Angiogenesis and cardioprotection after TNFα-inducer-Tolpa Peat Preparation treatment in rat’s hearts after experimental myocardial infarction in vivo

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Abstract

The aim of the presented work was to evaluate whether short subcutaneous (s.c.) administration of TNFα-inducer-Tolpa Peat Preparation (TPP or TPP batch 0210)® modulates the process of ischemic remodeling and spontaneous angiogenesis after experimental myocardial infarction (MI) in rats in vivo. The results obtained using three complementary and correlative methods: histological studies, Proliferating Cell Nuclear Antigen (PCNA) reaction and Lymphocytes Induced Angiogenesis (LIA) test showed a clear pro-angiogenic and cardioprotective effect of TPP administration after experimental MI.

TPP batch 0210 should be considered as an angiogenesis stimulating factor and consecutively as a cardioprotective preventing development of ischemic cardiomyopathy after MI in rats.

It might possibly be used as an adjunct to conventional therapy of coronary artery disease, including late phase after myocardial infarction or ischemic cardiomyopathy.

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Keywords: Tolpa peat preparation; Experimental myocardial infarction; Angiogenesis; Cardiomyopathy; TNFα-inducer

1. Introduction

The degree of left ventricle failure is the main reason for evaluation of prolonged prognosis after myocardial infarction (MI). It could be a direct consequence of developing tissue necrosis in the acute phase of myocardial infarction as well as the remodeling process. The immunocompetent cells infiltration of infarcted myocardium is one of the most important mechanisms of tissue repair. Angiogenesis, apoptosis, necrosis—all are considered as factors associated with immunocompetent cells activation creating the base of postinfarcted tissue remodeling. The prolonged or changed immunocompetent cells activity due to their apoptosis dysregulation in situ in postinfarcted tissue with upregulation of cytokines expression seems to be the crucial pathway of compensatory angiogenesis regulation.

Angiogenesis is normally under stringent control and occurs mainly during embryonic development, the female reproductive cycle and wound repair. The development of blood vessels is held in balance by proangiogenic and antiangiogenic factors (Folkman and Klagsbrun, 1987; Folkman and Shing, 1992; Fan and Brem, 1992). In many pathological conditions (e.g. solid tumor, rheumatoid arthritis, diabetic retinopathy and atherosclerosis), the disease itself appears to be accelerated by persistent...
upregulated vessel proliferation. Folkman (1972) hypothesized that tumour growth is angiogenesis-dependent and pioneered the idea that an antiangiogenesis strategy might represent a new therapeutic approach for the treatment of solid tumours. The first successful clinical treatment of an angiogenic disease was provided using interferon α-2a (INFα2a) during pulmonary hemangiomatosis (White et al., 1989).

Tolpa Peat Preparation (TPP)® is classified as an immunomodulator, which was found to be tumour necrosis factor (TNFα), interferon (INF) and several other cytokines inducer in human peripheral blood leukocytes (Inglot et al., 1993a). An inhibitory effect of TPP on spontaneous interleukin 1 (IL-1) release by monocytes of rheumatoid patients was observed (Skopinska-Rózewska et al., 1993). The stimulated low and suppressed abnormally high angiogenic activity of human mononuclear leukocytes was also found and possibly TPP might be used as an adjunct to conventional therapy of rheumatoid arthritis (Skopinska-Rózewska et al., 1993). In addition, theophylline, a phosphodiesterase inhibitor, suppresses stimulatory effect of TPP on human lymphocytes-induced angiogenesis (Skopinska-Rózewska et al., 1992).

We focused our attention on the potential proangiogenic benefit activity of TPP in postinfarcted tissue formation or to stimulate neovascularization using the previously established model of experimental MI in rats (Krzeminski et al., 1995).

The aim of the presented work was to show whether a short subcutaneous administration of TPP batch 0210 modulates the process of angiogenesis and remodeling after experimental MI in rats in vivo and whether it induces persistent angiogenic (in the late period after MI) and cardioprotective effect or short-lasting only?

