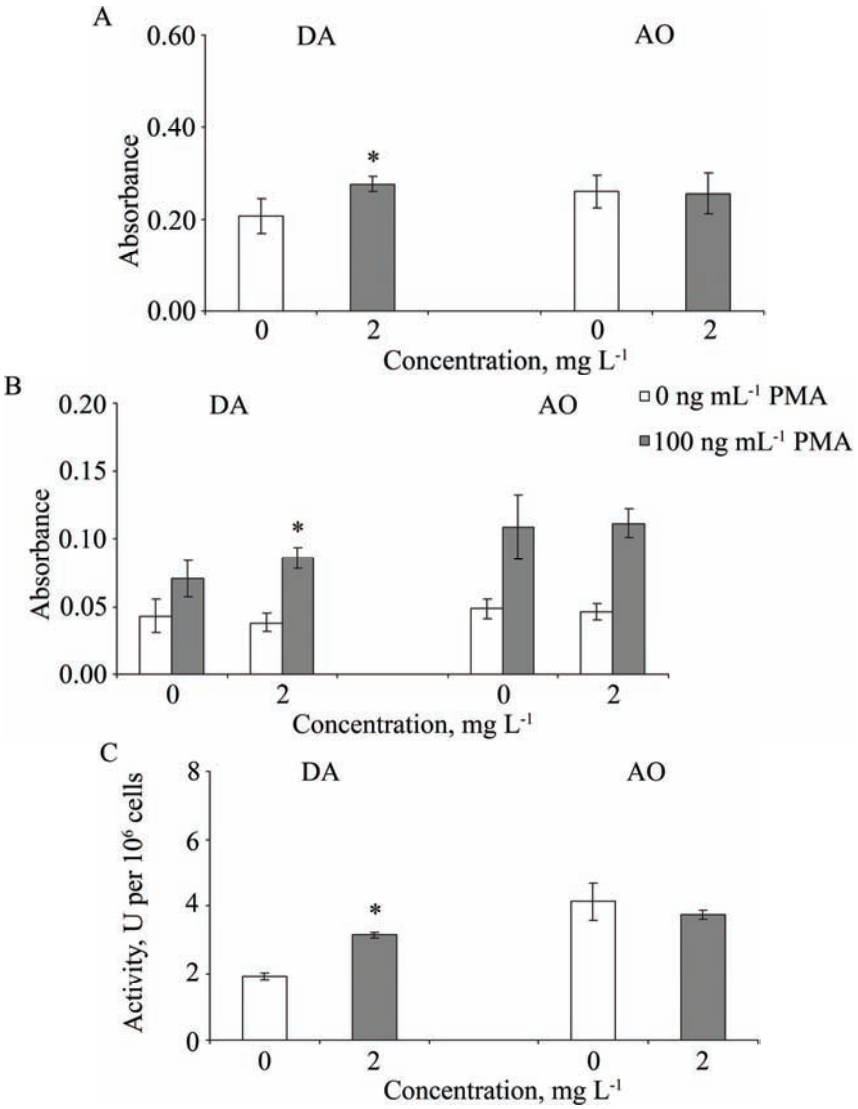

Fig. 3. The effect of warfarin intake on peripheral blood granulocyte viability and activity. A. Reduction of MTT. B. The spontaneous and PMA-stimulated reduction of NBT. C. Intracellular MPO activity. The results are expressed as mean values ± *SD* from two independent experiments with four to six animals per group. Significance at * $p < 0.05$ *vs.* controls (0 mg L^{-1} of warfarin) of the respective strain.

