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Therapy of the rat hemorrhagic cystitis induced by cyclophosphamide. Stable gastric pentadecapeptide BPC 157, L-arginine, L-NAME

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ABSTRACT

We focused on the cyclophosphamide-induced hemorrhagic cystitis (100 mg/kg/day intraperitoneally throughout three days) as a particular NO-system disturbance, and therapy possibilities. We demonstrated that it may be attenuated by subsequent administration of the NOS substrate L-arginine (100 mg/kg/day intraperitoneally), aggravated by NOS-blocker L-NAME (5 mg/kg/day intraperitoneally), all influenced by the stable gastric pentadecapeptide BPC 157 (10 μg/kg/day, 10 ng/kg/day, intraperitoneally or perorally, in drinking water). Regularly, cyclophosphamide dose- and time-dependently induced severe hemorrhagic cystitis lesions, gross lesions, and corresponding urothelial necrosis, vesical edema, erosion, hemorrhage, inflammation, and ulceration, microscopically. The bladder wet weight dramatically increased. Functionally, already after first cyclophosphamide administration, there is an increased leak point pressure. Until the second cyclophosphamide administration, L-arginine consistently attenuated regular cyclophosphamide-induced severe hemorrhagic cystitis lesions, grossly and microscopically, but not functionally. L-NAME aggravated these lesions and eradicated beneficial effect of L-arginine when combined. BPC 157 administration after cyclophosphamide, given in either dose or in either regimen markedly attenuated all cyclophosphamide lesions, grossly, microscopically. The increase of the bladder wet weight dramatically increased. Functionally, already after first cyclophosphamide administration, there is an increased leak point pressure. Until the second cyclophosphamide administration, L-arginine consistently attenuated regular cyclophosphamide-induced severe hemorrhagic cystitis lesions, grossly and microscopically, but not functionally. L-NAME aggravated these lesions and eradicated beneficial effect of L-arginine when combined. 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Similarly, because of a more prominent effect on the disturbances of the NO system, BPC 157 completely counteracted more severe stomach and duodenal cyclophosphamide lesions, which were further exacerbated by the nitric oxide synthase (NOS)-blocker L-NG-nitroarginine methyl ester (L-NAME) (cyclophosphamide + L-NAME-ulcers) (Luetic et al., 2017). Interestingly, these more severe cyclophosphamide + L-NAME-ulcers may also respond to L-arginine, while L-arginine could not affect ulcers induced by cyclophosphamide administered alone (Luetic et al., 2017).

However, unlike the NO-system aimed at maintaining the integrity of the stomach mucosa (Sikiric et al., 2014), NO plays a key role in cyclophosphamide-induced cystitis and NOS up-regulation (Alfieri et al., 2001). Consequently, the beneficial effect of L-NAME on cyclophosphamide-induced cystitis (Ribeiro et al., 2002; Souza-Fiho et al., 1997; Xu et al., 2001) may be distinctive from the worsening effect of L-NAME on gastrointestinal lesions (Amic et al., 2018; Belosic Halle et al., 2017; Djakovic et al., 2016; Drmic et al., 2018, 2017; Duzel et al., 2017; Lojo et al., 2016; Luetic et al., 2017). However, it has been reported that NO or L-arginine donors may be considered a possible treatment for cyclophosphamide-induced and other types of bladder inflammation (Aizawa et al., 2011; Ozawa et al., 1999).