2. Materials and methods

2.1. Animals and drug

All experiments were performed on Sprague–Dawley male rats (Central Animal Farm, Medical University of Silesia, Katowice, Poland), weighing 360 to 427 g, fed on a control rat fodder diet (Altrimin 1220, from Altrimin GmbH, Lage, Germany) with free access to water and housed individually under controlled conditions (ambient temperature 22–24°C, 45–60% relative humidity; with 12 h dark/light cycle).

Tolpa Peat Preparation (TPP batch 0210), a product of Torf Corporation (Fabryka Leków Sp. z o.o., ul. Fabryczna 11, 55-080 Katowice Torf Corporation, Poland) was dissolved in 0.9% NaCl and administered subcutaneously (s.c.) in a dose of 1 mg/kg of body weight (b.w.).

The study was performed following the Local Bioethical Committee’s approval (NN-210/97). All animal testing was carried out in accordance with NIH regulations of animal care as described in the “Guide for the Care and Use of Laboratory Animals” (released January, 1996).

2.2. Experimental myocardial infarction in vivo

The myocardial infarction was induced by left anterior descending coronary artery (LAD) occlusion for 11 and 28 days, these being the survival period of the animals. Transmural MI was confirmed: indirectly (during the experiment by the characteristic ECG changes e.g. ST-segment elevation) and directly—by qualitative estimation of infarcted myocardial tissue in histological examination. The rats were anesthetized with pentobarbital (Pentobarbital Sodium Salt, Sigma, Deisenhofen, Germany, 60 mg/kg intraperitoneally, i.p.). Surgical procedure was performed according to Selye et al. (1960), Guendelv (1977) and with own improvements described elsewhere (Krzeminski et al., 1995; Krzeminski, 1991).

Briefly, the trachea was incised longitudinally and cannulated to allow artificial ventilation. The chest was opened under ventilation with room air (Rodent VENTILATOR-UB 7025, Hugo Sachs Elektronik, March, Germany; stroke volume 2 ml/100 g body weight and rate 54 strokes/min) by left thoracotomy at the fifth intercostal space and the fifth and fourth ribs were sectioned approximately 2 mm from the left margin of the sternum. After opening the pericardium the heart was not exteriorized and a sling (6/0 prolene 0.7 suture attached to a 3/8 circled BV-1 9.3 mm atraumatic, reverse cutting needle, EH 7406H, Ethicon GmbH, Norderstedt, Germany) was placed around the LAD close to its origin. Then the ligature was passed through a plastic pad (0.5 mm long, 0.4 mm OD). The coronary artery was occluded by applying tension to the ligature while pressing the pad onto the surface of the heart. Tension was maintained by clamping a climb clip (Titan climb clip, LT-100, Ethicon). Successful occlusion was immediately confirmed by ischaemia-induced, characteristic alteration in ECG and observation of a pale arising ischemic zone below the climb clip. At the end of the operating procedure tissues were sutured in layers (4-0 Deklene TM-II, 1.5, D-5427, Ethicon) excluding pericardium. The rats woke a few hours after closing the thorax. The postoperative mortality rate of all rats was 15% (mainly caused by lethal arrhythmias and circulatory and/or respiratory insufficiency during the 1st day after MI).

2.3. Histology and immunocytochemistry (angiogenesis) studies

After 11 and 28 days, the surviving animals were again anesthetized with pentobarbital (60 mg/kg, i.p.), blood samples (1 ml) were taken directly from the left ventricle and centrifugated (the sera samples/0.5 ml/ were frozen/−15°C/within 20 min from collection and stored less than 3 weeks/−70°C/for further estimations) and subsequently the rats’ hearts were excised and fixed in 4% formalin with.
CaCO₃ addition (pH 7.4). After 48 h, the hearts were removed to the next dark bottles with formalin (pH 7.4). The control and infarcted hearts were cut 1–2 mm distally to the occlusion clamp and four micron paraffin slices were stained with hematoxylin and eosin.

