Прилози, Одд. биол. мед. науки, МАНУ, XXIX, 2, с. 167–183 (2008)
Contributions, Sec. Biol. Med. Sci., MASA, XXIX, 2, p. 167–183 (2008)
ISSN 0351–3254
УДК: 615.357:616.61-097.3]:615.277.06

ERYTHROPOIETIN REDUCES CUMULATIVE NEPHROTOXICITY FROM CISPLATIN AND ENHANCES RENAL TUBULAR CELL PROLIFERATION

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A b s t r a c t: Cisplatin, a heavy metal complex, is one of the most active drugs used in the treatment of several human malignancies. However, high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity.

The main objectives of this study were to determine the role of recombinant human erythropoietin (Epoetin alfa) in the prevention of nephrotoxicity induced experimentally in Wistar rats by long-term administration of cisplatin (2 mg/kg/b.w./week) over eight weeks, and an evaluation of its effect on renal tubular cell proliferation. The animals were randomly assigned into three groups, each including 25 rats. Group 1 (CP) received only cisplatin (2 mg/kg/b.w./week), group 2 (CP+EPO) received cisplatin (2 mg/kg/b.w./week) and epoetin alfa (150 IE/kg/b.w./three times a week), and group 3 (control group) received only saline. During the study, the following tests for the assessment of the renal function and renal damages were performed: determination of concentration of serum creatinine and BUN and determination of total protein quantity in 24-hour urine samples. At the end of the study, the abdomen was opened and both kidneys of the rats were removed and sent for histological and morphometric analysis. Ki-67 was used as a tool to determine a proliferative index. The results obtained have shown that epoetin alfa significantly reduced the functional renal failures and renal damages, and increased toleration of high doses of cisplatin. At the same time, our results with regard to tubular proliferative index have confirmed that one of the possible mechanisms by which erythropoietin accomplishes its renoprotective effect is stimulation of tubular cell proliferation and regeneration.

Key words: Erythropoietin, cisplatin, nephrotoxicity, rats.

Introduction

Cisplatin, a heavy metal complex, is one of the most active drugs used in the treatment of several human malignancies. The relatively moderate degree of haematologic toxicity associated with cisplatin makes it an ideal candidate to use in combination with agents whose primary dose-limiting toxicity is bone-marrow suppression [1]. Cisplatin-based combination chemotherapy regimens are currently used as front-line therapy in the treatment of testicular cancer, ovarian germ cell tumours, epithelial ovarian cancer, head and neck cancer, advanced cervical cancer, bladder cancer, mesothelioma, endometrial cancer, non-small cell lung cancer, malignant melanoma, carcinoids, penile cancer, adrenocorticol carcinoma and carcinoma of unknown primary [2]. Cisplatin-based chemotherapy is used with radiation therapy in the treatment of oesophageal cancer, localized cervical cancer and head and neck cancer [3]. It is used as consolidation therapy for many types of solid tumors that have failed standard treatment regimens. The therapeutic effects of cisplatin are significantly improved by dose escalation. However, high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity [4]. The nephrotoxicity of cisplatin is specific to the proximal tubule cells. The mechanism by which cisplatin kills the proximal tubule cells in the kidney has been the focus of intense investigation for many years. In tumours and other dividing cells, cisplatin-DNA crosslinks are thought to be the cytotoxic lesion [5]. Nonproliferating cells are less sensitive to the toxicity of DNA-damaging agents, yet the quiescent proximal tubule cells are selectively killed by cisplatin. High concentrations of cisplatin induce necrotic cell death in confluent monolayers of proximal tubule cell, whereas lower concentrations of cisplatin may induce apoptosis through a caspase-9-dependent pathway [6, 7].

Due to the cumulative nature of cisplatin nephrotoxicity, initial therapy is commonly limited to six cycles at doses of 75 to 100 mg/m^2, despite the fact that the patient may benefit from additional courses of treatment. Additionally, the relative permanence of the cumulative toxicities prohibit retreatment with cisplatin following disease recurrence, notwithstanding the proven potential of retained drug efficacy [8].

Its dose-limiting toxicities have spurred the development of the non-nephrotoxic derivative carboplatin and other platinum-based drugs. However, cisplatin is still the drug of choice in many platinum-based therapy regimens, and remains one of the most commonly used chemotherapy drugs.

