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INFLUENCE OF CARNOSINE ON THE CARDIOTOXICITY OF DOXORUBICIN IN RABBITS

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Influence of carnosine on the cardiotoxicity of doxorubicin in rabbits. R. ZIĘBA, M. WĄGROWSKA-DANILEWICZ. Pol. J. Pharmacol., 2003, 55, 1079–1087.

The aim of this study was to establish the effect of naturally occurring antioxidant carnosine (CAR) on the doxorubicin (DOX)-induced cardiotoxicity in a rabbit model. For this purpose, we evaluated the influence of DOX administration alone and in a combined therapy with CAR on the hemodynamic parameters and on the degree of cardiac muscle cell alterations in rabbits. Thirty one chinchilla rabbits were divided into four groups. One group of rabbits was injected *iv* with DOX at a dose of 2 mg kg^{-1} weekly for 7 weeks to induce congestive heart failure. Another group of rabbits received the same doses of DOX simultaneously with CAR at a dose of 100 mg kg^{-1} *po* daily for 9 weeks. Administration of CAR started 1 week prior to the first dose of DOX and ended one week after the administration of the last dose of DOX. The control groups of animals received 0.9% NaCl and CAR alone. The following hemodynamic parameters were estimated: heart rate (HR), mean arterial pressure (MAP), cardiac index (CI), stroke index (SI) and total peripheral resistance (TPR). Registration of the hemodynamic parameters in rabbits was performed by Doppler method (Hugo Sachs Elektronik Haemodyn). CAR normalized the values of MAP in rabbits receiving DOX and increased the values of CI and SI. The influence of CAR on TPR was not statistically significant, but there was a decreasing tendency. The degree of cardiac muscle cell alterations was examined by light microscopy using Mean Total Score (MTS) technique. The histopathological studies revealed smaller damage of cardiac muscle in rabbits which received DOX with CAR in comparison to animals receiving DOX alone. CAR seems to be cardioprotective during DOX administration.

Key words: *doxorubicin, cardiotoxicity, hemodynamic parameteres, histopathological changes, carnosine, cardioprotection*

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Abbreviations: CAR – *carnosine,* CI – *cardiac index,* DOX – *doxorubicin,* HR – *heart rate,* MAP – *mean arterial pressure,* MTS – *mean total score,* SI – *stroke index,* TPR – *total peripheral resistance*

INTRODUCTION

Doxorubicin (DOX) is an anthracycline antibiotic with a potent anticancer activity used widely in medical oncology. DOX is used against human neoplasms including a variety of solid tumors (e.g. head and neck tumors), breast cancer, bladder and testicular cancer, small cell lung cancer [39]. DOX is effective in acute lymphoblastic and non-lymphoblastic leukemias and malignant lymphomas [37].

The most dangerous, unique and often irreversible toxic effect of DOX that significantly limits its therapeutic use is dose-related cardiomyopathy, which occurs in ca. 20% of patients with a cumulative DOX dose of 450 to 500 mg m^{-2}. DOX may provoke a few types of cardiomyopathies. An acute form is due to rapid *iv* administration of DOX and is manifested by vasodilatation and hypotension. It is characterized by arrhythmias, disturbances in impulse conduction and congestive heart failure with pericardial effusion. Subacute cardiotoxicity develops during chemotherapy and is characterized by myocarditis and pericarditis. Chronic cardiotoxicity develops towards the end of therapy and after its termination and is manifested by dilated cardiomyopathy with loss of cardiac muscle cells, cytoplasmic vacuolization, subsequent replacement by fibrous tissue and congestive heart failure [12, 13].

The mechanism of DOX cardiotoxicity has not been precisely explained. The generation of reactive oxygen species through the interaction of the drug with iron and DOX reduction to C-13 alcohol metabolite *via* an intermediate semiquinone moiety play a very important role in the pathogenesis of cardiac toxicity, mainly of its chronic form [4, 16].

There have been attempts to reduce the cardiotoxicity of anthracycline antibiotics [42]. One of the applied methods to minimize the DOX cardiotoxicity is the use of cardioprotective substances which can selectively protect healthy cells against the cytotoxicity of DOX withought reducing the antitumor activity of the drug [35].

Carnosine (CAR), a dipeptide composed of L-histidine and β-alanine, is naturally occuring antioxidant found mainly in skeletal muscles (up to 20 mM), cardiac muscle (up to 10 mM), brain and other innervated animal and human tissues [21, 33, 40]. CAR plays several important functions. Under physiological conditions CAR inhibits lipid peroxidation and oxidative damage of protein in skeletal muscles (antioxidant and membrane-stabilizing activity) [36]. CAR can also inactivate highly reactive aldehydes from lipid peroxidation generated in muscle tissue during physical endurance and reducing sugars that cause non-enzymatic glycosylation of proteins (glycation) [1]. CAR acts as a mobile buffer in muscle tissue and a binder of divalent ions of transition metals. Besides, CAR can regulate calcium concentration in cardiac muscle cells and improve their contractility [40]. CAR can retard the senescence of cultured human diploid fibroblasts and restore a more juvenile phenotype [20].

