Full Paper

Doxorubicine-Congestive Heart Failure-Increased Big Endothelin-1 Plasma Concentration: Reversal by Amlodipine, Losartan, and Gastric Pentadecapeptide BPC157 in Rat and Mouse

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Received November 14, 2003; Accepted February 16, 2004

Abstract. Overall, doxorubicine-congestive heart failure (CHF) (male Wistar rats and NMRI mice; 6 challenges with doxorubicine (2.5 mg/kg, i.p.) throughout 15 days and then a 4-week-rest period) is consistently deteriorating throughout next 14 days, if not reversed or ameliorated by therapy (1/kg per day): a stable gastric pentadecapeptide BPC157 (GEPPPGKPADDAAGLV, MW 1419, promisingly studied for inflammatory bowel disease (Pliva; PL 10, PLD-116, PL 14736)) (10 μg, 10 ng), losartan (0.7 mg), amlodipine (0.07 mg), given intragastrically (i.g.) (once daily, rats) or in drinking water (mice). Assessed were big endothelin-1 (BET-1) and plasma enzyme levels (CK, MBCK, LDH, AST, ALT) before and after 14 days of therapy and clinical status (hypotension, increased heart rate and respiratory rate, and ascites) every 2 days. Controls (distilled water (5 ml/kg, i.g., once daily) or drinking water (2 ml/mouse per day) given throughout 14 days) exhibited additionally increased BET-1 and aggravated clinical status, while enzyme values maintained their initial increase. BPC157 (10 μg/kg) and amlodipine treatment reversed the increased BET-1 (rats, mice), AST, ALT, CK (rats, mice), and LDH (mice) values. BPC157 (10 ng/kg) and losartan opposed further increase of BET-1 (rats, mice). Losartan reduces AST, ALT, CK, and LDH serum values. BPC157 (10 ng/kg) reduces AST and ALT serum values. Clinical status of CHF-rats and -mice is accordingly improved by the BPC157 regimens and amlodipine.

Keywords: pentadecapeptide BPC157, big endothelin-1, doxorubicine-congestive heart failure, amlodipine, losartan

Introduction

Endothelin-1 (ET-1), a 21-amino acid peptide, is the predominant isoform of the endothelin family. ET-1 is ubiquitously expressed as a potent vasoconstrictor and promotor of cell proliferation. Most cardiovascular diseases, such as arterial hypertension, atherosclerosis, restenosis, congestive heart failure (CHF), idiopathic cardiomyopathy, pulmonary hypertension, and renal failure, are associated with local activation of the endothelin system (1–3). ET-1 is importantly involved in the functional and structural changes in the cardiovascular system (4–12), and its relationship with nitric oxide (NO)-system dysfunction is well recognized (12–15). Measuring big endothelin-1 (BET-1) plasma levels, the biologically less active precursor of ET-1 could suitably assess both the severity of CHF and the effects of therapy as well as the rate of ET-1 synthesis (4, 7, 16–18). Namely, while the stimulated endothelin system has gained special prognostic interest, the value of measuring plasma BET levels in patients with advanced disease left ventricular ejection fraction (LVEF) <20% is well established. Therefore, BET-1 assessment will be used for determination of doxorubicine-CHF and estimation of subsequent deterioration (4, 7, 17, 18).