In oncological practice, hydration protocols were developed to reduce the cumulative nephrotoxicity of cisplatin [9]. However, even with vigilant hydratation, approximately one-third of patients treated with cisplatin have elevation of blood urea nitrogen levels or other evidence of kidney damage in the

days following cisplatin treatment [10]. In recent years newer therapeutic strategies are being investigated aimed at minimizing cisplatin-induced nephrotoxicity while enhancing its antineoplastic efficacy. Such strategies may include inhibition of pathways leading to activation of cisplatin to a nephrotoxin, use of antioxidants to counter the ravages of reactive oxygen molecules, target inhibition of apoptotic mechanism activated by cisplatin specifically in kidney cells, uses of citoprotective agents that can protect normal cells, but not tumour cells, from cisplatin, and uses of agents that enhance cell proliferation and differenttiation.

For more than 30 years, the kidney has been known to be the primary site of erythropoietin production. Erythropoietin is a growth hormone whose effect may not be limited to bone-marrow progenitor cells. *In vitro*, recombinant human erythropoietin stimulates endothelial cells proliferation [11].

Previously, Vaziri *et al*. [12] suggested the potential effect of erythropoietin in enhancing the recovery after cisplatin induced acute tubular necrosis.

Aim

The main objectives of this study were to determine the role of epoetin alfa in prevention of nephrotoxicity induced experimentally in Wistar rats by long-term administration of cisplatin at a dose of 2 mg/kg/b.w./weekly for 8 weeks and to evaluate its effect on renal tubular cell proliferation.

Material and methods

Drugs: The following drugs were used in this study: Cisplatin (CP) – Bristol Myers Squib and Epoetin alfa (EPO) (Cilag AG).

Experimental animals: 75 male normotensive Wistar rats were used, aged 9–11 weeks, with body weight from 200 to 330 g. The rats were bred in the stall of the Preclinical and Clinical Pharmacology with Toxicology Institute.

Experimental protocols: to meet the objectives of this study, the rats were divided into 3 groups, each one containing 25 animals.

In order to evaluate the nephrotoxic effect of long-term cisplatin administration, the first group was injected with cisplatin intraperitoneally at a dose of 2 mg/kg/b.w./weekly for 8 weeks (CP-group). For evaluation of epoetin alfa effects on preventing nephrotoxicity induced by cisplatin, in the second group (EPO-group), epoetin alfa (150 IE/kg/b.w./3 times a week s.c.) was initiated concomitantly with cisplatin treatment (at the same dose as in the previous protocol). The third group was a control group, and these animals were given

saline in the same quantities and at the same time intervals as the groups of animals that received the examined drugs.

Measurements and analyses:

Functional tests: during the study, the following tests for assessment of the renal function in the examined animals were performed: biochemical analysis of the blood, that included determination of serum creatinine and urea concentrations, as well as determination of the quantity of total urinary proteins in 24-hour urine samples. These tests were performed prior to the beginning of the study (0-day), and after 2, 4, 6 and 8 weeks from the beginning of the study. In order to determine the level of creatinine and urea in serum, blood was taken by venepuncture from the orbital sinus of the rats under light ether anesthesia. Blood samples of 400 μl were taken for serum separation (200 μl). Metabolic cages were used for collecting 24-hour urine samples for determination of total urinary protein quantity.

Body weight: body weight of the examined animals was monitored throughout the entire study. It was measured once a week, prior to each application of cisplatin.

Histological examination and renal histology

At the end of the study, under general anesthesia induced by intraperitoneal injection of thiobarbitol the abdomen was opened and both kidneys were removed. They were promptly bisected and fixed in 10% buffered formalin and then embedded in paraffin; sections were cut at 4–6 μm and stained with haemaoxylin and eosin, PAS, silvermethenamine Jones and trichrome Masson.

A pathologist carried out a semiquantitative analysis of the kidney sections in a blinded fashion. Glomeruli and vessels were normal. Changes observed were limited to the tubules and interstitium, especially to the proximal straight S3 portion, the main site of cisplatin toxicity. Tubulo-interstitial lesions were graded as follows: 0 = no damage; 1 = area of tubular epithelial cell swelling, vacuolization, necrosis, desquamation less than 50%; 2 = lesion areas greater than 50% with or without focal involvement of the S3 segment in the medullar rays and moderate interstitial fibrosis; 3 = lesion areas 100% with diffuse involvement of the medullar rays and apoptosis in the tubular compartment.