The aim of this study was to investigate if CAR can reduce the cardiotoxic effect during the DOX administration in rabbits. For this purpose, we evaluated the influence of DOX administration alone and in a combined therapy with CAR on the hemodynamic parameters and on the degree of cardiac muscle cell alterations in rabbits.

MATERIALS and METHODS

Materials

Doxorubicin (Ebewe, Austria), carnosine (Cambridge Major Laboratories INC), urethane (ethyl carbamate, Sigma, USA), α-chloralose (Roth, Germany).

Animal experiments

The study was performed on thirty one outbred chinchilla rabbits of both sexes with body weight ranging between 2.5–3.5 kg, aged 15–20 weeks. The animals were housed in standard cages, one animal per cage, under a 12 h light/12 h dark cycle. The rabbits were fed on granulated mix "LSK" with free access to water. The weight of animals was checked weekly. All the experiments were carried out at room temperature between 9 and 12 a.m.

Experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiment. All the procedures in this experiment were approved by The Ethics Committee of the Medical University of Łódź (Poland).

The animals were divided into four groups of rabbits randomly allocated to every group, receiving the following doses of drugs:

Group 1: 0.9% NaCl, 1 ml kg^{-1} *iv* weekly for 7 weeks (control group, n = 6);

Group 2: DOX, 2 mg kg^{-1} weekly *iv* for 7 weeks (n = 10);

Group 3: DOX, 2 mg kg^{-1} weekly *iv* for 7 weeks and simultaneously CAR, 100 mg kg^{-1} *po* daily for 9 weeks (n = 9);

Group 4: CAR, 100 mg kg^{-1} *po* daily for 9 weeks (control group, n = 6).

DOX and 0.9% NaCl were administered into the marginal ear vein by bolus injection. CAR was administered by oral gavage in aqeous solution. Administration of CAR started 1 week prior to the first dose of DOX and ended one week after the administration of the last dose of DOX.

Surgery was performed two weeks after the administration of the last dose of DOX or after the same period of saline injections in the control group. The rabbits were placed on the operation table in a dorsal position. The animals were anesthetized with α-chloralose (40 mg kg^{-1}) and urethane (400 mg kg^{-1}) administered into the marginal ear vein. Anesthesia was maintained by additional bolus doses of urethane as needed during surgery. The level of anesthesia was demonstrated by lack of muscle movement and hemodynamic responses. Tracheostomy was needed because the thoracic cavity was opened. Ventilation was controlled using C.F. Palmer London LTD pump. Ventilation freqeuency was set at 30 bpm and tidal volume at 50–60 ml. ECG was recorded using Multicard E300. Extremity leads were registered to estimate ECG during surgery.

Hemodynamic studies

The registration of hemodynamic parameters in rabbits was performed by using Hugo Sachs Elektronik Haemodyn (Harvard Apparatus GmbH, March, Germany). A heparinized polyethylene catheter was placed into the dissected carotid artery and connected to an Isotec pressure transducer (HSE Harvard Apparatus) for arterial blood pressure measurement (SYS, DIA, MAP). Median sternotomy and pericardiotomy were performed to place the flow probe around the ascending aorta for aortic blood flow (AF_{min}, AF_{mean}, AF_{max}) measurement taken as an index of cardiac output. It was connected to an ultrasonic flow meter Transit Time Flowmeter TTFM Type 700 (HSE Harvard Apparatus and Transonic System Inc. USA). Heart rate (HR) was registered from the catheter placed in the carotid artery. All analog signals were amplified and recorded on a computer *via* an A/D converter (HSE-Haemodyn software for Microsoft Windows 95/98/NT) and they were evaluated according to the algorithms. The rabbits received a continuous infusion of normal saline into the marginal ear vein to compensate for the fluid loss during surgery.

The derivative hemodynamic parameters were calculated: cardiac index (CI), stroke index (SI), total peripheral resistance (TPR).

— **CI = CO/BW** (ml/min/kg), CO – cardiac output (ml/min), BW – body weight of rabbit (kg);

— **SI = SV/BW** (ml/beat/kg), SV – stroke volume (ml/beat), SV = CO/HR, HR – number of beats/min;

— **TPR = (MAP × 8)/CO** (kPa × s/l), MAP – mean arterial pressure (mmHg), 8 – coefficient for calculation of values in mm Hg and flow in l/min into kPa × s/l.

After surgery the animals were killed by overdosage of pentobarbital sodium (160 mg kg^{-1}) administered into the marginal ear vein.

Histopathological studies

Hearts were taken immediately postmortem, weighed and fixed in a buffered 10% solution of formalin for histopathological examination. Two segments from the right and left ventricle, interventricular septum and left papillary muscle were taken after macroscopic evaluation. Then, they were embedded in paraffin blocks. Four micron thick sections were obtained with microtome. After deparaffinating, carrying through a number of alcohols and washing in xylene, the sections were stained with hematoxylin and eosin with according to Masson's three-color method for connective tissue evaluation. Microscopic sections were examined under 400x magnification paying attention to the presence of vacuoles in cardiomyocytes cytoplasm, cardiomyocytes necrosis and cardiomyocytes and myocardium stroma edema. The intensity and extent of histological changes in sections from rabbits' hearts were evaluated semi-quantitatively using the modified mean total score (MTS) scale [11, 18, 38]. MTS scale is used to evaluate the intensity of changes in cardiomyocytes (N) on a scale from 1 to 2 and extent of changes (R) on a scale from 0 to 5 (Tab. 1).

