The influence of BPC 157 on nitric oxide agonist and antagonist induced lesions in broiler chicks

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Summary — We describe the effects of nitric oxide (NO) agonists and antagonists and the influence of a novel organoprotective pentadecapeptide BPC 157, on the development of pulmonary hypertension syndrome and tissue lesions in chicks. Acute toxicity, which includes single dose application of saline (1 mL intraperitoneally (ip)), BPC 157 (10 μg/kg bw), L-NAME (NO antagonist, doses 50, 100, 150 mg/kg bw) and L-arginine (NO agonist /100 mg/kg bw with their combination L-NAME + BPC 157, L-NAME + L-arginine) was investigated. In this experiment pathohistological examination of the spleen, heart, liver and lungs and hematological analysis was conducted. In the chronic toxicity experiment, the animals were treated daily for 5 weeks with L-NAME (10 mg/kg bw), L-arginine (100 mg/kg bw), BPC 157 (10 μg/kg bw) and their combinations (L-NAME + BPC 157; L-NAME + L-arginine) ip. Seven animals from each group, including controls (saline 1 mL ip) were killed every week. Application of L-NAME caused pulmonary hypertension syndrome (PHS) in the treated chicks, which was prevented by the simultaneous application of L-arginine and BPC 157. Pathohistological examination of both acute and chronic toxicity revealed that L-NAME caused severe tissue damage (myocardial and hepatic cell necrosis, necrosis of the lymphoid cells in the spleen) while L-arginine provoked predominantly congestion, edema and hemorrhages in all organs. The effect of L-NAME was successfully inhibited by the application of L-arginine and BPC 157 but the latter substance did not cause any tissue or organ damage. Hematological analysis shows significant hemoglobin and leukocyte number decrease in the L-NAME-treated groups of chicks.

pulmonary hypertension syndrome / chickens / nitric oxide / BPC 157

Introduction

Pulmonary hypertension syndrome (PHS) or ascites in broilers refers to the accumulation of the edematous fluid within the abdominal cavity as a consequence of the heart failure syndrome (HFS) (Scheele et al, 1991). HFS comprises abnormalities such as hypertrophy of the right ventricle, dilatation of the heart, hydropericardium, and congested and edematous lungs (Olander et al, 1967; Burton et al, 1968). However, it is generally accepted that HFS is a direct consequence of the pulmonary hypertension which is developing because of poor blood oxygenation or low partial oxygen pressures either because of increasing organism demands or high altitude, respectively. Chronic hypoxia has a significant influence on pulmonary vasomotor tone. Regional reductions in alveolar O2 tension constrict the nearby arterioles and thus prevent the return of poorly oxygenated blood to the left atrium by exploiting primarily the better ventilated alveoli (Scheid and Holle, 1978; Grover et al, 1983). In such a reduced pulmonary vascular bed, the heart has to respond by a more vigorous contraction to overcome the higher flow resistance which soon provokes the HFS followed by the rise of the venous pressure, especially of the portal vein and hepatic veins. The final results of all these changes is ascites (Levy, 1979). Julian et al (1987) found that the rapidly growing broilers are more susceptible to increased pulmonary arterial pressure than slowly growing broilers. Low ambient temperature can stimulate the metabolism and increase the oxygen requirement and thus trigger the mechanism of the pulmonary hypertension (Scheele and Frankenhaus, 1989). Considering these data, one can conclude that the broiler treatment with the pulmonary vasodilators would reduce the incidence of the PHS. Indeed, Wideman et al (1995) showed significant incidence decrease of this syndrome in chicks which had been supplemented with dietary L-arginine which is a source of nitric oxide (NO) a potent endogenous endothelial vasodilator (Cocks and Angus, 1990). The birds, unlike mammal can not de novo synthesize L-arginine and their plasma concentration of this amino acid is directly correlated with its dietary intake (Tamil and Ratner, 1963a, b). On the other hand, there is no literature data concerning the effects of nitric synthase (NOS) antagonists (derivatives of L-arginine), which are very strong vasoconstrictors, on the development of the PHS in broilers.