Immuno-histochemistry

Ki-67 (clone Mib-1) is a monoclonal antibody expressed in the nucleus strongly during all active phases of the cell cycle. Therefore, Ki-67 is used as a tool to determine a proliferative index. Immunostaining for Ki-67 was performed using the LSAB immunoperoxidase procedure (Dako, Denmark). Briefly, kidney sections were cut at 4 μm, mounted on silanized slides, and treated for 5 min in a microwave oven in a citrate buffer (pH = 6.0). Then, sections were incubated for 5 min with a blocking reagent to reduce non-specific background

staining, followed by incubation for 1h at room temperature with the specific antibody (Ki-67, Dako, Denmark), diluted in PBS (1 : 400). After a 10-min rinse in PBS, sections were incubated for 10 min at room temperature with a biotinylated secondary antibody, followed by the avidin/biotin/peroxidase complex and then revealed by 3,3' diaminobenzidine. The sections were counterstained with haematoxylin and mounted in Entelan (Merck, Germany). Negative controls were obtained by replacing specific antiserum with normal non-immune sera; no labelling was observed, indicating that the entire procedure and all reagents used resulted in a specific labelling. For Ki-67, lymph node specimens were used as positive controls.

Control sections of representative tissues were prepared by substitution of the primary antibody with dilutions of normal mouse serum or omission of the primary antibody. Morphometry was used to count the nuclei positive for Ki-67.

The results from morphometric analysis of the tissue samples from the analysed group stained for Ki-67 are expressed as a mean of the number of cells with positive nuclei per 1 high power field (× 400 HPF) from 10 analyzed HPF.

Statistics: All data were expressed as mean ± SD. To test more than two groups, Kruskal-Wallis variance analysis was used, followed by a Mann-Whitney U-test to determine which groups were significantly different. A p-value less than 0.05 was considered as statistically significant.

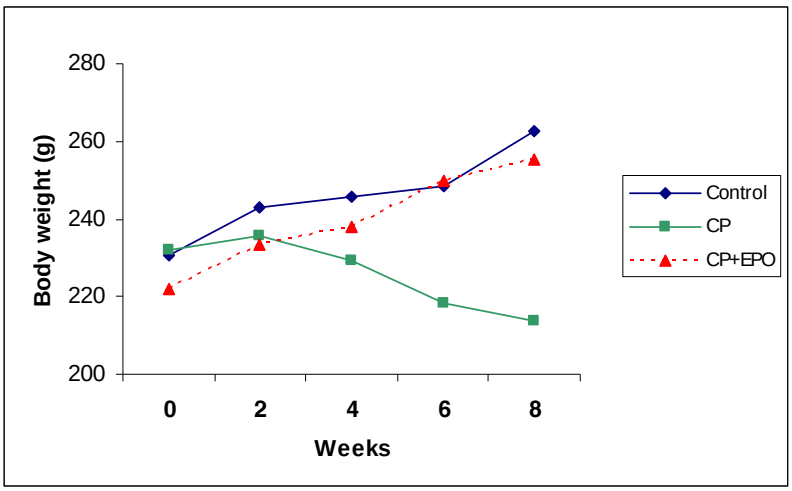
Results

Out of the total number of 75 rats included in the study (25 in each group) three rats died during the study, and they all belonged to the group that received only cisplatin (CP). In the remaining two groups of rats (CP+EPO and the control group) none of the animals died.

Body weight

One of the characteristics of the animals that were given only cisplatin (CP-group) was the progressive reduction of their body weight during the entire study. At the end of the study, after 8 weeks, the body weight in this group of animals was reduced by 8% ($p < 0.05$) as compared to the basal values. In the group of animals who received epoetin alfa in addition to cisplatin (CP+EPO group), there was neither stagnation in growth nor body weight reduction; on the contrary, there was an evident continual increase of body weight along with a relatively good general condition during the entire study. At the end, there was

an increase of the body weight by 15.01% in this group of rats in comparison to the basal values, which was significantly different ($p < 0.05$) than in the CP-group (Figure 1).



Control	230.56	242.78	245.56	248.33	262.78
CP	232.22	235.56	229.09	218.18	213.64
CP+EPO	222	233.5	238	250	255.33

Figure 1 – Body weight values in rats during the study

Слика 1 – Вредности на телесна тежина кај стаорците во текот на испитувањето

Serum creatinine and BUN

Serum creatinine and BUN concentrations in the rats were used as routine parameters for assessment of the renal function.

