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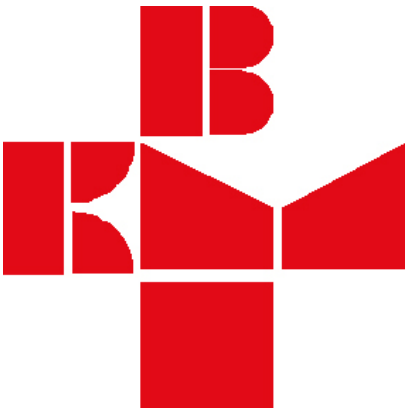

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Toxicity by NSAIDs. Counteraction by Stable Gastric Pentadecapeptide BPC 157

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Abstract: Stable gastric pentadecapeptide BPC 157 is an anti-ulcer peptidergic agent, proven in clinical trials to be both safe in inflammatory bowel disease (PL-10, PLD-116, PL 14736) and wound healing, stable in human gastric juice, with no toxicity being reported. Recently, we claim that BPC 157 may be used as an antidote against NSAIDs. We focused on BPC 157 beneficial effects on stomach, duodenum, intestine, liver and brain injuries, adjuvant arthritis, pain, hyper/hypothermia, obstructive thrombus formation and thrombolysis, blood vessel function, counteraction of prolonged bleeding and thrombocytopenia after application of various anticoagulants and antiplatelet agents and wound healing improvement. The arguments for BPC 157 antidote activity *(i.e., the role of BPC 157 in cytoprotection, being a novel mediator of Robert's cytoprotection and BPC 157 beneficial effects on NSAIDs mediated lesions in the gastrointestinal tract, liver and brain and finally, counteraction of aspirin-induced prolonged bleeding and thrombocytopenia)* obviously have a counteracting effect on several established side-effects of NSAIDs use. The mentioned variety of the beneficial effects portrayed by BPC 157 may well be a foundation for establishing BPC 157 as a NSAIDs antidote since no other single agent has portrayed a similar array of effects. Unlike NSAIDs, a very high safety (no reported toxicity (LD1 could be not achieved)) profile is reported for BPC 157. Also, unlike the different dosage levels of aspirin, as a NSAIDs prototype, which differ by a factor of about ten, all these beneficial and counteracting effects of BPC 157 were obtained using the equipotent dosage (μg, ng/kg) in parenteral or peroral regimens.

Keywords: NSAIDs-gastrointestinal tract lesions, NSAIDs-liver lesions, NSAIDs-brain lesions, aspirin-prolonged bleeding/ thrombocytopenia, stable gastric pentadecapeptide BPC 157, counteraction, antidote against NSAIDs.

INTRODUCTION

Stable gastric pentadecapeptide BPC 157 is an anti-ulcer peptidergic agent, proven in clinical trials to be both safe in inflammatory bowel disease (PL-10, PLD-116, PL 14736), wound healing and stable in human gastric juice, with no toxicity being reported [1-4].

Recently, we claim that BPC 157 may be used as an antidote against NSAIDs [5-7]. In this paper, we focused on the particular BPC 157 beneficial effect on stomach [[6,7], duodenum [6], intestine [[6,7], liver [5-7] and brain [5-7] injuries, adjuvant arthritis [8], pain [1], hyper/hypothermia [1,9], obstructive thrombus formation and removal and blood vessel function [10], counteraction of prolonged bleeding and thrombocytopenia after various applications of different anticoagulants and anti-platelet agents [11] and wound healing improvement [12,13].

However, this claim is complicated by the particular application of NSAIDs in the cytoprotection theory, showing several general drawbacks of NSAID use, which have been long ago recognized [14,15]. The consequential variety of side effects of using NSAIDS, have so far hindered any agent from being established as an effective antidote against NSAIDS. In support of our claim, we will review the arguments for BPC 157 antidote activity. First, the following should be a foundation for the role of BPC 157: in cytoprotection (i.e., novel mediator of Robert's cytoprotection) [1-4] *vs.* NSAIDs [14], its beneficial effects in the gastrointestinal tract, liver and brain which may all be particularly damaged by NSAIDs [5-7] and finally, a counteraction of aspirin-prolonged bleeding and thrombocytopenia [11].