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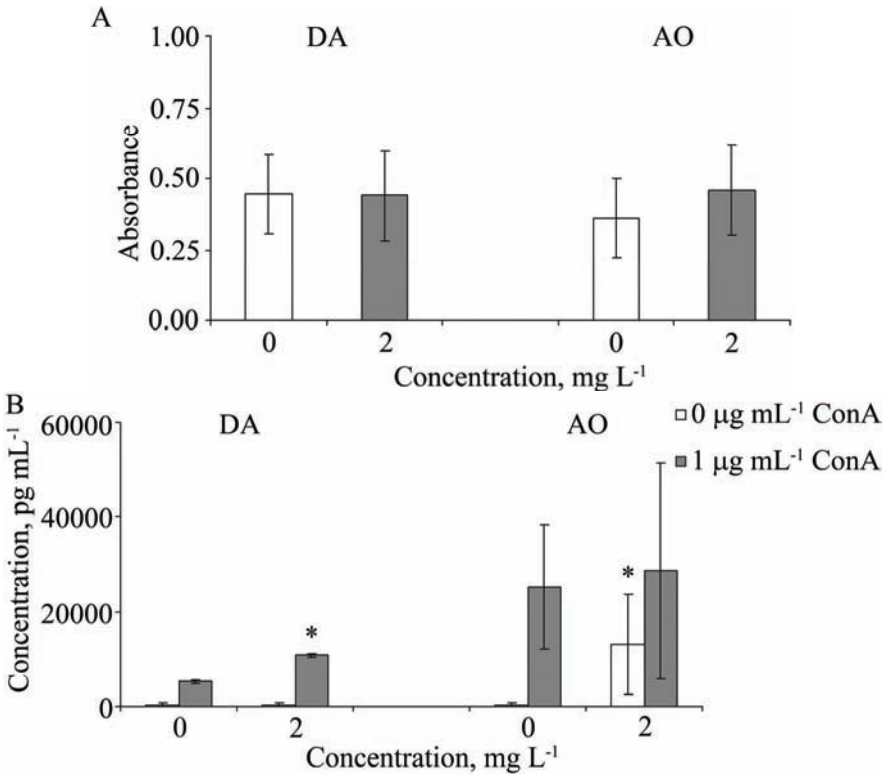

Fig. 4. The effect of warfarin intake on peripheral blood mononuclear cell viability and activity. A. Reduction of MTT. B. IFN-γ production. The results are expressed as mean values ± *SD* from two independent experiments with four to six animals per group. Significance at $*p < 0.05$ *vs.* controls (0 mg L^{-1} of warfarin) of the respective strain.

On the other hand, the differential effects of warfarin on some hematological parameters and peripheral blood leukocytes in the two strains imply that the effect of warfarin might depend on the parameter/activity examined. Indeed, the differential sensitivity of individuals of these two strains to warfarin was demonstrated by the hemoconcentration in AO rats (resulting from an increase in the number of red blood cells) following the consumption of the higher warfarin dose.

A decrease in the lymphocyte counts in the peripheral blood of rats that had consumed warfarin accounted for a drop in the leukocyte counts in both rat strains. This could not be ascribed to warfarin cytotoxicity, as there was a lack of changes in the viability of freshly isolated mononuclear cells. The underlying mechanisms of leuko/lymphopenia are presently unknown, but leukocyte migration to the focuses of hemorrhage in peripheral tissues might be responsible. Increases in immature bone marrow neutrophils imply that there is a need for these cells in the periphery and that this might be an underlying mechanism to uphold neutrophil

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numbers in DA rats. Neutrophil attendance in lungs (pronounced in AO rats) implies the exchange of neutrophils from circulation with the lung's intravascular reservoir of leukocytes (marginated pool).[31]