Administration of cisplatin at a weekly dose of 2 mg/kg/b.w. caused an increase of serum creatinine and BUN levels after 4 weeks, when the mean values of these parameters reached 64.75 ± 8.88 μmol/L (creatinine), and 10.04 ± 1.53 mmol/L (BUN), which were significantly higher ($p < 0.05$) than in the control group (creatinine = 38.55 ± 2.68 μmol/L; BUN = 7.58 ± 1.39 mmol/L) and the basal values. At the end of the study, the values of these two routine parameters used for assessment of the renal function were even more increased: 95.0 ± 23.15 μmol/L (creatinine) and 24.26 ± 10.72 mmol/L (BUN) (Table 1).

Pretreatment with epoetin alfa as a result of its nephroprotective characteristics has shown to be efficient in the prevention of the clinically significant increase of the values of these two parameters. Only at the end of the treatment,

after administration of the total cumulative dose of cisplatin (16 mg/kg/b.w.), was there a significant ($p < 0.05$) increase of these values in comparison to the basal ones (creatinine: 43.07 ± 10.44 μmol/L; BUN: 11.15 ± 3.05 mmol/L) and to the control group (Table 1).

Table 1 – Табела 1

Serum concentration of creatinine and BUN
Серумски концентрации на креатинин и уреа

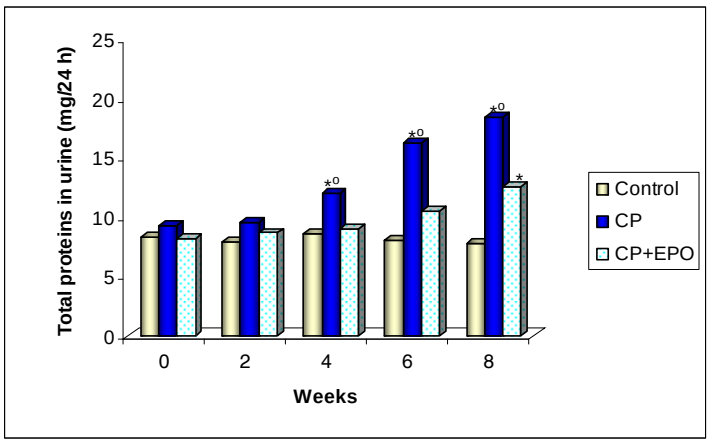
	Serum creatinine (μmol/L)					**BUN (mmol/L)**				
	Weeks of the treatment					*Weeks of the treatment*				
	0	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**
Control										
Mean	38.75	38.05	38.55	37.50	36.50	8.95	7.82	7.58	8.09	8.28
SD	2.43	2.82	2.68	2.19	3.20	0.86	1.55	1.39	1.42	1.15
CP										
Mean	39.00	41.00	64.75*^{o}	86.46*^{o}	95.00*^{o}	8.78	7.08	10.04*^{o}	22.10*^{o}	24.26*^{o}
SD	2.11	3.72	8.88	26.80	23.15	1.45	1.42	1.53	10.32	10.72
CP+EPO										
Mean	37.67	39.09	36.85	38.92	43.07*	8.74	7.16	6.94	9.55	11.15*
SD	2.77	1.51	6.63	5.90	10.33	1.30	1.47	1.17	2.52	3.05

* $p < 0.05$ vs. control group
^{o} $p < 0.05$ vs. EPO group

Total urinary proteins

Proteinuria (determination of total proteins in urine) was used as a specific parameter for assessment of the renal damage in the examined animals. Cisplatin in the CP-group produced a marked proteinuria with significantly ($p < 0.05$) increased values of total urinary proteins for 24 hours after 4 weeks (12.0 ± 2.03 mg/24 h). This was even more obvious after 6 weeks and after 8 weeks, when the values of total urinary proteins for 24 hours were almost twice as high as the basal values ($p < 0.01$) (18.45 ± 3.21 mg/24 h) (Figure 2).

Epoetin alfa has proved to be effective with reference to this parameter as it was regarding the others, too, thus preventing severe proteinuria in the rats. A mild significant ($p < 0.05$) increase of the total values of urinary proteins was registered only by the end of the study when the mean values were 12.54 ± 2.01 mg/24 h, but they were significantly lower ($p < 0.05$) in comparison to the group who received cisplatin alone (Figure 2).



