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Short communication

Acute doxorubicin nephrotoxicity in rats with malignant neoplasm can be successfully treated with fullerenol $C_{60}(OH)_{24}$ *via* suppression of oxidative stress

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Abstract:
Oxidative stress has an important role in the pathogenesis of doxorubicin (DOX)-induced nephrotoxicity. The aim of this study was to investigate the nephroprotective effects of fullerenol (FLR), an antioxidant agent, on DOX-induced nephrotoxicity.
The investigation was carried out on adult female Sprague Dawley outbred rats with chemically induced breast cancer (1-methyl-1-nitrosourea; 50 mg/kg; *ip*). Rats were divided into the following groups: control healthy, control cancer, DOX alone (8 mg/kg, *ip*, cancer), DOX plus FLR as a pre-treatment (8 mg/kg and 100 mg/kg, respectively, *ip*, cancer), and FLR alone (100 mg/kg, *ip*, cancer). At the end of the 2nd day after drug administration, blood and kidney tissues were taken for analysis. The activity of lactate dehydrogenase and α-hydroxybutyrate dehydrogenase as serum enzymes, as well as level of malondialdehyde, glutathione, glutathione peroxidase, glutathione reductases, catalase and superoxide dismutase, were determined.
DOX caused nephrotoxicity, but FLR pre-treatment prevented oxidative stress, lipid peroxidation and the disbalance of GSH/GSSG levels in kidney tissue caused by DOX. Our results confirm satisfactory nephroprotective efficacy of FLR in the acute phase of toxicity and encourage further studies regarding its use as a potential nephroprotector.

Key words:
doxorubicin, nephrotoxicity, fullerenol, oxidative stress, kidney, rats, mammary carcinomas

Abbreviations: α-HBDH – alpha-hydroxybutyrate dehydrogenase, BUN – blood urea nitrogen, CAT – catalase, DOX – doxorubicin, FLR – fullerenol, GR – glutathione reductase, GSH – glutathione, GSH-Px – glutathione peroxidase, GSSG – oxidized glutathione, GST – glutathione-S-transferase, LDH – lactate dehydrogenase, MDA – malondialdehyde, MNU – 1-methyl-1-nitrosourea, NO – nitric oxide, NOS – nitric oxide synthase, ROS – reactive oxygen species, SCr – serum creatinine, SOD – superoxide dismutase, TP – total protein concentration

Introduction

Since the introduction of doxorubicin (DOX) for the treatment of cancer in 1969, this compound has demonstrated high antitumor efficacy. DOX's cytotoxic effect on malignant cells, as well as its toxic effects on various organs is thought to be related to its DNA intercalation and cell membrane lipid binding activities. It has been suggested, that DOX-induced apoptosis may be an integral component of the cellular mechanism of action responsible for its therapeutic effects, toxicities, or both [18, 21].

DOX's use in chemotherapy has been limited largely due to its diverse toxicities, including cardiac, renal, pulmonary, hematological and testicular toxicity. DOX-induced changes in the kidneys of rats include increased glomerular capillary permeability and glomerular atrophy. Although the exact mechanism of DOX-induced nephrotoxicity remains unknown, it is believed that the toxicity is mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules, and membrane lipid peroxidation [14].

In animal trials, DOX demonstrated nephrotoxic activity and produces chronic progressive glomerular disease. In rats with DOX-induced nephropathy, heavy proteinuria associated with swelling and vacuolation of epithelial cells were reported in short-term experiments. DOX-induced nephrosis provides a well-characterized model of progressive renal damage, induced by a uniform challenge at a single point in time. This results in proteinuria and subsequent structural renal damage with a relatively large variability among individual animals. Severe renal damage, extensive glomerular lesions, tubular dilatation, vacuolization of renal glomeruli, protein deposits in tubular lumen, and stromal fibrosis have been observed in long-term studies. These experiments indicated that DOX-induced nephropathy has chronic and self-perpetuating continual effects leading to terminal renal failure. The dose and the duration of DOX required to induce renal diseases vary among investigations. It was demonstrated that a 3 mg/kg dosage of DOX induced renal damage after 6 weeks. On the other hand, nephrotoxicity can be induced by 25 mg/kg of DOX after only 2 days [8, 9, 14, 15, 17, 20, 25, 26].