In addition, BPC 157 showed therapeutic potentials in stress urinary incontinence (SUI) in rats (Jandric et al., 2013), and the recovery of leak point pressure as a particular rescue effect in the failed leak point pressure (Jandric et al., 2013). Consistently, besides recovering the function of the urinary sphincter (Jandric et al., 2013), BPC 157 maintains the functions of other sphincters (Belosic Halle et al., 2017; Djakovic et al., 2016; kokot et al., 2016; Vidaic et al., 2017) or transacted striated muscles (Breic et al., 2009; Novinscak et al., 2008; Pevec et al., 2010; Staresinic et al., 2006). The beneficial effect on muscle integrity was specially reviewed (Gwyer et al., 2019). In addition, BPC 157 counteracts fecaluria and defecation through the vagina in the healing of rat colovesical and rectovaginal incontinence (Jandric et al., 2013), BPC 157 completely counteracted more severe stomach and duodenal cyclophosphamide lesions, which were further exacerbated by the nitric oxide synthase (NOS)-blocker L-NG-nitroarginine methyl ester (L-NAME) (cyclophosphamide + L-NAME-ulcers) (Luetic et al., 2017). Interestingly, these more severe cyclophosphamide + L-NAME-ulcers may also respond to L-arginine, while L-arginine could not affect ulcers induced by cyclophosphamide administered alone (Luetic et al., 2017).

2.1. Animals

Wistar Albino female rats (200 g b.w.) were randomized to the experiments (10 animals for each experimental group), all of which were approved by the Local Ethics Committee (School of Medicine, University of Zagreb; Case number: 380 -59- 10106-16-20/187). In addition, all the experiments were carried out under a blind protocol, and the effect was evaluated by examiners who were completely unaware of the given protocol.

2.2. Drugs

The pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, M.W. 1419) (Diagen, Ljubljana, Slovenia) dissolved in saline, was used in all experiments. The peptide, BPC 157, is part of the sequence of the human gastric juice protein, BPC, and is freely soluble in water at pH 7.0 and saline. It was prepared as described above with a purity of 99% high pressure liquid chromatography (HPLC), expressing the 1-de-Gly peptide as an impurity (Cesarec et al., 2013; Seiwerth et al., 2018, 2014; Sikiric et al., 2018, 2017, 2016, 2014, 2013, 2012, 2011, 2010). L-NAME, L-arginine and cyclophosphamide were purchased commercially (Sigma-Aldrich) and used accordingly (Luetic et al., 2017).

2.3. Experimental protocol

Hemorrhagic cystitis model and medication protocol. The rats were injected intraperitoneally with 100 mg/kg of cyclophosphamide once a day for the next three days. The medication (per kg intraperitoneally) (BPC 157 10 μg, 10 ng, L-NAME 5 mg, L-arginine 100 mg alone or in combination) was administered immediately after the cyclophosphamide, while the controls received simultaneously an equivalent volume of saline solution (5 ml/kg intraperitoneally) (Luetic et al., 2017). Alternatively, BPC 157 was administered orally in drinking water (per day) (10 μg/kg, 10 ng/kg, 0.16 μg/ml, 0.16 ng/ml, 12 ml/rat) until killing, while controls received simultaneously potable water (12 ml/rat/day). Hemorrhagic cystitis was evaluated 24 h after each application of cyclophosphamide, at 24, 48 and 72 h of the experimental period, macroscopically, microscopically, functionally, as follows, and by oxidative stress and NO-level in the bladder tissue.

2.4. Assessment

Gross evaluation. Immediately after the killing, all bladder specimens were excised. Briefly, as described (Chow et al., 2007), the appearance of the gross damage of the bladders (gross edema, hemorrhage) was scored (0–3). Gross edema: 0 - none (normal); 1 - mild (between moderate and normal); 2 - moderate (fluid confined to the internal mucosa); 3 - severe (fluid inside and outside the bladder wall). Gross hemorrhage: 0 - none (normal); 1 - mild (telangiectasia or dilatation of the vessels of the bladder); 2 - moderate (mucosal hematomas); 3 - severe (intravesical clots).