Control	8.3	7.86	8.61	8.03	7.75
CP	9.29	9.61	12	16.24	18.45
CP+EPO	8.18	8.67	8.97	10.52	12.54

* $p < 0.05$ vs. control group
° $p < 0.05$ vs. CP+EPO group

Figure 2 – Total urinary proteins for 24 hours
Слика 2 – Вкупни протеини во урина за 24 часа

Histological examinations

CP-group: at the end of the study, histological examination with a light microscope of the rat kidneys that had received cisplatin alone revealed signs of subacute and chronic renal damage characteristic of the long-term use of cisplatin. These changes were mainly localized in the cortico-medullar region that is in the tubular compartment and to a lesser degree in the interstitium, whereas there were no significant deviations in the glomeruli.

Changes in the tubular compartment were expressed to a greater extent in the proximal tubules and to a lesser degree in the distal tubules. Pathohistological findings of the tubules included: a moderate to severe degree of tubule dilation, signs of increased intratubular pressure, apparent vacuolization of the tubular epithelium, presence of protein deposites, necrotic cells, degenerative changes of the tubules, cystic dilation with flattening of the epithelium and sporadic presence of apoptotic cells (Figure 3).

There were signs of chronic renal damage in the interstitium, including dilated interstitium, interstitial fibrosis with increased collagenous fibers (Figure 4).

CP+EPO-group: contrary to the CP-group, in the rats from the CP+EPO-group who in addition to cisplatin preventively received erythropoeitin during the study, histological examinations of the kidneys, especially of the

tubular compartment, showed no significant renal failure architecture. Light microcopy detected the presence of mildly expressed vacuolization of the tubules, and sporadic presence of a flattened epithelium with rare foci and polyploid nuclei (Figure 5). Mild residual fibrosis, distinct proliferation and dominant regeneration were noticed in the interstitium (Figure 6).

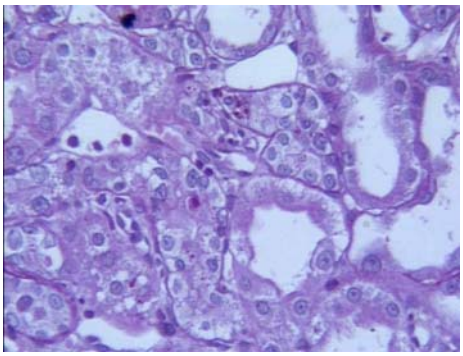


Figure 3 – Histological changes in the tubular compartment (× 400 PAS) (CP)
Слика 3 – Хистолошки промени во тубуларниот компартман (× 400 PAS) (CP)

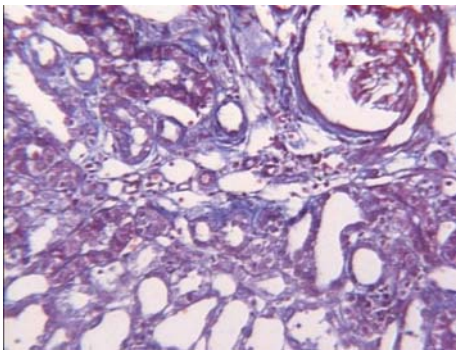


Figure 4 – Histological changes in the Interstitium (× 200 Trichrom Masson) (CP)
Слика 4 – Хистолошки промени во интерстициумот (× 200 Trichrom Masson) (CP)

A characteristic feature of the histological finding for this group of animals was the presence of signs of distinct proliferation and dominant regeneration.

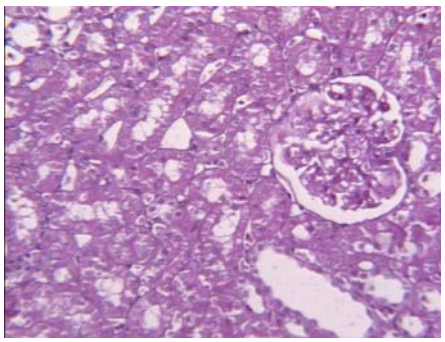


Figure 5 – Histological changes in the tubular compartment (× 200 H.E) (CP+EPO)
Слика 5 – Хистолошки промени во тубуларниот компартман (× 200 H.E) (CP+EPO)

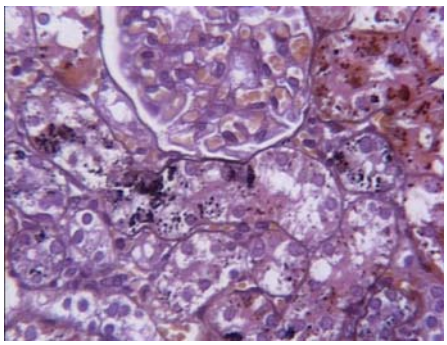


Figure 6 – Histological changes in the interstitium (× 400 Silver. Jones) (CP+EPO)
Слика 6 – Хистолошки промени во интерстициум (× 400 Silver. Jones) (CP+EPO)