TOXICITY BY NSAIDS

Considering aspirin as a NSAID prototype, with different dosage levels, differing by a factor of about ten (i.e., platelet aggregation<headache and other pains<rheumatic arthritis or lupus erythematodes) [15], makes it difficult to imagine, how a single molecular mechanism, i.e., the inhibition of cyclooxygenase could be responsible for the toxicity by NSAIDs. Likewise, the essential question remains unresolved [15], besides the claim that different cyclo-oxygenases display different sensitivities to drugs, why then, for example, do drugs such as amidopyrine and paracetamol, which are more potent than sodium salicilate as inhibitors of bradykinin-induced bronchoconstriction in the guinea-pig, as antinociceptive agents [16] or as inhibitors of prostaglandin biosynthesis [17,18], fail to show anti-rheumatic action [15]? In addition, inflammation is a complex and dynamic process involving a myriad of cellular and humoral responses and many of them can be suppressed by NSAIDs at mM concentration or higher, presumably due to their strong protein binding and membrane interaction properties [15]. Thus, a list of earlier proposals includes uncoupling of oxidative phopshorylation, inhibition of protein denaturation, stabilization of lysosomal and cellular membranes, inhibition of proteases, inhibition of complement activation, fibrinolytic activity and inhibition of protein kinase [15].

Therefore, it seems to us that for defining a NSAIDs-antidote, a clear demonstration of counteraction of the induced various lesions should be the most practical approach. Particular NSAIDs toxicity that is well defined, in the gastrointestinal tract, liver, brain and in platelet aggregation [14,15,19,20] should reveal the BPC 157 antidote activity based on its direct counteracting potential on all these NSAIDs-injuries (i.e., [5-7,11]).

STOMACH

The relations between the gastric ulcer, NSAIDs and stable gastric pentadecapeptide BPC 157 [1-4] could all be explained in terms of the Robert's concept of cytoprotection [14]. Generally, NSAIDs are known to produce stomach lesions and these lesions were termed to be cytotoxic lesions, not related to gastric acid secretion and agents that prevent these lesions are cytoprotective agents [14]. However, an alternative way may be a *protective* effect of NSAIDs [14, 21]. Specifically, in Robert's cytoprotection, by collaborating with endogenous adaptive processes, aspirin protects against indomethacin-gastric lesions [14, 21] and then, due to Rob-

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ert's stomach memory, the more and longer aspirin is used the longer the protection against ulceration by strong irritants [21]. Therefore, it is particularly important that BPC 157 may be a novel mediator of Robert's cytoprotection and thereby, particularly related to the NSAID induced gastric lesions [1-4]. Initially, BPC 157 was shown to be involved in both cytoprotective response and adaptive cytoprotective response against both endogenous and exogenous strong irritants [22,23]. Consequently, it is not surprising with gastric ulcer therapy that BPC 157 cytoprotective effects cure the gastric lesions induced by a variety of NSAIDs, i.e., aspirin, indomethacin, diclofenac and ibuprofen [5-7,23,24]. In addition, BPC 157 also nullified the harmful effect of systemic corticosteroid application [12,25] as may be seen in rats with persistent gastrocutaneous fistulas [25]. In severely burned animals with severe stomach ulcers, BPC 157 equally cured skin and stomach lesions and alleviated systemic corticosteroid-healing impairment and immunosupression [13,14]. Thus, it may be that BPC 157 as a cytoprotective agent [1-4] essentially combines the processes responsible for both stomach and skin ulcer healing [25]. Therefore, it may have an exceptionally wide range of beneficial effects against different gastric lesion models [1-4], gastric acid non-dependent, gastric acid dependent, besides the models commonly thought to be prostaglandin related [1-4,6-8,24,26] and in turn, with PGs-system failure [1-4] in the models that implicate dopamine [27-30], glucose (hyperglycemia or hypoglycemia) [31,32], as well as those that involve NO-system disbalance [33]. Therefore, besides NSAIDs-counteraction, on the base of counteraction of acute and chronic alcohol lesions [22-24,33-35], the cytoprotective and adaptive cytoprotective BPC 157 activities (noted also *in vitro* studies [36,37]) even in the longer-term [22-24,33-35], are obviously a part of a larger array of beneficial effects of BPC 157 administration (note, BPC 157 counteracts gastric stress lesions [22,24,29]). BPC 157 also inhibits pylorus-ligation-induced gastric lesions [26]. For the dopamine-blockade-gastric ulcer model, BPC 157 particularly affects the substantia nigra by alpha-[14C]methyl-L-tryptophan (alpha-MTrp) portrayed by the autoradiographic method [38], BPC 157 may also counteract haloperidol-, reserpine-, 1-methyl-4-phenyl-1,2,3,6-tetrahydrophyridine (MPTP)-lesions [27-30,39]. Interestingly, the beneficial effect against gastric lesions is in conjunction with the antagonization of central dopamine system disturbances [27-30,39]. BPC 157 also antagonizes alloxan-gastric lesions [32] as well as heals the diabetic alloxan skin ulcer [40] and also, given in the same dose, counteracts insulin-over-dose-gastric lesions and convulsions [31]. BPC 157 counteracts capsaicin-gastric lesions [24] and capsaicin-nasal lesions [41] and consequently, somatosensory neuron failure [24].