The protective effect of different agents such as dihydropyridine calcium antagonists (nifedipine, nitrendipine, amlodipine), melatonin, lycopene, caffeic acid phenethyl ester, low molecular weight heparin on DOX-induced toxicity has been described [4, 8, 14, 25, 26].

Additionally, polyhydroxylated fullerenes, like their parent compounds, have demonstrated high antioxidant activity *in vitro* that is comparable to or even higher than that of natural biological antioxidants like ascorbic acid and vitamin E. The high antioxidative potential of these compounds is proposed to be a consequence of their ability to react with highly reactive oxygen radical species, such as superoxide ($O_2^{\bullet -}$) and hydroxyl ($OH^{\bullet}$) radicals, which may damage many biologically important macromolecules [5, 24].

In vitro and *in vivo* studies have shown that the water soluble fullerenol (FLR) $C_{60}(OH)_{24}$ has strong antioxidative potential. It may function as a free radical scavenger and it strongly suppresses the cytotoxicity of DOX in animal models. Both *in vitro* and *in vivo* studies have shown that polyhydroxylated C_{60} fullerene derivatives, fullerenols, can be potential antioxidative agents and free radical scavengers in biological systems. This has been shown in cases of oxidative stress induced by xenobiotic factors as well as radioactive exposure [2, 5, 7, 10–12, 16, 23, 24].

The scope of this experiment was to investigate the potential nephro-protective effects of FLR, $C_{60}(OH)_{24}$, on the kidneys of rats with mammary carcinomas by assessing oxidative status after a single dose of DOX [10, 11].

Materials and Methods

Chemical and preparation

FLR ($C_{60}(OH)_{24}$) was synthesized and characterized from the polybromine derivative $C_{60}Br_{24}$, which was synthesized by the reaction of C_{60} and Br_2 with $FeBr_3$ as a catalyst [6]. FLR $C_{60}(OH)_{24}$ (Novi Sad, Serbia) was dissolved (20 mg/ml) in a sterilized and apyrogenic mix-solution (0.9% NaCl in water : Tween 80 (80:20; w/w)). DOX (Adriablastina®, Pharmacia & Upjohn, Milan, Italy), was dissolved in a sterilized and apyrogenic 0.9% NaCl solution (3 mg/ml). MNU (1-methyl-1-nitrosourea) was obtained from Sigma (Deisenhofen, Germany). It was dissolved in a sterilized and apyrogenic 0.9% NaCl solution (14 mg/ml).

All chemicals were dissolved just before administration in a laminar flow cabine.

Animals

The experiments were carried out on female Sprague-Dawley outbred rats (Harlan, Italy). The animals had free access to food (Altromin, Germany) and water and were kept at a constant room temperature (22 ± 1°C) and relative humidity (70 ± 10%), under a 12 h light/dark cycle (3–4 rats per cage). Experimental protocols were approved by the local Ethic Committee (National Animal Ethical Committee of Republic of Slovenia – license number 3440-138/2006) and met the guidelines of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123).

Drug administration

All 32 rats (II–V groups) received *ip* applications of MNU (50 mg/kg – carcinogenicity was induced chemically) on the 50th and 113th day of age [13]. When rats reached 160 days of age, they were treated with DOX and/or FLR. The animals of both control groups received sterilized and apyrogenic 0.9% NaCl solution. Two days after saline, DOX and/or FLR application rats were sacrificed using CO_2.

Animals were randomly divided into five groups (eight per group) as follows:

I) Untreated control group – rats received saline only (*ip*);
II) Cancer control group – rats received MNU (*ip*) and saline (*ip*);
III) DOX group – rats received MNU (*ip*) and DOX 8 mg/kg (*ip*);
IV) DOX/FLR group – rats received MNU (*ip*) and FLR 100 mg/kg (*ip*) 30 min before DOX 8 mg/kg (*ip*);
V) FLR group – rats received MNU (*ip*) and FLR 100 mg/kg (*ip*) [7, 10, 11, 24].

All rats (n = 40) survived before sacrificing.

Coefficient of kidney weight

The coefficient of kidney to body weight was calculated as the ratio of kidney tissues (wet weight, mg) to body weight (g).

Light microscopy of tissue

The kidneys were fixed into 10% neutral buffered formaldehyde for 10 days, embedded in paraffin wax and automatically processed. Sections (3 μm in thickness) of the embedded tissue were stained with hematoxylin-eosin and Periodic Acid Schiff (PAS) for light microscopy observations.