Recently, we identified and partially synthesized a 15 amino acid fragment of the new gastric juice
peptide with wide cyto- (Bódis et al, 1996) and organoprotective properties (Paré and Klucyznski, 1992; Sikiric et al, 1993a, b, c, 1994, 1996a, b; Veljaca et al, 1994a, b, 1995) code-named BPC 157. Therefore, in this work we wanted to investigate the effects of chronic and acute administration of NO agonists and antagonists in broilers and in the same time to describe the effects of simultaneous treatment with BPC 157.

**Materials and methods**

**Chronic toxicity**

In the experiment we used 180 1-day-old broiler male Ross chicks that were kept in metal wire cages. Food and water were supplied ad libitum. On the 1st day of life the chicks were vaccinated against Marek disease (Marikal SPF, Pliva), intramuscularly (im) in the thigh muscle and on the 15th day of life they were vaccinated against Newcastle disease (Pes-

| Table I. Pathohistological heart changes. |
|----------------|----------------|----------------|----------------|----------------|
| **Group** | **I (weeks)** | **II (weeks)** | **III (weeks)** | **IV (weeks)** | **V (weeks)** |
| K | Perivascular edema; mild parenchymatous degeneration | No visible changes | Slight perivascular and interfibrillar edema | Focal hyperplasia of mononuclear cells; parenchymatous degeneration | Thickened vessel wall |
| LN | Prominent perivascular edema | Severe parenchymatous degeneration; mild perivascular edema | Severe perivascular and interfibrillar edema | Most prominent perivascular subendothelial edema; severe myocardiolysis | Severe interfibrillar edema; Hyperaemia; Hemorrhage |
| LA | Slight perivascular edema; Severe parenchymatous degeneration; Hemorrhage | Perivascular interfibrillar edema; hyperemia | Mild edema | Mild hyperaemia; Hemorrhages; Mild edema | Mild hyperaemia; Edema |
| BPC | Severe edema perivascular and interfibrillar; mild parenchymatous degeneration | Mild edema; mild interstitial mononuclear hyperplasia | Without any visible changes | Mild interfibrillar edema | Slight perivascular and interfibrillar edema |
| BPC | Severe parenchymatous degeneration | Severe perivascular edema | Moderate parenchymatous degeneration | Moderate parenchymatous degeneration | Moderate parenchymatous degeneration |
| BPC | Mild perivascular edema; Severe parenchymatous degeneration | Focal mononuclear hyperplasia | Severe perivascular edema and parenchymatous degeneration | Moderate parenchymatous degeneration | Moderate parenchymatous degeneration |
Acute toxicity

For the investigation of the acute toxicity 30 30-day-old male broilers, Avian 34, were divided into six groups and treated with the following substances (treatment/no of chicks): 1) control: K, 0.5 mL/kg saline; five chicks; 2) LN50: L-NAME, 50 mg/kg; five chicks; 3) LN100: L-NAME, 100 mg/kg; five chicks; 4) LN150: L-NAME, 150 mg/kg; five chicks; 5) LN150/1A: L-NAME, 150 mg/kg + L-arginine, 100 mg/kg; five chicks; and 6) LN150/BPC 157: L-NAME, 150 mg/kg + BPC 157, 10 μg/kg; five chicks.

During the 6 h of the experiment the chicks did not receive any food or water. After 6 h the animals were killed under chloroform anesthesia and necropsied. The organs (heart, liver, lungs, spleen) were taken for histopathological examination. Whole blood samples were taken for hematological analysis. The erythrocyte, leukocyte (lymphocyte and heterophyle) number, hemoglobin, hematocrit and mean corpuscular volume value were determined. The hematological parameter data were statistically analyzed by Mann-Whitney U-test between the two independent groups. The level of significance was P < 0.05.

