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INFLUENCE OF DIFFERENT GLUCOCORTICOSTEROID TREATMENT REGIMENS ON PATHOHISTOLOGICAL CHANGES IN HEARTS OF RATS POISONED WITH T-2 TOXIN

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In this study the protective effect of methylprednisolone (soluble form, Lemod-solu® and depot form, Lemod-depo®) (40 mg/kg im) on pathohistological changes in hearts of Wistar rats poisoned with T-2 toxin (0.23 mg/kg sc) was examined. Pathohistological, quantitative and morphometric analysis was based on the haematoxylin and eosin (HE) method. Animals were sacrificed after the end of day 1, 3, 5 and 7 of the study. In the hearts of poisoned animals T-2 toxin caused massive, diffuse degenerative and vascular changes associated with gross necrotic areas. The described changes could be found only sporadically in poisoned rats protected with tested methylprednisolone formulation. The best protective effect was produced by the soluble form of methylprednisolone and the least one with a combination of both tested formulations of the drug. The pathohistological alterations in the methylprednisolone protected animals varied from parenchymatous dystrophy to hyaloid degeneration, hyperaemia and haemorrhages with mononuclear cell infiltration. These histological deformations of the myocardial architecture were focal. Based on these results, it could be concluded that methylprednisolone formulations, both short and long-acting ones, exert a significant protection of rat hearts from T-2 toxin- induced pathohistological changes.

Key words: trichothecenes, mycotoxins, T-2 toxin, methylprednisolone, pathohistology, heart

INTRODUCTION

It is well established that 12, 13-epoxy-trichothecene mycotoxin (T-2 toxin) is one of the most potent cytotoxic metabolites produced by *Fusarium* fungi (Ueno, 1984; Visconti *et al*., 1991). Its biological activity is closely related to its lethal toxicity to eukaryotic cells (Pestka *et al*., 2004). After ingestion of contaminated food, T-2 mycotoxin affects actively dividing cells, cells of tissues with a lower mitotic index and permanent or irreversible post-mitotic cells (Grizzle *et al*., 2004).

In these cells cytomorphological changes such as karyorrhexis, karyopyknosis and karyolysis have been demonstrated (Jaćević *et al.*, 2001; Jaćević *et al*., 2002).

Macrocyclic trichothecene mycotoxins exert the highest cytotoxicity, followed by type A trichothecenes such as T-2 toxin and type B trichothecenes. Administration of quantities close to LD-50 values has a potentially inhibitory effect on protein synthesis in all eukaryotic cells (Larsen *et al*., 2004). A dose-dependent inhibition of incorporation of amino acids into proteins of eukaryotic cells is a very important part of the mechanism of their cytotoxicity (Karppanen *et al*., 1989). This inhibitory action of trichothecenes was observed in various animal tissues, such as rat liver preparations (Bergmann *et al*., 1994), lymphocytes (Nagata *et al*., 2001) and cell cultures (Albarenque and Doi, 2005). An early evidence of this is the breakdown of the polyribosome profile at the cellular level (Speijers and Speijers, 2004). T-2 toxin is one of the inhibitors of the initial step of protein synthesis, belonging thus to type I group of trichothecene mycotoxins (Koch, 2004). The most important findings about the toxins of type I group are their high affinity for 60 S ribosome subunits (Matsumoto *et al*., 1978), inhibition of termination of polypeptide synthesis and of peptidyl transferase activity. The activity of this enzyme is localised in the 60 S ribosome subunit. After T-2 toxin poisoning the active centre is presumed to be masked by the binding of the toxin molecule. Probably, this mechanism is responsable for the potent inhibition of DNA and RNA syntheses in the cells. The inhibition of nucleic acid synthesis may secondarily cause the impairment of the protein synthesis mechanism. However, some damage in the organisation of the cell may have important effects on nucleic acid synthesis (Meloche and Smith, 1995).

Considering these facts, one of the most important target organs in T-2 toxin poisoning is the heart. Several authors have suggested that T-2 toxin may be involved in the aetiology of some forms of human and animals cardiomyopathy (Pang *et al*., 1987b). Some authors showed that during acute T-2 toxin poisoning signs of inflammation, degeneration and rapid loss of normal cell architecture can be found in the heart of humans and animals. If exposure to the toxin was long enough, pathohistological and ultarastructural changes in heart of T-2 toxin poisoned rats ranged from intracellular and interstitial edema to parenchymal and hyaline degeneration with focal accumulation of mononuclear cells. Thickening of blood vessels with vacuolisation of endothelial cells was also observed. At the final step of poisoning, necrosis of the muscle cells was found, too (Sinovec, 1996; Jaćević, 2002).

