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Vesna M. Jaćević[1], *Aleksandra S. Bočarov-Stančić*[2], *Radmila D. Resanović*[3], *Snežana B. Đorđević*[1], *Dubravko R. Bokonjić*[1], *Miloš P. Stojiljković*[4]

[1] National Poison Control Centre, Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia, petmedic@net.yu
[2] Hold. Comp. "Center for Bio-Ecology", Petra Drapšina 15, 23000 Zrenjanin, Serbia, microb.bec@beotel.net
[3] Institute for Poultry Disease, Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia, mimares@eunet.yu
[4] Actavis Trading Ltd., Representative Office, Omladinskih Brigada 21, 11000 Belgrade, Serbia, mstojiljkovic@health.sdu.dk

BASIC MECHANISMS OF THE CELLULAR ALTERATIONS IN T-2 TOXIN POISONING: INFLUENCE ON THE CHOICE AND RESULT OF THE THERAPY

ABSTRACT: T-2 mycotoxin, secondary metabolite of *Fusarium* fungi, is one of the most potent cytotoxic representatives of trichothecene mycotoxin type A. After ingestion, T-2 toxin affects actively dividing cells and irreversible post-mitotic cells. In our experiments, the best protective effects were produced by dexametasone (PI = 3.37) and different methylprednisolone formulations (PI = 2.43—2.64). Significant protective efficacy was shown by nimesulide (PI = 1.44) and N-acethyilcistein (PI = 1.29), but their values were higher in a combination with methylprednisolone (PI = 2.16—2.34). Radioprotector amifostine (WR-2721) expressed good protective effects (PI = 1.26) or/and different absorbent formulations, such as: activated charcoal (PI = 1.13) and variuos Min-a-zel® powder compounds, which are a well known zeolite clinoptilolite absorbents. Among the five zeolite regimens investigated, only Min-a-zel Plus® showed a significant protective effect (PI = 1.77). In summary, the steroidal anti-inflammatory drugs could be recommended as a regimen of choice for treatment of acute T-2 toxicosis, while nonsteroidal anti-inflammatory compounds, different absorbent formulations and their combinations with antioxidants or radioprotectors, could be important for the treatment of subacute and chronic T-2 toxin poisonings.

KEY WORDS: T-2 toxin, citotoxicity, inflamation, therapy

INTRODUCTION

T-2 toxin, as one of the most potent cytotoxic trichothecene mycotoxin type A, produces sometimes fatal toxic reactions in humans and animals (Pang et al., 1988; Anonymous, 2003; Josephs, 2004).

Acute high dose of T-2 toxin has a potentially inhibitory effect on the membrane structure, which consequently stimulates lipid peroxidation. T-2 toxin affects actively dividing cells and irreversible post-mitotic cells (Grizzle et al., 2004; Pestka 2004), interacts with a number of targets, including the ribosome and the mitochondria (Speijers and Speijers, 2004), and inhibits protein synthesis (Ueno, 1984) in all eukaryotic cells (Larsen et al., 2004). Pro-inflamatory actions of the T-2 toxin are probably the most important mechanisms of its acute toxicity, especially in permanent cells (Bondy and Pestka, 2000). They showed that trichothecene-mediated elevations in cytokines, chemokines, and other immune related proteins are preceded by up-regulation of mRNAs for these genes. The underlying mechanism for such effects is induction of COX-2 (Islam et al., 2002; Pestka et al., 2004). Also, in the heart tissue, T-2 toxin activates a large number of mast cells (Jaćević et al., 2001b; Jaćević, 2005; Jaćević et al., 2006), synthesis and degranulation of numerous mediators which play an important role in the pathogenesis of T-2 mycotoxicosis (Bondy and Pestka, 2000).

The aim of this study was to investigate the protective effects of different antidotes and their combinations on 24 hour survival and pathohistological changes in the gut and heart of the rats acutely poisoned with 1.0 LD_{50} T-2 toxin (T2).

MATERIAL AND METHODS

The experiment was performed on adult Wistar rats, weighing 180—220 g (Animal House, Military Medical Academy, Belgrade, Serbia). The animals were housed in plastic cages, under standard laboratory conditions (21°C, 12/24 hour light/dark cycle, commercial food and tap water *ad libitum*), before being randomized into experimental groups. One day before the experiment, animals were fasting. During the subsequent experiment, they were fed with standard laboratory food *ad libitum*. They were allowed access to fresh tap water *ad libitum*.

In order to obtain the optimal doses of dexamethasone (DM), methylprednisolone (Lemod-solu®, LS; Lemod-depo®, LD; and their combination, LS + LD), nimesulide (NM), N-acetylcysteine (Fluimucil®; NAC), Amifostine (WR-2721, AMI), Activated charcoal (AC) and different Min-a-zel® formulations (M, MP, MD, M32 and M+), a range of their doses was previously tested (Jovanović, 1992; Jaćević et al., 2001a; Jaćević et al., 2002; Jaćević et al., 2003; Jaćević et al., 2006) (Table 1).

