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S. ILIC, D. DRMIC, K. ZARKOVIC, D. KOLENC, M. CORIC, L. BRCIC, R. KLICEK, B. RADIC, M. SEVER, V. DJUZEL, M. IVICA, A. BOBAN BLAGAIC, Z. ZORICIC, T. ANIC, I. ZORICIC, S. DJIDIC, Z. ROMIC, S. SEIWERTH, P. SIKIRIC

HIGH HEPATOTOXIC DOSE OF PARACETAMOL PRODUCES GENERALIZED CONVULSIONS AND BRAIN DAMAGE IN RATS. A COUNTERACTION WITH THE STABLE GASTRIC PENTADECAPEPTIDE BPC 157 (PL 14736)

Department of Pharmacology and Pathology, Medical Faculty University of Zagreb, Zagreb, Croatia

We focused on stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419, an anti-ulcer peptide efficient in inflammatory bowel disease trials (PL 14736), no toxicity reported) because of its hepatoprotective effects. We investigate a particular aspect of the sudden onset of encephalopathy with extreme paracetamol overdose (5 g/kg intraperitoneally) so far not reported: rapidly induced progressive hepatic encephalopathy with generalized convulsions in rats. BPC 157 therapy (10 µg, 10 ng, 10 pg/kg, intraperitoneally or intragastrically) was effective (µg-ng range) against paracetamol toxicity, given in early (BPC 157 immediately after paracetamol, prophylactically) or advanced stage (BPC 157 at 3 hours after paracetamol, therapeutically). At 25 min post-paracetamol increased ALT, AST and ammonium serum values precede liver lesion while in several brain areas, significant damage became apparent, accompanied by generalized convulsions. Through the next 5 hour seizure period and thereafter, the brain damage, liver damage enzyme values and hyperammonemia increased, particularly throughout the 3-24 h post-paracetamol period. BPC 157 demonstrated clinical (no convulsions (prophylactic application) or convulsions rapidly disappeared (therapeutic effect within 25 min)), microscopical (markedly less liver and brain lesions) and biochemical (enzyme and ammonium serum levels decreased) counteraction. Both, the prophylactic and therapeutic benefits (intraperitoneally and intragastrically) clearly imply BPC 157 (µg-ng range) as a highly effective paracetamol antidote even against highly advanced damaging processes induced by an extreme paracetamol over-dose.

Key words: *paracetamol, hepatic encephalopathy, convulsions, pentadecapeptide BPC 157, hyperammonemia, fatty liver*

INTRODUCTION

We investigate a particular aspect of the sudden onset of encephalopathy with paracetamol overdose (for review see 1-3) so far not reported: may paracetamol rapidly induce hepatic encephalopathy with generalized convulsions in rats and if so, the possible therapy effect in early and advanced stage. Supporting may be a dramatic decrease of glutathione levels in the rat brain after a paracetamol oral overdose (3.0 g/kg) (4) and brain tissue susceptibility to oxidative stress (high content of peroxidizable unsaturated fatty acids, high oxygen consumption per unit weight, but poorly developed antioxidative defense mechanisms) (5). So far, paracetamol 750 mg/kg per os (6) induced brain edema in rats but no behavioral changes. On the other hand, paracetamol did not influence convulsion or death that may be induced by pentetrazol (7), quinolones (8) or febrile seizures (9) or alternatively, it prevented pentylenetetrazol-induced seizures (10).

A solution to induce an acute hepatic toxicity and a rapidly progressing encephalopathy with severe seizures in rats may be a single paracetamol overdose (5 g/kg intraperitoneally) that excided the regimens previously used (1, 2, 6, 11). A counteracting agent may be the stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419, an anti-ulcer peptide per-orally active (12-15) that may also affect many central disturbances (16-23), efficient in inflammatory bowel disease trials (PL 14736) (24, 25) and various wound treatment (26-28), no toxicity reported (12, 13, 24, 25)) which showed various hepatoprotective effects (29-31). BPC 157 is stable in human gastric juice (more than 24 h), unlike rapidly degraded standard peptides such as h-EGF and h-TGF (12, 13), and is recognized to be a basal protectant in saliva and gastric juice (12, 13).

To demonstrate possible BPC 157 therapy capability as a paracetamol antidote in early and advanced stage of paracetamol toxicity, BPC 157 was applied intraperitoneally or intragastrically (12, 13) (i) prophylactically, immediately after paracetamol or (ii) therapeutically, after 3 hours elapsed. Presenting the generally known significance of paracetamol toxicity (1-3, 6, 11), this may reveal the role of BPC 157 (*i.e.*, a free radical scavenger (17, 29)) against the early paracetamol lesions development, and even more importantly, when the original damaging process induced by an extreme paracetamol over-dose was highly advanced.

MATERIALS AND METHODS

Animals

Male Albino Wistar (200 g) rats, were used in all of the experiments approved by the Local Ethic Committee (at least 10 rats per each experimental group per each period), assessed by observers naive about the given treatment.

Drugs

Medication, without a carrier or peptidase inhibitor, includes pentadecapeptide BPC 157 (a partial sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline; peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity, manufactured by Diagen, Ljubljana, Slovenia, GEPPPGKPADDAGLV, M.W. 1419 (12,13)) and paracetamol (Plicet, Pliva).

Drugs protocol and assessment

Paracetamol (5 g/kg) was intraperitoneally applied. Then, we applied BPC 157 (dissolved in saline, 10.0 μg, 10.0 ng, 10 pg/kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically as follows: (i) prophylactically, immediately after or (ii) therapeutically, after 3 hours elapsed. Histology and biochemistry assessment was carried out immediately after sacrifice after paracetamol challenge at 25 min (general seizure initiation), 3 h (at the time of maximal convulsion, used as the point of therapeutic application of BPC 157 regimens) and at 24 h post-paracetamol. Intensity of behavioral disturbances and seizure presentation was accordingly assessed. Assessment was (i) at 10, 20, 25, 30, 35, 60, 120, 240, 300, 360, 420, 480, and 1440 min following paracetamol when prophylactic regimen (BPC 157 immediately after paracetamol) was studied or (ii) when therapeutic regimen (BPC 157 at 180 min after paracetamol) was studied, the initial assessment was before therapy application, at 120 min and 180 min after paracetamol (*i.e.*, in conditions of generalized convulsions). Then, after BPC 157 or saline therapy, the subsequent assessment was in few next minutes' intervals at 190, 200, 210, 220, 230, 235, 240, 300, 360, 420, 480 and 1440 min following paracetamol (i.e., 10, 20, 30, 40, 50, 55, 60, 120, 180, 240, 300, 1260 min after delayed application of BPC 157 or saline therapy).