Biochemical analysis

Blood for analysis was taken via heart puncture after opening the thoracic region. Serum was used for analysis of enzymatic activity. The assay for lactate dehydrogenase (LDH) was carried out according to the methods described in the commercial kits Chema Diagnostica (Jesi, Italy). α-Hydroxybutyrate dehydrogenase (α-HBDH) activity was determined with a commercial kit obtained from Dialab (Vienna, Austria). Samples were stored at –80°C before use.

Each kidney was quickly removed from the sacrificed rat, placed in an ice-cold solution and the adipose tissues were trimmed off. Finally, each organ was minced and homogenized in a Tris-buffer solution (pH 7.4; organ : buffer 1:10; w/w) and divided into two portions. One was used for malondialdehyde (MDA; Chromsystems Diagnostic, Munchen, Germany) determination, and the second was centrifuged at 13,000 × g for 20 min at 4°C (Beckman refrigerated, Ultracentrifuge). The supernatant was used for the assays of total protein concentration (TP; Sentinel Diagnostics, Milan, Italy), glutathione (GSH; Chromsystems Diagnostic, Munchen, Germany), glutathione peroxidase (GSH-Px; Ransel, Crumlin, UK), glutathione reductase (GR; Crumlin, UK), catalase (CAT; [1]), LDH, and superoxide dismutase (SOD; Ransod, Crumlin, UK).

Statistics

Data were analyzed by one-way ANOVA followed by LSD *post-hoc* test (SPSS 15.0 for Windows). The effects were considered significant when $p < 0.05$.

Results

The results are summarized in Figures 1–3, as well as in Table 1. The differences in the weight of the rats

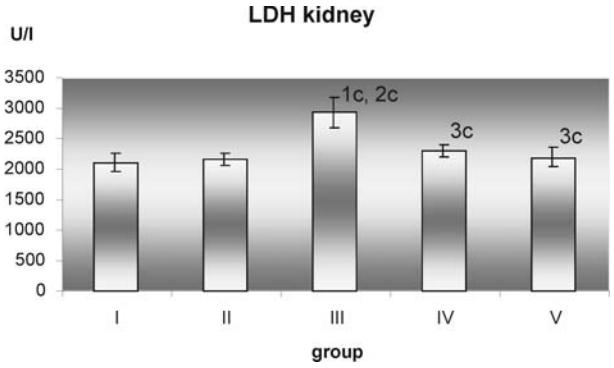

Fig. 1. Biochemical evaluation of LDH levels in kidney tissue samples (n = 8 per group). Significant difference from the corresponding group 1, 2, 3 – groups (^{c} $p < 0.001$)

and coefficient of kidney weight after sacrificing were not significant with exception of body weight between FLR and untreated groups ($p < 0.05$). In the acute phase of nephrotoxicity no definitive histopathological alteration were found in the kidney tissues of rats treated with DOX and/or FLR when examined by hematoxylin and eosin staining at the light microscopic level (data not shown).

The activity of serum (Tab. 1) and tissue (Fig. 1) enzyme LDH was higher in the DOX group than the other groups ($p < 0.05$). Serum α-HBDH levels did not show any significant differences among all of the investigated groups.

Tissue levels of GSH, GSSG, MDA and the activities of the antioxidant enzymes SOD, GSH-Px, GR and CAT were measured. Administration of DOX by *ip* route produced a marked increase in the kidney MDA level ($p < 0.001$; $p < 0.01$) as well as GR ($p < 0.05$; $p < 0.05$) and SOD ($p < 0.01$; $p < 0.001$) activity compared to both control groups (untreated and cancer group, respectively). This was accompanied by an increase in the GSSG level over the cancer control group ($p < 0.001$) and significant decrease of GSH ($p < 0.001$; $p < 0.01$) and GSH-Px ($p < 0.05$; $p < 0.01$) levels over both control groups. A significant increase ($p < 0.05$) in the activity of CAT was observed in the DOX intoxicated rats, while those mitochondrial enzymes were maintained to almost normal levels with FLR pre-treatment. Both the FLR and the DOX/FLR groups were significantly lower compared to the DOX group ($p < 0.01$). Pre-treatment with FLR at a dose of 100 mg/kg produced a marginal change in