Tab. 1 — Effects of various methylprednisolone regimens on 24-hour survival in rats poisoned with T-2 toxin

Regimens	T-2 toxin LD_{50} (mg/kg *sc*)	95% confidence limits	$f(LD_{50})$	Protective index (PI)
DM	1.65	1.35—1.80	1.67	3.37
LS	0.44	0.35—0.55	1.25	2.43
LD	0.48	0.36—0.63	1.32	2.64
LS + LD	0.45	0.30—0.45	1.48	2.48
NM	1.53	1.39—1.69	1.10	1.44
NAC	1.22	1.19—1.27	1.19	1.29
LS + NM	1.55	0.47—0.30	1.15	2.34
LS + NAC	0.76	0.51—1.12	1.48	2.16
NM + NAC	1.24	1.57—2.22	1.15	1.22
AMI	1.95	1.56—2.42	1.25	1.26
AC	1.31	0.95—1.74	1.27	1.13
M	1.31	1.05—1.33	1.37	1.17
MP	1.21	0.95—1.53	1.27	1.33
MD	0.95	0.75—1.19	1.26	1.04
M32	0.67	0.49—0.93	1.38	0.74
M+	1.61	1.42—1.81	1.13	1.77

The rats were randomly allocated to 18 groups, each of them consisting of 10 animals. Their treatments were:

(1) The control group, (2) T2, (3) T2 + DM, (4) T2 + LS, (5) T-2 + LD, (6) T-2 + LS + LD, (7) T2 + NM, (8) T2 + NAC, (9) T2 + LS + NM, (10) T2 + LS + NAC, (11) T2 + NM + NAC, (12) T2 + AMI, (13) T2 + AC, (14) T2 + M, (15) T2 + MP, (16) T2 + MD, (17) T2 + M32 and (18) T2 + M+.

Following the registration, 24 hour survival and pathohistological changes were monitored after 28 days. General health condition of the animals was monitored daily, throughout the whole experimental period (four weeks).

Study protocol was based on the Guidelines for Animal Study no. 282--12/2002 (Ethics Committee of the Military Medical Academy, Belgrade, Serbia).

T-2 toxin (T2) used in these experiments was produced under the laboratory conditions from *Fusarium sporotrichoides* fungi, cultivated on synthetic GPY medium (glucose 5%, peptone 0.1%, yeast extract 0.1%, pH 5.4). 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) (R o m e r, 1987). T-2 toxin was preliminarily tested an animals in order to obtain its LD_{50} value (L i t c h f i e l d and W i l c o x o n, 1949; J a ć e v i ć et al., 2001a). It was thereafter used in the current experiment as a single dose of 0.23 mg/kg sc (1 LD_{50}).

Dexamethasone was used in a single dose of 20 mg/kg *sc*. Commercially available formulation of methylprednisolone, Lemod-solu® or/and Lemod-depo®, was used in a single dose of 40 mg/kg *sc*. A dose of 30 mg/kg of nimesulide was dissolved in 1 ml of 0.9% NaCl before *im* application. Fluimucil® contains 200 mg of N-acetylcysteine in 1 ml. It was a single injected *im* dose in these experiments. Activated charcoal in a dose of 1 g/kg was dissolved in

1 ml of 0.9% NaCl before *po* application. Amifostine (WR-2721) was synthesized of the Chemical Department of Military Technical Institute, Belgrade. Amifostine, in a dose of 50 mg/kg, was also dissolved in 1 ml of 0.9% NaCl immediately before *im* use. Five different commercially available formulations of Min-a-zel® absorbents were used in these experiments. These absorbents were dissolved in 1 ml of 0.9% NaCl before *po* administration.

Animals were sacrificed, after 28 ended treatment days. The gut and heart were excised and their samples were fixed in 10% neutral formalin for 5 days. Transmural tissue samples were dehydrated in graded alcohol, xylol and embedded in paraffin blocks. Finally, 2-μm of thick paraffin sections were stained by haematoxylin and eosin (H & E) method and analyzed (Olympus-2 microscope).

RESULTS AND DISSCUSION

Registration of 24 hour survival rates revealed that all the regimens significantly antagonized the lethal effects of T-2 toxin. Based on the results shown in Table 1, it could be seen that the highest protective index was obtained with DM and LD, respectively.

During the 4 week period, in the gastrointestinal tract, T-2 toxin caused diffuse epithelium deficit, erosions, ulcerations, hyperemia, transmural edema, atrophy of intestinal villi, cystic deformation of the stomach, and small intestine glands with diffuse accumulation of polymorphonuclear cells (Figure 1).