Histology assessment

Four-micron sections of formalin-fixed liver, embedded in paraffin were stained with haematoxylin and eosin. Two to three complete transections of each liver were reviewed at four levels by a board-certified pathologist who was blinded to treatments and strains. Steatosis, congestion, and necrosis were graded separately over the entire submitted tissue as previously described (11). The grading system used to measure the extent of damage was the same for steatosis, necrosis, and congestion as follows: score 0: normal, no abnormality; score 1: mild, <30% of cells or lobule affected; score 2: moderate, 30-60% of cells or lobule affected; and score 3: severe, >60% of cells or lobule affected. Criteria for necrosis included karyorrhexis (loss of nucleus) and/or degeneration of cytoplasm with either coagulative or liquefactive changes. Steatosis was defined as either microvesicular, if the vacuoles were multiple within the cytoplasm and did not indent the nucleus, or macrovesicular, if there was a single vacuole with displacement and distortion of the nucleus. Steatosis progresses from microvesicular to macrovesicular with severity; however, our criteria for evaluation were the number of cells affected, not the size of the vacuole within the cell. Congestion was identified by the expansion of the sinusoids with blood cellular elements. Inability to view hepatocytes because of congestion was not equated with necrosis (11).

The brain was fixed in 10% formalin during two days. Upon fixation, the brain was grossly inspected and cut by consecutive coronal sections. Brain slabs were dehydrated in graded ethanol and embedded in paraffin. Paraffin blocks were cut into 5 μm thin slices. Paraffin slices were deparaffinated in xylene, rehydrated in graded ethanol and stained with haematoxylin and eosin. Intensity and distribution of brain lesions (red neurons) (swollen or ballooned neurons are a future of the axon reaction and a variety of disease in which perikaryal changes occur independently of axonal damage; histologically, they appear as distended, weakly-staining cells with large, realtively clear nuclei) and brain edema were described and evaluated semiquantitatively (22). While 0 generally indicated no changes, the lesions were subsequently scored as follows: 0-3, edema (1- weak diffuse and/or perifocal; 2- moderate; 3- strong and generalized); 0-4, red neurons (1- <5% red neurons, 2- 5–30% red neurons, 3- 30–50% red neurons, 4- 50% red neurons).

Biochemistry assessment

To determine serum values (IU/l) of aspartate transaminase (AST), alanine transaminase (ALT), and ammonium blood samples were centrifuged for 15 min at 3000 rpm, immediately after death. All tests were measured on an Olympus AU2700 analyzer, with original test reagents (Olympus Diagnostica, Lismeehan, Ireland).

Intensity of behavioral disturbances and seizure presentation assessment

After paracetamol application, intensity of progressing behavioral disturbances and seizure presentation was continuously monitored, and assessed and scored (score 0 - normal, score 1 - rearing on hind legs, shaking, 2 - sleepy, tottering walk, 3 - sleepy, tottering walk and repeatedly falling down, 4 - sleepy, tottering walk and repeatedly falling down, jerking of the extremities, 5 - lying down, generalized convulsions) at particular time points depending on the regimen used, as described before.

Statistical analysis

Statistical analysis of the quantified data was performed by analysis of variance (ANOVA). Post hoc comparisons were appraised using the conservative Bonferroni/Dunn test. Data are presented as mean±standard deviation (SD). Non parametric statistic analysis was performed for categorical data using Kruskal-Wallis and post hoc Mann-Whitney U test. Values are expressed as min/med/max. Values of $P<0.05$ were considered statistically significant.

RESULTS

Liver lesions and biochemistry assessment

In controls, the grading system used to microscopically measure the extent of liver damage (the same for steatosis, necrosis, and congestion) showed no lesion presentation at the earliest point of 25 minutes after paracetamol (*Table 1*), but liver enzymes and ammonium serum values were already increased (*Table 2*). Then, after 3 hours, we found moderate lesions, and finally, after 24 hours severe lesions (*Table 1, Fig. 1, Fig. 2*) accordingly with further increase of liver enzymes and ammonium serum values (*Table 2*), unless BPC 157 was given within μg-ng range, intraperitoneally or intragastrically (*Table 1, Table 2, Fig. 1,*

Table 1. Prophylactic effect of pentadecapeptide BPC 157 on paracetamol liver injury in rats. BPC 157 (10.0 µg, 10.0 ng, /kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically. Microscopy presentation (score 0-3), Min/Med/Max, **vs*. saline (control), at least P<0.05, assessed at 25 min, 3 hours and 24 hours post-paracetamol.

Assessment time after paracetamol (5 g/kg i.p.)	Route of medication	Medication immediately after paracetamol	Paracetamol liver injury in rats		
			Microscopical presentation of liver lesion (score 0-3)		
			Steatosis	Necrosis	Congestion
25 min	i.p.	**Saline**	**0/0/0**	**0/0/0**	0/0/0
		BPC 157 µg	0/0/0	0/0/0	0/0/0
		BPC 157 ng	0/0/0	0/0/0	0/0/0
		BPC 157 pg	0/0/0	0/0/0	0/0/0
	i.g.	**Saline**	**1/2/3**	**1/2/3**	**1/2/3**
		BPC 157 µg	0/1/2*	0/1/1*	0/1/1*
		BPC 157 ng	0/1/3*	0/1/2*	0/1/2*
		BPC 157 pg	2/2/3	1/2/23	1/2/3
3 h	i.p.	**Saline**	**1/2/3**	**1/2/3**	1/2/3
		BPC 157 µg	0/1/2*	0/1/1*	0/1/1*
		BPC 157 ng	0/1/3*	0/1/2*	0/1/2*
		BPC 157 pg	1/2/3	1/2/3	2/2/3
	i.g.	**Saline**	**2/2/3**	**1/2/3**	1/2/3
		BPC 157 µg	0/1/2*	1/1/2*	0/1/1*
		BPC 157 ng	1/1/3*	1/1/3*	1/1/2*
		BPC 157 pg	1/2/3	0/2/3	1/2/3
24 h	i.p.	**Saline**	**2/3/3**	**2/3/3**	2/3/3
		BPC 157 µg	0/1/2*	1/1/2*	0/1/1*
		BPC 157 ng	1/1/2*	1/1/2*	1/1/2*
		BPC 157 pg	1/3/3	2/3/3	1/3/3
	i.g.	**Saline**	**2/3/3**	**2/3/3**	3/3/3
		BPC 157 µg	0/1/2*	1/1/2*	0/1/1*
		BPC 157 ng	0/1/2*	1/1/2*	1/1/2*
		BPC 157 pg	2/3/3	1/3/3	2/3/3