Tubulo-interstitial score

The results obtained from the tubulo-interstitial score displayed significant differences ($p < 0.05$) between the examined groups of animals. The worst tubulo-interstitial score was found in the CP-group of rats. It was 2.36 ± 0.56 and was significantly higher than that in the control group (0.3 ± 0.47). This score demonstrated moderate to severe renal damage in this group of animals. In the CP+EPO-group of rats, although the tubulo-interstitial score was worse than in the control group, it was significantly lower than that determined in the CP-group (0.85 ± 0.745) yielding moderate renal damages (Table 2).

Table 2 – Табела 2

Tubulo-interstitial score
Тубуло-интерстицијален скор

Tubulo-interstitial score			
	Control	**CP**	**CP+EPO**
Mean	0.3	2.3	0.85
SD	0.470	0.571	0.745
Min	0	1	0
Max	1	3	2

* $p < 0.05$ vs. control group
° $p < 0.05$ vs. EPO group

Tubular proliferative index

The results of the morphometric analysis of the tubular proliferative index (Ki-67) showed significant differences ($p < 0.05$) between the groups of animals examined. The highest proliferative index was registered in the CP+EPO-group of rats (4.06 ± 1.638) and it was significantly higher ($p < 0.05$) in comparison both to the control group (1.5 ± 0.503) and to the CP-group of rats (0.5 ± 0.674). In the CP-group of rats, the tubular proliferative index was not only significantly lower compared to the CP+EPO-group, but it was also significantly lower than the control group ($p < 0.05$). These values of the tubular proliferative index presented distinct proliferation and regeneration in the CP+EPO-group of rats and inhibited proliferation in the CP-group (Table 3, Figure 7 and 8).

Table 3 – Табела 3

Tubular proliferative index
Тубуларен пролиферативен индекс

	Proliferative index		
	Control	**CP**	**EPO**
Mean	1.5	0.5	4.01
SD	0.503	0.674	1.487
Min	1	0	2
Max	2	2	8

* $p < 0.05$ vs. control group
° $p < 0.05$ vs. EPO group

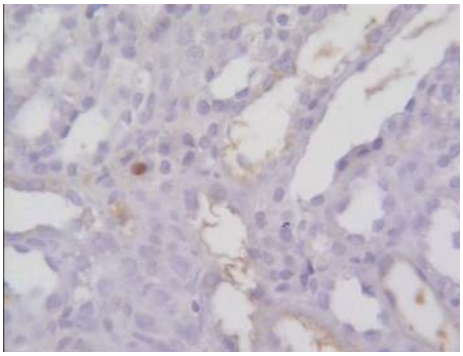


Figure 7 – Tubular proliferation (CP)
Слика 7 – Тубуларна пролиферација (CP)

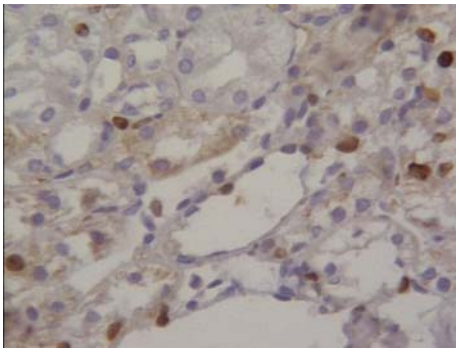


Figure 8 – Tubular proliferation (CP+EPO)
Слика 8 – Тубуларна пролиферација (CP+EPO)

Discussion

The results obtained confirm the cumulative nephrotoxicity of cisplatin. Administration of cisplatin at a dose of 2 mg/kg/b.w./weekly resulted in moderate or severe functional kidney abnormalities in all the animals in this group. The changes were obvious and significant in comparison with the basal values and the control group 4 weeks after the initiation of cisplatin administration, after administration of a total cumulative dose of 8 mg/kg/b.w. This nephrotoxicity was also reflected in the general condition of the animals examined, manifested in growth stagnation, body weight loss and reduced motor activity in the animals examined. In the further course of the investigation, by increasing the

total cumulative dose of cisplatin, the symptoms of nephrotoxicity and general poor condition were even more expressed in the CP-group, which pointed to the cumulative character of cisplatin nephrotoxicity. At the end of the investigation, as a result of the toxic effect of cisplatin, 3 out of 25 rats died (12%).