Myocardial alterations detected in the poisoned animals ranged from degeneration to diffuse necrosis of all the myocardiocytes and included massive vascular changes, too. Such areas were most prominent in the inner part of the

Fig. 1. — The small intestine of rats treated by T-2 toxin, 28 day (HE, 20x)

myocardium, and in all layers of endocardium. The most striking finding was the presence of haemorrhagic foci in the interstitium that separates the bundles and fibres of myocardium. This haemorrhage appeared uniformly in each of the examined sections, and was located in the middle of myocardial or subendocardial areas (Figure 2).

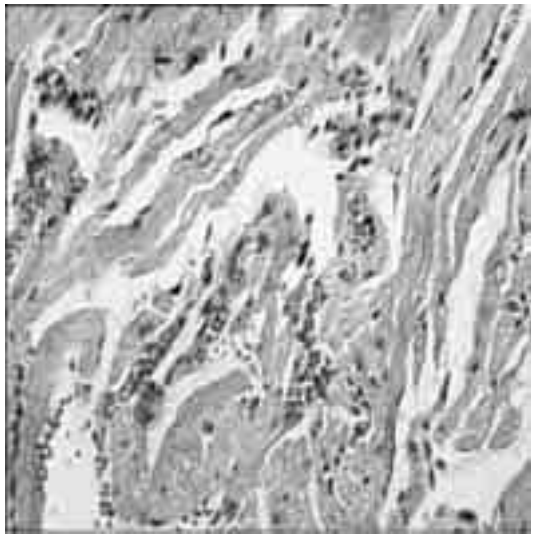

Fig. 2. — Miocardium of rats treated by T-2 toxin, 28 day (HE, 20x)

The histological changes observed from the gut section of these animals varied from intracellular edema to focal necrosis of the epithelial cells and mild hemorrhagic infiltration. These areas were present in the focal part of *the tunica mucosa* and some layers of *the tunica submucosa*. Dissolution and granularity of cytoplasm were observed in 50 percent of the stomach and small intestine. The presence of polymorphonuclear cell infiltration, diffuse hyperemia and hemorrhagic foci were more prominent in the poisoned group treated with LD, LS + LD, NM, NAC, AMI, AC, MD and M32. In the group of poisoned animals protected with LS, LS + NM or NAC, AMI + AC and M+, described histological changes were the smallest. After the 4 week period, the guts of rats treated with combination of LS and NM, or AMI + AC, and especially LS and M+ alone (Figure 3) had histological structure similar to the those of the control group.

The quality of pathohistological changes in this experimental group was similar to that observed in the poisoned rats, protected with solu form of methylprednisolone. However, the intensity of degeneration and vascular infiltration was stronger in NM, NAC, AMI, AC, MD and M32 groups. The presence of mononuclear cell infiltration, diffuse hyperaemia and haemorrhagic foci was more prominent in the inner part of the myocardium and all layers of endocardium. Single injection of LS in poisoned rats showed significant cardi-

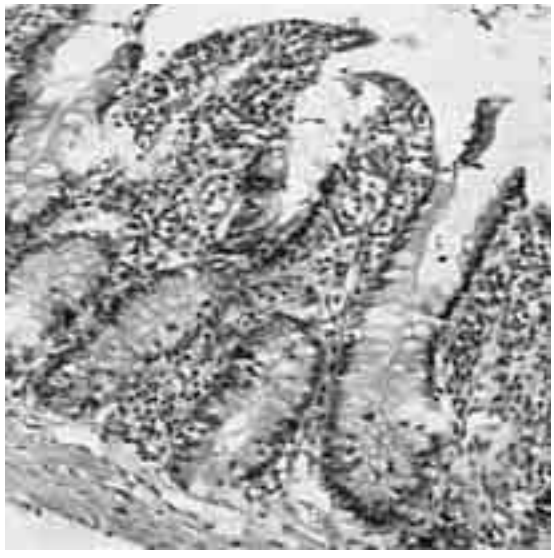

Fig. 3. — The small intestine of rats treated by T-2 toxin and M+, 28 day (HE, 20x)

oprotective efficiacy, in comparison with animals that received T-2 toxin only (Figure 4). During the whole experimental period, these values remained significantly higher than those in the control animals. Cardioprotective efficiacy was registered in the other protected groups, but the values obtained were significantly less than in the control group and LS.