To this end, the reversal of doxorubicine-induced CHF, still presenting a potential therapeutic problem,
was studied in advanced status of failing heart in rats and mice by BET-1 plasma level. The focus was the stable gastric pentadecapeptide BPC157 (GEPPPGK PADDAGLV, M.W. 1419), currently in clinical trials for inflammatory bowel disease (PL 10, PLD-116, PL 14736; Pliva, Zagreb, Croatia). Initially, as a gastric pentadecapeptide, BPC157 opposes a variety of gastrointestinal lesions (19 – 21). Later, it aids in healing of different wounds (19 – 26). Also, it has an angiogenic effect (26) and modulates NO-synthesis (21). Besides, it reduces duration of arrhythmias during hypoxia and reoxygenation in isolated guinea pig hearts (27). Given before or simultaneously with doxorubicine, it markedly reduces doxorubicine-induced cardiotoxicity (28). Likewise, it also inhibits L-N-nitro-L-arginine methylester (l-NAME) blood pressure increase and reverses already established l-NAME hypertension, as well as blood pressure decrease induced by L-arginine application in rats. Similarly, it also inhibits lesions of various organs induced by l-NAME application in birds, unlike L-arginine (29, 30). Furthermore, BPC157 induces and modulates NO-release from stomach slides in vitro, an effect resistant to blockade with l-NAME (21). Even though endothelium-derived NO is considered to be primarily an important determinant of vascular tone and platelet activity, the modulation of myocardial metabolism by NO may be one of its most important roles critical for the regulation of tissue metabolism. A decrease in NO production is involved in the pathophysiological modifications that occur in heart failure and diabetes, disease states associated with altered cardiac metabolism that contributes to the evolution of the disease process. In contrast, several drugs (e.g., angiotensin-converting enzyme inhibitors, amlodipine, and statins) can restore or maintain endogenous production of NO by endothelial cells, and this mechanism may explain part of their therapeutic efficiency (8). Conversely, doxorubicine, with potent cardiotoxicity, may variably affect NO-production in vitro (31, 32). Therefore, considering the high ET-1 values as an implication of NO-disarrangement, which likely occurs in doxorubicine-induced CHF, we hypothesized BPC157 interference with ET-1 production in rats with doxorubicine-induced CHF (21, 29, 30). High stability (19 – 25, 33 – 35) (i.e., no degradation in human gastric juice even for 24 h, unlike rapidly degraded human transforming growth factor (h-TGF), and human epidermal growth factor (h-EGF)) (36, 37) can be used without any carrier (19 – 25, 33 – 35) and can be given by intragastrical (i.g.) or peroral (p.o.) application in drinking water. Together, this means that unlike limited delivery of other peptides (38 – 41), this stable pentadecapeptide is highly resistant to otherwise inescapable degradation of peptides. Likewise, acting alone, without carrier, it presents a healing potential of its own and may be suitable for therapy of complex disturbances such as CHF (19 – 25, 33 – 35).

Agents commonly used in CHF, but so far not investigated in doxorubicine-induced CHF, amlodipine, a Ca2+ channel blocker, and losartan, non-peptide antagonist of angiotensin 1 (AT1) receptors, were also used for further comparison (42 – 54).

Materials and Methods

Animals

Male Wistar Albino rats (200 – 250 g) or NMRI mice (20 – 25 g), randomly assigned, were used for all of the experiments, which were approved by Local Ethic Committee.

Drugs

Pentadecapeptide BPC157 (Diagen, d.o.o., Ljubljana, Slovenia) is a partial sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline. It was prepared as described before (19 – 25, 33 – 35). Peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity), dissolved in saline, was used in all of the experiments (19 – 25, 33 – 35). Doxorubicine (Adriablastina RD; Farmitalia, Milan, Italy); amlodipine (Amlopin; Lek, Ljubljana, Slovenia); and losartan (Cozaar; MSD, Whitehouse Station, NJ, USA) were commercially purchased.

Experimental protocol

For CHF, a procedure previously described by Kawasaki and coworkers (55) regularly producing CHF was followed. Doxorubicine regimen (total dose of 15 mg/kg, given intraperitoneally (i.p.) at six time points, every 3rd day during 14 days) with 4 weeks of rest was used. The control animals were sacrificed to establish the initial values in CHF-animals. Thereafter, all the animals were randomly assigned for subsequent protocols for the next 14 days.