Table 2. Serum liver enzymes and ammonium values (IU/L) in rats that receive BPC 157 (10.0 µg, 10.0 ng/kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically immediately after paraceteamol. Min/Med/Max, **vs*. saline (control), at least P<0.05, assessed at 25 min, 3 hours and 24 hours post-paracetamol.

Assessment time after paracetamol (5 g/kg i.p.)	Route of medication	Medication immediately after paracetamol	Paracetamol liver injury in rats		
			Liver serum enzyme and ammonium values (IU/L)		
			ALT	AST	Ammonium
25 min	i.p.	**Saline**	**95/100/107**	**122/180/210**	**264/285/310**
		BPC 157 µg	41/45/48*	18/21/34*	101/122/153*
		BPC 157 ng	39/44/49*	19/24/41*	98/125/148*
		BPC 157 pg	90/95/109	112/171/220	95/119/145
	i.g.	**Saline**	**94/103/110**	**129/180/205**	258/279/313
		BPC 157 µg	40/46/49*	19/22/29*	105/130/150*
		BPC 157 ng	39/44/49*	19/24/41*	119/140/174*
		BPC 157 pg	89/113/120	135/188/215	256/298/300
3 h	i.p.	**Saline**	**380/414/430**	**121/161/179**	287/**310/333**
		BPC 157 µg	140/168/182*	42/50/62*	115/150/179*
		BPC 157 ng	256/270/295*	42/58/69*	125/144/173*
		BPC 157 pg	374/403/444	119/173/223	275/299/321
	i.g.	**Saline**	**370/418/459**	**127/161/188**	287/**310/333**
		BPC 157 µg	144/168/182*	48/50/66*	167/189/220*
		BPC 157 ng	245/270/292*	47/58/69*	170/178/213*
		BPC 157 pg	362/458/449	134/168/189	2288/312/344
24 h	i.p.	**Saline**	**619/714/785**	**142/161/189**	**350/440/480**
		BPC 157 µg	249/268/303*	60/70/89*	198/220/265*
		BPC 157 ng	331/370/405*	58/78/92*	188/219/256*
		BPC 157 pg	622/703/775	132/171/200	331/420/475
	i.g.	**Saline**	**630/714/810**	**139/161/190**	363/433/477
		BPC 157 µg	221/268/333*	59/70/93*	187/240/278*
		BPC 157 ng	311/370/419*	66/78/95*	178/232/281*
		BPC 157 pg	622/700/808	144/155/181	319/415/463

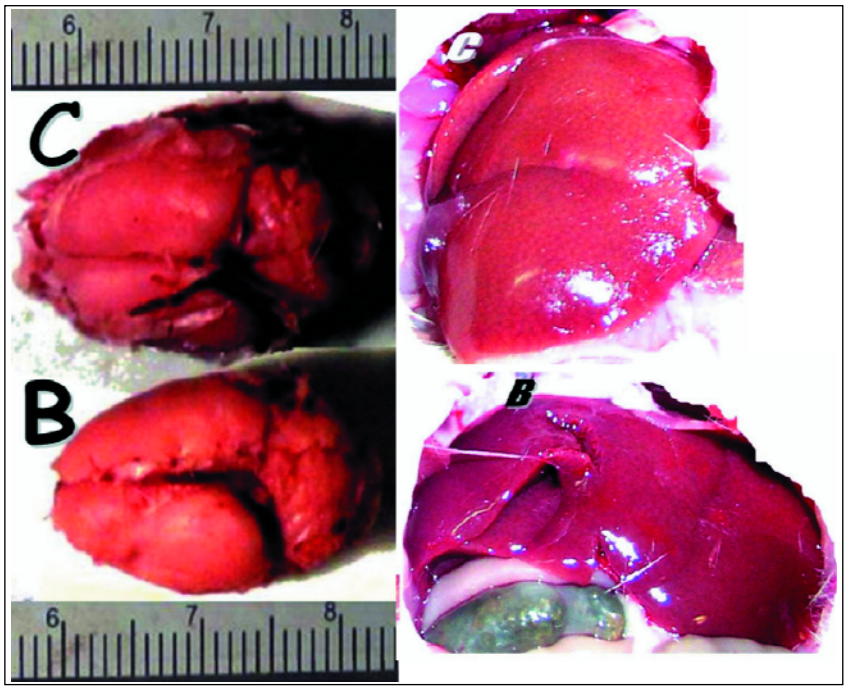

Fig. 1. Prophylactic effect of pentadecapeptide BPC 157 on paracetamol liver and brain injury in rats. Characteristic yellowish liver (C) and edematous brain (C) presentation in situ in control (saline (5.0 ml/kg)) or liver (*B*) and brain (B) presentation close to normal in BPC 157 (B) treated rats at 24 h after paracetamol application. Gross liver and brain appearance was not influenced by large *vs*. small dose of BPC 157 (10.0 µg, 10.0 ng/kg b.w.) or or intraperitoneal *vs*. intragastrical application.