In the last decade, some authors showed that pro-inflammatory effects of T-2 toxin probably are the most important mechanism of its acute cardiotoxicity. T-2 toxin activates a large number of mast cells (Jaćević *et al*., 2003), synthesis and degranulation of numerous mediators, such as histamine, serotonin, proteoglycans, leukotrienes, NO, PAF and prostaglandins (Castells, 2000). Intensive synthesis of pro-inflammatory mediators involves the migration and activation of macrophages and production of IL-1, IL-2, IL-6, IL-8 and TNF-alpha, which play an important role in the pathogenesis of T-2 mycotoxicosis (Bondy and Pestka, 2000).

The important role of prostaglandins in the pathogenesis of T-2 toxicosis is confirmed by the immunosuppressive efficiency of glucocorticoid hormones as antidotes against T-2 toxin (Mutoh *et al*., 1988). The best protection was obtained by dexamethasone (protective index 3.37) and methylprednisolone (protective index 2.14) (Jovanović, 1992). Glucocorticoids suppress the migration and activation of macrophages and mast cells, as well as a synthesis of their pro-inflammatory mediators (leukotrienes, prostaglandins, IL-1, IL-2, IL-6, IL-8 and TNF-alpha) (Zucker *et al*., 1994).

The aim of this experiment was to investigate the effects of soluble and depot forms of methylprednisolone (Lemod-solu® and Lemod-depo®) and their combination on pathohistological alterations in the hearts of rats acutely poisoned with 1 LD_{50} of T-2 toxin (0.23 mg/kg *sc*). The rationale for this experimental study was our previous study and finding that a single administration of methylprednisolone significantly alleviated the general toxic effects of T-2 toxin in rats (Stojiljković *et al.,* 2001).

MATERIAL AND METHODS

Experimental animals

Eighty female Wistar rats 4-6 weeks old, weighing 200-250 g, were used in this experiment. Animals were poisoned with a single *sc* injection of 1 LD_{50} of T-2 toxin (0.23 mg/kg). After registration of the 24-hour survival rate, pathohistological changes were monitored over a 7 day period. Rats were randomly allocated to eight groups, each of them consisting of 8 animals. Their treatments were: I - saline only (control group), II - Lemod-depo® 40 mg/kg *im*, III - Lemod-solu® 40 mg/kg *im*, IV - Lemod-solu® 40 mg/kg *im* and Lemod-depo® 40 mg/kg *im*, V - T-2 toxin 0.23 mg/kg *sc* ("the poisoned control"), VI - T-2 toxin and Lemod-depo® 40 mg/kg *im*, VII - T-2 toxin and Lemod-solu® 40 mg/kg *im* and VIII - T-2 toxin, Lemod-solu® 40 mg/kg *im* and Lemod-depo® 40 mg/kg *im*.

After randomisation, rats were housed in individual plastic cages. One day before the experiment, animals were fasted. During the subsequent experiment, they were fed with standard laboratory food *ad libitum*. They were allowed access to fresh tap water *ad libitum*.

T-2 toxin

T-2 toxin that was used in this experiment was produced under laboratory conditions from *Fusarium sporotrichoides* fungi and cultivated on synthetic GPY (glucose 5%, peptone 0.1%, yeast extract 0.1%, pH 5.4) medium. Extraction and crude purification of the toxin were performed by filtration, while definite purification and determination of T-2 toxin content were performed by gas chromatography with electron capture detection (GC-ECD) (Romer *et al*., 1987). T-2 toxin was previously tested on animals in order to obtain its LD_{50} value, (Litchfield *et al*., 1949). It was thereafter used in the current experiment as a single dose of 0.23 mg/kg *sc*.

Methylprednisolone

Two commercially available formulations of methylprednisolone were used in this experiment: Lemod-solu® and Lemod-depo®. The former one contains methylprednisolone sodium succinate dissolved in 1 ml of 0.9% benzyl alcohol, while the latier one contains methylprednisolone acetate. Both formulations contain 40 mg of the active substance per 1 ml solution. The dose administered in this experiment was 40 mg/kg *im*.

Pathohistological procedure

Animals were sacrificed after the end of treatment days 1, 3, 5 and 7, respectively. Heart samples were fixed in 10% neutral formalin for 15 days. After the process of fixation they were dehydrated in graded alcohol, xylol and paraffin wax. Finally, 2 μm thick paraffin slices were stained by haematoxylin and eosin (HE) method.

Quantitative analysis

The type, degree and intensity of pathohistological changes and an amount of inflammatory cells in the heart's samples were counted in 10 randomly selected visual fields, magnified by 40x. Myocardial changes were scored according to the grades showed in Table 1.