Differential effects of warfarin consumption on leukocyte functional activity were observed in these strains. While no effects on granulocyte viability were observed in AO rats, an increase in the MTT reducing capacity by cells from warfarin-treated DA rats was observed. As the MTT assay depends on mitochondrial activity in viable cells and is influenced by their metabolic activity, it actually reflects the overall functional state of granulocytes.[29,35] A cytochemical assay for the respiratory burst, which is a measure of cellular capacity to reduce NBT *via* tetrazolium reducing respiratory burst phagocyte oxidase, is often employed as an *in vitro* measure of peripheral blood granulocyte activation.[36,37] Similar levels of spontaneous NBT reduction by granulocytes between controls and rats that had consumed warfarin imply the lack of the capacity of this chemical to activate granulocytes. However, significantly higher NBT reduction provoked by PMA in the granulocytes from warfarin-treated DA rats compared to the controls indicated to a primed state of these cells, *i.e.*, a state of increased responsiveness to exogenous stimulation.[38] An increase in intracellular MPO content, which along with phagocyte oxidase, is a source of oxidant activity in phagocytes,[39] emphasises the effect of warfarin intake on the oxidative activities of granulocyte in peripheral blood. The priming of granulocytes of DA rats for the respiratory burst and the higher intracellular MPO content imply the higher responsiveness of these rats to warfarin, compared to AO rats.

The lack of an effect of warfarin intake on the peripheral blood mononuclear cell production of IFN-γ (the main mononuclear cell cytokine under conditions of systemic inflammation) in DA when compared to AO rats implies the differential susceptibility of these cells to warfarin. In addition, the lack of priming of mononuclears from warfarin-treated AO rats (similar levels of IFN-γ production in response to ConA stimulation) in contrast to the increased levels of cytokine produced in DA rats shows refractoriness of the mononuclear cells to exogenous stimulation. These data also suggest that not only peripheral blood neutrophils, but also mononuclear cells present targets for warfarin. The underlying mechanisms responsible for differential granulocyte and mononuclear cell responsiveness to oral warfarin might be ascribed to differences in signaling pathways and/or sensitivity to chemical signals, such as pro-inflammatory cytokines, known to modulate leukocyte activities. These assumptions warrant future attention.

CONCLUSIONS

The present study has demonstrated strain-related differential responsiveness to oral warfarin intake in rats not only in terms of anticoagulation, but in certain hematological parameters and in inflammation–relevant peripheral blood gra-

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nulocyte and mononuclear cell activity as well. Recognition of differences in quantitative and qualitative effects of oral warfarin on processes other than hemostasis might be of relevance for those humans who are on warfarin therapy.

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ИЗВОД

СОЈНЕ РАЗЛИКЕ У ТОКСИЧНОСТИ АНТАГОНИСТЕ ВИТАМИНА К ВАРФАРИНА КОД ПАЦОВА

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Варфарин (3-α-ацетонилбензил)-4–хидроксикумарин) је антагонист витамина К (ВК) који инхибира процесе зависне од овог витамина, укључујући коагулацију крви. Осим тога, он испољава и активности које не зависе од витамина К као што су антитуморска и имуномодулаторна активност. У овом раду је испитан ефекат субакутног (30 дана) оралног уноса варфарина на хематолошке параметре и активност леукоцита периферне крви код два соја пацова *Albino Oxford* (АО) и *Dark Agouti* (DA) који се разликују у осетљивости на исте хемијске агенсе. Код јединки АО соја запажена је већа смртност након конзумирања дозе од 4 mg L^{-1} као и већа осетљивост на антикоагулантно дејство варфарина при нижим дозама (2 mg L^{-1}) које је праћено повећањем неких хематолошких параметара. Иако код јединки оба соја долази до повећања броја неутрофилних леукоцита периферне крви при дози од 2 mg L^{-1}, промене у основним проинфламаторним активностима ових ћелија су запажене само код јединки DA соја. Промене у броју неутрофилних леукоцита у крви DA јединки су праћене повећањем броја гранулоцитних прекурсора у коштаној сржи, док присуство неутрофила у плућима АО јединки указује на размену ћелија између периферне крви и плућног интраваскуларног пула ћелија. Диференцијалне сојно–зависне промене у активности мононуклеарних ћелија периферне крви су такође запажене. Разлике у ефекту орално унетог варфарина могу да имају импликације за особе на оралној варфаринској терапији.

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