On the other hand, in the second group animals who received epoetin alfa (CP+EPO) in addition to cisplatin, although symptoms and signs of nephrotoxicity were not completely prevented, they were significantly less expressed in comparison to the CP-group. Only in 4 out of 25 rats, were mild to moderate functional kidney abnormalities found. As a result of the well-known effects of epoetin alfa on anaemia, growth and increase of oxygenic capacity, there was no stagnation in the growth nor was there body weight loss. It should be especially pointed out that none of the animals from this group died during the study, showing their increased tolerance to high doses of cisplatin.

The nephroprotective effects of epoetin alfa were unequivocally confirmed by pathohistological examinations of the rats' kidneys at the end of the investigation. Signs of subacute and chronic kidney damage were evident in the group of rats that received cisplatin. These changes were predominantly located in the tubular compartment and mainly in the proximal tubules (distinct vacuolezation of the tubular epithelium, cystic dilatation with flattening of the epithelium, presence of protein deposits, degenerative changes, necrotic cells, apoptotic cells) and, to a lesser degree, in the interstitium (fibrosis and collagenosis), whereas there were no clear significant damages in the glomeruli. On the contrary, in the group of rats that were given erythropoietin as prevention concomitantly with the cisplatin given during the study, pathohistological analysis of the kidneys, particularly of the tubular compartment, showed no significant renal architecture failure. At this level of light microscopy, the only registered changes were the sporadic presence of a flattened epithelium with rare foci with the presence of polyploid nuclei with mild vacuolization, a manifest proliferation and dominant regeneration.

The findings of several *in vivo* experimental studies have clearly shown that erythropoietin stimulates tubular cell regeneration and accelerates recovery from acute renal insufficiency.

Vaziri *et al*. [12] has shown that erythropoietin can enhance the recovery from acute tubular necrosis induced by cisplatin. Later Bagnis *et al*. [13] conducted a study with rats, in which an acute renal insufficiency was induced by a single dose of cisplatin (6 mg/kg) and found that erythropoietin enhanced the recovery, but at the same time significantly increased the tubular cell regeneration. Vesey DA *et al*. [14] demonstrated that erythropoietin had renal protective effects in *in vitro* and *in vivo* models of ischaemic acute renal insufficiency. Erythropoeitn increased tubular epithelial regeneration and stimulated the recovery of renal function in conditions of hypoxic or ischaemic acute renal insufficiency.

One of the main objectives of this study was an assessment of erythropoietin's effects on proliferation of endothelial cells in the tubular compartment during long-term administration of cisplatin. The results obtained in this study concerning the examination of proliferative index by quantifying the proliferative index with immunohistochemical detection of Ki 67 (Mib-1) have shown that erythropoietin significantly increases proliferation and regeneration in comparison with the rats that were not given erythropoietin.

These results have undoubtedly proved that eryhtropoetin, although not completely, to a large extent reduces cisplatin-induced nephrotoxicity and has an extremely favourable effect on the general condition of the animals. Our results are in agreement with recent investigations, demonstrating that epoetin alfa has an important role not only in therapeutic aims for the correction of different types of anaemia, but it can also be efficient as a neuroprotective drug [15] (particularly in peripheral cisplatin-induced neurotoxicity), hepatoprotective [16], cardioprotective [17] and particularly as a nephroprotective drug in nephrotoxicity induced by platinum-based drugs.

The nephroprotective effects of erythropoietin (epoetin alfa), have not been completely elucidated. There are several hypotheses that suggest various possible mechanisms for the nephroprotective effects of erythropoietin. It is known that erythropoietin is a cytokine which specifically regulates the differrentiation and proliferation of erythroid progenitor cells. The proliferating effects of erythropoietin have also been documented in other cell types, such as endothelial cells [10]. This proliferating effect of erythropoietin is most probably due to the fact that endothelial cells also possess erythropoietin receptors [11, 12]. Recent data have suggested that tubular and mesengial cells express authentic Epo-receptor mRNA [13] – advocating erythropoietin to be a renotropic cytokine.

The influence of erythropoietin on cell proliferation may be considered as one of the many possible mechanisms of the effect of erythropoietin in preventing cisplatin-induced nephrotoxicity. This is particularly important when larger doses are used when cisplatin manifests its cytotoxic effect, inducing more serious changes in the proximal tubules. Erythropoietin, with its stimulating effect on the differentiation, proliferation and regeneration of tubular epithelial cells, may have a favourable influence on this type of nephrotoxicity.