Thereafter, for the next subsequent 14 days, the animals received medication: losartan (0.7 mg/kg), amlodipine (0.07 mg/kg), or BPC157 (10 ng/kg and 10 μg/kg) given i.g. once daily (rats) or in drinking water (mice, 2 ml/mouse daily) with following concentrations: losartan (7 μg/ml), amlodipine (70 ng/ml), BPC157 (0.1 ng/ml and 0.1 μg/ml), while controls received an equivalent of distilled water (rats, 5.0 ml /kg, i.g.) or drinking water (mice). The animals were sacrificed at 24 h after the end of the therapy period.
Biochemical analysis

BET-1 was measured in plasma by ELISA (Biomedica, Graz, Austria) with cross reactivity to big ET-2 and big ET-3, ET-1, 2, 3, and mouse ET-2 less than 0.1%. Serum enzymes (aspartate aminotransferase, AST; alanin aminotransferase, ALT; creatine phosphokinase CK; myocardial fraction, MBCK; and lactate dehydrogenase, LDH) were assessed using autoanalyzer AU-800 (Olympus, Tokyo).

Assessment of clinical parameters

Clinical signs of CHF (tail systolic blood pressure (rats), respiratory rate (rats, mice), heart rate (rats)) were monitored before therapy initiation and thereafter, every 2 days. In mice, only respiration rate was determined. In rats, tail systolic blood pressure and pulse were measured by blood pressure recorder Technical Science Equipment 8002 (TSE, GmbH, Bazel, Switzerland), and respiration rate was assessed. In generally, the basal data correlated with the normal values.

Histopathologic study

For histopathologic study, organs (heart, liver, lungs, and kidneys) were flushed with 10% buffered formaline, fixed for 48 h, and cross dissected. After routine processing and hematoxylin and eosin (HE) staining, the analysis was performed on an optical microscope, using a 40× objective (Leitz Daplan, Wetzlar, Germany).

Statistical analysis

Statistical analysis was performed using non-parametric Kruskal-Wallis ANOVA and subsequent Mann-Whitney U-test to compare groups. Values of \( P<0.05 \) and less were considered statistically significant. Also, Spearman’s correlation test was used.

Results

At presentation at 4 weeks following doxorubin regimen, all rats and mice have elevated BET-1 (Table 1) and plasma enzyme values (Table 2) with pronounced CHF (edema, dyspnea, acrocyanosis, but still no increased heart rate and respiratory rate and still normotensive) (Table 3). Next 2 weeks present a worsening: further increase of BET-1 plasma values parallel with a downhill course of CHF, with statistically correlations: hypotension (rats, \( r = -0.46 \)), dyspnea with increased heart rate (rats, \( r = 0.63 \)), and respiratory rate (rats, \( r = 0.53 \); mice, \( r = 0.65 \)). Meanwhile, the enzyme values maintained initial increases until the end of the experiments, showing no correlation with CHF clinical deterioration (Table 2).

These observed correlations indicate the effectiveness of the therapy. Some dose regimens (BPC157-ng (mice), losartan (mice, rats)) counteract the further increase of BET-1 plasma values, which is otherwise inevitable in control mice and rats. Moreover, others (BPC157-\( \mu \)g (mice, rats), BPC157-ng (rats), amlodipine (mice, rats)) even lead to reversal of the previously increased values. These could be further seen in distinctively reversed clinical deterioration by tested agents, but the improvement seems to be at the best expressed in the BPC157-treated animals. Although losartan and amlodipine as antihypertensive agents did not induce further hypotension aggravation, hypotension and dyspnea with increased heart rate and respiratory rate persist in these compounds-treated animals. Contrary, hypotension was absent in BPC157-treated animals. Likewise, dyspnea with increased heart rate and respiratory rate were fully counteracted (Table 3).