Table 1. Pathohistologic grades of myocardial toxicity in rats treated with T-2 toxin

Grade	Definition
0	Normal histological structure of myofibrils
1	Minority cells with early myofibrillar loss and normal nuclear architecture
2	Groups of cells (more than 50%) with marked myofibrillar loss and citoplasmatic vacuolisation
3	Majority cells with homogenization and hyalinization of cytoplasm with karyopicnosis and focal accumulation of inflammatory cells
4	Diffuse discoidal fragmentation of citoplasm with karyorrhexis or karyolysis and diffuse acumulation of inflammatory cells

Morphometric analysis

Myocardial changes were examinated with a standard microscope connected to a computerized video system and analyzed with Image-analysis software (Laboratory IMP, Belgrade).

Statistical analysis

The data were analyzed using commertial statistical software (Sigma Stat for Windows, R.4.5, Stat Soft, Inc., USA, 1993). Results were showed as mean ($\times$) $\pm$ standard deviation (SD). For comparation of data Student's test was used. For comparation of multiple groups one-way analysis on ranks, post-hock analysis and Tuckey's test were performed.

RESULTS

General condition of experimental animals

During the 7 day period of observation, no significant changes in general health and appearance of the surviving animals could be seen between groups. All the rats were in good shape. Their hair, skin, visible mucoses, muscle tonicity were without changes and their movements and co-ordination were preserved and comparable to the control animals.

Effects of methylprednisolone on 24-hour survival rate in rats poisoned with T-2 toxin

Registration of 24-hour survival rates revealed that all the glucocorticoid regimens significantly decreased the lethal effects of T-2 toxin. Based on the results shown in Table 2, it can be seen that the highest protective index (2.64) was obtained with the depot form of methylprednisolone, although this value is not significantly different from those of the remaining two corticosteroid regimens (Table 2).

Table 2. Effects of various methylprednisolone regimens on 24-hour survival in rats poisoned with T-2 toxin

Methylprednisolone	T-2 toxin LD_{50} (mg/kg *sc*)	95% confidence limits	f(LD_{50})	Protective index
Lemod-solu®	0.44	0.35-0.55	1.25	2.43
Lemod-depo®	0.48	0.36-0.63	1.32	2.64
Lemod-solu® + Lemod-depo®	0.45	0.30-0.45	1.48	2.48

Pathoanatomy examination

All the sacrificed rats were in good shape. Their hair, skin, visible mucosa and muscle tonicity were without any changes.

Pathohistological analysis of the heart in the control groups

On microscopic examination of the heart control in the animals (Figure 1) and those treated by Lemod-solu® or/and Lemod-depo® showed no pathological changes (Figure 2, 3, 4).

Pathohistological analysis of the heart in the T-2 toxin group

Myocardial alterations detected in poisoned animals ranged from degeneration to diffuse necrosis of myocardiocytes to massive vascular changes. Such areas were most prominent in the inner part of the myocardium and in all layers of the endocardium. In areas where wavy myocardial fibres are present an extensive myofibrillar fragmentation can be seen. In the animals sacrificed on the third day of the experiment, degenerative changes were predominant, including intracellular oedema, parenchymal and hyaline degeneration. These irregular,

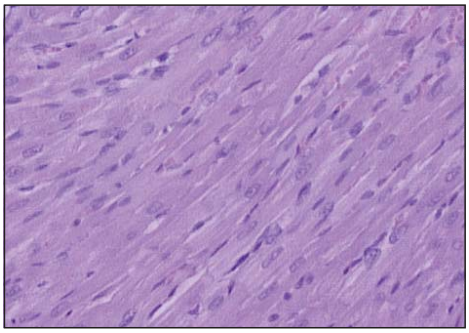

Figure 1. Myocardium of control rats (HE, 20x)

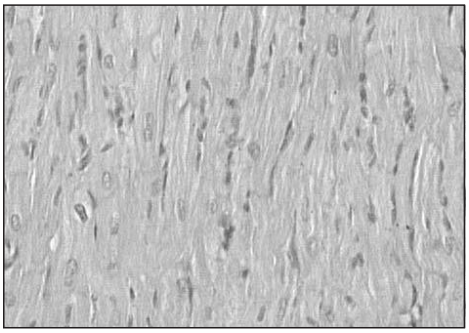

Figure 2. Normal histological structure of myofibrils in rats treated with Lemod-solu® (HE, 20x)

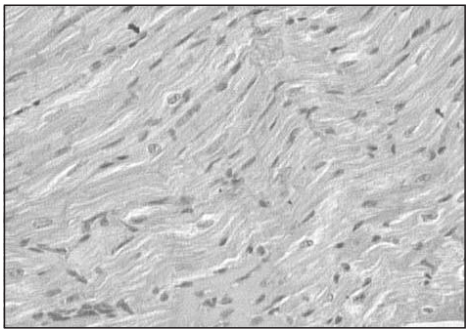

Figure 3. Inner part of myocardium in rats treated with Lemod-depo® (HE, 20x)