Histological evaluation. The bladders were excised, embedded in paraffin and stained with hematoxylin and eosin. The following scoring system was used as described previously (Chow et al., 2007): 0 - normal (normal epithelium, no infiltrate of inflammatory cells or ulcers); 1 - mild (decreased epithelial cells, flattening with submucosal edema, mild hemorrhage, few ulcerations); 2 - moderate (erosion of the mucosa, infiltrate of inflammatory cells, fibrin deposition, hemorrhage and multiple ulcerations); 3 - severe (erosion of the mucosa, infiltrate of inflammatory cells, fibrin deposition, multiple ulcerations and transmural hemorrhage with severe edema).

Measurement of vesical edema. Vesical vascular edema was quantified by wet bladder as wet bladder weight (mg)/100 g of animal weight as described (Chow et al., 2007).

In normal female rats, the of the wet bladder weights were evaluated as 39 ± 3 mg/100 g of body weight (data not shown specifically).

2.5. Leak point pressure assessment

In a separate group of animals, as described previously (Jandric et al., 2013) under anesthesia with urethane (1.2 mg/kg intraperitoneally, SIGMA-Aldrich Chemie GmbH, Steinheim, Germany) used to maintain physiological urethral responses (Jandric et al., 2013), the bladder was exposed through an abdominal incision in the midline and manually emptied. A 24 G transvesical catheter, connected to an infusion pump (Green Stream VO-P ARGUS 414, Argus Medical AG, Heimberg, Switzerland) and a monitor with an invasive pressure transducer module (model 90309, Spacelabs Medical Inc., Redmond, Washington, USA) through a 3-way stopcock, was inserted and secured...
in the dome of the bladder, and the abdominal wall was temporarily closed with sutures. The intravesical pressures [mmHg] referred to the air pressure at the level of the bladder and were observed continuously as the bladder was subsequently filled with saline at room temperature at a rate of 5 ml/h. At half the capacity of the bladder (~0.4 ml), the infusion was stopped and gentle pressure was applied on the bladder until the first drop of fluid was observed in the urethral meatus. The intravesical pressure recorded at that point was considered as the pressure of the leak point. In normal female rats, the leak point pressure was evaluated as 25 ± 3 mmHg (data not shown specifically).

2.6. Oxidative stress in bladder tissue

At the end of the experimental period of 24, 48 and 72 h, oxidative stress was evaluated in bladder tissue samples collected by quantifying thiobarbituric acid reactive species (TBARS) as malondialdehyde equivalents (MDA). The tissue samples were homogenized in PBS (pH 7.4) containing 0.1 mM butyated hydroxytoluene (BHT) (TissueRuptor, Qiagen, USA) and sonicated for 30 s in an ice bath (ultrasonic bath, Branson, USA). Trichloroacetic acid (TCA, 10%) was added to the homogenate, the mixture was centrifuged at 2200 g for 15 min, and the supernatant was collected. Then, 1% TBA was added and the samples were boiled (95 °C, 60 min). The tubes were kept on ice for 15 min. After centrifugation (10,000 g, 10 min), the absorbance of the mixture was determined at the wavelength of 532 nm. The MDA concentration was read from a standard calibration curve plotted with 1,1,3,3-tetraethoxypropane (TEP). The extent of lipid peroxidation was expressed as MDA using a molar extinction coefficient of MDA of 1.56 × 10 mol/L/cm. The protein concentration was determined using a commercial kit. The results are expressed in nmol per mg of protein.

2.7. NO determination in bladder tissue

At the end of the experimental period of 24, 48 and 72 h, we determined NO levels in bladder tissue samples using the Griess reaction (Griess reagent system, Promega, USA). Sulfanilamide was added to the homogenized tissue, the mixture was incubated and N-(1-naphthyl) ethylenediamine hydrochloride was added. The Griess reaction is based on the diazotization reaction in which the acidified nitrite reacts with the diazonium ions and, in a further step, is coupled to the N-(1-naphthyl) ethylenediamine dihydrochloride, forming a chromophoric azo derivative. Absorbance was measured at 540 nm, using a sodium nitrite solution as standard. NO levels are reported in μmol/mg of protein. Protein concentrations were determined using a commercial kit (BioRad Protein DR Assay Reagent Kit, USA).