Clinical outcome does not correlate with the course

<table>
<thead>
<tr>
<th>Table 1. Elevated BET-1 plasma values at presentation at 4 weeks following doxorubin regimen in all rats and mice and subsequent changes following amlodipine, losartan, and gastric pentadecapeptide BPC157 (daily dose) given intragastrically (once daily, rats) or in drinking water (2 ml/mouse per day) at the end of 2-week medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big endothelin-1 plasma values (fmol/ml)</td>
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<tr>
<td></td>
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<tr>
<td>rats</td>
</tr>
<tr>
<td>mice</td>
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<tr>
<td>Four weeks following doxorubin regimen before medication (Control-1)</td>
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<tr>
<td>At the end of medication for 2 weeks</td>
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<tr>
<td>BPC157 (10 ng/kg, i.g.) (0.1 ng/ml, p.o.)</td>
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<tr>
<td>BPC157 (10 ( \mu )g/kg, i.g.) (0.1 ( \mu )g/ml, p.o.)</td>
</tr>
<tr>
<td>Losartan (700 ( \mu )g/kg, i.g.) (7 ( \mu )g/ml, p.o.)</td>
</tr>
<tr>
<td>Amlodipine (70 ( \mu )g/kg, i.g.) (70 ng/ml, p.o.)</td>
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<td>Control-2</td>
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</table>

Control-2 received an equivalent of distilled water (rats, 5 ml/kg, i.g.) or drinking water (mice). *\( P<0.05 \) vs control-1, *\( P<0.05 \) vs control-2. means ± S.D.
mean following amlodipine, losartan, and gastric pentadecapeptide BPC157 (daily dose) given intragastrically (once daily, rats) or in drinking water (2 ml/mouse per day) at the end of 2-week medication.

BPC157-losartan (AST, ALT, LDH, CK); and in rats given BPC157-losartan (AST, ALT, LDH, CK), and gastric pentadecapeptide BPC157 (daily dose) given intragastrically (once daily, rats) or in drinking water (mice).

Table 2. Elevated serum enzyme values at presentation at 4 weeks following doxorubicine regimen in all rats and mice and subsequent changes following amlodipine, losartan, and gastric pentadecapeptide BPC157 (daily dose) given intragastrically (once daily, rats) or in drinking water (2 ml/mouse per day) at the end of 2-week medication.

<table>
<thead>
<tr>
<th></th>
<th>AST (IU/L) rats</th>
<th>ALT (IU/L) rats</th>
<th>LDH (IU/L) mice</th>
<th>CK (IU/L) mice</th>
<th>MBCK (IU/L) mice</th>
<th>% CK mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four weeks following doxorubicine regimen (Control-1)</td>
<td>173.7 ± 1.4</td>
<td>230.28 ± 2.11</td>
<td>78.2 ± 13.3</td>
<td>63.71 ± 8.7</td>
<td>2559.1 ± 125.3</td>
<td>2290.42 ± 486.3</td>
</tr>
<tr>
<td></td>
<td>2047.2 ± 101.6</td>
<td>248 ± 11.3</td>
<td>202.3 ± 7.72</td>
<td>10 ± 1.6%</td>
<td>26 ± 5.4%</td>
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</table>

Medication for 2 weeks

Control-2 received an equivalent of distilled water (rats, 5 ml/kg, i.g.) or drinking water (mice). *P<0.05 vs control-1, **P<0.05 vs control-2. mean ± S.D.

Table 3. Clinical findings at presentation at 4 weeks following doxorubicine regimen in all rats and mice and subsequent changes following amlodipine, losartan, and gastric pentadecapeptide BPC157 (daily dose) given intragastrically (once daily, rats) or in drinking water (2 ml/mouse per day) at the end of 2-week medication.

<table>
<thead>
<tr>
<th>Regimen in rats for 2 weeks intragastrically once daily</th>
<th>Blood pressure (mmHg), rats</th>
<th>Respiratory rate (breaths/min), rats</th>
<th>Heart rate (beats/min), rats</th>
<th>Regimen in mice for 2 weeks in drinking water</th>
<th>Respiratory rate (breaths/min), mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before therapy</td>
<td>After therapy</td>
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BPC157

(10 ng/kg, i.g.) (0.1 mg/ml, p.o.)