For immunocytochemical study, the hearts were kept in formalin less than 7 days, dehydrated in alcohol, soaked in xylene and shortly (<2 h) impregnated in paraffin stocks (50 °C). Five micron paraffin slices were stained for monoclonal mouse anti–Proliferating Cell Nuclear Antigen Antibodies (PCNA, Dako, USA) (Roos et al., 1993; Yu and Filippe, 1993).

2.4. Angiogenic activity study

The angiogenic activity of tested sera was evaluated with the lymphocytes-induced angiogenesis (LIA) test of Sidky and Auerbach (1975) with slight modifications (Kamiński et al., 1981; Majewski et al., 1987; Polakowski et al., 1988). LIA test is a model of xenogenic local graft versus host reaction, where the number of newly formed blood vessels induced by angiogenic lymphokines released from lymphocytes can be measured. It is a simple quantitative method for evaluation of the cytokines level responsible for angiogenesis. Briefly, the test was performed as follows: the suspension of rat’s spleen cells (0.1 ml) was injected intradermally in shave flanks of each mouse. The cells suspension was supplemented with 0.05 ml/ml 0.01% trypan blue in order to facilitate recognition of injection sites later on. Before performing injections the mice (6–10 week old Balb/c inbred mice) were anaesthetized with 3.6% chloralhydrate (0.1 ml per 10 g of b.w.) injected i.p. For performing the cell injections easily, both flanks of each mouse were finally shaved by razor and on each flank 2–3 injections were localized. On the day of the cells injection and on the subsequent two days the mice received 0.1 ml s.c. studied serum probes (obtained from blood samples taken directly from the left ventricle) or saline phosphate buffer (PBS, control). After 72 h the mice were killed, their skin was separated from underlying tissues, injection sites (stained with trypan blue) were localized on the inner skin side and the newly formed blood vessels were counted. Counting was performed in a circle surrounding the injection site (the area of this circle was equal to half the area visible under surgical microscope at 32 magnification). All blood vessels directed toward the injection site were counted according to previously proposed criteria (Kamiński et al., 1981; Majewski et al., 1987; Polakowski et al., 1988). These vessels differed from the background vasculature due to their tortuosity and bifurcations.

In addition, the influence of tested serum on leukocytes-induced angiogenesis was estimated. The mean of the absolute number of the new vessels counted in the place of the suspension of rats’ spleen cells injection was calculated. These means values were replaced with so-called factors of angiogenetic activity (WAA) according to the formula: WAA=a/b (a-number of newly formed vessels in control probe; b-mean of newly formed vessels in control estimated after PBS injections/the value of 1.0±0.02/).

2.5. Experimental protocol

MI-surviving animals were collected and divided into six (controls and treated with TPP) groups 11 and 28 days after MI for:

a) histological study—in each group,

b) PCNAstudy—to confirm the proliferative effect of the studied preparation in the treated groups,

c) LIAtest as a model of xenogenic local graft versus host reaction—in each group.

The experimental follow up is presented in Table 1.

2.6. Statistical analysis

The mean of absolute numbers of the new vessels was calculated and compared to the control values using Mann–Whitney U test. Differences were considered as significant at P<0.05. All other results are expressed as mean values ± SEM, median, minimal and maximal values.

3. Results

3.1. Histological studies

Normal myocardium, without any pathology was observed in the intact group. The small vessels in fibrous tissue without signs of cardiocyte hypertrophy, cardiomyopathy or inflammation were visible in the C-int-TPP group.