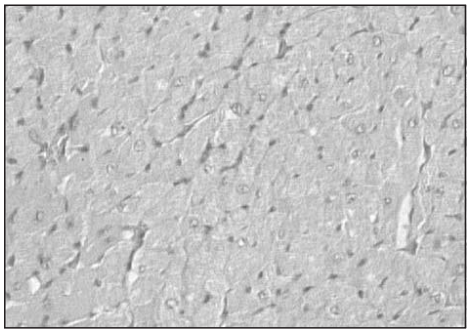

Figure 4. Heart of rats treated with Lemod-solu and Lemod-depo® (HE, 20x)

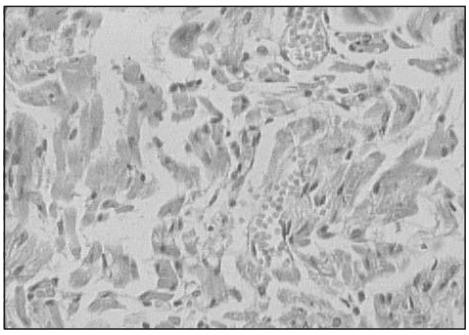

Figure 5. Myocardium of rats poisoned with T-2 toxin, day 7 (HE, 20x)

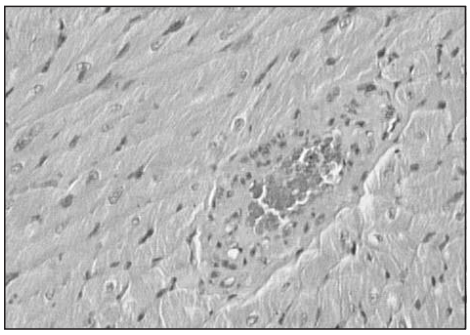

Figure 6. Heart of poisoned rats treated with Lemod-solu®, day 7 (HE, 20x)

round to ovoid cells developed dissolution of cytoplasm with granularity of myofilaments, consistent with myofibrillar degeneration. In the majority of these myocardial cells nuclear polymorphism was present, with large, round to rectangular shapes and prominent nucleoli. Retractile, slightly basophilic material was deposited at one pole of the nucleus in all the myofibrils. Thickening of blood vessels with vacuolisation of endothelial cells was observed. Massive necrosis was accompanied by mononuclear infiltration. The most striking finding was the presence of haemorrhagic foci in the interstitium that separates the bundles and fibres of myocardium. This interstitial haemorrhage appeared uniformly in each of the examined sections, and is located in the middle myocardial or subendocardial areas. Described pathohistological changes were most intensive in the heart of rats sacrificed on day 7 of the study (Figure 5).

Pathohistological analysis of the heart in T-2 toxin and Lemod-solu® group

The histological changes observed in the heart sections of these animals varied from intracellular oedema to mild parenchymal degeneration of myocardial cells and mild haemorrhagic infiltration. These areas were distributed focally in the myocardium and some layers of the endocardium. Fragmentation of myofibrils was observed in about 20-30 percent of the myocardiocytes. The minority of these cells were irregular, round to ovoid with dissolution of cytoplasm or granularity of myofilaments. A small number of myocardial cells with nuclear polymorphism and large, round to rectangular shapes and prominent nucleoli were seen. In some sections of myofibrils basophilic material at one pole of the nucleus was deposited. Thickening of blood vessels with vacuolisation of endothelial cells was observed. Focal accumulation of mononuclear cells was found in the vicinity of blood vessels. The most interesting finding was focal hyperaemia in the interstitium without massive haemorrhage. These pathohistological changes were mildest in the myocardium of rats sacrificed on day 7 of the study (Figure 6).

Pathohistological analysis of hearts in T-2 toxin and Lemod-depo® group

The quality of pathohistological changes in this experimental group was similar to the ones observed in the poisoned rats protected with the soluble form of methylprednisolone. However, the intensity of degeneration and vascular infiltration was strongest in the Lemod-depo® group. The presence of mononuclear cell infiltrates, diffuse hyperaemia and haemorrhagic foci were most prominent in the inner part of the myocardium and in all layers of the endocardium. The cytoplasm of degenerated cells contained granulated myofilaments and large,