Finally, for counteraction of NSAIDs-gastric lesions, BPC 157 was also compared with sucralfate, proving that BPC 157 has both a local effect on mucosal integrity (i.e., stable in human gastric juice [1-4]) and endothelium injury [22] (used to further demonstrate a strong angiogenic effect [42-44])). BPC 157 exerts a powerful angiogenic effect and new granulation tissue formation, more than H2-blockers, omeprazole and even sucralfate [43] and maintains and recovers AMP-ADP-ATP storage in the stomach mucosa [45]. Also, in the same dose range, it counteracts the ulcerogenic effects of NO-system blockade, by NOS-blocker L-NAME, better than that of the NOS-substrate L-arginine [33]. Finally, BPC 157 has a simultaneous rescuing effect on both stomach and skin defects [13,14,25], as it was directly shown in rats with gastrocutaneous fistulas that would hardly spontaneously heal [25]. This may suggest an essential wound healing capability of BPC 157 [1-4] that in stomach ulcer therapy may overwhelm the essential failure of healing, triggering the healing of persistent ulcers that could be hardly cured and largely contributes to its other effects.

Duodenum

Considering NSAIDs duodenal lesions, mepirizole is particularly used to produce a duodenal ulcer [46]. Likewise, for BPC 157 protection against duodenal lesions, we also noticed with diclofenac that most of the lesions were located close to the pylorus and these lesions were completely counteracted with BPC 157 application. In addition, BPC 157 has a particular effect on the pyloric sphincter in healthy and in ill conditions [47-49] along with an effect on the lower esophageal sphincter [47-49]. Likewise, BPC 157 counteracts the failed sphincter pressure that would otherwise be produced by different NSAIDs, i.e., aspirin, ibuprofen, diclofenac, paracetamol and celebrex [50]. Thus, considering celebrex and paracetamol which produce no or less gastrointestinal lesions as opposed to other NSAIDs, these effects of NSAIDs on the pyloric sphincter are particular and this counteraction may therefore be a particular beneficial effect. Furthermore, when considering the debilitating cysteamine effect on motility [51] and that cysteamine for duodenal ulcers mimics human conditions [51], where BPC 157 consistently counteracts cysteamine lesions [22,52,53], it may be argued that such a beneficial effect of BPC 157, combining the achieved recovery of pyloric sphincter function is particularly important for its successful healing of duodenal ulcers.

Finally, in rats with duodenocutaneous fistulas [54], the simultaneous rescuing effect of BPC 157 on both duodenum and skin defects [54] may suggest that the described BPC 157 wound healing in the gastrocutaneous fistula rats [25], may be accordingly applied as an addition to its beneficial effect on duodenal lesions.

INTESTINE

Small intestinal ulceration is a frequent and potentially serious condition associated with nonselective cyclooxygenase 1/2 inhibitors (nonsteroidal anti-inflammatory drugs, NSAIDs), presenting with diclofenac as an agent prototype [55]. So far pentadecapeptide BPC 157 was tested in inflammatory bowel disease patients [1-4] and thereby, the presented BPC 157 intestinal protection [6,8,56-61] particularly against NSAIDs, should be of particular use [6,8,61]. Of note, BPC 157 was shown to protect against upper gastrointestinal tract lesions that were induced by various NSAIDs and thereby, the consistent beneficial effect that was noted after indomethacin [8] and diclofenac [6,61], with both peroral and parenteral application of BPC 157 should be perceived as an extension from the upper to the lower part of the gastrointestinal tract and BPC 157 as a novel therapy for lesions of both the upper and lower gastrointestinal tract.