MTS = Σ (N × R) / number of sections evaluated in each rabbit.

Statistics

Statistical analysis of hemodynamic parameters was performed using the Statistica version 5 StatSoft program. The normality of distribution was checked by means of Kolmogorov-Smirnov test with Lillieforce correction. If the data were normally distributed, homogenity of variance was tested by Bartlett's test. The statistical evaluation was performed using analysis of variance (ANOVA) and *post-hoc* comparisons were performed by means of NIR test. If the data were not normally distributed, statistical evaluation was performed by using ANOVA test (Kruskal-Wallis) and U Mann-Whitney's test.

The histopathological MTS technique results were compared using Student's *t*-test between groups of rabbits, preceded by analysis of distribution and analysis of variance.

All parameters were considered statistically significantly different if $p < 0.05$.

Table 1. The modified mean total score (MTS) scale for semi-quantitative microscopic evaluation of cardiac muscle lesion

Intensity of changes (N)	1	microvacuolic degeneration of cardiomyocytes and/or edema of cardiomyocytes and interstitial edema
	2	macrovacuolic degeneration of cardiomyocytes or atrophy, necrosis, proliferation of interstitial fibrous connective tissue, endocardium lesion and thrombi
Extent of changes (R)	0	without changes
	0.5	changes involve not more than 10 cardiomyocytes
	1	single damaged cardiomyocytes
	2	changes involve single scattered groups of cardiomyocytes
	3	changes involve several groups of cardiomyocytes
	4	changes involve large groups of cardiomyocytes
	5	most cardiomyocytes are damaged

RESULTS

Results of hemodynamic studies

The results of hemodynamic studies are shown as the values of five parameters: HR, MAP, CI, SI and TPR and have been presented in Table 2.

Heart rate

HR did not differ in a statistically significant way between the examined groups of rabbits, however, there was a trend towards a decrease in HR in rabbits receiving DOX.

Mean arterial pressure

The value of MAP in the control group of rabbits did not differ statistically significantly from the value of MAP registered in the group of rabbits receiving CAR. DOX administration to rabbits resulted in a statistically significant decrease in MAP. CAR administration to the group of rabbits receiving DOX caused a statistically significant increase in MAP. This value of MAP did not differ signifi-

Table 2. The values of heart rate (HR), mean arterial pressure (MAP), cardiac index (CI), stroke index (SI) and total peripheral vascular resistance (TPR) in rabbits

Group	Drugs	HR (beats/min)	MAP (mmHg)	CI (ml/min/kg)	SI (ml/beat/kg)	TPR (kPa × s/l)
1	0.9%NaCl	285 ± 30	97.8 ± 12.0	79.0 ± 4.1	0.29 ± 0.03	3.17 ± 0.13
2	DOX	256 ± 68	61.7 ± 27.7[a.b]	37.5 ± 3.9[a.b]	0.15 ± 0.02[a.b]	4.16 ± 0.31
3	DOX + CAR	273 ± 48	97.0 ± 23.0[c]	59.4 ± 5.4[c]	0.22 ± 0.03[d]	3.92 ± 0.29
4	CAR	294 ± 31	96.5 ± 8.2	73.4 ± 4.7	0.26 ± 0.03	3.08 ± 0.20

Each value represents mean ± SD, DOX – doxorubicin, CAR – carnosine; [a] statistically significant difference in comparison with group 1 ($p < 0.05$), [b] statistically significant difference in comparison with group 4 ($p < 0.05$), [c] statistically significant difference in comparison with group 2 ($p < 0.05$), [d] statistically significant difference in comparison with group 1 ($p < 0.05$)

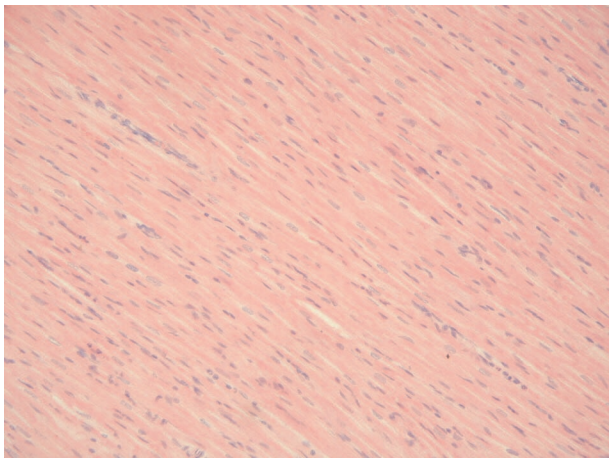

Fig. 1. Left ventricle without microscopic changes in cardiomyocytes in rabbit from control group. H&E staining, magnification 200x