In the C-inf-11th MI group granulation tissue with mainly young vessels and scanty inflammatory infiltration

<table>
<thead>
<tr>
<th>Experimental protocol</th>
<th>Number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control-int group, 1 ml/kg 0.9% NaCl s.c. during 4 days (C-int)</td>
<td>10</td>
</tr>
<tr>
<td>2. Control-int group, TPP 0210 1 mg/kg s.c. during 4 days (C-int-TPP)</td>
<td>10</td>
</tr>
<tr>
<td>3. Control-infarcted group, 1 mg/kg 0.9% NaCl s.c. from 5th till 9th day after MI</td>
<td>8</td>
</tr>
<tr>
<td>4. Treated group, TPP 0210 1mg/kg s.c. from 5th to 9th day after MI</td>
<td>8</td>
</tr>
<tr>
<td>5. Control-infarcted group, 1 mg/kg 0.9% NaCl s.c. from 21st till 25th day after MI</td>
<td>8</td>
</tr>
<tr>
<td>6. Treated group, TPP 0210 1mg/kg s.c. from 21st to 25th day after MI</td>
<td>10</td>
</tr>
</tbody>
</table>

The rats were sacrificed on 28th day after MI (TPP-inf-28th MI)
was observed, also without cardiomyopathic pattern. Mainly young vessels, and numerous fibroblasts were found in the TPP-inf-11th MI group (Fig. 1) as well as a decrease of inflammatory reaction density in comparing to C-inf-11th MI; in contrast, vessels amount and vascular walls (maturity) were comparable. However, cardiomyopathic morphology in the C-inf-28th MI group was observed. Furthermore, small fibrocytic and partly fibroblastic scars were present in myocardial tissue, but the vessels were predominantly young (70%) and partly mature (30%).

After TPP treatment (TPP-inf-28th MI group) no myocardial pathology, or myocardial fibrosis was found (Fig. 2). Moreover, young vessels were predominant in this group and these vessels formed two populations: young capillaries lined with endothelial cells and more maturated with the presence of differentiated walls containing muscular fibres.

Briefly, it should be pointed out that delicate myocardial fibrosis was observed in 80% of the animals in all groups receiving TPP 0210. Additionally, in animals receiving TPP 0210 after MI, reduced inflammatory infiltrations compared with the non-treated MI group were found. The presence of differentiated small vessels after treatment, while absent in hearts from rats without TPP administration, suggests that the process of revascularization, or accelerated maturation of granulation tissue in damaged myocardium is affected by TPP. The cardiomyopathic morphology, visible in the C-inf-28th MI group, was absent in the analogous group which received TPP.

In summary, short-lasting later period after MI secondary prevention with TPP reduced the inflammatory reaction, stimulated revascularization and prevented the onset of postinfarctive cardiomyopathy.

3.2. Immunocytochemical study

PCNA immunostaining of the hearts TPP-inf-11th MI showed positive reaction in some fibroblasts and capillary endothelial cells localized in the infarct scar (Fig. 3), while in the group TPP-inf-28th MI the cells reacting positively were numerous (Fig. 4).
In summary, the infarcted rats’ hearts stimulation with TPP 0210 revealed positive reaction in a greater amount of the fibroblasts and to a lesser extent the endothelial cells.

### 3.3. Angiogenic activity study

The results are presented in Tables 2 and 3. The C-inf-11th MI group differs significantly (higher value of WAA index) from the C-int group (Mann–Whitney test, 1 vs. 3, p = 0.019) as well as the C-inf-11th MI from C-inf-28th MI group (Mann–Whitney test, 3 vs. 5, p = 0.008).

Furthermore, a significant difference was also found between only one treated group with TTP after MI and control (C-inf-28th MI vs. TPP-inf-28th MI; Mann–Whitney test, 5 vs. 6, p = 0.039).

### 4. Discussion

Recent stages of MI in rats closely resemble those of humans. The dynamic of MI in rats is faster than in humans, the resorption processes and vascularization are accelerated with end-point at the second week. Greater restitution of damaged tissue takes place in rats; it may be accelerated with end-point at the second week. Greater restitution of damaged tissue takes place in rats; it may be associated with a significant decrease in left ventricle (LV) fractional shortening, a rise in LV pressure, a significant increase in LV end-diastolic dimension/body weight and LV end-diastolic pressure (Nakamura et al., 2003). In both mice and rats, MI induces a time-dependent impairment of heart function with subsequent development of heart failure. The hemodynamic consequences after 4 weeks are characterized by reduced LV pressure and increased right ventricular (RV) pressure (Deten and Zimmer, 2002).