141.28 ± 3.45 | 108.57 ± 4.75* | 89.42 ± 7.89 | 91.42 ± 3.77* | 400 ± 4.36 | 335.71 ± 14.45* | 131.7 ± 8.19 | 134.57 ± 7.36* |

Control

(50 ng/kg, i.g.) (0.1 mg/ml, p.o.)

118.33 ± 6.61 | 111.11 ± 8.93* | 93.55 ± 4.87 | 84.88 ± 7.28* | 393.33 ± 3.33 | 312.22 ± 7.41* | 128.28 ± 5.21 | 130.57 ± 7.45* |

Losartan

(700 ng/kg, i.g.) (7 mg/ml, p.o.)

117.5 ± 4.18 | 101.66 ± 6.05* | 90.00 ± 6.06 | 104.33 ± 4.96* | 393.33 ± 4.21 | 360 ± 16.32 | 127.42 ± 7.63 | 142.85 ± 6.51* |

Amlodipine

(70 ng/kg, i.g.) (7 mg/ml, p.o.)

118.5 ± 5.29 | 98.5 ± 4.11* | 92.2 ± 7.59 | 88.4 ± 10.1* | 382.22 ± 2.22 | 333.33 ± 15.89* | 127.42 ± 5.74 | 137.14 ± 3.02* |

Control

119.37 ± 5.62 | 99.37 ± 4.95* | 87.25 ± 5.65 | 101.25 ± 5.84* | 388.88 ± 12.29 | 382.0 ± 22.0 | 128.66 ± 7.96 | 152.33 ± 9.58* |

Control received an equivalent of distilled water (rats, 5 ml/kg, i.g.) or drinking water (mice). *P<0.05 vs before therapy, **P<0.05 vs control. mean ± S.D.

of the enzyme values, but they were markedly lowered by the given medication, an effect more pronounced in mice than in rats (Table 2): (in mice treated with BPC157-μg (AST, ALT, LDH, CK), BPC157-ng (AST, ALT, LDH), amlodipine (AST, ALT, LDH, CK), and losartan (AST, ALT, LDH, CK); and in rats given BPC157-μg (AST, ALT, LDH, CK) or amlodipine (AST, ALT, CK).

Discussion

At presentation at 4 weeks following doxorubicine regimen, the elevated BET-1 plasma values correlate with pronounced CHF (edema, dyspnea, acrocyanosis, but still no increased heart rate and respiratory rate and still normotensive) (56 – 58). Likewise, a sharp additional increase of BET-1 plasma values throughout.
the two subsequent weeks correlates with the downhill course of CHF (i.e., CHF-hypotension, dyspnea with increased heart rate and respiratory rate). Accordingly, along with many tests used in clinical practice to monitor the effect of various therapeutic efforts and extracardiac neurohormonal systems such as the sympathetic system, the renin-angiotensin-aldosteron system, and the endothelial system up-regulated in heart failure contributing to disease progression, the stimulated endothelin system has gained special prognostic interest, and the value of measuring plasma BET levels in patients with advanced disease (LVEF <20%) is well established. Therefore, our data using BET-1 is a suitable method for determining doxorubicine-CHF and estimation of subsequent deterioration (4, 7, 16, 17).

Meanwhile, the enzyme values maintained their increased level until the end of the experiments, without correlation with CHF clinical deterioration (59, 60). Therefore, the elevated BET-1 values, and thereby increased ET-1 synthesis rate could be clearly used for better evaluation of worsening of doxorubicine-induced CHF. Thus, reversal of otherwise prominently increased BET-1 plasma values, counteraction of progressing CHF manifestations, such as increased heart rate and respiratory rate, hypotension, were all presented in BPC157-treated animals. Importantly, since all together counteracted with BPC157 regimen, these could be indicative for a useful therapy.