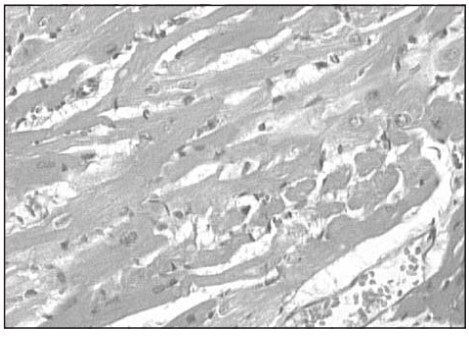

Figure 7. Myocardium of poisoned rats treated with Lemod-depo®, day 7 (HE, 20x)

ovoid, round or rectangular prominent nuclei. In the majority of these myocardial cells basophilic material deposits were present. Thickening of blood vessels with vacuolisation of endothelial cells was observed. The pathohistological changes described were most intensive in the heart of rats sacrificed on day 1 and 3 of the study (Figure 7).

Pathohistological analysis of hearts in T-2 toxin and Lemod-solu® and Lemod-depo® group

The myocardial alterations detected in this group of rats were similar to those in the T-2 toxin treated group. These histological changes varied from parenchymatous and hyaline degeneration to focal necrosis of myocardial cells, and diffuse hyperaemia or massive haemorrhagic infiltration. These interstitial haemorrhages appeared in each of the examined sections, and were located in the middle myocardial or subendocardial areas. In areas of wavy myocardial fibres, myofibrillar fragmentation was seen, as well. The altered cells showed dissolution of cytoplasm with granularity of myofilaments, consistent with myofibrillar degeneration. In all of these damaged cells nuclear polymorphism, with large, round, ovoid or rectangular nuclei and granular or dissoluted cytoplasm were present. In the minority the cells nuclei could not be seen at all. In the inner part of the myocardium blood vessels with vacuolisation of endothelium were observed. The described pathohistological changes were most intensive in the myocardium and endocardium of rats sacrificed on day 3 and day 7 of the study (Figure 8).

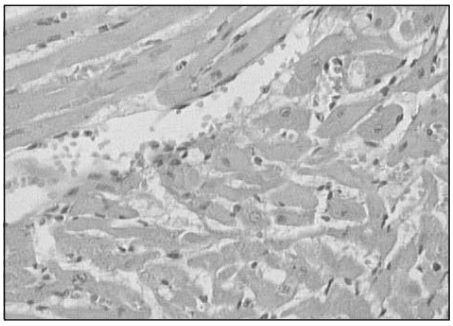

Figure 8. Myocardium and endocardium of T-2 toxin poisoned rats treated with Lemod-solu® and Lemod-depo®, day 7 (HE, 20x)

Effects of methylprednisolone on the degree of heart's damage in rats treated with T-2 toxin

In animals treated with T-2 toxin only, over the period of the first week after poisoning a gross degenerative damage in all parts of the heart could be seen. These values remained significantly higher than those in the control animals. In the endocardium, and especially in the myocardium, the intensity of these pathohistological changes significantly increased during the whole experimental period. A single injection of different formulations of methylprednisolone in poisoned rats showed a significant cardioprotective effect in comparison with the animals which received T-2 toxin only. During the whole experimental period, these values remained significantly higher than those in the control animals. The best cardioprotective effect was obtained with the soluble form of methylprednisolone, which was most prominent on the first day of the experiment.

Table 3. Influence of different treatments on the degree of damage in different structures of the heart of rats ($\times \pm$ SD)