However, the BPC 157 beneficial effect against diclofenac intestinal lesions may be essential when considering other organ lesions. Namely, the more extensive the intestinal lesions occurred, the more amplified was the toxin presentation [62,63], with an altered brain penetration and an increased brain-to-plasma diclofenac concentration ratio [64], which may then aggravate diclofenac-induced encephalopathy secondary to liver damage. In this context, the evidence that BPC 157 may both prevent and counteract liver and brain lesions, therefore clearly suggests that the maintenance of small intestine mucosal integrity by BPC 157 is an immediate well directed antidote effect [6]. Even more, an equipotent beneficial effect was also present in rats with a short-bowel after massive small intestine resection in which, after small intestine removal, post-operative application of diclofenac would extend the existing lesions and new extensive lesions would appear in the colon [61]. Alternatively, with respect to NSAIDs toxicity, stomach-small intestine-colon-liver-brain protection may be taken as a well-balanced effect that affects all of the organs equally and may define the same effective counteracting dosage range for all combined organs, including for instance, in the case of diclofenac, the stomach, intestine, liver and brain lesions in the present study [6].

Along with the extension of NSAID-lesions in the stomach, the intestine and colon, goes with the extension of BPC 157 protection and BPC 157 general protection. Cysteamine enema, as the model of ulcerative colitis in rats, suitably extends cysteamine toxicity from a duodenal ulcer to ulcerative colitis [52] and BPC 157 consis-

tently protects and rescues both cysteamine-duodenal and -colonic lesions.

Finally, the importance of such generalization supports BPC 157 curing of colocutaneous fistulas [59], along with other intestinal fistulas [25,54,59,61,65] and other severe inflammatory bowel disease complications [58,60,61,66] (i.e., BPC 157 is efficacious in healing intestinal anastomoses [58,60,61], colo-colonic [66], ileo-ileal [58] and jejuno-ileal termino-terminal anastomoses [60,61]). Likely, these may also be related to BPC 157's wound healing capability [1-4].

BPC 157 increases adaptative capability of the remaining intestine in all intestinal layers, fully reverses short-bowel syndrome in rats after massive intestine resection, reduces body weight loss post-operatively, induces consistent body weight gain and finally increases the weight of rats with intact intestine [60].

Thus, the extent of BPC 157 beneficial effects in the intestine is obviously large, and may fully support its potential to counteract the intestinal damage induced by NSAIDs.

LIVER

For BPC 157 and NSAID-hepatotoxicity, BPC 157 consistently protects against paracetamol-, diclofenac- and ibuprofen-hepatotoxicity [5-7].

Here, it is important to emphasize that more than with gastrointestinal lesions, all NSAIDs present with a various degree of hepatoxicity (paracetamol/diclofenac>ibuprofen) and hepatotoxic background (i.e., NSAIDs are classified as intrinsic (aspirin, acetaminophen, phenylbutazone), immunoallergic idiosyncratic (ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac) and metabolic idiosyncratic (benoxaprofen, diclofenac, indomethacin, naproxen)) (for review see, i.e., [19]). Thus, BPC 157 counteraction of paracetamol-, diclofenac- and ibuprofen-hepatotoxicity [5-7] obviously does imply distinctive hepatic lesions (i.e., perivenular necrosis (paracetamol)) and quite different mechanisms. For instance, paracetamol hepatotoxicity is attributed to its transformation into a highly reactive metabolite, *N*-acetyl-p-benzoquinone imine (NAPQI), by microsomal enzymes of the P450 family[67]. NAPQI is detoxified by conjugation with glutathione (GSH). Once GSH is depleted, NAPQI covalently binds to proteins, causing alterations in intracellular homeostasis that result in cell necrosis [67–69]. The diclofenac toxicity has consequently been categorized as metabolic idiosyncrasy. Both oxidative stress (caused by putative diclofenac cation radicals or nitroxide and quinone imine-related redox cycling) and mitochondrial injury (protonophoretic activity and opening of the permeability transition pore), alone or in combination, have been implicated in diclofenac toxicity [19]. In some cases, immune-mediated liver injury is involved, as inferred from inadvertent rechallenge data and from a number of experiments demonstrating T cell sensitization [19]. Ibuprofen is a compound that may induce a particular type of cholestasis, which leads to vanishing bile ducts in humans [70,71].