2.8. Statistical analysis

We use Statistica 12.1 for Windows to perform the statistical analysis. The data were expressed as arithmetic mean ± standard deviation (S.D.) and minimum/median/maximum. The statistical significance of the differences between the groups was analyzed by means of a one-way ANOVA followed by the post-hoc Student–Newman–Keuls’ test and the Kruskal-Wallis test, and finally with the post-hoc Mann–Whitney U test (where appropriate). The differences were considered statistically significant if P < 0.05.

3. Results

In general, our studies indicated that the application of BPC 157 is an effective therapy for hemorrhagic cystitis during cyclophosphamide applications (Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Table 1). It was administered intraperitoneally after cyclophosphamide or orally in drinking water in rats receiving cyclophosphamide. The most important thing is that BPC 157 in drinking water attenuated the increase in MDA levels in the bladder of cyclophosphamide rats and normalized NO levels in the bladder (Figs. 1 and 2).

To further perform the hemorrhagic cystitis induced by cyclophosphamide as a particular disturbance of the NO system, we revealed the stable gastric pentadecapeptide BPC 157, L-arginine, versus L-NAME, which were faced together with the preceded applications of cyclophosphamide (Figs. 2, Fig. 3, Fig. 4, Figs. 5 and 6, Table 1). Regularly, cyclophosphamide dose- and time-dependently increased severe hemorrhagic cystitis lesions, gross lesions, corresponding urethelial necrosis, vesical edema, erosion, hemorrhage, inflammation, and ulceration microscopically (Figs. 3, Fig. 4, Fig. 5, Fig. 6, Table 1). The bladder wet weight increased dramatically (Fig. 3). Functionally, already after the first administration of cyclophosphamide, there is an increased leak point pressure. It remained constantly increased after subsequent administrations (Fig. 4).

L-arginine consistently attenuated the regular cyclophosphamide severe hemorrhagic cystitis lesions, grossly and microscopically (Figs. 3 and 5).
and 4), until the second administration of cyclophosphamide. However, it was not effective against the third cyclophosphamide as seen with the wet bladder weights (Fig. 3). In contrast, L-arginine is not functionally effective (i.e., the leak point pressure is increased as in severe hemorrhagic cystitis lesions with control cyclophosphamide) (Fig. 4).

L-NAME aggravated the lesions. The aggravation of lesions grossly and microscopically precedes an even more dramatic increase in the bladder wet weight after subsequent administrations. As a result, there is a further increase in the leak point pressure evaluated (Figs. 3, Fig. 4, Fig. 5, Fig. 6, Table 1).

When combined (L-arginine + L-NAME), L-NAME and L-arginine antagonize each other’s effect. L-NAME eradicated the beneficial effect of L-arginine previously observed grossly and microscopically, while L-arginine counteracted the aggravating effect of L-NAME that was observed when increasing the leak point pressure (Figs. 3, Fig. 4, Fig. 5 Fig. 6, Table 1).

Administration of BPC 157 after cyclophosphamide, administered at any dose or regimen, markedly attenuated all cyclophosphamide lesions grossly and microscopically. The increase in the bladder wet weight was constantly attenuated. Functionally, the increased leak point pressure was reversed to the values recorded in normal rats. Similar findings were observed in rats that received BPC 157 together with L-NAME or L-arginine, administered alone or in combination...
4. Discussion