Days	Parts of the heart	TREATMENT							
		Control	LS	LD	LS+LD	T-2	T-2+LS	T-2+LD	T-2+ LS+LD
1.	EPI	0.0 ± 0.0	0.3 ± 0.5 **a**	0.4 ± 0.5 **a**	0.2 ± 0.4 **a**	2.5 ± 0.5 **a**	1.7 ± 0.5 **ab**	2.3 ± 0.5 **a**	2.5 ± 0.5 **a**
	MYO	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.5 **a**	0.6 ± 2.9 **a**	2.7 ± 0.5 **a**	1.7 ± 0.5 **ab**	2.3 ± 0.5 **a**	2.5 ± 0.5 **a**
	END	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.5 **a**	0.6 ± 0.9	2.9 ± 0.5 **a**	1.7 ± 0.5 **ab**	2.3 ± 0.5 **a**	2.5 ± 0.5 **a**
3.	EPI	0.0 ± 0.0	0.3 ± 0.5 **a**	0.4 ± 0.5 **a**	0.2 ± 0.4 **a**	2.7 ± 0.5 **a**	1.5 ± 0.5 **ab**	2.2 ± 0.4 **a**	2.4 ± 0.5 **a**
	MYO	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.5 **a**	0.6 ± 0.5 **a**	3.0 ± 0.0 **a**	2.0 ± 0.0 **ab**	2.4 ± 0.5 **ab**	3.0 ± 0.0 **a**
	END	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.5 **a**	0.6 ± 0.5 **a**	3.0 ± 0.0 **a**	1.6 ± 0.5 **ab**	2.4 ± 0.5 **ab**	2.7 ± 0.5 **a**
5.	EPI	0.0 ± 0.0	0.4 ± 0.5 **a**	0.5 ± 0.5 **a**	0.4 ± 0.5 **a**	3.0 ± 0.0 **a**	1.5 ± 0.5 **ab**	2.0 ± 0.0 **ab**	2.1 ± 0.3 **ab**
	MYO	0.2 ± 0.4	0.4 ± 0.4 **a**	0.5 ± 0.5 **a**	0.8 ± 0.4 **a**	3.3 ± 0.5 **a**	2.0 ± 0.0 **ab**	2.4 ± 0.5 **ab**	2.5 ± 0.5 **ab**
	END	0.2 ± 0.4	0.4 ± 0.4 **a**	0.5 ± 0.5 **a**	0.8 ± 0.0 **a**	3.0 ± 0.0 **a**	2.0 ± 0.0 **ab**	2.3 ± 0.5 **ab**	2.6 ± 0.5 **a**
7.	EPI	0.0 ± 0.0	0.4 ± 0.5 **a**	0.6 ± 0.5 **a**	0.8 ± 0.4 **a**	3.5 ± 0.5 **a**	1.8 ± 0.4 **ab**	2.0 ± 0.0 **ab**	2.0 ± 0.0 **ab**
	MYO	0.2 ± 0.4	0.4 ± 0.5 **a**	0.6 ± 0.5 **a**	0.8 ± 0.4 **a**	3.5 ± 0.5 **a**	2.0 ± 0.0 **ab**	2.4 ± 0.5 **ab**	2.7 ± 0.5 **ab**
	END	0.2 ± 0.4	0.4 ± 0.5 **a**	0.6 ± 0.5 **a**	0.8 ± 0.4 **a**	3.3 ± 0.5 **a**	1.8 ± 0.4 **ab**	2.0 ± 0.0 **ab**	2.6 ± 0.5 **ab**

EPI – Epicardium; MYO – Myocardium; END – Endocardium
a – $p<0.05$ versus control group; **b** – $p<0.05$ versus T-2 toxin group

The histological structure of rat's heart from that group was close to the control group. The cardioprotective effect was registered in the other two corticosteroid groups, but the values obtained were significantly less than in the control and soluble methylprednisolone-treated groups. In animals treated with Lemod-solu® or/and Lemod-depo® *per se* statistical significance was examined in comparison with the control group (Table 3, Figure 9).

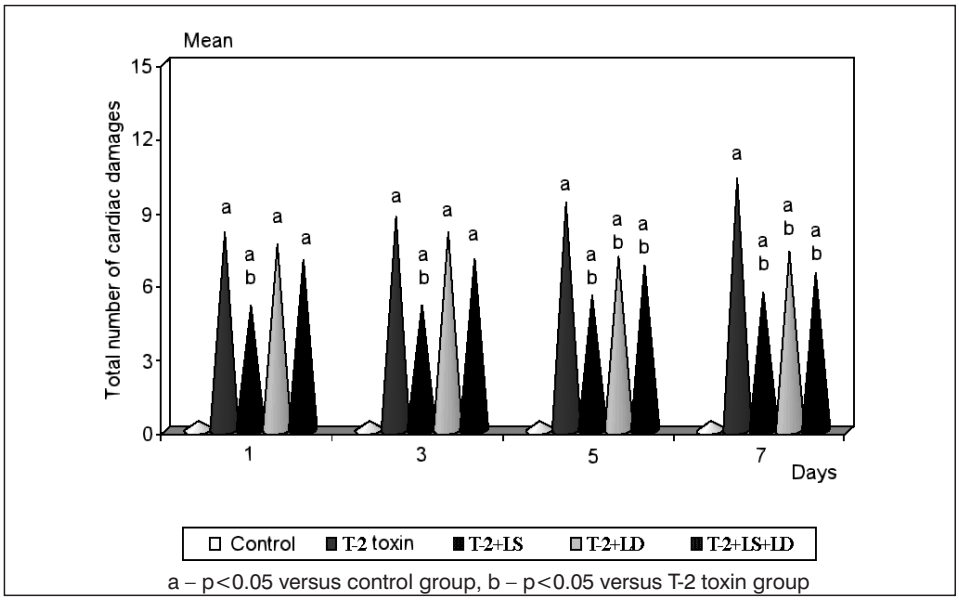

Figure 9. Influence of different treatments on the total degree of damage in the heart of rats (× ± SD)

DISCUSSION

Based on these results, and on the those obtained in our previous studies (Jaćević *et al*., 2001; Stojiljković *et al*., 2001), it is clear that T-2 toxin is one of the most important and strongest mycotoxins that induces extremely harmful changes in the organism of both humans and animals (Anonimus 2003). The observed changes were followed by clinical symptoms, the intensity of which did not correlate with the degree of the alterations found in various organs. The observed changes depend on the animal species, age and gender of the individual, route of administration, quantity of the toxin applied and length of its persistence in the organism (Mutoh *et al*., 1988).