Thus, BPC 157 hepatoprotection is an extensive one, and shown to be practically applicable, given parenterally or per-orally (in drinking water or intragastrically), acutely or chronically, even in the advanced stages of the liver lesions [5-7]. Furthermore, BPC 157 liver protection is consistently combined with gastrointestinal tract protection (diclofenac, ibuprofen), regardless of whether the NSAIDs gastrointestinal tract lesions were severe and extensive (diclofenac), or only mild (ibuprofen) [6,7]. BPC 157 therapy connects NSAID-liver and NSAID-gastrointestinal lesions (i.e., the more extensive intestinal lesions, the more amplified toxin presentation [62,63]). Combined BPC 157 gastrointestinal/liver protection [5-7] against NSAID gastrointestinal/liver toxicity may also be important considering the original cytoprotective background of both NSAIDs and BPC 157 (gastric lesions induction *vs.* gastric lesions counteraction) [1-4] and the evidence that the beneficial effect of classic cytoprotective agents (i.e., PGs) or standard anti-ulcer agents on stomach lesions was hardly extended to liver protection against NSAIDs [72,73]. For instance, the addition of cimetidine therapy to standard N-acetylcysteine treatment did not provide additional hepatoprotection in acutely acetaminophen poisoned patients [73].

Initially, BPC 157 counteracted CCl4-, bile duct+hepatic ligation- and restraint stress-induced liver lesions [74]. Likewise, we demonstrated that the antagonization has an even broader range, i.e., chronic ethanol administration counteracted by BPC 157 involves counteraction of portal hypertension and liver and gastric lesions [35,75]. In support of these studies, there is also evidence that BPC 157 antagonizes stomach–liver–brain lesions induced by an overdose of insulin [31]. BPC 157-treated rats showed no fatal outcome and most were without hypoglycemic seizures, but they did have apparently higher blood glucose levels (glycogen was still present in hepatocytes); normal liver weight; a less fatty liver; a counteracted increase in ALT, AST and amylase serum values; markedly fewer damaged neurons in the brain and only occasional small gastric lesions [31].

Thus, BPC 157-rats, despite huge paracetamol over-dose, repeated diclofenac treatment, or continuous ibuprofen application, recover liver function, specifically shown by almost completely normalized (ALT) or markedly reduced (AST) enzyme serum values (i.e., diclofenac) [5-7]. They also reestablished mucosal integrity (i.e., abolished or reduced gastric and intestinal lesions) and thereby had a cessation of further challenge (i.e. normal removal of toxic substances by the liver). Thus, as demonstrated, an absence of deterioration in brain function with paracetamol-, diclofenac- and ibuprofen-hepatotoxicity was consistently reported [5-7].

Encephalopathy

BPC 157 counteracting capability in encephalopathy is consistently evident when given intraperitoneally, intragastrically or per-orally in drinking water [5-7]. Seen from our viewpoint of the realization of counteraction of toxicity of NSAIDs to a full extent, BPC 157 counteracting capability presented that throughout the paracetamol-, diclofenac- and ibuprofen-intoxication course [5-7], BPC 157 eventually counteracted all NSAID-encephalopathies. Thus, paracetamol-, diclofenac- and ibuprofen-encephalopathy, all counteracted in an equally consistent way [5-7], should certainly be a worthy hallmark. Besides antagonizing hyperammonemia, i.e., in paracetamol encephalopathy, BPC 157 interacts with several neurotransmitters in the central nervous system (i.e., dopamine [27-30,39,76,77], serotonin [38], opioid [78], GABA [79,80]); specifically, when given peripherally it affects serotonin synthesis in particular brain areas, especially increasing serotonin release in the substantia nigra [38] that may be particularly beneficial in the prevention and treatment of hepatic encephalopathy [81].

In addition, it seems that the consistent beneficial effect of BPC 157 on paracetamol-, diclofenac- and ibuprofen-encephalopathy [5-7] may markedly contribute to solve some inconsistencies in the general evidence considering NSAIDs-brain damage such as which, may be the rapidly induced paracetamol or diclofenac hepatic encephalopathy. For instance, the consistent demonstration of a sudden onset of encephalopathy with paracetamol overdose [5] along with a dramatic decrease of glutathione levels in the rat brain after a paracetamol oral overdose (3.0 g/kg) [82], the susceptibility of brain tissue to oxidative stress (high content of peroxidizable unsaturated fatty acids, high oxygen consumption per unit weight, but poorly developed antioxidative defense mechanisms) [83] or in the case where paracetamol 750 mg/kg per os [84] induced brain edema in rats, all practically resolve the previous controversies such as the claim that there are no behavioral changes [84], no influence on convulsion or death that may be induced by pentetrazol [85], quinolones [86] or febrile seizures [87], prevention of pentylenetetrazol induced seizures [88].