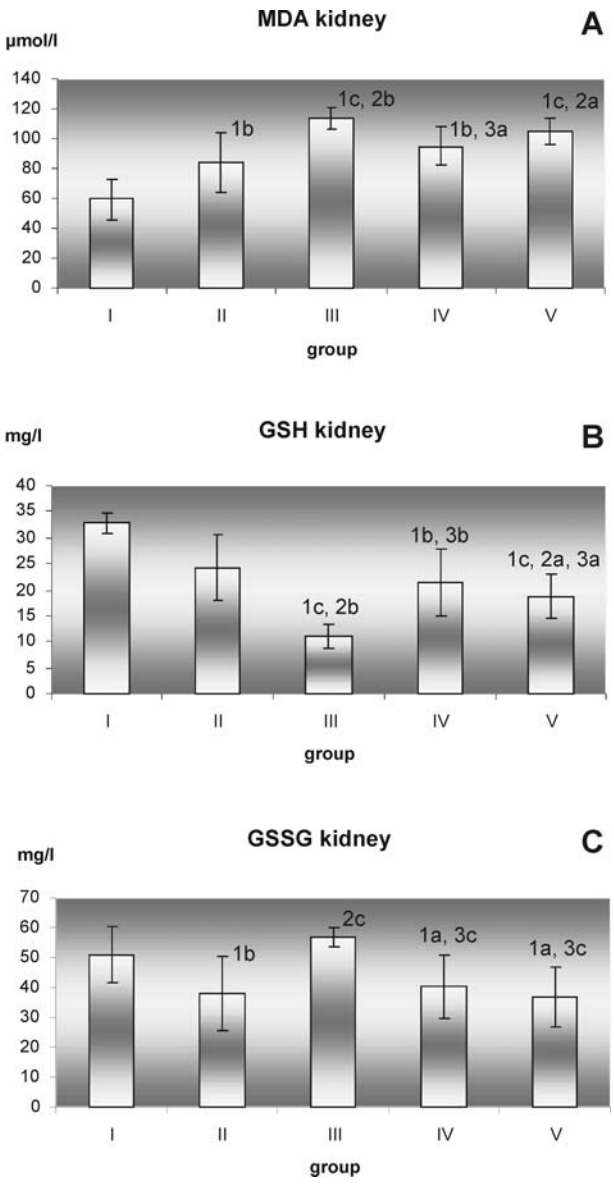

Fig. 2. Biochemical evaluation of **(A)** MDA, **(B)** free GSH, and **(C)** GSSG levels in kidney samples (n = 8 per group). Significant difference from the corresponding group 1, 2, 3 – groups (^{a} $p < 0.05$; ^{b} $p < 0.01$; ^{c} $p < 0.001$)

the oxidative stress parameters (Fig. 2 and 3). However, all parameters in DOX/FLR and FLR groups (IV and V) were similar to the results of both control groups and therefore significantly different from the DOX treated group ($p < 0.05$).

Histopathological examination revealed that all treated rats developed mammary tumors. The tumor incidence was 4.9 ± 0.6 per rat.