Considering these facts, and the T-2 toxin general toxicity (Speijers and Speijers, 2004), one of the most important target organs in T-2 toxin poisoning is the heart (Yarom *et al*., 1983). Clinical signs of its damage are similar in both humans and animals. In our study, signs of inflammation, degeneration and rapid

loss of normal cell architecture could be found in the hearts of T-2 toxin treated rats sacrificed after the end of day 1 of the trial. Similar pathohistological alterations, vacuolisation and hyaline degeneration of myocytes with karyopicnosis, have been reported in the heart of humans and animals during acute T-2 toxin poisoning (Sudakin, 2003). If the disease persisted long enough (in our experiment this was a 7 day period) pathohistological changes in the heart of T-2 toxin-poisoned rats ranged from intracellular and interstitial oedema to parenchymal and hyaline degeneration with focal accumulation of mononuclear cells. Thickening of blood vessels with vacuolisation of endothelial cells was observed, too. It was suggested that T-2 mycotoxin may have a direct toxic effect on the capillaries and increase their permeability thus leading to diffuse infiltration with mononuclear cells (Jaćević, 2001). Furthermore, in the heart tissue, electron microscopy analysis showed hydraulic degeneration of mitochondria with peripheral condensation of the nuclear chromatin and lysis of sarcolemma. Finally, necrosis of the muscle cells could be found, as well (Sinovec, 1996).

In our experiment, a single administration of methylprednisolone significantly decreased the toxic effects of T-2 toxin which is in accordance with our earlier results and corroborates by the antidotal efficacy of prednisolone and of dexamethasone in rats poisoned with T-2 toxin (Jovanović 1992). These facts confirmed the hypothesis that the pro-inflammatory mechanism of T-2 toxin is the most important factor of its toxic effect on irreversibile post-mitotic or permanent cells, as well as myocytes (Jaćević *et al*., 2002; Jaćević, 2003). Methylprednisolone induced the decrease of gross pathohistological alterations in the heart of T-2 toxin poisoned rats, supports the assumption that glucocorticoids have a complex role in the protection of animals against T-2 toxin (Tremel *et al*., 1985). The best protective effects were produced by the soluble form of methylprednisolone. In these heart sections histological changes observed varied from focal intracellular oedema to parenchymal and hyaline degeneration of myocardial cells and mild haemorrhagic infiltration. Fragmentation of myofibrils was observed in about 50 percent of the myocardiocytes. Although the degree of intensity and quality of these pathohistological changes was similar to the ones observed in poisoned rats protected with the soluble form of methylprednisolone, the intensity of degeneration and vascular infiltration was stronger in the Lemod-depo® group. This result is probably a consequence of the fact that even the soluble form of methylprednisolone acts long enough to cover the peak of the T-2 toxin harmful effects, while, on the other hand, the depot form of methylprednisolone starts to act soon enough to counteract the first T-2 toxin-induced manifestation of poisoning. On the other hand, the acute focal myocardial changes caused by the T-2 toxin are not specific. These types of cardiac lesions can be caused by a number of cardiotoxic drugs (Dragojević-Simić, 2004). The pathogenic mechanism of these drugs is different and often multifunctional like the pro-inflammatory mechanism of T-2 toxin (Bondy and Pestka, 2000; Meky *et al*., 2001). Numerous investigations showed that corticosteroids have powerful anti-inflammatory and immunosuppressive effects. Glucocorticoids inhibit both the early and the late manifestation of inflammation. They can suppress the

production of prostanoids and COX-2 expression, generation of cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, TNF-γ and cell adhesion factors), reduce the concentration of complement components in the plasma, generate the induction of nitric oxide, activate macrophages of mast cells and IgG production (Barnes and Adcock, 1993; Smith, 1996; Sugita-Konishi and Pestka, 2001).