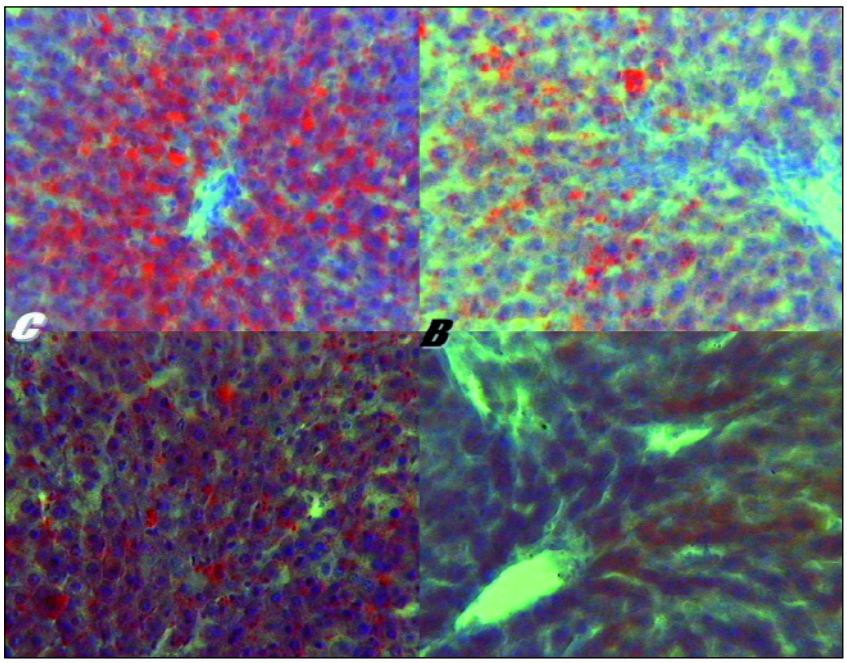

Fig. 2. Effect of pentadecapeptide BPC 157 on paracetamol liver injury in rats. Characteristic liver histology presentation in control (*C*) at 24 hours (upper) or at 3 hours (lower) post-paracetamol. Characteristic liver histology presentation at 24 hours post-paracetamol in BPC 157 rats (B) when BPC 157 had been given immediately after paracetamol (upper), or at 3 h after paracetamol (lower)). Suddan staining, 25x. BPC 157 (10.0 µg, 10.0 ng/kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically. Histologic appearance was not influenced by large versus small dose of BPC 157 (10.0 µg, 10.0 ng/kg b.w.) or or intraperitoneal *vs*. intragastrical application.

Fig. 2). In rats that received pentadecapeptide BPC 157 immediately after paracetamol, we found no lesions after 25 minutes or 3 hours post-paracetamol, and only mild lesions after 24 hours (*Table 1*) and consistently less liver enzymes and ammonium serum values (*Table 2*). When pentadecapeptide BPC 157 therapy was given in already advanced stage of paracetamol toxicity, such as at 3 hours after initial paracetamol application, only mild liver lesions (*Table 3, Fig. 2*) and markedly less liver enzymes and ammonium serum values (*Table 4*) could be noted at the final 24 hour post-paracetamol point.

Intensity of behavioral disturbances and seizure presentation

Regularly, paracetamol toxicity is presenting with progressing intensity of behavioral disturbances and seizure presentation, unless BPC 157 was given.

Shortly after paracetamol application, the rats started to rear on hind legs and shake. After 10 minutes, they became sleepy, with a tottering walk, repeatedly falling down while after 20 min they started to exhibit jerking of the extremities. After 25 minutes all rats were lying down, and all presented generalized convulsions, a status lasting the next 5 hours. Then, they regained posture, but were still sleepy, with a tottering walk, repeatedly falling down for next 2 hours. Then, rats started to drink excessively, then to eat and repeatedly drink water for a period of a few minutes. The rest of the time (7-24 h) the paracetamol rats survived normally although exhausted (*Table 5, Table 6*).

This course was markedly changed when BPC 157 was given within µg-ng range, intraperitoneally or intragastrically. Generally, BPC 157 rats had a markedly attenuated course completely preventing convulsions as well leading to full reversal when applied in rats exhibiting generalized

Table 3. Paracetamol liver injury in rats that received BPC 157 (10.0 µg, 10.0 ng/kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically at 3 hours after paracetamol challenge. Microscopy presentation (score 0-3), Min/Med/Max, *vs*. saline (control), at least P<0.05, assessed at the final interval (24 hours post-paracetamol).

Assessment time after paracetamol (5g/kg i.p.)	Route of medication	Medication at 3 hours after paracetamol	Paracetamol liver injury in rats		
			Microscopical presentation of liver lesion (score 0-3)		
			Steatosis	Necrosis	Congestion
24 h min	i.p.	**Saline**	**2/3/3**	**1/3/3**	**1/3/3**
		BPC 157 µg	0/1/2*	0/1/2*	0/1/2*
		BPC 157 ng	0/1/2*	0/1/3*	1/1/3*
		BPC 157 pg	1/2/2	2/3/3	1/3/3
	i.g.	**Saline**	**1/3/3**	**2/3/3**	**2/3/3**
		BPC 157 µg	0/1/2*	0/1/2*	0/1/1*
		BPC 157 ng	1/1/2*	0/1/3*	0/1/3*
		BPC 157 pg	2/3/3	1/3/3	1/3/3

Table 4. Serum liver enzyme and ammonium values (IU/L) in rats that received BPC 157 (10.0 µg, 10.0 ng, /kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically at 3 hours after paracetamol challenge. Min/Med/Max, *vs*. saline (control), at least P<0.05, assessed at the final interval (24 hours post-paracetamol).