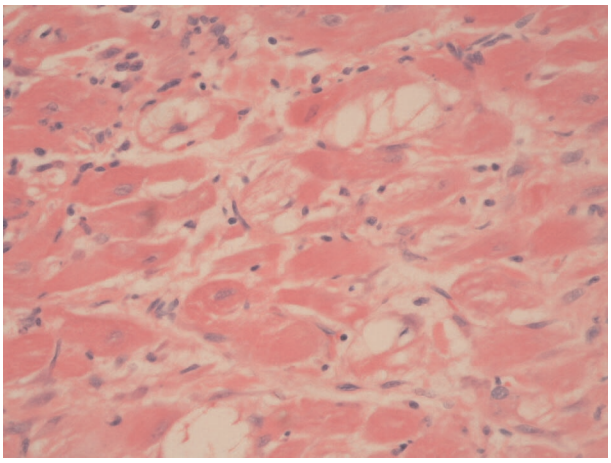

Fig. 2. Left ventricle from rabbit treated with doxorubicin. Vacuolar degeneration of myocardial muscle fibers and focal necrosis of cardiomyocytes. H&E staining, magnification 400x

cantly from the value of MAP registered in the control group and in the group receiving CAR.

Cardiac index

The value of CI in the control group of rabbits did not differ statistically significantly from the value of CI calculated for the group of rabbits receiving CAR. DOX administration to rabbits caused a statistically significant decrease in CI. CAR administration to the group of rabbits receiving DOX resulted in a statistically significant increase in CI. This value of CI did not differ significantly from the value of CI calculated for the control group and for the group receiving CAR.

Stroke index

The value of SI in the control group of rabbits did not differ statistically significantly from the value of SI calculated for the group of rabbits receiving CAR. DOX administration to rabbits caused a statistically significant decrease in SI. CAR administration to the group of rabbits receiving DOX caused a statistically significant increase in SI. This value of SI differed significantly from the value of SI calculated for the control group and for the group receiving DOX, but it was not significantly different from the value of SI calculated for the group receiving CAR.

Total peripheral resistance

TPR did not differ statistically significantly in the examined groups of rabbits, although there was a trend towards an increase in TPR in the group of rabbits receiving DOX. The calculated values of TPR were not significantly lower after simultaneous administration of DOX and CAR.

Results of histopathological studies

In rabbits from the control group (n = 6) and from the group receiving CAR (n = 6) histopathological evaluation of sections from the left and right ventricle, interventricular septum and papillary muscle of the left ventricle did not reveal any pathological changes (Fig. 1).

In all rabbits receiving DOX (n = 10) microscopic examination of sections from hearts revealed macrovacuolic and microvacuolic changes in the cytoplasm of cardiomyocytes and interstitial edema. In 7 animals, additionally necrosis of cardiomyocytes was found (Fig. 2). The extent of changes varied from small lesions, of not more than 10 cardiomyocytes in a preparation, to large lesions forming foci of damaged heart muscle cells (Fig. 3). In no case, there was proliferation of interstitial fibrous connective tissue.

In rabbits receiving simultaneously DOX and CAR (n = 9), macrovacuolic and microvacuolic changes in cardiomyocytes and interstitial edema were also observed (Fig. 4). However, in comparison with rabbits receiving DOX alone, the extent of changes was small and concerned single cells (Fig. 5). In heart sections of rabbits from this group, there was no necrosis in cardiomyocytes,

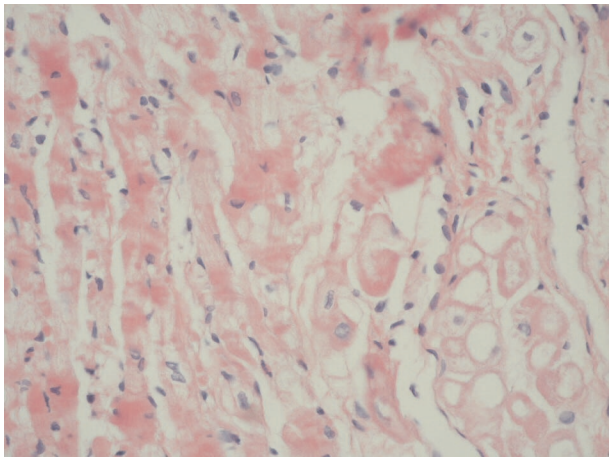

Fig. 3. Confluent groups of affected myocardial fibers in rabbit treated with doxorubicin. Severe vacuolar degeneration and myocardial atrophy. H&E staining, magnification 400x