Results

Chronic toxicity

Necropsy findings

The post-mortem necropsy examination of the animals did not reveal notable differences between control and treated groups. However, five animals from the L-NAME group older than 4 weeks died from PHS. The same cause of death was noticed in one animal in the L-NAME and BPC 157 group.

Pathohistological changes

Examination of the heart revealed very slight pathohistological changes in the control group of ani-

Photo 1. Severe myocardial cell necrosis and interfibrillar and endomysial edema in the chronic toxicity experiment in the chicks after 4 weeks of treatment with L-NAME. HE, x 10.

Photo 2. L-arginine treated group of chicks (4th week, chronic toxicity); severe intramyocardial hemorrhages. HE, x 25.

Photo 3. Mild edema and hyperemia of the myocardium; animals treated with L-arginine and BPC 157 (4th week, chronic toxicity). HE, x 10.

Photo 4. Severe perivascular hemorrhages and hyperemia in the chick lungs treated with L-arginine (5th week, chronic toxicity). HE, x 25.
mals (table I). However, the lesions were very much more pronounced in the group of animals treated with L-NAME. The changes were most striking after the 3rd week and they were characterized by edema and myocardiolysis (photo 1). On the contrary, the changes in the animals treated with L-arginine were characterized by severe intramyocardial hemorrhages (photo 2). In the groups of chicks treated with BPC 157 either alone or in combination with L-arginine and L-NAME (photo 3) none of these changes were found.

In the lungs (table II) the most striking findings in the treated groups of chicks were edema and parenchymatous hemorrhages, especially pronounced in the LA group (photo 4). In the BPC 157-
treated group of chicks, the lesions were mild hyperemia and edema (photo 5).

The liver changes (table III) were predominantly reversible degenerative lesions in all groups but one, namely, in the group of chicks treated with L-NAME severe perivascular hepatocellularysis (photo 6) was found.

### Acute toxicity

The first results of the clinical examination were obtained after the period of 6 h and the highest degree of the skin, subcutis and breast muscle cyanosis were noted in the chicks of the groups LN150 and

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**Table II.** Pathohistological lung changes.

<table>
<thead>
<tr>
<th>Group</th>
<th>I (weeks)</th>
<th>II (weeks)</th>
<th>III (weeks)</th>
<th>IV (weeks)</th>
<th>V (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>Erythrocytes in parabronchial space</td>
<td>Interlobular hyperemia</td>
<td>Mild hyperemia</td>
<td>Mild hyperemia and perivascular edema</td>
<td>Mild hyperaemia and perivascular edema</td>
</tr>
<tr>
<td>LN</td>
<td>Severe congestion; perivascular edema</td>
<td>Severe edema and hyperemia</td>
<td>Mild hyperemia</td>
<td>Severe perivascular edema; hyperemia</td>
<td>Severe perivascular and interstitial edema</td>
</tr>
<tr>
<td>LA</td>
<td>Mild hyperemia, perivascular edema</td>
<td>Severe hyperemia; hemorrhages</td>
<td>Severe hyperemia</td>
<td>Severe hyperemia; perivascular fibrinoid fluid</td>
<td>Severe hyperemia; hemorrhages</td>
</tr>
<tr>
<td>BPC</td>
<td>Slight perivascular edema</td>
<td>Mostly no visible changes</td>
<td>Mild hyperemic; perivascular edema</td>
<td>Mild hyperemia; perivascular edema</td>
<td>Mostly no visible changes</td>
</tr>
<tr>
<td>BPC</td>
<td>Slight perivascular edema</td>
<td>Mild to severe hyperemia; hemorrhage</td>
<td>Mild hyperemia</td>
<td>Mild hyperemia; edema</td>
<td>Mild hyperemia; edema</td>
</tr>
<tr>
<td>LA</td>
<td>Mild perivascular edema</td>
<td>Mild hyperemia</td>
<td>Mild edema and hyperemia</td>
<td>Mild hyperemia; edema</td>
<td>Mild to severe hyperemia; edema</td>
</tr>
<tr>
<td>BPC</td>
<td>Mild perivascular edema</td>
<td>Mild hyperemia</td>
<td>Mild edema and hyperemia</td>
<td>Mild hyperemia; edema</td>
<td>Mild to severe hyperemia; edema</td>
</tr>
</tbody>
</table>
Table III. Pathohistological liver changes.