Assessment time after paracetamol (5 g/kg i.p.)	Route of medication	Medication at 3 hours after paracetamol	Paracetamol liver injury in rats		
			Liver serum enzyme and ammonium values (IU/L)		
			ALT	AST	Ammonium
24h	i.p.	**Saline**	**714±37**	**161±21**	**410/480/530**
		BPC 157 µg	268±28*	70±18*	210/254/273*
		BPC 157 ng	370±41*	78±19*	198/235/281*
		BPC 157 pg	699±36	171±18	404/462/511
	i.g.	**Saline**	**738±54**	**151±16**	387/450/512
		BPC 157 µg	268±43*	70±13*	188/234/288*
		BPC 157 ng	370±28*	78±17*	222/260/311*
		BPC 157 pg	**719±36**	**162±22**	**381/422/467**

Table 5. Prophylactic effect of pentadecapeptide BPC 157 on paracetamol convulsions in rats. BPC 157 (10.0 µg, 10.0 ng, /kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically. Intensity of behavioral disturbances and seizure presentation, Min/Med/Max, *vs*. saline (control), at least P<0.05, assessed throughout the post-paracetamol period.

Medication (i.p. or i.g.) immediately after paracetamol 5 g/kg i.p.	Seizure presentation, score 0-5, Min/Med/Max, after therapy Post-paracetamol time (min)													
	10	20	25	30	35	60	120	180	240	300	360	420	480	1440
Saline i.p.	**1/1/2**	**2/3/3**	**4/5/5**	**4/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**2/3/4**	**1/3/3**	**0/0/0**	**0/0/0**
BPC 157 µg i.p.	0/1/2	1/1/2*	2/3/3*	1/1/2*	0/1/2*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
BPC 157 ng i.p.	1/1/2	1/1/2*	2/3/3*	1/1/2*	0/1/2*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
BPC 157 pg i.p.	1/1/2	2/3/3	4/5/5	4/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	2/3/4	1/3/3	0/0/0	0/0/0
Saline i.g.	**1/1/2**	**1/3/3**	**3/5/5**	**4/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**3/3/4**	**1/2/3**	**0/0/0**	**0/0/0**
BPC 157 µg i.g.	0/1/1	1/1/2*	1/3/3*	1/1/2*	0/1/1*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
BPC 157 ng i.g.	1/1/2	1/1/2*	1/3/3*	1/2/2*	0/1/2*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
BPC 157 pg i.p.	1/1/2	2/3/3	4/5/5	4/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	2/3/4	1/3/3	0/0/0	0/0/0

convulsions. When pentadecapeptide BPC 157 was given immediately after paracetamol, BPC 157 rats started to rear on hind legs and shake shortly after paracetamol application while after 10 minutes, they became sleepy, with a tottering walk, after 35 min they regained normal behavior, drank (but not excessively) and then ate and showed no behavioral abnormalities until their sacrifice (*Table 5*).

Moreover, presenting that the most threatening period may be the seizures-period, and the regular duration estimated to be 5 hours, and that at the time point of 3 hours following

Table 6. Paracetamol convulsions in rats that received BPC 157 (10.0 µg, 10.0 ng/kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically after 3 hours had elapsed following paracetamol challenge. Intensity of behavioral disturbances and seizure presentation, Min/Med/Max, before (120-180 min) and after (190-1440 min) therapy, **vs.* saline (control), at least P<0.05, assessed throughout post-paracetamol period.

Intensity of behavioral disturbances and seizure presentation BEFORE and AFTER therapy (score 0-5) and assessment time (min) *(post-paracetamol time (min) (paracetamol (time 0); post-therapy time (min) (therapy (time 0))*													
Seizure presentation BEFORE therapy		Medication (i.p. or i.g.) at 3 hours after paracetamol 5 g/kg i.p.	Seizure presentation, score 0-5, Min/Med/Max, AFTER therapy *Post-paracetamol time (min) (paracetamol (time 0))*										
120	*180*		*190*	*200*	*210*	*220*	*230*	*235*	*240*	*300*	*360*	*420*	*480*	*1440*
5/5/5	**5/5/5**	**Saline i.p.**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**3/3/4**	**1/2/3**	**0/0/0**	**0/0/0**
5/5/5	**5/5/5**	BPC 157 µg i.p.	3/5/5	2/3/3*	2/3/3*	1/3/3*	1/2/3*	0/0/1*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
5/5/5	**5/5/5**	BPC 157 ng i.p.	3/5/5	2/3/3*	2/3/3*	2/3/3*	2/2/3*	0/0/1*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
5/5/5	**5/5/5**	BPC 157 pg i.p.	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	3/3/4	1/2/3	0/0/0	0/0/0
5/5/5	**5/5/5**	**Saline i.g.**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**5/5/5**	**2/3/4**	**1/2/2**	**0/0/0**	**0/0/0**
5/5/5	**5/5/5**	BPC 157 µg i.g.	3/5/5	1/3/3*	1/3/3*	1/3/3*	1/2/3*	0/0/1*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
5/5/5	**5/5/5**	BPC 157 ng i.g.	4/5/5	2/3/3*	1/3/3*	1/3/3*	1/2/3*	0/0/1*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0	0/0/0
5/5/5	**5/5/5**	BPC 157 pg i.p.	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	3/3/4	1/2/3	0/0/0	0/0/0
		Medication (i.p. or i.g.) at 3 hours after paracetamol 5 g/kg i.p.	*10*	*20*	*30*	*40*	*50*	*55*	*60*	*120*	*180*	*420*	*160*	*1260*
			Post-therapy time (min) (therapy (time 0)) Seizure presentation, score 0-5, Min/Med/Max, AFTER therapy											