One of the alternative hypotheses that explain the favourable effect of erythropoietin on renal damage includes also its haemodynamic effects, although they have not been clearly demonstrated. Literature reports have shown that erythropoietin increases the nephron glomerular filtration of cortical nephrons, without increasing the total filtration rate [21].

On the other hand, the registered anti-apoptotic effect of erythropoietin on bone marrow, that is its stimulating effect on *bcl-2*, which is for now the

only identified intracellular inhibitor of apoptosis, makes it a very interesting and attractive drug to be used in other indications, too. The anti-apoptotic effect of erythropoietin might be useful for the inhibition of apoptosis in other body tissues and organs, especially in the prevention of drug toxicity in which the mechanism of toxicity apoptosis has an important influence. Being aware of the mechanism of the nephrotoxic effect of cisplatin, which it is assumed is due, to a high degree, to the induction of apoptosis during its chronic administration, a logical assumption is that eryhtropoetin could be effective in preventing nephrotoxicity by inhibition of cisplatin-induced apoptosis, as well.

Conclusion

The results obtained in this study have confirmed the cumulative nephrotoxicity of cisplatin. Cisplatin administration at a total cumulative dose of 16 mg/kg/b.w. for 8 weeks in a group of rats that received only cisplatin resulted in moderate or severe functional renal failure and pathohistological findings characteristic of subacute and chronic renal damage, predominantly located in the tubular compartment.

Erythropoietin (epoetin alfa), although not completely, to a large extent prevented nephrotoxicity induced by long-term cisplatin administration. Erythropoietin significantly reduced functional renal failures and renal damage, and increased tolerance to high doses of cisplatin.

At the same time, our results with regard to the tubular proliferative index have confirmed that one of the possible mechanisms by which erythropoietin accomplishes its renoprotective effect is stimulation of tubular cell proliferation and regeneration.

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Резиме

ЕРИТРОПОЕТИНОТ ЈА НАМАЛУВА КУМУЛАТИВНАТА НЕФРОТОКСИЧНОСТ НА ЦИСПЛАТИН И ЈА ЗГОЛЕМУВА ТУБУЛАРНАТА КЛЕТОЧНА ПРОЛИФЕРАЦИЈА

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Цисплатинот е комплекс на тежок метал и е еден од најактивните лекови кои се користат во третманот на голем број хумани малигни заболувања. Меѓутоа, високодозната терапија со цисплатин е ограничена од неговата кумулативна нефротоксичност.

Главни цели на оваа студија беа да се утврди ефектот на рекомбинантниот хуман еритропоетин (епоетин алфа) во превенцијата на нефротоксичност експериментално индуцирана кај Wistar стаорци со долготрајна администрација на цисплатин (2 mg/kg/t.t./недела) во текот на осум недели и да се процени неговиот ефект на бубрежната тубуларна клеточна пролиферација. Со рандомизација животните беа поделени во три групи, во секоја по 25 стаорци. Групата 1 (CP) примаше само цисплатин (2 mg/kg/t.t./недела), групата 2 (CP+EPO) примаше цисплатин (2 mg/kg/t.t./недела) и епоетин алфа (150 IE/kg/t.t./ три пати неделно), а групата 3 (контролната група) примаше само физиолошки раствор. Во текот на студијата за процена на бубрежната функција и бубрежните оштетувања беа спроведени следните испитувања: одредување на концентрацијата на креатинин и уреа во серум и одредување на вкупната количина на протеини во примероци од 24 часовна урина. На крајот од испитувањето, абдоменот на стаорците беше отворен и двата бубрега беа отстранети и пратени за хистолошка и морфометриска анализа. Ki–67 беше користен за одредување на пролиферативниот индекс.

Добиените резултати од оваа студија покажуваат дека епоетин алфа сигнификантно ги ублажува функционалните бубрежни нарушувања и бубрежните оштетувања, зголемувајќи ја толерабилноста кон повисоки дози на цисплатин.

Истовремено, добиените резултати во однос на тубуларниот пролиферативен индекс потврдуваат дека еден од можните механизми преку кој еритропоетинот го остварува своето ренопротективно дејство е стимулација на тубуларната клеточна пролиферација и регенерација.

Клучни зборови: еритропоетин, цисплатин, нефротоксичност, стаорци.

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