Thus, as it has not been previously reported that such an ubiquitous counteraction has been achieved, for paracetamol-, diclofenac- and ibuprofen-encephalopathy [5-7] it should be most relevant, that a paracetamol overdose, 5 g/kg intraperitoneally (i.e., rapidly induced hepatic encephalopathy, generalized convulsions, a particular aspect of the sudden onset of encephalopathy in rats) outgrades most of the known toxicity of paracetamol [89]; thereby, these extreme conditions undoubtedly prove a beneficial effect of BPC 157 with respect to the whole paracetamol pathology. Likewise, the same could be suggested for the sudden onset of diclofenac encephalopathy and a corresponding beneficial effect of BPC 157 that we just demonstrated in rats in our very recent study. Also, we demonstrated that chronic ibuprofen treatment, in addition to causing hepatomegaly [90] might be inducing hepatic encephalopathy, explaining the evidence that high doses of ibuprofen could induce drowsiness, dizziness, headache, tinnitus, nystagmus, severe seizures and coma [91]. This allowed us to further define the potential of this agent in the reversal of side effects caused by NSAIDs.

Evidently, the BPC 157 counteraction of encephalopathy was supported by the corresponding counteracting effect on all peripheral lesions whether they may be associated with or concomitantly presented with encephalopathy [5-7]. Here, considering the particularities of paracetamol, diclofenac and ibuprofen application(s) [5-7], encephalopathy seems to be inherent to NSAIDs. Obviously, encephalopathy is induced in a particular way by each of the NSAIDs and thereby, antagonized by BPC 157 in a particular way as well, providing that the concurrent presentation of the gastrointestinal lesions varies from no lesion (paracetamol) [5] to mild (ibuprofen) [6] to severe (diclofenac) [7]; likewise, the presentation of the liver lesions varies from severe (paracetamol; diclofenac) to mild (ibuprofen) [5-7].

Specifically, from a brain-viewpoint (i.e., paracetamol preferentially inhibited brain cyclooxygenases [17]) at 25 min post-paracetamol, in convulsing rats, the brain seems to be affected more quickly and more extensively than the liver, where significant damage soon became apparent in several brain areas, accompanied by generalized convulsions while increased ALT, AST and ammonium serum values precede liver lesions [5]. 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. Specifically, BPC 157 therapy (10 μg, 10 ng, 10 pg/kg, intraperitoneally or intragastrically) was effective (μg-ng range) against all paracetamol toxicity, liver and brain, given in the early (BPC 157 immediately after paracetamol, prophylactically) or advanced stage (BPC 157 at 3 hours after paracetamol, therapeutically). BPC 157 demonstrated a 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 [5]. Thereby, it is possible that this consistent BPC 157 beneficial effect on the rat's pathology [5] underlies the close BPC 157 therapeutic potential in the sudden onset encephalopathy with paracetamol overdose, as well as in paracetamol overdose which is considered as a leading cause of acute liver failure in patients [92].

Likewise, in diclofenac induced brain injury in rats [6]; brain edema and cyanosis were particularly present in the cerebral cortex and cerebellum, more often in white than in gray matter. Damaged (ballooned) red neurons without any inflammation were expressed particularly in the cerebral cortex and cerebellar nuclei, in the Purkinje cells and to a lesser extent in the hippocampal neurons. In accordance with the complete absence of sedation, BPC 157- treated rats, intraperitoneally or per-orally, commonly had less edema or damaged (ballooned) red neurons [6].

In particular, ibuprofen toxicity [7] showed a pathognomonic brain edema, particularly in the cerebellum, with the white matter being more affected than gray matter. In addition, we noted damaged and red neurons, in the absence of anti-inflammatory reaction, particularly in the cerebral cortex and cerebellar nuclei and also present although to a lesser extent in the hippocampus, dentate nucleus and Purkinje cells. Inhibited pathology seen otherwise with ibuprofen was using the same protocol as before with diclofenac intoxication (10 μg, 10 ng/kg), when given intraperitoneally, immediately after NSAID daily or when given in drinking water (0.16 μg, 0.16 ng/ml, 12 ml/rat/day). All adverse effects such as hepatic encephalopathy were counteracted. Specifically for the effect on encephalopathy, a moderate edema, ballooned and red neurons were present in the cerebellum. Ischemic neurons were present more often in the gray matter but edema was present more often in the white matter. Damaged swollen and ischemic neurons, without any inflammatory reaction, were present in all animals. They were particularly present in the cerebral cortex and cerebellar nuclei and less common in the hippocampal neurons, the neurons of the dentate nucleus and the Purkinje cells. The same number of damaged neurons was present in the pons and mesencephalon, particularly in the tegmentum of these structures [7].