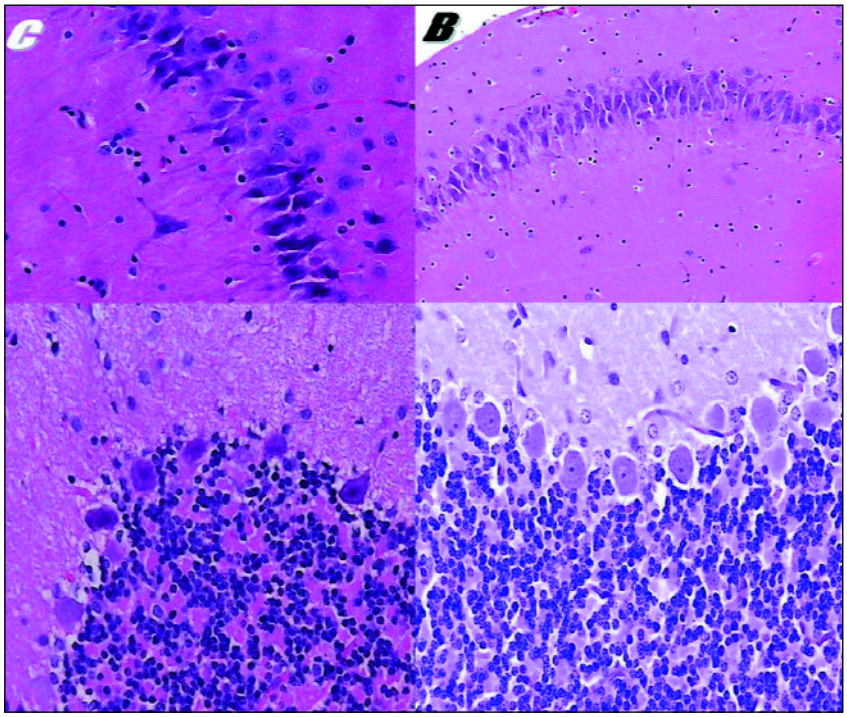

Fig. 3. Effect of pentadecapeptide BPC 157 on brain injury in rats. Characteristic brain histology presentation at 24 hours in control (C) and BPC 157 rats (B). Hippocampal red neurons (C, x 400), some hippocampal neurons are red (B, x 200) (upper) Purkinje cells are acidophilic (C, x 400) Regular morphology of Purkinje cells (B, x 400) (lower). HE. Histologic brain appearance was not influenced by large versus small dose of BPC 157 (10.0 µg, 10.0 ng/kg b.w.) or intraperitoneal *vs.* intragastrical application.

paracetamol the rats would exhibit generalized convulsions for the next two subsequent hours, it is interesting that after BPC 157 application the generalized convulsions disappeared within the next 25 minutes and the rats regained posture, still sleepy, with a tottering walk for the next 50 minutes. Then, the rats started to drink excessively, then to eat and repeatedly drink water for a period of a few minutes after which they regained normal behavior, drank and then ate and showed no behavioral abnormalities until their sacrifice (*Table 6*).

Brain lesions assessment

Regularly, after 24 hours we found a heavy interstitial edema in all rat brains, particularly in the cerebellum and more so in

Table 7. Prophylactic effect of pentadecapeptide BPC 157 on paracetamol brain injury in rats. BPC 157 (10.0 μg, 10.0 ng/kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically. Microscopy presentation, intensity and distribution of brain lesions (red neurons (0-4) and brain edema (0-3) were described Min/Med/Max, *_vs_. saline (control), at least P<0.05, assessed at 25 min, 3 hours and 24 hours post-paracetamol.

Assessment time after paracetamol (5 g/kg i.p.)	Route of medication	Medication immediately after paracetamol	Microscopical presentation of paracetamol brain injury in rats			
			Cerebral cortex (red neurons)	Hippocampus (red neurons)	Purkinje cells and cerebellar nn. (red neurons)	Edema
25 min	i.p.	**Saline**	**1/3/3**	**2/3/3**	**1/3/3**	**1/2/3**
		BPC 157 μg	0/0/1*	0/0/1*	0/0/1*	0/0/1*
		BPC 157 ng	0/0/1*	0/0/1*	0/0/1*	0/0/1*
		BPC 157 pg	2/3/3	1/3/3	2/3/3	2/2/3
	i.g.	**Saline**	**1/3/3**	**2/3/3**	**1/3/3**	**1/2/3**
		BPC 157 μg	0/0/1*	0/0/1*	0/0/1*	0/0/1*
		BPC 157 ng	0/0/1*	0/0/1*	0/0/2*	0/0/2*
		BPC 157 pg	1/3/3	1/3/3	1/3/3	1/2/3
3 h	i.p.	**Saline**	**1/3/3**	**1/3/4**	**1/3/3**	**2/2/3**
		BPC 157 μg	0/0/1*	0/0/1*	0/0/1*	0/0/1*
		BPC 157 ng	0/0/1*	0/0/1*	0/0/1*	0/0/2*
		BPC 157 pg	1/2/3	2/3/4	2/3/3	1/2/3
	i.g.	**Saline**	**2/3/4**	**2/3/4**	**1/3/4**	**2/2/3**
		BPC 157 μg	0/0/1*	0/0/1*	0/0/1*	0/0/1*
		BPC 157 ng	0/0/1*	0/0/1*	0/0/1*	0/0/1*
		BPC 157 pg	**1/3/4**	**1/2/4**	**1/3/4**	**2/2/3**
24 h	i.p.	**Saline**	**2/4/4**	**2/4/4**	**2/4/4**	**2/3/4**
		BPC 157 μg	0/1/2*	0/1/2*	0/1/2*	0/0/1*
		BPC 157 ng	0/1/2*	0/1/2*	/0/1/2*	0/0/1*
		BPC 157 pg	2/4/4	2/4/4	2/4/4	3/4/4
	i.g.	**Saline**	**3/4/4**	**3/4/4**	**2/4/4**	**2/3/4**
		BPC 157 μg	0/1/2*	0/1/2*	0/1/2*	0/0/1*
		BPC 157 ng	0/1/2*	0/1/2*	0/1/2*	0/0/1*
		BPC 157 pg	2/4/4	2/4/4	3/4/4	3/3/4

white than in gray mater. Neurons presented with severe damage. Severely damaged red neurons, without any inflammatory reaction, were present in all animals, in more then one half of hippocampal neurons, neurons of the dentate nucleus, Purkinje cells and other cerebellar nuclei. Other damaged neurons were in the pons and mesencephalon, particularly in the tegmental areas of these structures. Less than one third of neurons in the lateral geniculate body were changed like the red neurons. Interestingly, moderate edema was already present at 25 minutes following paracetamol application, at the time of initiation of the generalized convulsions. Damaged red neurons, without any inflammatory reaction, were present in all animals in more than 30% but less than 50% of hippocampal neurons, neurons of the dentate nucleus, Purkinje cells and other cerebellar nuclei. The severity of lesions in neurons further progressed after a 3 hour period leading to additional worsening, noted at the final 24 hour post-paracetamol point (*Table 7, Table 8, Fig. 1, Fig. 3*).