TNFα as well as INFγ are considered to be the crucial cytokines in an immunoresponse after MI. The expression of mRNA for TNFα, INFγ and other cytokines such as IL-1β, IL-2, IL-6, TGF-β1 utilizing the reverse transcriptase–polymerase chain reaction amplification technique was studied. Increased cardiac mRNA levels for all studied cytokines, except IL-6 and INF-γ, were measurable within 15 to 30 min of LAD occlusion and increased levels were observed for 3 h. Cytokine mRNA levels returned to baseline levels at 24 h, while TNFα, TGF-β1, IL-1β expression were significantly increased at 7 days only in animals with LAD occlusion without reperfusion (Hershkowitz et al., 1995).

Furthermore, Deten et al. (2003) showed strong induction of pro-inflammatory cytokines expression in the myocytes of the infarct area 6 h after MI, thereby suggesting that cytokines (IL-1β and IL-6) produced by ischemic myocytes may be connected with the initiation of the necrotic area restitution. TNFα action seems to be multidirectional in myocardial remodeling pathophysiology. It is well known that TNFα may promote the fibrosis of myocardium. One of the potential mechanisms is due to angiotensin type 1 receptor (AT1) activation, localized in cardiac fibroblasts. AT1 receptors upregulated by TNFα mediate most Ang II effects, e.g. H3-proline incorporation and specific protein produc-

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**Table 2** Descriptive statistics of WAA index in analyzed groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Number of rats</th>
<th>Mean ± SEM</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-int</td>
<td>9</td>
<td>0.888 ± 0.056</td>
<td>0.85</td>
<td>0.71</td>
<td>1.18</td>
</tr>
<tr>
<td>C-int-TTP</td>
<td>10</td>
<td>1.011 ± 0.078</td>
<td>0.99</td>
<td>0.58</td>
<td>1.33</td>
</tr>
<tr>
<td>C-inf-11th MI</td>
<td>5</td>
<td>1.120 ± 0.068</td>
<td>1.17</td>
<td>0.94</td>
<td>1.29</td>
</tr>
<tr>
<td>TPP-inf-11th MI</td>
<td>5</td>
<td>0.994 ± 0.100</td>
<td>0.905</td>
<td>0.73</td>
<td>1.40</td>
</tr>
<tr>
<td>C-inf-28th MI</td>
<td>8</td>
<td>0.770 ± 0.044</td>
<td>0.76</td>
<td>0.62</td>
<td>0.86</td>
</tr>
<tr>
<td>TPP-inf-28th MI</td>
<td>10</td>
<td>1.073 ± 0.075</td>
<td>1.14</td>
<td>0.74</td>
<td>1.39</td>
</tr>
</tbody>
</table>

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**Table 3** Comparisons of WAA indexes between analyzed groups

<table>
<thead>
<tr>
<th>Compared groups</th>
<th>Probability (p)</th>
<th>Mann–Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs. 2</td>
<td>0.211</td>
<td></td>
</tr>
<tr>
<td>1 vs. 3</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>1 vs. 4</td>
<td>0.541</td>
<td></td>
</tr>
<tr>
<td>1 vs. 5</td>
<td>0.297</td>
<td></td>
</tr>
<tr>
<td>1 vs. 6</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>2 vs. 3</td>
<td>0.439</td>
<td></td>
</tr>
<tr>
<td>2 vs. 4</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>2 vs. 5</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>2 vs. 6</td>
<td>0.684</td>
<td></td>
</tr>
<tr>
<td>3 vs. 4</td>
<td>0.621</td>
<td></td>
</tr>
<tr>
<td>3 vs. 5</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>3 vs. 6</td>
<td>0.859</td>
<td></td>
</tr>
<tr>
<td>4 vs. 5</td>
<td>0.435</td>
<td></td>
</tr>
<tr>
<td>4 vs. 6</td>
<td>0.459</td>
<td></td>
</tr>
<tr>
<td>5 vs. 6</td>
<td>0.039</td>
<td></td>
</tr>
</tbody>
</table>
tion. These events have been found to be approximately 2-fold greater in nonpretreated fibroblasts than after TNFα treatment, thus promoting fibrosis in nonpretreated hearts (Peng et al., 2002). However, some beneficial TNFα effect was also observed. Excessive TNFα stimulates TNF-RII and enhances migration of multipotential cells in vitro. Activation of protein p38 and c-Jun amino-terminal kinase is required for TNFα-enhanced multipotential cell migrations which are able to regenerate infarcted myocardium (Chen et al., 2003). Nevertheless, it should be stressed that these effects were observed only in vitro study. The anti-TNFα therapy using soluble TNFα receptor 1 (sTNFR1) may be a recommended tool for treatment of MI. It has been shown that direct injection of a sTNFR1 plasmid DNA (the TNFα antagonist) to the infarcted myocardium reduces infarct size in experimental MI, as well as apoptosis of cardiomyocytes (Sugano et al., 2002).