In the rat, cyclophosphamide-induced the complete syndrome of hemorrhagic cystitis (dysfunctional bladder, wet bladder weight gain, macro/microscopically, urothelial necrosis, vesical edema, erosion, hemorrhage, inflammation, ulceration, increased NO and MDA levels in the bladder). As in the case of the worsening of the stomach and duodenal lesions induced by cyclophosphamide (Luetic et al., 2017), L-NAME deteriorated the hemorrhagic cystitis syndrome. In particular, the worsening occurs in the late period when the cyclophosphamide lesions worsen spontaneously. The L-arginine produces a partial and temporary attenuation (the leak point pressure remained increased, all the beneficial effects disappear with the prolongation of the cyclophosphamide cycle). BPC 157 has a more prominent complete curative effect, which is permanent (Kang et al., 2018; Seiwerth et al., 2018, 2014; Sikiric et al., 2018, 2017, 2016, 2014, 2013, 2012, 2011, 2010). The effect of the therapy appears as an intraperitoneal bolus immediately after cyclophosphamide or administered continuously in drinking water. Therefore, it can appear rapidly (in intraperitoneal bolus) and act in a sustainable and beneficial way (when administered in drinking water) against the increase in harmful toxicity of the bladder by cyclophosphamide.

This can be a particular advantage since BPC 157 is stable in human gastric juice for more than 24 h, unlike standard peptides, which are rapidly destroyed (Veljaca et al., 1995). Conceptually, this characteristic allows a useful intragastric application in our studies (for a review, see Kang et al., 2018; Seiwerth et al., 2014, 2018; Sikiric et al., 2018, 2017, 2016, 2014, 2013, 2012, 2011, 2010). As well as in other group studies (Xue et al., 2004). Consequently, that application in drinking water could be successful in the present and other studies (i.e., in the therapy of cirrhosis induced by bile duct ligation in rats (Sever et al., 2019)). This interesting point of the BPC 157 activity was specially reviewed (Seiwerth et al., 2018). By in situ hybridization and immunostaining, BPC 157 was found in the human gastrointestinal mucosa, the pulmonary bronchial epithelium, the epidermal layer of the skin and the kidney glomeruli (Seiwerth et al., 2018). In accordance with the initial findings (Sikiric et al., 1993), these data suggest that in addition BPC has been isolated from gastric juice and probably acts mainly in the gastrointestinal system, it may have additional regulatory functions in human lung, kidney and skin (Seiwerth et al., 2018). Essentially, the established ratio of BPC 157-NO in several experimental models and species (Sikiric et al., 2014) demonstrates that it could interfere in the toxicity of cyclophosphamide with the effects of the application of the NOS-blockade or NOS-substrate agent (Luetic et al., 2017). Consequently, it also works against cyclophosphamide-induced bladder toxicity as a more severe disturbance of the NO- system aggravated by the addition of L-NAME (Kang et al., 2018; Seiwerth et al., 2018, 2014; Sikiric et al., 2018, 2017, 2016, 2014, 2013, 2012, 2011, 2010). The administration of BPC 157 cancels the strong aggravating effect of L-NAME.

Similarly, cyclophosphamide increased the levels of NO and MDA in the bladder tissue. The increase is very similar to the previous one in the stomach and duodenum (Luetic et al., 2017). These increased levels of NO and MDA decreased when the rats were cured with the administration of BPC 157 (Luetic et al., 2017). In addition, in the bladder, instead of the levels increased at 24 h, the healthy values of the MDA and NO levels appear in the BPC 157 rats. Interestingly, patients with carcinomas (Luetic et al., 2017) have increased NO and MDA levels in plasma and tissue samples. It has been suggested that MDA itself, due to its high cytotoxicity and inhibitory action on protective enzymes, acts as a tumor promoter and cocarcinogenic agent (Luetic et al., 2017). Therefore, as previously emphasized (Luetic et al., 2017), these may be the excessive release of NO generated by the inducible isozyme, which damages the vascular wall and other cells of the tissues, especially in combination with reactive oxygen intermediates and defective endothelial production (Chow et al., 2007; Matthys and Bult, 1997; Yousefipour et al., 2005). Therefore, there is an additional aggravation of L-NAME. This aggravation was reduced to the control values with L-arginine (i.e., attenuation of L-NAME with L-arginine). However, it was reverted to a positive effect with the administration of BPC 157 (i.e., L-NAME annulled with BPC 157) (Luetic et al., 2017).