When pentadecapeptide BPC 157 was given immediately after paracetamol (μg-ng range, intraperitoneally or intragastrically), in accordance with a complete absence of convulsions, BPC 157 rats commonly had all alterations markedly less expressed 24 hours after paracetamol, reduced interstitial edema (only mild) and markedly less damaged neurons, without inflammatory reaction. Interestingly, these rats presented consistently less damage at the earliest points *i.e*., 25 minutes or 3 hours following paracetamol application, there was no edema and no damaged red neurons, without any inflammatory reaction (*Table 7, Fig. 3*).

Finally, for therapy effect of BPC 157 on paracetamol brain injury in rats that received pentadecapeptide BPC 157 later in an advanced paracetamol toxicity stage, we should consider that at the time point of 3 hours following paracetamol, as previously mentioned, after therapy these rats had initially exhibited generalized convulsions and had significant brain damage. However, they presented with markedly attenuated brain lesions at the final 24 hour post-paracetamol point. Notably, they exhibited lesser interstitial edema (only mild), markedly less damaged neurons than corresponding controls and had no inflammatory reaction (*Table 8*).

DISCUSSION

In an attempt to elucidate the sudden onset of encephalopathy with paracetamol overdose (3), this study established a consistent BPC 157 beneficial effect in paracetamol-rats, given in early or advanced stage of paracetamol toxicity. Also, an essential convulsive effect of paracetamol overdose may be a prime rapid and then sustain hallmark of paracetamol toxicity. With generalized convulsions, severe brain lesion and hyperammonemia and increased serum enzyme values already at 25 min post-paracetamol, it seems that toxins can rapidly reach the brain and affect its function, even before major liver lesions might appear, unless BPC 157 was given. Moreover, once started, following jerking of the extremities and coarse tremors (*i.e.*, they had to be preceded by the already advanced significant damage in the brain), the seizures lasted for a few hours throughout the progressing brain and liver damage (as seen with worsening throughout the 3-24 h post-paracetamol period). Again, the course was completely changed with BPC 157 application (*i.e.*, absence and reversal of

Table 8. Paracetamol brain injury in rats that received therapy BPC 157 (10.0 μg, 10.0 ng/kg b.w.) or saline (5.0 ml/kg) intraperitoneally or intragastrically at 3 hours after paracetamol challenge. Microscopy presentation, intensity scored (red neurons (0-4) and brain edema (0-3)) and distribution of brain lesions were described, Min/Med/Max, **vs*. saline (control), at least $P<0.05$, assessed at the final interval (24 hours post-paracetamol).

Assessment time after paracetamol (5 g/kg i.p.)	Route of medication	Medication at 3 hours after paracetamol	Microscopical presentation of paracetamol brain injury in rats			
			Cerebral cortex (red neurons)	Hippocampus (red neurons)	Purkinje cells and cerebellar nn. (red neurons)	Edema
24 h	i.p.	**Saline**	**2/4/4**	**2/4/4**	**2/4/4**	**2/3/4**
		BPC 157 μg	0/1/2*	0/1/2*	0/1/2*	0/0/1*
		BPC 157 ng	0/1/2*	0/1/2*	1/1/3*	0/0/1*
		BPC 157 pg	1/3/4	2/4/4	3/4/4	1/3/4
	i.g.	**Saline**	**3/4/4**	**2/4/4**	**3/4/4**	**1/3/4**
		BPC 157 μg	0/1/2*	1/1/2*	0/1/3*	0/0/1*
		BPC 157 ng	1/1/3*	1/1/3	0/1/3*	0/0/1*
		BPC 157 pg	2/4/4/	1/3/4	2/4/4	2/3/4

convulsion). Thus, this paracetamol over-dose toxicity, therefore, would be an effective means for the progressive hepatic encephalopathy and convulsions course in rat relation, at least. Thereby, it is possible that this consistent BPC 157 beneficial effect on rat's pathology underlies the sudden onset of encephalopathy with paracetamol overdose as well as paracetamol overdose as the leading cause of acute liver failure in patients (3). Also, this BPC 157 effect on the background of antagonization of high paracetamol regimen may be relevant for anatagonization of comparably lower doses of paracetamol.

Likely, the prime commencing of generalized seizures may be related to the largely described molecular paracetamol processes underlying the progressive hepatotoxicity (1, 2) (*i.e*., the covalent binding of NAPQI to the thiol groups of critical cellular proteins (following glutathione depletion), oxidative stress induced directly by NAPQI or *via* coproduction of reactive oxygen species (superoxide radical and hydrogen peroxide) through redox cycling between the paracetamol semiiminoquinone radical and NAPQI) (1, 2), and *vice versa*, these processes counteraction may be involved in BPC 157 beneficial effects. Thus, by being both more rapid and persistent in causing neurological abnormalities, these processes are even more destructive for brain (*i.e.*, paracetamol preferentially inhibited brain cyclooxygenases (32)), thereby generalized convulsions, particularly presenting brain tissue's high susceptibility to oxidative stress, leaving the glutathione in the brain defenseless to depletion by paracetamol (4, 5), unless BPC 157 was applied. Early concentrations of paracetamol in the brain (33) appear to be well-timed with the period of the rising concentration in various brain structures (33) and coincide with the noted brain damage and with the appearance of generalized seizures in the present study.

Therefore, the severe interstitial edema, more expressed in white than in gray mater, and severe neuron loss in hippocampal neurons, neurons of dentate nucleus, Purkinje cells and other cerebellar nuclei and less severe damage in the pons, the mesencephalon and lateral geniculate body could be a specific paracetamol effect and these territories could be involved in the occurrence of maximal paracetamol induced seizures. Besides, consistently higher paracetamol regimen may minimize all problems related to possible variations from animal to animal and from time to time in a given animal and invariably link paracetamol action with seizures, severely damaged brain tissue, hepatomegaly, fatty liver and necrosis, breakdown of liver function with profoundly increased ammonia, AST and ALT levels. Also, it may be more relevant for extreme paracetamol toxicity in patients (3).