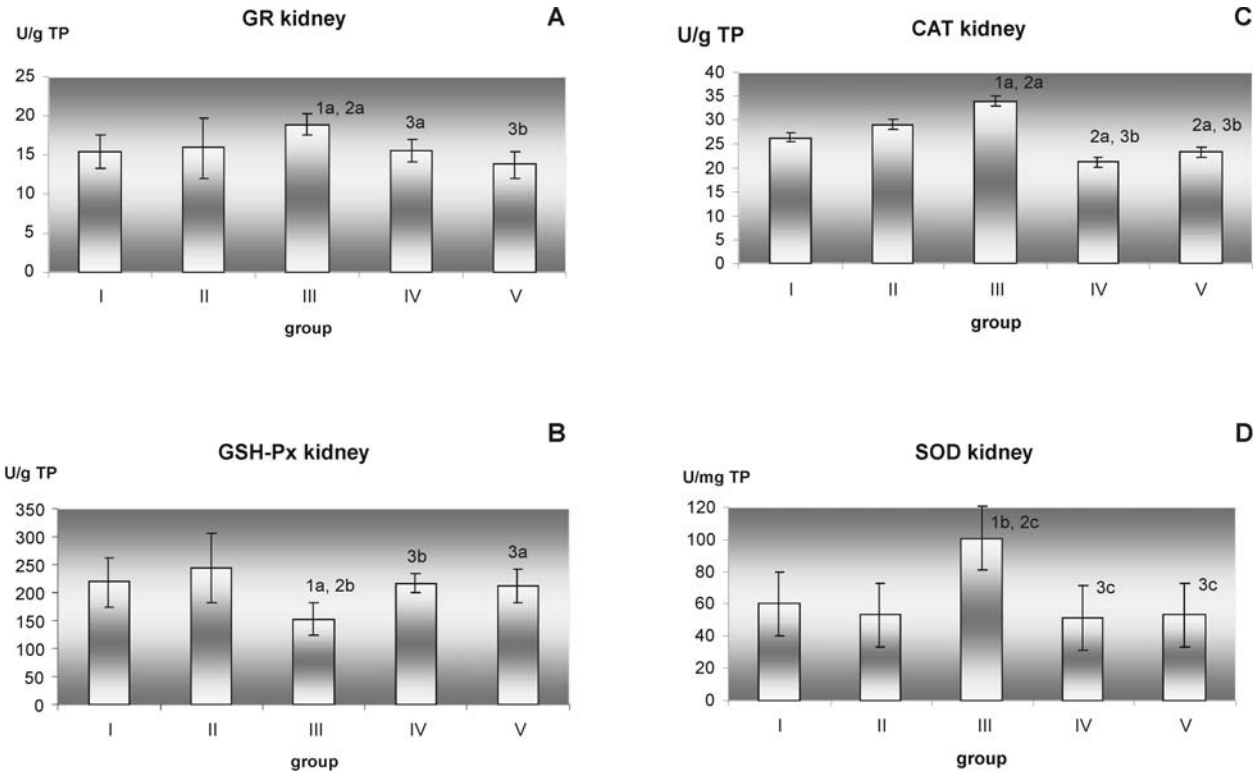

Fig. 3. Biochemical evaluation of **(A)** GR, **(B)** GSH-Px, **(C)** CAT, and **(D)** SOD levels in kidney samples (n = 8 per group). Significant difference from the corresponding group 1, 2, 3 – groups (^{a} $p < 0.05$; ^{b} $p < 0.01$; ^{c} $p < 0.001$)

Tab. 1. Body weight of rats and coefficient of kidney weight after sacrifice. Levels of serum enzymes LDH and α-HBDH (n = 8 per group)

Group	Body weight (g)	Kidneys (mg/g)	LDH (U/l)	α-HBDH (U/l)	α-HBDH/LDH
I	271.2 ± 12.8	7.2 ± 0.3	861.5 ± 132.0	265.9 ± 26.8	0.31
II	262.5 ± 22.0	7.5 ± 1.0	817.4 ± 253.6	207.2 ± 44.9	0.25^{1c}
III	257.0 ± 15.8	7.5 ± 0.4	$1127.5 \pm 107.2^{1a,2a}$	243.3 ± 48.3	$0.22^{1c,2c}$
IV	262.0 ± 13.8	7.0 ± 0.4	818.7 ± 223.5^{3b}	272.0 ± 89.9	$0.31^{2c,3c}$
V	251.5 ± 14.4^{1a}	7.2 ± 0.3	789.5 ± 155.1^{3b}	254.8 ± 74.6	$0.32^{2c,3c}$

1, 2, 3 – Represents significant difference from the corresponding group (^{a} $p < 0.05$; ^{b} $p < 0.01$; ^{c} $p < 0.001$)

Discussion

DOX is a very important agent in the treatment of cancer patients although its use may be complicated by the presence of acute and chronic side effects. Despite the wide use of DOX in the treatment of cancer, its mechanism of action is still not well known and has often been the subject of controversy [18]. Many antioxidants have been assayed with very different results. Among these molecules, metal ion chelators, like transferrins, metallothionein, desferrioxamine or proteins that oxidize ferrous ions, such as ceruloplasmin, have been widely investigated in relation to