In accordance with a complete absence of sedation, ibuprofen treated rats that had also received BPC 157 had normal brains [7].

Thus, counteracting the side effects that appear after application of the huge dose of paracetamol, diclofenac and ibuprofen [5-7] and thereby, different NSAID's damaging axes liver-brain of paracetamol [5], stomach-duodenum-small intestine-liver-brain of diclofenac [6], stomach-brain of ibuprofen [7], we consistently defined the potential of BPC 157 in the reversal of side effects caused by NSAIDs. The congruence of all of these effects naturally may raise an important question about the physiological significance of BPC 157, that may likely be used in further therapy as well [5-7].

Arthritis, Pain, Temperature

Namely, besides the counteracting potential for side effects of NSAIDs [5-8,61], which makes likely that BPC 157 would participate in variety of actions related to the effects of NSAIDs (i.e., platelet aggregation, headache and other pains, rheumatoid arthritis [15]), BPC 157 also antagonized rat adjuvant arthritis [8], both counteracted the development of and reversed the already established adjuvant arthritis. In the adjuvant arthritis (tail-application of 0.2 mL of Freund's adjuvant) studies (14 days, 30 days, 1 year), BPC 157 (10μg/kg i.p. or 10 ng/kg i.p.) was effective both prophylactically and therapeutically, in both short-term and long-term regimens, given as a single application (at 1 h either before or following the application of Freund's adjuvant) or in a once daily regimen (0-14th day, 14-30th day, 14th day-1 year) [8]. Likewise, pentadecapeptide BPC 157 successfully antagonized several models of acute, non-specific inflammation (i.e., carrageean, turpentine, cotton pellet) as well as DNFB-injuries) [1]. Consistently, BPC 157 antagonized inflammatory pain (acetic acid-writing), prostaglandin-dependent, and non-inflammatory pain ($MgSO_4$-writing), prostaglandin-non-dependent [1]. It is also interesting, however, that naloxone and BPC 157 counteracted morphine-analgesia [78]. While BPC 157 counteraction was slower than that of naloxone, it may be seen with a very small dose as well [78]. Likewise, BPC 157 antagonized temperature changes, both for decreased (i.e., water immersion-test) and increased (yeast-induced) temperature [1]. Also interesting, in counteracting severe serotonin syndrome, gastric pentadecapeptide BPC 157 (alone, no behavioral or temperature effect) has a beneficial activity, which is likely particular and mostly related to a rather specific counteraction of 5-HT2A receptor phenomena [9] with an ability to counteract both hypothermia and

hyperthermia that would both appear sequentially throughout a non-opposed serotonin syndrome [9].

BPC 157 Effect on Obstructive Thrombus Formation in Abdominal Aorta and Aspirine-Prolonged Bleeding and Thrombocytopenia

Now it seems that BPC 157 may antagonize the side effects of NSAIDs, but it may also mimic at least some of their potentially beneficial effects. Recently, it has been shown that the stable gastric pentadecapeptide BPC 157 prevents obstructive thrombus formation in the rat abdominal aorta terminoterminal-anastomosis [9]. This effect was ascribed to BPC 157 endothelium protection [10], repeatedly shown in cytoprotective studies, providing that endothelium protection is an essential part of cytoprotection, and BPC 157 is implemented to be a novel mediator of Robert's cytoprotection [1-4]. Even more importantly, BPC 157 also rapidly destroys an already formed obstructive thrombus [10]. In rats, BPC 157 (10μg/kg, 10ng/kg) improved bleeding disorders, always reducing the bleeding time and the amount of blood loss after (tail) amputation only and after amputation associated with application of heparin, warfarin and aspirin. It also counteracted consequent thrombocytopenias [11]. Finally, besides a very high safety profile (no reported toxicity (LD1 could be not achieved)) [1-4], other advantages may be the suitable application ways of BPC 157, parenteral or peroral, reversing effects of the parenteral and peroral anticoagulants or antiplatelet agent associated bleeding and thrombocytopenia [10,11].