<table>
<thead>
<tr>
<th>Group</th>
<th>I (weeks)</th>
<th>II (weeks)</th>
<th>III (weeks)</th>
<th>IV (weeks)</th>
<th>V (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>Severe parenchymatous degeneration</td>
<td>No changes</td>
<td>Mild edema; parenchymatous degeneration</td>
<td>Mild edema; parenchymatous degeneration</td>
<td>Severe parenchymatous degeneration</td>
</tr>
<tr>
<td>LN</td>
<td>Mild interstitial edema; hyperemia</td>
<td>Severe interstitial edema</td>
<td>Severe interstitial edema; mild hyperemia</td>
<td>Perivascular hepatocellularis; hyperemia; edema</td>
<td>Severe edema; hyperemia</td>
</tr>
<tr>
<td>LA</td>
<td>Severe perivascular and parenchymatous degeneration; hyperemia</td>
<td>Perivascular fibroplasia; mononuclear hyperplasia</td>
<td>Perivascular hemorrhages; hyperemia</td>
<td>Severe hyperemia</td>
<td>Mild edema; severe hyperemia; parenchymatous degeneration</td>
</tr>
<tr>
<td>BPC</td>
<td>Mild perivascular edema; hyperemia</td>
<td>Mild edema; mild parenchymatous degeneration</td>
<td>Mild edema; perivascular mononuclear hyperplasia</td>
<td>Lymphoid foci; hyperemia; mild parenchymatous degeneration</td>
<td>Mild hyperemia; edema</td>
</tr>
<tr>
<td>BPC LA</td>
<td>Perivascular fibroplasia; mononuclear hyperplasia</td>
<td>Mild hyperemia</td>
<td>Hemorrhages; edema</td>
<td>Mild hyperemia</td>
<td>Mild hyperemia</td>
</tr>
</tbody>
</table>

Fig 1. Mean heterophile/lymphocyte index values in the control and treated groups of chicks.
K, 0.5 mL of saline ip; LN50, L-NAME: 50 mg/kg bw ip; LN100, L-NAME: 100 mg/kg bw ip; LN150, L-NAME: 150 mg/kg bw ip; LA, L-arginine: 100 mg/kg bw ip; BPC, BPC 157: 10 μg/kg bw ip.
LA/LN150. Mild cyanosis of the breast area was observed in all other treated groups of chicks.

Necropsy findings
There were slight edemas in the subcutis of the breast area, hydropericardium and increased peritoneal fluid in the chicks of the groups LN50, LN150/BPC, LN150/LA, and LN100. In the chicks of the control group there were no visible pathological changes.

Hematological analysis
Mean hemoglobin value of the group LN50, LN100 and LN150/LA were significantly lower than other group values (fig 3). Mean erythrocyte value (fig 2) of the LN150/BPC 157 group was significantly higher than the other group values. Mean leukocyte (fig 4) values of the LN150/BPC 157, LN100/LA group were significantly lower than the other group values. Mean hematocrit value (fig 5) of the LN150/BPC 157 group was significantly higher than the other group values. The level of significance was $P < 0.05$. There were no significant differences in the mean heterophile/lymphocyte index value (fig 1), the mean granular leukocyte value (fig 6), the mean non-granular leukocyte value (fig 7) and the mean corpuscular volume value (fig 8) between the control and the treated groups of chicks.

Pathohistological examination
The most prominent lesions were found in the hearts of the chicks treated with higher doses of L-NAME (table IV). The lesions were very similar to those observed in the chronic toxicity experiment. The spleen was also significantly affected by necrosis of lymphoid cells. Both lesions were successfully prevented by BPC 157 application. The histopathological examination of the vessels revealed mild edema and hemorrhage in the chicks of the group LN150/LA.