DOX. Also, numerous studies have been developed using low molecular-mass agents that scavenge reactive oxygen species (ROS) and that are synthesized *in vivo* as bilirubin, sex hormones, melatonin, uric acid, and lipoic acid [8, 19]. The most commonly investigated agents are antioxidants that are derived from the diet such as vitamin E, vitamin C, vitamin A, coenzyme Q, flavonoides, selenium, and antioxidant components of virgin olive oil [3, 19]. Recently, some publications have dealt with the protective effects of dihydropyridine calcium antagonists [14], lycopene [26], and caffeic acid phenethyl ester [25] on DOX-induced nephrotoxicity in rats. Work published by our group and others on the use of FLR $C_{60}(OH)_{24}$ as an antioxidant have showed significant influence on heart and liver protection against DOX-induced toxicity [2, 5, 7, 10–12, 16] and heart, liver, small intestine, lung, kidney and spleen protection in irradiated rats [23, 24].

DOX causes tissues injury in the kidney and this damage was demonstrated by the biochemical evaluation performed in the present study. DOX toxicity is attributed to its pro-oxidant action. Our study demonstrated that DOX induced GSH/GSSG disbalance and lipid peroxidation in kidney tissue samples. It is well known that MDA and GSSG are indexes of biomarkers of lipid peroxidation and increasing oxidative stress, respectively [18]. ROS including superoxide radical ($O_2^{\bullet-}$), hydroxyl radical, hydrogen peroxide (H_2O_2), and singlet oxygen can cause cellular injury when they are generated excessively. ROS attack polyunsaturated fatty acids within membrane lipids as well as proteins and genetic materials. Superoxide dismutase catalyzes dismutation of the superoxide anion ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), which is then detoxified to H_2O by CAT. GSH, GSSG, and MDA levels with antioxidant capacity have been important biochemical components to detect tissue damage after DOX toxicity in the kidney tissue.

The present data indicate that DOX-induced kidney damage by a possible oxidative injury. This is in agreement with several reports. Liu et al. [14] found that administration of DOX (6.5 mg/kg, *iv*) caused an increase in MDA, apoptosis rate, urinary protein, BUN (blood urea nitrogen), NO (nitric oxide), NOS (nitric oxide synthase), and SCr (serum creatinine), as well as decreases in GSH, SOD, and GST (glutathione-S-transferase) in rats when compared to the control group. According to their results they concluded that nifedipine, nitrendipine, and amlodipine are not equally effective in terms of their effects on kidney histology and antioxidants apoptosis in DOX-induced nephrotoxicity. Yagmurca et al. [25] demonstrated that a 20 mg/kg (*ip*) single injection of DOX caused increased SOD, MDA, NO, XO (xanthine oxidase), and MPO (myeloperoxidase) in kidney tissues in rats 10 days after administration. According to their results, caffeic acid phenethyl ester as a pre-treatment agent protected renal tissues against DOX-induced nephrotoxicity. Caffeic acid phenethyl ester may prevent these renal lesions with its antioxidant and anti-inflammatory activities [25]. Yilmaz et al. [26] established that 4 mg/kg of lycopene 10 and 2 days before or 3 days after *ip* administration of 10 mg/kg DOX may be used to prevent cardiotoxicity and nephrotoxicity during DOX therapy. Their results are very similar to previous two studies [14, 25] on MDA, GSH, CAT, and GSH-Px levels, as well as our present results. Dzięgiel et al. [8] found that melatonin has a protective effect with respect to daunorubicin and doxorubicin toxicity. Histological evaluation after both types of intoxication, acute and subchronic, revealed less pronounced renal lesions after anthracycline treatment when melatonin was given. Unfortunately, in our study, during acute DOX-induced toxicity (over two days), we did not find any pathohistological changes in the DOX-treated group or the other investigated groups.

It has been indicated that FLR pre-treatment prevented various oxidative stress models in rats. In our recently published papers [7, 10–12] it was shown that 100 mg/kg *ip* FLR pre-treatment 30 min before DOX administration (*ip*) attenuated the DOX-induced heart and liver injury in rats (both with breast cancer [10, 11] and healthy [7, 12]).

Both LDH and α-HBDH are often used as markers of cardiovascular, liver or kidney damage, while the α-HBDH/LDH ratio can be calculated for differentiation between liver and heart diseases. Comparison of all groups with the healthy control group led to the conclusion that in cases with lower α-HBDH/LDH ratios, parenchymal liver damage is stronger. In contrast, groups pretreated with $C_{60}(OH)_{24}$ have almost normal α-HBDH/LDH ratios compared to the healthy control group. Very high levels of LDH activity in the serum without significant changes in α-HBDH activity shows strong tissue damage (liver, heart, kidney), after DOX administration, which could be controlled and prevented by application of FLR half an hour beore DOX-treatment. This confirms, once again, the

results obtained during our experiments with FLR and DOX [2, 5, 7, 10–12].