Moreover, the sustainedly raised serum enzyme values in controls were also consistently counteracted by BPC157 regimens. Consistent with this findings are the results obtained during hypoxia and reoxygenation experiment-reduced arrhythmias, prolonged periods of sinus rhythm, as well as prevention and reversal of acute doxorubicine cardiotoxicity (27, 28).

Amlodipine and losartan have comparable effects on BET-1 and enzyme values, but unlike normotensive-BPC157 rats, the animals remain hypotensive like the controls, but not more so, which is consistent with the potential anti-hypertensive effect of (amlodipine and losartan). Thus, presenting blockade of either calcium channel (i.e., amlodipine), or AT1-receptors (i.e., losartan) with BPC157, they share some other common points. Presenting hypotension as an implication of CHF in rats, BPC157 seems to be more successful in CHF-correction. With respect to losartan, a more consistent lowering effect on BET-1 concentrations was found in animals treated with amlodipine. Therefore, it could be speculated that in the given dosage, Ca\(^{2+}\) blockade seems to be more effective than AT1 receptor blockade (61 – 64).

Although many peptides are implicated in CHF-pathophysiology (i.e., BNP, TNF-\(\alpha\), ANP) or therapy, in vitro or in vivo studies, so far no peptide is reported to be beneficial in doxorubicine-induced CHF. BPC157 is stable in human gastric juice even for 24 h (36, 37). Importantly, no carrier was used in the previous (19 – 25, 33 – 35) and present studies. Together, this means that unlike limited delivery of other peptides (38 – 41, 65, 66), this stable pentadecapeptide is highly resistant to otherwise inescapable degradation of peptides. Consistently, BPC157 is given systemically and/or locally (19 – 25, 33 – 35) along with the positive effect following i.g. applications. Likewise, acting alone, without carrier (unlike other peptides), it presents a salutary potential of its own (19 – 25, 33 – 35) and may be suitable for therapy of a complex disturbance such as the CHF. Besides, this gastric pentadecapeptide likely controls functions of collagen fragments (19, 41).

Showing consistent endothelium protection and angiogenesis promotion in healing, of special interest for its effect in CHF may be its interaction with the NO-system. It opposes L-arginine NO-synthesis over-expression in gastric mucosa from rat stomach tissue homogenates, a finding relevant for various tissues. Interestingly, BPC157 induces by itself a generation of NO that could be not inhibited by L-NAME (similar direct evidence is still lacking for other growth factors (21)). Showing NO-restoration needed for CHF-recovery, BPC157 both prevents and reverses L-NAME-hypertension, along with ulcerogenesis antagonization. Similarly, it also inhibits lesions of various organs induced by L-NAME application in birds, unlike L-arginine (29, 30). Along with reversal of hypotension in CHF-animals, BPC157 antagonizes L-arginine-hypertension (21, 29).

Since several lines of evidence indicate for NO-ET relationship that NO impairs ET production presenting increased BET serum values as NO-failure, the reversal of otherwise increased BET serum values thereby likely indicates the restored balance and NO-function. Likewise, increase of NO release in CHF- and hypertension-models is fully recognized and extensively documented for angiotensin-converting enzyme inhibitors, losartan and other AT1 inhibitors, as well as for amlodipine (6, 15, 42, 44, 45, 49 – 53, 61 – 64, 67 – 73). For instance, amlodipine significantly increased nitrite production from coronary microvessels in both normal and failing dog hearts. Moreover, it releases NO even after heart failure and this may be partly responsible for its favorable effect in the treatment of heart failure (61 – 64, 71 – 73).

Thus, most likely, exerting the activity throughout the healing process, gastric pentadecapeptide BPC157 reaches the treatment aim to induce and then maintain healing. In addition, the toxicology studies indicate a
very safe profile. Therefore, these findings clearly suggest its possible use in further therapy of CHF.

References

synthesis in rat cardiac cells that is inhibited by iron supplementation. Toxicol Appl Pharmacol. 2002;185:85–90.