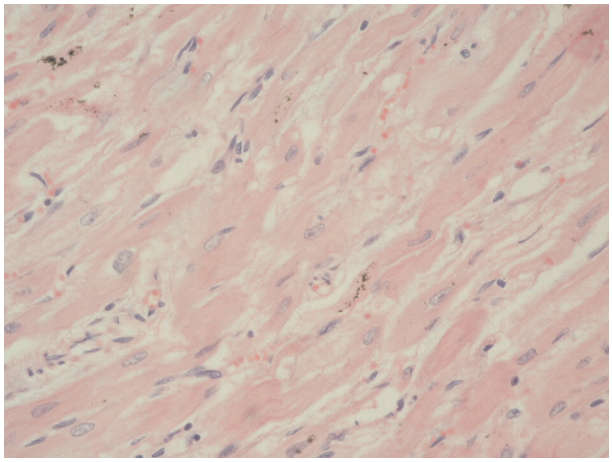

Fig. 4. Left ventricle from rabbit receiving doxorubicin and carnosine. Slight degree of myocardial muscle fibers vacuolization and focal interstitial edema in myocardium. H&E staining, magnification 200x

either. In 2 animals, there were extensive devascularizations under the epicardium and endocardium of both right and left ventricles. The abovementioned changes were not typical of DOX-induced lesions and, thus, they were not included in microscopic studies and in statistical analysis of results. In no case, there was proliferation of interstitial fibrous connective tissue.

Table 3 presents mean values of MTS for rabbits receiving DOX and DOX with CAR.

The intensity and extent of histological changes in sections from rabbit hearts receiving DOX were statistically significantly larger than in rabbits receiving DOX with CAR.

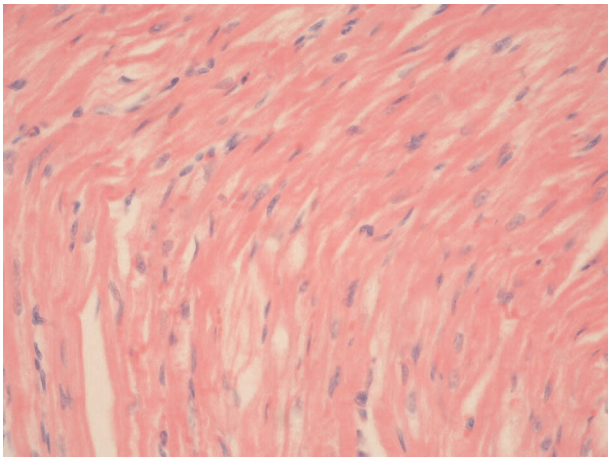

Fig. 5. Scattered single affected myocardial fibers in left ventricle in rabbit treated with doxorubicin and carnosine. Vacuolar degeneration and interstitial edema. H&E staining, magnification 200x

Table 3. The intensity and extent of microscopic changes in rabbits' heart muscle evaluated using the modified Mean Total Score (MTS) scale

Group	Drugs	MTS
2	DOX	5.41 ± 1.37
3	DOX + CAR	3.54 ± 1.36[a]

Each value represents mean ± SD; DOX – doxorubicin, CAR – carnosine, [a] statistically significant difference in comparison with group 2 ($p = 0.014$)

DISCUSSION

Numerous mechanisms of cardiotoxic activity of anthracycline antibiotics have been proposed. The most important and probable seems to be the mechanism based on the generation by DOX of oxidative stress in the heart muscle and stimulation of apoptosis in cardiomyocytes [9]. Because of poor antioxidant protection in cardiac myocytes (low concentration of superoxide dismutase, catalase and glutathione peroxidase) reactive oxygen species cause lipid peroxidation and mitochondrial destruction [41].

There are some ways of reducing the cardiotoxicity of DOX: monitoring chemotherapy, modifying the schedule of administration, patients selection considering risk factors, liposomal encapsulation of DOX, using less toxic DOX analogues [7, 30]. An important method of decreasing the cardiotoxicity of DOX is administration of this drug with

cytoprotective agents, which selectively protect normal cells against toxic effect of the drug. Dexrazoxane (Cardioxane) was synthesized as a specific water-soluble cardioprotective agent which protects against iron-dependent oxygen free radical-mediated anthracycline-induced cardiotoxicity [22]. Unfortunately, dexrazoxane does not reduce gastrointestinal toxicity and myelotoxicity of DOX [10]. Besides, the influence of dexrazoxane on antitumor activity of DOX is not quite clear [32, 45, 46]. New cytoprotective compounds are being investigated. Researchers concentrate on numerous antioxidative compounds, such as melatonin, coenzyme Q, amifostin, flavonoid compounds, essential fatty acids, L-carnitine, selenium [3, 7, 31].

In our research, we concentrated on CAR, a dipeptide commonly occurring in human body, which also shows antioxidative properties. CAR is a water-soluble free radical scavenger and prevents lipid peroxidation of the cell membranes [29, 36, 48]. CAR may act not only as a scavenger of reactive oxygen species, but also may prevent their formation. Under *in vitro* conditions, CAR prevents the generation of strongly oxidating hydroxyl radical by chelating of transitional metals ions (Cu, Zn, Fe) [23]. It also prevents the generation of peroxynitril radical, and, thus, it may exert a protective effect on the endothelium [14]. Moreover, CAR, an antioxidant well soluble in water, prevents modification and loss of activity of proteins responsible for sequestration of metals in blood plasma (for example ceruloplasmin) due to effect of free radicals or toxic products of lipids peroxidation [28].