Thus, when given to paracetamol-rats, BPC 157 would be confronted with the all processes simultaneously occurring that eventually lead to all mentioned disturbances in paracetamol over-dose-rats (1, 2). However, we shown that pentadecapeptide BPC 157, as an antiulcer peptide (12-15), may consistently counteract all paracetamol disturbances. This may also indicate that these disturbances are also interconnected throughout BPC 157 background. Moreover, considering the used paracetamol (5 g/kg i.p.)/BPC 157 (10 μg, 10 ng, 10 pg/kg i.p. or i.g., and effectiveness within μg-ng range) ratio (12, 13), it may be reasonably to assume that these therapy effects may indicate a likely role of BPC 157 in controlling, and then, counteracting one or more causative process(es).

Unfortunately, the more specific targets (1, 2) for these BPC 157 effects remained outside of the present investigation, but we should assume that in few hours period such a high paracetamol over-dose may be able also to reflect the disturbances that would otherwise require much longer period. Thus, the targets of BPC 157 counteraction may be at least partly approached within the frame of obtained paracetamol-damages. For instance, the most likely possibility may be, as mentioned before, the liver presenting BPC 157 beneficial effect on various liver lesions including CCl_4 hepatotoxicity (29-31). Counteracted hepatomegaly, fatty liver and necrosis and pronounced elevation of liver enzymes (AST and ALT) and hyperammonemia, and consequently, counteraction of paracetamol seizures and brain damages by BPC 157 may be also perceived in this context. Besides, BPC 157 as an anti-ulcer peptide antagonizes hepatomegaly, fatty liver, increased AST, ALT and amylase serum values, breakdown of liver glycogen with profound hypoglycemia, along with calcium deposition after huge insulin over-dose application (31). Also, in chronically alcohol drinking rats, BPC 157 may prevent and reverse portal hypertension (30). However, another mechanism may be that the pentadecapeptide BPC 157 can directly protect against paracetamol induced brain damage.

What's more, the premise that when given peripherally, BPC 157 may have a particular beneficial effect on CNS (*i.e*., markedly less damaged neurons in most severely injuried areas) is in accord with: its neuroprotective properties (22, 34), consistent antagonization of different central disturbances (16-23, 34), brain 5-HT synthesis and antagonization of serotonin-syndrome in rats based on region-specific influence on the brain given peripherally either acutely or chronically (*i.e.*, dorsal thalamus, hippocampus, lateral geniculate body, hypothalamus, dorsal raphe nucleus, substantia nigra, medial anterior olfactory nucleus, lateral caudate, accumbens nucleus, superior olive)) (shown by the very precise alpha-[14C]methyl-L-tryptophan (alpha-MTrp) autoradiographic method) (16, 20). Presenting the suggested significance of substantia nigra for controlling seizures (35), it may be important

that BPC 157/substantia nigra relation may be particularly substantiated: the increased 5-HT synthesis in substantia nigra was the most prominent one (20), counteracted 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) parkinsonian syndrome as well as the lethal outcome due to particular substantia nigra damage (17), maintained dopamine system function (BPC 157 may counteract akinesia, catalepsy induced by neuroleptics or reserpine as well as amphetamine-stereotypy behavior) (17-19). Also, BPC 157 was found to have anti-convulsive properties (16, 17, 21). Also very recently, with the respect of K^+-ATP channels in the substantia nigra as a predisposing factor for seizure development, BPC 157 inhibits K^+ conductance in WT HEK293 cells (36). Besides, after an induced traumatic brain injury in mice, BPC 157 regimens (corresponding to those used in the present study) demonstrated a marked attenuation of damage with an improved early outcome and a minimal postponed mortality throughout a 24 h post-injury period. Ultimately, the traumatic lesions (subarachnoidal and intraventricular haemorrhage, brain laceration, haemorrhagic laceration) were less intense and consecutive brain edema had considerably improved (22).

In conclusion, we showed the progressive hepatic encephalopathy, accompanied by severe seizures with a very early onset, in paracetamol-rats (*i.e.*, the full threatening circuit in paracetamol's acute hepatic toxicity). This was consistently counteracted by the stable gastric pentadecapeptide BPC 157, showing this peptide as an effective antidote therapy (*i.e.*, given in µg- and ng-dose regimens in either stage of paracetamol intoxication). Also, when given parenterally or per-orally (*i.e.*, stable in human gastric juice for more than 24 h (12, 13)), in the same dose-regimens, BPC 157 protected against various agents or procedures that would otherwise have lead to severe liver lesions (29-31) or CNS disturbances (16-23). It was effectively applied either immediately (prophylactically) after paracetamol challenge or (therapeutically) administered after a 3 h period had elapsed (note, the maximum depletion of hepatic glutathione occurred 3 h after acute paracetamol dosing (37)). Finally, BPC 157 counteracted a particular overdose toxicity that excided regular paracetamol regimens (*i.e.*, 1, 2, 6, 11), at either of the assessed intervals (*i.e.*, 25 min, 3 hours and 24 hours). On the other hand, the demonstration of paracetamol's progressive hepatic encephalopathy with severe seizures at least partly overruled the general discordance of NSAIDs' adverse effects and therapeutic application, paracetamol in particular as previously mentioned (1-10). Also, the significance of this peptide's therapeutic benefit against amplified and advanced paracetamol toxicity remains to be further postulated, particularly considering the extensive investigation of gastrointestinal peptides in gastrointestinal and liver physiology (38), physiological mediators in NSAIDs-induced impairment of gastric mucosal defense and adaptation (39), and brain-gut peptide regulation (40). However, very recent novel evidence suggested that just the stable gastric pentadecapeptide BPC 157 may have possible significance and implications for novel mediator of both Robert's cytoprotection and adaptive cytoprotection (41), and thereby potential to counteract paracetamol and other NSAIDs (over)-toxicity.

Conflict of interests: None declared.

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Author's address: Prof. Predrag Sikiric, MD, PhD, Professor, Department of Pharmacology, Medical Faculty University of Zagreb, 11 Salata Street, POB 916, 10000 Zagreb, Croatia; Phone: 385-1-4566-833; Fax: 385-1-42-0050; E-mail: sikiric@mef.hr