In our study, it was found that oxidative stress increased in kidney tissue after DOX administration. Tissue damage after DOX-induced nephrotoxicity was established by changes in the activity of MDA, GSH, GSSG, SOD, CAT, GR, GSH-Px, and α-HBDH, as well as serum and tissue LDH levels. In addition, the potential nephroprotective influence of FLR as a pre-treatment agent in DOX therapy in acute phase was evaluated. The main advantage of FLR over the other investigated protectors is its dual action. It is well known that anticancer therapy includes either radio- or chemo-therapy, which are harmful to healthy tissue. In that manner, FLR may act as a cardioprotector [7, 10], hepatoprotector [11, 12], nephroprotector and radioprotector [23, 24], and thus has benefits beyond the other antioxidants described in literature. The cytoprotective effect of amifostine in the treatment of childhood neoplastic diseases as a radio- and chemoprotective agent has been reported by Stolarska et al. [22], but the advantages of FLR over amifostine were well described in a recently published work by Trajkovic et al. [24].

Conclusion

The present work describes the protection afforded by FLR against acute renal damage induced by DOX on the basis of oxidant-antioxidant system management, and was confirmed by biochemical examination. The results demonstrate that single *ip* injection of DOX at a dosage of 8 mg/kg to Sprague-Dawley rats with chemically-induced mammary carcinomas caused renal injury. FLR, as a pre-treatment agent in doses of 100 mg/kg half an hour before DOX administration, may prevent DOX caused damage by its antioxidant action. On the other hand, before a conclusive statement on the potential efficacy of FLR as an adjunct to DOX therapy may be made, there is a need for further investigations, including studies on chronic DOX-induced nephrotoxicity, the use of another animal model species and, finally, human trials.

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References:

1. Aebi H: Catalase in vitro. Methods Enzymol, 1984, 105, 121–126.
2. Bogdanovic G, Kojic V, Djordjevic A, Canadanovic-Brunet J, Vojinovic-Miloradov M, Baltic V: Modulating activity of fullerenol $C_{60}(OH)_{22}$ on doxorubicin induced cytotoxicity. Toxicol In Vitro, 2004, 18, 629–637.
3. Chattopadhyay A, Bandyopadhyay D: Vitamin E in the prevention of ischemic heart disease. Pharmacol Rep, 2006, 58, 179–187.
4. Deepa PR, Varalakshmi P: Biochemical evaluation of the inflammatory changes in cardiac, hepatic and renal tissues of adriamycin-administered rats and the modulatory role of exogenous heparin-derivative treatment. Chem Biol Interact, 2005, 156, 93–100.
5. Djordjevic A, Canadanovic-Brunet J, Vojinovic-Miloradov M, Bogdanovic G: Antioxidant properties and hypothetical radical mechanism of fullerenol $C_{60}(OH)_{24}$. Oxi Commun, 2004, 27, 806–812.
6. Djordjevic A, Vojinovic-Miloradov M, Petranovic N, Devecerski A, Lazar D, Ribar B: Catalytic preparation and characterization of $C_{60}Br_{24}$. Fullerene Sci Technol, 1998, 6, 689–694.
7. Djordjevic-Milic V, Djordjevic A, Dobric S, Injac R, Vuckovic D, Stankov K, Dragojevic Simic V, Suvajdzic Lj: Influence of fullerenol $C_{60}(OH)_{24}$ on doxorubicin induced cardiotoxicity in rats. Mater Sci Forum, 2006, 518, 525–529.
8. Dzięgiel P, Suder E, Surowiak P, Jethon Z, Rabczynski J, Januszewska L, Sopel M, Zabel M: Role of exogenous melatonin in reducing the nephrotoxic effect of daunorubicin and doxorubicin in the rat. J Pineal Res, 2002, 33, 95–100.
9. Hahn H, Park YS, Ha IS, Cheong HI, Choi Y: Age-related differences in adriamycin-induced nephropathy. Pediatr Nephrol, 2004, 19, 761–766.
10. Injac R, Perse M, Boskovic M, Djordjevic-Milic V, Djordjevic A, Hvala A, Cerar A, Strukelj B: Cardioprotective effects of fullerenol $C_{60}(OH)_{24}$ on a single dose doxorubicin-induced cardiotoxicity in rats with malignant neoplasm. Technol Cancer Res Treat, 2008, 7, 15–26.
11. Injac R, Perse M, Obermajer N, Djordjevic-Milic V, Prijatelj M, Djordjevic A, Cerar A, Strukelj B: Potential hepatoprotective effects of fullerenol $C_{60}(OH)_{24}$ in doxorubicin-induced hepatotoxicity in rats with mammary carcinomas. Biomaterials, 2008, 29, 3451–3460.
12. Jacevic V, Djordjevic-Milic V, Dragojevic-Simic V, Radic N, Govedarica B, Dobric S, Srdjenovic B et al.: Protective effects of fullerenol $C_{60}(OH)_{24}$ on doxorubicin-induced hepatotoxicity in rats: pathohistological study. Toxicol Lett, 2007, 172S, S146.
13. Liska J, Galbavy S, Macejova D, Zlatos J, Brtko J: Histopathology of mammary tumors in female rats treated with 1-methyl-1-nitrosourea. Endocr Regul, 2000, 34, 91–96.
14. Liu LL, Li QX, Xia L, Li J, Shao L: Differential effects of dihydropyridine calcium antagonists on doxorubicin-