CAR inactivates *in vitro* dangerous highly reactive lipid peroxidation products (malondialdehyde, 4-hydroxynonenal, acrolein) [1]. Reactive aldehydes and reducing sugars that cause glycation attack biomolecules and cause the accumulation of carbonyl groups on proteins and formation of advanced glycosylation end products (AGEs). CAR, an effective antiglycation factor, prevents carbonylation of biomolecules by entering a reaction with reactive aldehydes and eliminates AGEs in the process of carnosylation of modified proteins [6]. CAR protects against cross-linking of proteins [20]. Accumulation of AGEs is a biochemical manifestation of the ageing process of cells. Antioxidant and antiglycating properties of CAR may be responsible for its antiaging properties [19, 34]. CAR may also inhibit mitochondrial apoptosis pathways [24].

Besides, *in vitro* studies revealed that CAR can increase contractility of isolated cardiac muscle of rat by directly acting on the ryanodine receptor and releasing calcium. CAR can play a role of modulator of calcium-regulated proteins in cardiac muscle cells [40].

CAR was administered to animals and humans in several experiments. The efficacy of 1% N-acetylcarnosine in the form of eye drops in the treatment of cataracts in people was demonstrated in a randomized placebo-controlled study. N-acetylcarnosine improved the visual acuity and glare sensitivity and reduced lens opacity [2].

Zinc L-CAR (polaprezinc) acts as a gastric mucosal protector against various irritant factors. Orally administered polaprezinc at a dose of 100 mg kg^{-1} attenuates *Helicobacter pylori*-induced gastric mucosal inflammation in Mongolian gerbils [44]. Polaprezinc (150 mg kg^{-1} for 7 days) in combination with lansoprazole, amoxycillin and clarithromycin increased the effectiveness of *Helicobacter pylori* eradication during 7-day therapy in patients [25]. Polaprezinc (3–10 mg kg^{-1} *po* twice daily for 14 days) improved the impaired healing of chronic gastric ulcers in arthritic rats [27]. The beneficial effects of polaprezinc on gastric mucosal injury may be due to its antioxidative properties. Polaprezinc was effective in acetic acid-induced stomatitis in hamsters. This suggests that polaprezinc might be applicated in severe stomatitis induced by anticancer drugs [26].

CAR was investigated for antiischemic activity. CAR reduced mortality of animals during experimental cerebral ischemia and decreased the consequences of cardiac ischemia. It protected cardiomyocytes and enhanced the contractility of the heart muscle [43].

CAR can be a neuropotective agent. The effect of CAR supplementation was investigated in children with autistic spectrum disorders in a double-blind, placebo-controlled study. CAR at a dose of 800 mg administered for 8 weeks enhanced neurologic function [8].

We have tested possible cardioprotection by CAR during DOX administration in rabbits. Rabbits received 2 mg kg^{-1} of DOX weekly *iv* for seven weeks to induce congestive heart failure [5] and CAR *po* at a dose of 100 mg kg^{-1}. CAR administered *po* is absorbed mainly in the small intestine. During absorption, partial hydrolysis of dipeptide to β-alanine and L-histidine takes place [15, 17,

47]. This fact was taken into consideration, CAR was administered at the dose of 100 mg kg^{-1}, i.e. 5-times higher than the dose administered parenterally to rats and rabbits.

CAR reduced the disadvantageous hemodynamic changes in rabbits during DOX administration. CAR normalized MAP in rabbits receiving DOX, and increased the values of CI and SI. The effect of CAR on the value of TPR was not statistically significant, but resistance tended to decrease. The results of hemodynamic studies may indicate a positive interaction between CAR and DOX in decreasing the cardiotoxicity of the antitumor drug.

The conducted histopathological studies revealed smaller damage to the heart muscle in rabbits which received DOX in combination with CAR, as compared to those receiving DOX alone. It may indicate that CAR decreases the cardiotoxicity of DOX in rabbits.

In conclusion, CAR seems to show cardioprotective effect during DOX administration.