Quantitative and morphometric analysis of degenerative changes in the heart of poisoned rats trated with soluble or/and depo form of methylprednisolone, sacrificed at different time intervals, have been similar in all parts of the heart, but their intensity was decreased at the end of the study. Namelly, therapeutical use of different formulations of methylprednisolone showed a highly significant cardioprotective efficacy in poisoned non-treated rats. These antiinflammatory drugs successfully stopped the development of toxic and inflammatory myofibrilar damages, which probably evolve due to the influence of T-2 toxin on decreased sintesis of ATP and activation of phospholipase (Sinovec, 1996). A single injection of metylprednisolone increased the sintesis of ATP and indirectly decreased an acute *intumesentio opaca* of myocytes which is one of the first signs of hypoxia caused by T-2 toxin. In the next few days, therapeutical use of methylprednisolone, especially its soluble form, inhibed further development of ishaemia, dissolution of ER, dissotiation of polysomes monosomes and irreversible pathohistological changes (Pestka *et al*., 2004; Larsen *et al*., 2004). In our experiment, not only intracellular oedema and parenchymal degeneration of myocardial cells were less intensive, but also the fragmentation of myofibrils was less evident, in poisoned rats protected with Lemod solu® compared with T-2 poisoned but unprotected rats.

Therefore, one of the most interesting and most important findings in our experiment was the observation that the myocardial alterations detected in the group of poisoned animals protected with a combination of Lemod-solu® and Lemod-depo® were similar to those in the unprotected T-2 toxin treated group. These histological changes varied from parenchymatous and hyaline degeneration to focal necrosis of all myocardial cells and diffuse hyperaemia or massive haemorrhagic infiltration. Such finding is probably the consequence of the influence of the combination of Lemod-solu® and Lemod-depo® on the heart of rats observed in intact animals. Namely, in these animals the combination of the two tested formulations of methylprednisolone produced damages of the myocardium, such as parenchymatous and hyaline degeneration, focal necrosis of myocardial cells and massive haemorrhagic infiltrations. Accordingly, we suppose that a tested dose of 80 mg/kg of methylprednisolone could have a cardiotoxic effect itself. The presence of myocardial necrosis suggests that the highest doses of glucocorticosteroides produced toxic effects. These cytotoxic effects of glucocorticosteroids were supported by the toxic influence of T-2 mycotoxin. These results confirmed that glucocorticoid hormones have an important and complex role in the pathogenesis of T-2 toxicity (Tremel *et al*., 1985; Klaassen, 2001).

Although some differences could be seen among the three corticosteroid regimens investigated, they were not conclusive enough to recommend only one of them as a regimen of choice for the treatment of T-2 toxicosis. Therefore, due to

the specific toxicokinetics and time-dependency of T-2 toxin action, the choice of a glucocorticoid is not crucial for the efficacy of the treatment of acute T-2 toxin poisoning (Jaćević, 2004). However, the choice could be important for the treatment of repeated or subacute poisonings (Jaćević, 2005).

It can be concluded that methylprednisolone, soluble and depot formulations, exert a significant protective effects on the heart of rats from T-2 toxin induced histological changes.

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UTICAJ RAZLIČITIH GLUKOKORTIKOSTEROIDNIH TERAPIJSKIH REŽIMA NA PATOHISTOLOŠKE PROMENE U SRCU PACOVA TROVANIH T-2 TOKSINOM

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SADRŽAJ

U okviru ovog rada praćen je uticaj različitih oblika metilprednizolona (Lemod-solu® i Lemod-depo®) apikovanih u dozi od 40 mg/kg *im* na patohistološke promene u srcu Wistar pacova akutno trovanih citotoksičnim trihotecenskim mikotoksinom, T-2 toksinom (0.23 mg/kg *sc*). Patohistološka, kvantitaivna i morfometrijska analiza histoloških preparata je vršena primenom nakon njihovog bojenja hematoksilin-eozinom (HE). Ispitivane životinje su žrtvovane 1, 3, 5. i 7. dana eksperimenta. U srcu trovanih pacova T-2 toksin je izazvao masivne, difuzne, degenerativne i vaskularne promene koje su bile okružene sa velikim nekrotičnim poljima. Opisane promene bile su najmanje izražene kod trovanih životinja koje su primale preparat Lemod-solu®. Ove fokalne patohistološke promene varirale su od parenhimatozne distrofije do hijaline degeneracije, hiperemije, hemoragija i mononuklearnog ćelijskog infiltrata. Kod malog broja ćelija citoplazma je bila razložena, a njihova jedra su imala normalan izgled ili su bila blago izdužena ili okrugla. Prisustvo bazofilnog, granuliranog ćelijskog detritusa moglo se uočiti na jednom polu malog broja miofibrila. Veoma je važno naglasiti da su ove promene normalne histološke arhitekture miokarda bile isključivo fokalnog karaktera. Na osnovu prikazanih rezultata može da se zaključi da metilprednizolonske formulacije - i kratko- i dugo delujuća - proizvode značajne antidotske efekte kod pacova trovanih T-2 toksinom.