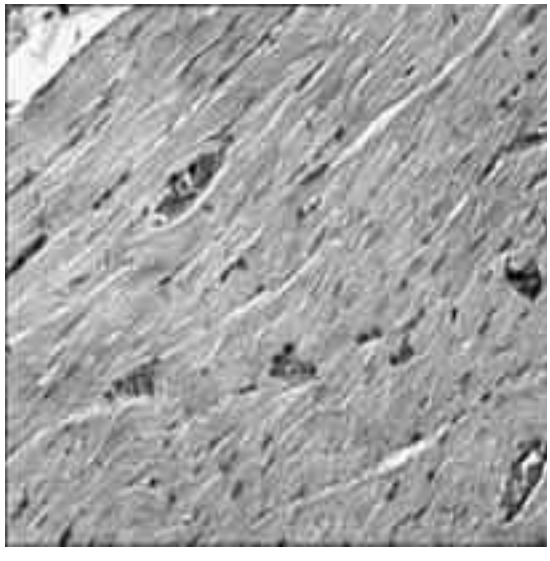

Fig. 4. — Myocardium of rats treated by T-2 toxin and LS, 28 day (HE, 20x)

CONCLUSION

In summary, the steroidal anti-inflammatory drugs could be recommended as a regimen of choice for treatment of acute T-2 toxicosis, while the nonsteroidal anti-inflammatory compounds, different absorbent formulations and their combinations with anti-oxidants or radioprotectors could be important for the treatment of subacute and chronic T-2 toxin poisonings.

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ОСНОВНИ МЕХАНИЗМИ ОШТЕЋЕЊА ЋЕЛИЈА КОД ТРОВАЊА Т-2 ТОКСИНОМ: УТИЦАЈ НА ИЗБОР И ИСХОД ТЕРАПИЈЕ

Весна М. Јаћевић[1], Александра С. Бочаров-Станчић[2], Радмила Д. Ресановић[3], Снежана Б. Ђорђевић[1], Дубравко Р. Бокоњић[1], Милош П. Стојиљковић[4]

[1] Центар за контролу тровања, Војномедицинска академија, Црнотравска 17, 11000 Београд, Србија, petmedic@net.yu

[2] А.Д. „Био-еколошки центар", Петра Драпшина 15, 23000 Зрењанин, Србија, microb.bec@beotel.net

[3] Институт за болести живине, Факултет ветеринарске медицине, Булевар Ослобођења 18, 11000 Београд, Србија, mimares@eunet.yu

[4] Здравље Actavis Compani, представништво, Омладинских бригада 21, 11000 Београд, Србија, mstojiljkovic@health.sdu.dk

Резиме

Т-2 микотоксин, секундарни метаболит гљивица из рода *Fusarium*, један је од најтоксичнијих представника трихотеценских микотоксина типа А. Његове основне особине, првенствено велика стабилност у природи, јефтина производња, тешка детекција и још увек непостојање адекватног антидота, чине га веома добрим потенцијалним бојним отровом. После уношења, у организму отроване јединке Т-2 токсин се у ћелијама везује за рецепторе на рибозомима и покреће серију каскадних реакција које за последицу имају смањење стабилности гРНК и повећану експресију проинфламаторних гена који су између осталог одговорни за настанак анорексије, губитак телесне масе, имуносупресију, аутоимуних ефеката и оштећење већине ткива. Токсично оштећење циљних органа, настало под дејством Т-2 токсина, последица је његовог цитотоксичног ефекта на лабилне ћелије и проинфламаторног ефекта на стабилне ћелије у организму животиња и

људи. С обзиром на напред изнете чињенице, јасно је што је у нашим истраживањима најбољи терапијски ефекат, код акутног тровања Т-2 токсином, постигнут применом антиинфламаторних лекова стероидне структуре, првенствено дексаметазона (ZI = 3,37) и различитих облика метилпреднизолона (ZI = 2,43—2,64). Осим тога, антиинфламаторни лекови нестероидне структуре испољили су значајан терапијски ефекат, нимесулид (ZI = 1,44) и N-acetlilcistein (ZI = 1,29), али се њихово заштитно дејство потенцира у комбинацији са метилпреднисолоном (ZI = 2,16—2,34). Терапијску ефикасност испољили су радиопротектор амифостин (WR-2721) (ZI = 1,26) и/или различити апсорбенси. Од примењених апсорбенаса, као што су активни угаљ (ZI = 1,13) и различити облици Мин-а-зел-а®, највећи протекивни ефекат испољио је Мин-а-зел Плус® облик клиноптиолинског зеолита (ZI = 1,77). На основу приказаних резултата, а у складу са чињеницом да је цитотоксично и проинфламаторно дејство Т-2 токсина у директној сразмери са његовом акутном токсичношћу, у потпуности је оправдано коришћење високих доза антиинфламаторних лекова стероидне структуре у терапији акутног тровања Т-2 токсином. Са друге стране, у терапији субакутних или хроничних тровања Т-2 токсином, препоручује се употреба антиинфламаторних лекова нестероидне структуре, различитих апсорбенаса, или њихове комбиноване примене са антиоксидансима или радиопротекторима.