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REFERENCES

1. Aldini G., Granata P., Carini M.: Detoxification of cytotoxic alpha,beta-unsaturated aldehydes by carnosine: characterization of conjugated adducts by electrospray ionization tandem mass spectrometry and detection by liquid chromatography/mass spectrometry in rat skeletal muscle. J. Mass Spectrom., 2002, 37, 1219–1228.
2. Babizhayev M.A., Deyev A.I., Yermakova V.N., Semiletov Y.A., Davydova N.G., Doroshenko V.S., Zhukotskii A.V., Goldman I.M.: Efficacy of N-acetylcarnosine in the treatment of cataracts. Drugs RD., 2002, 3, 87–103.
3. Bagchi D., Ray S.D., Bagchi M., Preuss H.G., Stohs S.J.: Mechanistic pathways of antioxidant cytoprotection by a novel IH636 grape seed proanthocyanidin extract. Indian J. Exp. Biol., 2002, 40, 717–726.
4. Barnabe N., Zastre J.A., Venkataram S., Hasinoff B.B.: Deferiprone protects against doxorubicin-induced myocyte cytotoxicity. Free Radical Biol. Med., 2002, 33, 266–275.
5. Blake D., Way D., Trigg L., McGrath B.: Regional blood flow effects of dopexamine versus enalaprilat during propofol anaesthesia in rabbits with experimental chronic heart failure. Cardiovasc. Res., 1994, 28, 710–714.
6. Burcham P.C., Kaminskas L.M., Fontaine F.R., Petersen D.R., Pyke S.M.: Aldehyde-sequestering drugs: tools for studying protein damage by lipid peroxidation products. Toxicology, 2002, 181–182, 229–236.
7. Catino A., Cruciia E., Latorre A., Sambiasi D., Calabrese P., Lorusso V.: Amifostine as chemoprotectant in metastatic breast cancer patients treated with doxorubicin. Oncol. Rep., 2003, 10, 163–167.
8. Chez M.G., Buchanan C.P., Aimonovitch M.C., Becker M., Schaefer K., Black C., Komen J.: Double-blind, placebo-controlled study of L-carnosine supplementation in children with autistic spectrum disorders. J. Child. Neurol., 2002, 17, 833–837.
9. Childs A.C., Phaneuf S.L., Dirks A.J., Phillips T., Leeuweburgh C.: Doxorubicin treatment in vivo causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity and Bcl2:Bax ratio. Cancer Res., 2002, 62, 4592–4598.
10. Dorr R.T.: Cytoprotective agents for anthracyclines. Semin. Oncol., 1996, 23, 23–34.
11. Dziegiel P., Jethon Z., Suder E., Sopel M., Rabczynski J., Surowiak P., Zabel M.: Role of exogenous melatonin in reducing the cardiotoxic effect of daunorubicin and doxorubicin in the rat. Exp. Toxicol. Pathol., 2002, 53, 433–439.
12. Ferrans V.J.: Overview of cardiac pathology in relation to anthracycline cardiotoxicity. Cancer Treat. Rep., 1978, 62, 955–961.
13. Ferrans V.J., Clark J.R., Zhang J., Yu Z.X., Herman E.H.: Pathogenesis and prevention of doxorubicin cardiomyopathy. Tsitologiia, 1997, 39, 928–937.
14. Fontana M., Pinnen F., Lucente G., Pecci L.: Prevention of peroxynitrite-dependent damage by carnosine and related sulphonamido pseudodipeptides. Cell. Mol. Life Sci., 2002, 59, 546–551.
15. Gardner M.L., Illingworth K.M., Kelleher J., Wood D.: Intestinal absorption of the intact peptide carnosine in man and comparison with intestinal permeability to lactulose. J. Physiol., 1991, 439, 411–422.
16. Gille L., Kleiter M., Willmann M., Nohl H.: Paramagnetic species in the plasma of dogs with lymphoma prior to and after treatment with doxorubicin. An ESR study. Biochem. Pharmacol., 2002, 64, 1737–1744.
17. Hama T., Tamaki N., Miyamoto F.: Intestinal absorption of beta-alanine, anserine and carnosine in rats. J. Nutr. Sci. Vitaminol., 1976, 22, 147–157.
18. Herman E.H., Ferrans V.J.: Timing of treatment with ICR-187 and its effect on chronic doxorubicin cardiotoxicity. Cancer Chemother. Pharmacol., 1993, 32, 445–449.
19. Hipkiss A.R., Brownson C.: Carnosine reacts with protein carbonyl groups: another possible role for the anti-ageing peptide? Biogerontology, 2000, 1, 217–223.