Discussion
Chronic application of nitric oxide synthase inhibitor (L-NAME) used in a dose (10 mg/kg bw) that produces vasoconstriction and blood pressure elevation (Rees et al, 1989) could produce PHS in broilers. This disease was not developing in the
### Table IV. Pathohistological changes in the selected organs of the chicks treated with the different doses of BPC 157, L-NAME and L-arginine.

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen</th>
<th>Liver</th>
<th>Heart</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>No visible changes</td>
<td>No visible changes</td>
<td>Mild perivascular edema; erythrocytes in parabronchial space: mild hyperemia</td>
<td></td>
</tr>
<tr>
<td>LN₅₀</td>
<td>Necrosis of the lymphoid cells</td>
<td>Mild hyperemia</td>
<td>Mononuclear hyperplasia; mild perivascular edema</td>
<td>Mild perivascular edema</td>
</tr>
<tr>
<td>LN₁₀₀</td>
<td>Necrosis of the lymphoid cells</td>
<td>Mild hyperemia; severe edema</td>
<td>Severe hyperemia; severe perivascular, interfibrilar and subendothelial edema and myocardiolysis</td>
<td>Mild perivascular edema</td>
</tr>
<tr>
<td>LN₁₅₀</td>
<td>Severe hyperemia</td>
<td>Mild hyperemia</td>
<td>Perivascular and interfibrilar edema</td>
<td>Perivascular prominent edema</td>
</tr>
<tr>
<td>LN₁₅₀/LA</td>
<td>No changes</td>
<td>A highest degree of edema</td>
<td>As LN₁₀₀</td>
<td>Severe hyperemia; edema</td>
</tr>
<tr>
<td>LN₁₅₀/BPC</td>
<td>No change</td>
<td>Mild parenchymatous degeneration</td>
<td>Mild hyperemia; perivascular mild edema</td>
<td>Severe hemorrhages; perivascular edema</td>
</tr>
</tbody>
</table>

**Fig 3.** Mean hemoglobin values ($\times 10^\text{g/L}$) in the control and treated groups of chicks.

K, 0.5 mL of saline ip; LN₅₀, L-NAME: 50 mg/kg bw ip; LN₁₀₀, L-NAME: 100 mg/kg bw ip; LN₁₅₀, L-NAME: 150 mg/kg bw ip; LA, L-arginine: 100 mg/kg bw ip; BPC, BPC 157: 10 μg/kg bw ip. * $P < 0.05$ vs control, Mann-Whitney U-test.
**Fig 4.** Mean leucocytes values (x 10^9/L) in the control and treated groups of chicks. K, 0.5 mL of saline ip; LN50, L-NAME: 50 mg/kg bw ip; LN100, L-NAME: 100 mg/kg bw ip; LN150, L-NAME: 150 mg/kg bw ip; LA, L-arginine: 100 mg/kg bw ip; BPC, BPC 157: 10 μg/kg bw ip. * P < 0.05 vs control, Mann-Whitney U-test.

**Fig 5.** Mean hematocrit values (%) in the control and treatment groups of chicks. K, 0.5 mL of saline ip; LN50, L-NAME: 50 mg/kg bw ip; LN100, L-NAME: 100 mg/kg bw ip; LN150, L-NAME: 150 mg/kg bw ip; LA, L-arginine: 100 mg/kg bw ip; BPC, BPC 157: 10 μg/kg bw ip. * P < 0.05 vs control, Mann-Whitney U-test.
Fig 6. Mean granular leucocytes (granulocytes) values (%) in the control and treated groups of chicks. K, 0.5 mL of saline ip; LN50, L-NAME: 50 mg/kg bw ip; LN100, L-NAME: 100 mg/kg bw ip; LN150, L-NAME: 150 mg/kg bw ip; LA, L-arginine: 100 mg/kg bw ip; BPC, BPC 157: 10 μg/kg bw ip.