This possible special aggravating point (L-NAME versus L-arginine) may explain how the dysfunction of NO production is causal to or result of the formation of bladder lesion by cyclophosphamide. It could also explain how the toxicity of the bladder with cyclophosphamide could be affected by the application of the agents (i.e., L-arginine affects the earlier period, L-NAME affects the later period). However, when co-administered, BPC 157 reversed all effects of NO agents towards constant beneficial effects. These illustrate particular relationships: L-NAME + BPC 157-rats; L-arginine + BPC 157-rats; L-NAME + L-arginine + BPC 157-rats and permanent beneficial effect. On the other hand, we have L-NAME-rats (constant aggravation); L-arginine-rats (the effect of L-arginine alone disappears with increased toxicity by cyclophosphamide); L-NAME + L-arginine-rats (the control values presented illustrate that L-arginine constantly antagonizes the aggravation of L-NAME, unlike the shorter effects of L-arginine itself in

(Figs. 3, Fig. 4, Fig. 5, Fig. 6, Table 1).
The lesions were evaluated macroscopically (bladder edema, bladder hemorrhagic lesions) or microscopically (severity of bladder lesions) were scored 0–3, Min/Med/Max. The rats were injected intraperitoneally with 100 mg/kg of cyclophosphamide (CY) once a day for the next three days. The medication (kg intraperitoneally) (BPC 157 10 μg, BPC 157 10 ng (BPC L-NAME 5 μg), L-arginine 100 mg (A alone and/or in combinations) was administered immediately after the cyclophosphamide while controls simultaneously received an equivalent volume of saline (5 ml/kg intraperitoneally) (CY + S). Alternatively, BPC 157 was administered perorally in drinking water (daily) (10 μg/kg, 10 ng/kg, 16 μl/ml, 12 ml/rat/day) until killing, while controls received potable water simultaneously (12 ml/rat/day) (CY + DW). The evaluation was at 24 h after each application of cyclophosphamide, at 24, 48 and 72 h of the experimental period.

* P < 0.05, vs. control.

cyclophosphamides-rats). Therefore, BPC 157, throughout the period, could act and interfere with the effects of the application of the NOS-blocking agent or NOS-substrate (Sikirić et al., 2014). As with the double role of the NO-system (L-NAME vs. L-arginine), it can act with the NO immobilized system (L-NAME vs. L-arginine vs. combination). Given together (L-NAME + L-arginine), they regularly attenuated or antagonized the response of each other (for a review, see Moncada et al., 1991; Sikirić et al., 2014; Whittle et al., 1992); the remaining pathology was finally counteracted by the administration of BPC 157. Therefore, as before (Luetic et al., 2017; Sikirić et al., 2014), BPC 157 could consolidate the stimulating and inhibiting effects of the NO-system. NO has either antioxidant or pro-oxidant properties and, together with other reactive oxygen species, induces cytotoxicity and cytostasis (Bakan et al., 2002). The consolidation may be towards greater curative efficacy (i.e., the markedly mitigated lesions in rats subjected to cyclophosphamide) (Luetic et al., 2017; Sikirić et al., 2014). Illustratively, BPC 157, by itself, can induce the release of NO in vitro, in the gastric mucosa of rat stomach tissue homogenates, and counteracts the opposite adverse effect of L-NAME (i.e., hypertension; lack of NO-release in vitro) and L-arginine (i.e., hypotension, over-release of NO in vitro) (Sikirić et al., 1997).