20. Hipkiss A.R., Brownson C., Bertani M.F., Ruiz E., Ferro A.: Reaction of carnosine with aged proteins: another protective process? Ann. NY Acad. Sci., 2002, 959, 285–294.
21. Horinishi H., Grillo M., Margolis F.L.: Purification and characterization of carnosine synthetase from mouse olfactory bulbs. J. Neurochem., 1978, 31, 909–919.
22. Imondi A.R., Della-Torre P., Mazue G., Sullivan T.M., Robbins T.L., Hagerman L.M., Podesta A., Pinciroli G.: Dose-response relationship of dexrazoxane for prevention of doxorubicin-induced cardiotoxicity in mice, rats, and dogs. Cancer Res., 1996, 56, 4200–4204.
23. Kang J.H., Kim K.S., Choi S.Y., Kwon H.Y., Won M.H., Kang T.C.: Protective effects of carnosine, homocarnosine and anserine against peroxyl radical-mediated Cu,Zn-superoxide dismutase modification. Biochim. Biophys. Acta, 2002, 1570, 89–96.
24. Kang K.S., Yun J.W., Lee Y.S.: Protective effect of L-carnosine against 12-O-tetradecanoylphorbol-13-acetate- or hydrogen peroxide-induced apoptosis on v-myc transformed rat liver epithelial cells. Cancer Lett., 2002, 178, 53–62.
25. Kashimura H., Suzuki K., Hassan M., Ikezawa K., Sawahata T., Watanabe T., Nakahara A., Mutoh H., Tanaka N.: Polaprezinc, a mucosal protective agent, in combination with lansoprazole, amoxycillin and clarithromycin increases the cure rate of Helicobacter pylori infection. Aliment. Pharmacol. Therapeut., 1999, 13, 483–487.
26. Katayama S., Nishizawa K., Hirano M., Yamamura S., Momose Y.: Effect of polaprezinc on healing of acetic acid-induced stomatitis in hamsters. J. Pharm. Pharm. Sci., 2000, 3, 114–117.
27. Kato S., Tanaka A., Ogawa Y., Kanatsu K., Seto K., Yoneda T., Takeuchi K.: Effect of polaprezinc on impaired healing of chronic gastric ulcers in adjuvant-induced arthritis rats – role of insulin-like growth factors (IGF)-1. Med. Sci. Monit., 2001, 7, 20–25.
28. Kim K.S., Choi S.Y., Kwon H.Y., Won M.H., Kang T.C., Kang J.H.: The ceruloplasmin and hydrogen peroxide system induces alpha-synuclein aggregation in vitro. Biochimie, 2002, 84, 625–631.
29. Klebanov G.I., Teselkin Yu.O., Babenkova I.V., Lyubitsky O.B., Rebrova O.Yu., Boldyrev A.A.: Effect of carnosine and its components on free-radical reactions. Membr. Cell. Biol., 1998, 12, 89–99.
30. Lacko A., Włodarska I., Zymliński R., Mazur G., Wróbel T., Gisterek I.: Cardiac toxicity in cancer therapy (Polish). Pol. Merkuriusz Lek., 2002, 13, 79–85.
31. Liu X., Chen Z., Chua C.C., Ma Y.S., Youngberg G.A., Hamdy R., Chua B.H.: Melatonin as an effective protector against doxorubicin-induced cardiotoxicity. Amer. J. Physiol.-Heart Circ. Phy., 2002, 283, H254–H263.
32. Lopez M., Vici P.: European trials with dexrazoxane in amelioration of doxorubicin and epirubicin-induced cardiotoxicity. Semin. Oncol., 1998, 25, 55–60.
33. Margolis F.L., Grillo M., Hempstead J., Morgan J.I.: Monoclonal antibodies to mammalian carnosine synthetase. J. Neurochem., 1987, 48, 593–600.
34. McFarland G.A., Holliday R.: Retardation of the senescence of cultured human diploid fibroblasts by carnosine. Exp. Cell. Res., 1994, 212, 167–175.
35. Muggia F.M.: Cytoprotection: concepts and challenges. Support. Care Cancer, 1994, 2, 377–379.
36. Nagasawa T., Yonekura T., Nishizawa N., Kitts D.D.: In vitro and in vivo inhibition of muscle lipid and protein oxidation by carnosine. Mol. Cell. Biochem., 2001, 225, 29–34.
37. Ogura M.: Adriamycin (doxorubicin) (Japanese). Gan To Kagaku Ryoho, 2001, 28, 1331–1338.
38. Podesta A., Della-Torre P., Pinciroli G., Iatropoulos M.J., Brughera M., Mazue G.: Evaluation of 4'-iodo-4'-deoxydoxorubicin-induced cardiotoxicity in two experimental rat models. Toxicol. Pathol., 1994, 22, 68–71.
39. Ragab A.H., Sutow W.W., Komp D.M., Starling K.A., Lyon G.M., George S.: Adriamycin in the treatment of childhood solid tumors. A Southwest Oncology Group Study. Cancer, 1975, 36, 1567–1576.
40. Roberts P.R., Zaloga G.P.: Cardiovascular effects of carnosine. Biochemistry, 2000, 65, 856–861.
41. Seifert C.F., Nesser M.E., Thompson D.F.: Dexrazoxane in the prevention of doxorubicin-induced cardiotoxicity. Ann. Pharmacother., 1994, 28, 1063–1072.
42. Speyer J., Wasserheit C.: Strategies for reduction of anthracycline cardiac toxicity. Semin. Oncol., 1998, 25, 525–537.
43. Stvolinsky S.L., Dobrota D.: Antiischemic activity of carnosine. Biochemistry, 2000, 65, 849–855.
44. Suzuki H., Mori M., Seto K., Miyazawa M., Kai A., Suematsu M., Yoneta T., Miura S., Ishii H.: Polaprezinc attenuates the Helicobacter pylori-induced gastric mucosal leucocyte activation in Mongolian gerbils – a study using intravital videomicroscopy. Aliment. Pharmacol. Therapeut., 2001, 15, 715–725.
45. Swain S.M.: Adult multicenter trials using dexrazoxane to protect against cardiac toxicity. Semin. Oncol., 1998, 25, 43–47.
46. Swain S.M., Whalez F.S., Gerber M.C., Weisberg S., York M., Spicer D., Jones S.E., Wadler S. et al.: Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. J. Clin. Oncol., 1997, 15, 1318–1332.
47. Tamaki N., Ikeda T., Fujimoto S., Mizutani N.: Carnosine as a histidine source: transport and hydrolysis of exogenous carnosine by rat intestine. J. Nutr. Sci. Vitaminol., 1985, 31, 607–618.
48. Tamba M., Torreggiani A.: Hydroxyl radical scavenging by carnosine and Cu(II)-carnosine complexes: a pulse-radiolysis and spectroscopic study. Int. J. Radiat. Biol., 1999, 75, 1177–1188.

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