induced nephrotoxicity in rats. Toxicology, 2007, 231, 81–90.
15. Manabe N, Kinoshita A, Yamaguchi M, Furuya Y, Nagano N, Yamada-Uchio K, Akashi N et al.: Changes in quantitative profile of extracellular matrix components in the kidneys of rats with adriamicin-induced nephropathy. J Vet Med Sci, 2001, 63, 125–133.
16. Mirkova SM, Djordjevic AN, Andricb NL, Andric SA, Kostic TS, Bogdanovic GM, Vojinovic-Miloradov MB, Kovacevic RZ: Nitric oxide-scavenging activity of polyhydroxylated fullerenol, $C_{60}(OH)_{24}$. Nitric Oxide, 2004, 11, 201–207.
17. Oteki T, Nagase S, Yokoyama H, Ohya H, Akatsuka T, Tada M, Ueda A et al.: Evaluation of adriamycin nephropathy by an in vivo electron paramagnetic resonance. Biochem Biophys Res Commun, 2005, 332, 326–331.
18. Priestman T: Cancer Chemotherapy in Clinical Practice. Springer-Verlag, London Ltd., UK, 2008.
19. Quiles JL, Huertas JR, Battion M, Mataix J, Ramirez-Tortosa CM: Antioxidant nutrients and adriamycin toxicity. Toxicology, 2002, 180, 79–95.
20. Rook M, Lely AT, Kramer AB, Goor H, Navis G: Individual differences in renal ACE activity in healthy rats predict susceptibility to adriamycin-induced renal damage. Nephrol Dial Transplant, 2005, 20, 59–64.
21. Stańczak A, Ferra A: Prodrugs and soft drugs. Pharmacol Rep, 2006, 58, 599–613.
22. Stolarska M, Młynarski W, Zalewska-Szewczyk B, Bodalski J: Cytoprotective effect of amifostine in the treatment of childhood neoplastic diseases – a clinical study including the pharmacoeconomic analysis. Pharmacol Rep, 2006, 58, 30–34.
23. Trajkovic S, Dobric S, Djordjevic A, Dragojevic-Simic V, Milovanovic V: Radioprotective efficiency of fullerenol in irradiated mice. Mater Sci Forum, 2005, 494, 549–554.
24. Trajkovic S, Dobric S, Jacevic V, Dragojevic-Simic V, Milovanovic Z, Djordjevic A: Tissue-protective effects of fullerenol $C_{60}(OH)_{24}$ and amifostine in irradiated rats. Coll Surf B, 2007, 58, 39–43.
25. Yagmurca M, Erdogan H, Iraz M, Songur A, Ucar M, Fadillioglu E: Caffeic acid phenethyl ester as a protective agent against doxorubicin nephrotoxicity in rats. Clin Chim Acta, 2004, 348, 27–34.
26. Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S: Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology, 2006, 218, 164–171.

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