In addition, the function retained (or recovered) as a final net result can be supportive. Rats that underwent cyclophosphamide showed an increased leak point pressure, and even more with the administration of L-NAME. Consequently, we have the increase or even more increase in the bladder wet weight due to necrosis, vesical edema, erosion, hemorrhage, inflammation, a dramatic increase in obstruction of the outflow of the bladder as noted (Andersson and Arner, 2004), and it is likely to be areflexic bladder. Note that L-arginine alone did not show any effect on leak point pressure and, with obviously shorter and weaker activity (Sikirić et al., 2014), did not produce any beneficial effect as did BPC 157. In contrast, BPC 157 rats showed a leak point pressure in the range of healthy rats. A leak point pressure close to the level of healthy rats was noted also in BPC 157-rats that underwent prolonged vaginal dilation or transabdominal urethrolysis (Jandric et al., 2013). It is noteworthy that a recent understanding of the cytoprotective mechanism and the application of BPC 157 in the potential therapy for enteric neural lesions and gastrointestinal ulcers documents that BPC 157 increased the survival of cultured enteric neurons (Wang et al., 2019).
Other studies should determine the importance of the findings that BPC 157 affects several molecular pathways (Cesarec et al., 2013; Chang et al., 2014, 2011; Hsieh et al., 2017; Huang et al., 2015; Tkáčević et al., 2007; Vukojević et al., 2018) and several receptors activation (i.e., VEGFR2 (Hsieh et al., 2017) and growth hormone (Chang et al., 2014)). In this, rats with occluded inferior caval vein, direct vein lesions, thrombosis, thrombocytopenia, and prolonged bleeding were counteracted (Vukojević et al., 2018).

In addition, the rapid presentation of collaterals and the redistribution of otherwise trapped blood volume (bypassing through the left ovarian vein and other veins), with venous hypertension, arterial hypotenison, and tachycardia counteraction were shown (Vukojević et al., 2018). The peculiar point may be its strong angiogenic effect, more expressed than that of other standard anti- ulcer agents (Sikiric et al., 1999), associated with its curative effect (Brcic et al., 2009; Cesarec et al., 2013; Chang et al. 2014, 2011; Hsieh et al., 2017; Huang et al., 2015; Kang et al., 2018; Radeljak et al., 2004; Seiwerth et al., 2015; Sikiric et al., 2014, 2017, 2016, 2013, 2012, 2011, 2010; Tkáčević et al., 2007; Vukojević et al., 2018). Especially, the curative effect of BPC 157 is combined with the increased expression and internalization of VEGFR2, the activation of the VEGFR2-Akt-eNOS signaling pathway without the need for other known ligands or shear stress (Hsieh et al., 2017). Similarly, BPC 157 inhibits the tumor promoting effect of VEGF via the MAPK pathway in the human melanoma cell line (Radeljak et al., 2004). Recently, in a cancer cachexia representative mice model bearing C-26 colon adenocarcinoma cell, BPC 157 therapy antagonized TNF-α and IL-6 pivotally involved in cachexia (Kang et al., 2018). In addition, BPC 157 prolonged survival and counteracted weight loss, muscle wasting by significantly correcting muscle proliferation and myogenesis, the changes in the expression of FoxO3a, p-AKT, p-mTOR and P-GSK-3β (Kang et al., 2010).

In summary, cyclophosphamide induces a wide range of toxicity (i.e., in cardiac tissue, a single dose of cyclophosphamide-induced a significant increase in MDA and NO (Makou et al., 2015)). Therefore, this particular therapeutic aspect includes the pleiotropic efficacy of BPC 157, particularly against cyclophosphamide (Luetic et al., 2017) and cyclophosphamide-induced hemorrhagic cystitis, managed the effects of the NO agents (L-NAME and L-arginine) alone/combined, and eliminates or attenuates the enormous increase in MDA and NO levels in the bladder. Therefore, the therapeutic aspect includes BPC 157 as a very safe antupeptide (LD1 not achieved), including oral application (Kang et al., 2018; Seiwerth et al., 2018, 2014; Sikiric et al., 2015, 2016, 2014, 2013, 2012, 2011, 2010), along with other applications of cyclophosphamide.

Disclosure of conflict of interest

The authors state that they have no conflicts of interest.

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