Fig 7. Mean non-granular leucocytes (lymphocytes) values (%) in the control and treated groups of chicks. K, 0.5 mL of saline ip; LN50, L-NAME: 50 mg/kg bw ip; LN100, L-NAME: 100 mg/kg bw ip; LN150, L-NAME: 150 mg/kg bw ip; LA, L-arginine: 100 mg/kg bw ip; BPC, BPC 157: 10 μg/kg bw ip.
Fig 8. Mean mean corpuscular volumes (MCV) values (fl) in the control and treated groups of chicks.
K, 0.5 mL of saline ip; LN50, L-NAME: 50 mg/kg bw ip; LN100, L-NAME: 100 mg/kg bw ip; LN150, L-NAME: 150 mg/kg bw ip; LA, L-arginine: 100 mg/kg bw ip; BPC, BPC 157: 10 μg/kg bw ip.

Group of chicks treated simultaneously with the L-arginine; also, BPC 157 inhibited the above mentioned condition a little less successfully. This effect of L-arginine in preventing PHS is already described in the literature (Wideman et al., 1995). However, our result shows that this substance may have detrimental effects, especially on the myocardium. Namely, severe hemorrhages noted in the L-arginine-treated group are probably the consequence of the dilated and damaged coronary blood vessels. BPC 157 did not have such a worsening effect, either applied alone or in combination with the other drugs used. Notably, both experiments revealed very interesting findings concerning the myocardial lesions. Cardiotoxicity as observed in the chicks treated with the L-NAME is an unexpected finding and we could not find similar literature data. It must be emphasized that very similar findings were found in the acute toxicity experiment. BPC 157 in both cases (chronic and acute toxicity) successfully prevented the myocardial necrosis. These organoprotective effects of BPC 157 have already been described in the literature using other animal models of diseases (Sikiric et al., 1993a, b, c).

Very significant perivascular hepatocellular lysis, interstitial edema and hyperemia were found in the chick liver of the L-NAME treated group. The same lesions, except for hepatocellular lysis, were followed by hyperemia and hemorrhages in the chick liver of the group LA. The very same findings were found in the heart. Generally speaking it could be noted that L-NAME caused severe irreversible lesions of the heart and liver, while L-arginine induced severe hyperemia and hemorrhages in the three organs (heart, liver, lung). BPC 157 very efficiently decreased the severity of the above mentioned lesions.

In the acute toxicity experiment, an important finding was the necrosis of the lymphoid cells around a lienis that was found in the spleens of the animals treated with two smaller doses of L-NAME (50 and 100 mg/kg bw). This finding is difficult to interpret although it is very well known from the literature (James, 1995) that NO serves not only as blood pressure mediator but also has a notable physiological role in the cellular defense mechanisms against intracellular parasites.

Myocardial lesions in the chicks of the groups LN100, LN150, LN150/BPC and LN150/LA were characterized by some degree of perivascular, interfibrillar and subendothelial edema and hyperemia, but in the LN150 group, as already noted, a myocardial
cell necrosis very similar to those described in the chronic toxicity experiment, was found.

Concerning the hematology data in the acute toxicity experiment, some findings are interesting. This goes primarily for the hemoglobin and leukocyte values. Namely, the decreasing of the leukocyte number was partially supported by the pathohistological finding of the lymphoid cell necrosis, but the decreased hemoglobin amount is unexpected especially in such a short experiment (6 h).

As a conclusion, we emphasize that the results of this investigation show that in the whole field of tissue lesions induced by inadequate balance of NO in the birds are in the most cases successfully diminished by simultaneous application of BPC 157.

Acknowledgments

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References


Burton RR, Besch EJ, Smith AH (1968) Effect of chronic hypoxia of tissue lesions induced by inadequate balance of NO in the birds are in the most cases successfully diminished by simultaneous application of BPC 157.