Thus, although BPC 157 was shown to consistently counteract the NSAIDs'- gastric, intestinal, liver and brain lesions [5-7,23,24] thereby, the counteraction of the aspirin prolonged bleeding and thrombocytopenia would likely be expected as well. This obviously departs from a general pharmacological point of view that it was quite safe to speculate that measures intended to correct a bleeding disorder may enhance the risk of developing thrombosis and vice versa [93]. This may be particularly interesting since the equipotent counteraction was also noticed with heparin and warfarin prolonged bleeding and thrombocytopenia, and thereby a more general concept should be envisaged [10,11].

Conceptually, to solve this intriguing issue that may involve both the attenuation of the development of thrombosis, obstructive thrombus breakdown and blood vessel function, with counteraction of prolonged bleeding and thrombocytopenia after application of various anticoagulants and anti-platelet agents, we claim that this beneficial effect on bleeding disorders also coincides with BPC 157's wound healing improvement [12,16,19,25,40,42,54,58-61,65,66,94-107]. This beneficial effect was perceived as a brief "repetition" of the agents beneficial efficacy on wound healing acceleration, implicating an effect on vascular constriction, platelet plug stability, fibrin mesh structure and formation which insures the stability of the platelet plug (to counteract the prolonged bleeding and thrombocytopenia after application of various anticoagulants and anti-platelet agents), dissolution of the clot inherent to a successfully exerted wound healing effect (attenuating thrombosis development, breakdown of obstructive thrombus, blood vessel function reestablishing) [10,11]. It should be noted that BPC 157's "wound healing effect" [1-4] obviously includes different tissues, skin [12,40,42,94,95], gastrointestinal tract [16,19,25,54,58-61,65,66], cornea [96], muscle [97-99], tendon [100-102], ligament [103], nerve [104,105], blood vessel [10], bone [106,107] (note, chronic BPC 157 application has potent antiinflammatory effects on periodontal tissue in rat periodontitis [107]). It could be likely assumed that this could happen only if all of the 4 major events (vascular constriction, platelet plug stability, fibrin mesh to insure stability of platelet plug, dissolution of the clot) that occur in a set order following the loss of vascular integrity, had been realized, step by step [11]. Thereby, not only in theory, an agent implemented in wound healing and shown to be generally effective in wound healing, should be particularly effective in counteracting both developing thrombosis and bleeding disorders [11].

CONCLUSION

Thus, for demonstration of counteraction of NSAID-side effects, as stable gastric pentadecapeptide, BPC 157, with verified prominent effects on wound healing [1-4] may be of both theoretical and practical value [10,11]. These findings may be a practical demonstration that the wound healing concept as implemented by BPC 157 application, could be an additional successful background in allowing that aspirin-prolonged bleeding and thrombocytopenia [11] like various other side effects of NSAIDs (gastrointestinal tract, liver and brain-lesions) could be accordingly counteracted.

Finally, although this study was highly focused on the direct demonstration of particular counteraction of NSAID-lesions by BPC 157, it as a very safe peptide (LD1 could be not achieved) [1-4] and as a novel mediator of Robert's cytoprotection [1-4] has also revealed its capability to cope with NSAIDs side effects while maintaining some of their beneficial effects on arthritis, pain and increased temperature [1,8,9]. There are some other points which should also be mentioned. For instance, besides stimulating the expression of the early growth response 1 (egr-1) gene [108], responsible for collagen and blood vessel formation, BPC 157 has been shown to stimulate the expression of the egr-1 repressor nerve growth factor 1-A binding protein-2 (nab2) [108]. Therefore, it is possible that BPC 157 and nab2 [109] are part of a feedback mechanism that serves to regulate egr-1-mediated gene transcription and that this may be an effective axis in BPC 157 healing potential. Namely, the egr-1 gene, largely involved in the BPC 157 healing effect [108] is also critical for the development of liver lesions, chronic ethanol-induced steatosis [110], hepatic injury during acute inflammation and/or hepatitis [111], gastrointestinal (cysteamine) ulcers, while NSAIDs delay ulcer healing and hinder angiogenesis by inhibiting egr-1 [62,63,112]. A final argument may be the different dosage levels of aspirin, as a NSAID prototype, differing by a factor of about ten, making it difficult to imagine a single molecule mechanism assuming all responsibility [15], while all these beneficial and counteracting effects of BPC 157 were obtained using an equipotent dosage (μg, ng/kg) in parenteral or peroral regimens [1-4,5-12].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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