As mentioned above, TPP is an immunomodulator which has been found to be INFα, INFγ, TNFα and several other cytokine inducers in human peripheral blood leukocytes. However, the most reliable angiogenic factors till now are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).

Previously we described that TNFα among other cytokine expressions such as iNOS and VEGF was assessed by reverse transcription polymerase chain reaction during experimental MI in rats in vivo (Heba et al., 2001a,b). The expression of TNFα, iNOS, VEGF (164) and VEGF (188) was observed in part at the same time-points after MI as in the present study (1, 4, 11, 28 and 40 days), whereas VEGF (120) was found only on day 1 and 4. The most intense immunostaining for TNFα was observed at the border zone. The expression of TNFα and iNOS genes with the concomitant occurrence of a decrease in VEGF (120), VEGF (188) and VEGF (164) protein could be related to insufficient angiogenesis and may suggest the possible involvement of these events in remodeling after MI (Heba et al., 2001a). Therefore TNFα should be considered as a multifacetal cytokine.

There exists the possibility that TPP throughout the induction of TNFα production stimulates angiogenesis due to upregulation of VEGF (164) expression, which overweight decrease of other VEGF forms. Our observation was focused on an extended period after experimental MI, where defense cells infiltration are the main mechanism concerning potential angiogenesis, remodeling and eventual heart failure development. These results indicate that there exists restricted time between two points, where compensatory angiogenesis is supported/prolonged after 4 days’ TPP treatment. We could assume that in our experiment this point is between the 11th and 28th day of MI and this should be considered in further investigation as the most proper moment for pharmacological intervention for revascularisation induction. It is suggested, that angiogenesis during ischemic remodeling is probably modulated by TPP administration.

It is possible that angiogenesis stimulation is the effect of TNFα and also angiogenesis stimulation is the effect of INFα and also angiogenesis stimulation is the effect of INFα and also angiogenesis stimulation is the effect of INFα and also angiogenesis stimulation is the effect of INFα. It has been shown that INFγ inhibits in vitro capillary formation (Skopińska-Różewska et al., 1992; Krzemiński et al., 1995).

Interferon-dependent angiogenesis regulatory action is indirect. Interferon-inducible protein 10 (IP-10) is a potent angiostatic factor. In the first hours of reperfusion, TNFα-released from mast cells may induce IP-10 synthesis in the microvascular endothelium, whilst after the first 24 h of reperfusion, TGFβ-mediated IP-10 down-regulation shifts the balance towards angiogenesis. In addition, simultaneously monocyte-derived macrophages, mast cells, and probably myofibroblasts secrete proteases and growth factors necessary for neovessel formation and optimal repair (Frangogiannis et al., 2001).

In the TPP treated volunteers tolerance developed during three weeks and it lasted one to two weeks (Yu and Filippe, 1993). The phenomenon of hyporeactivity or tolerance is characteristic of every IFN inducer described so far. In contrast to INFs, after intermittent or continuous administration of 5 mg TTP batch 0210 for 3 weeks, the clear cut tolerance of PBL (Peripheral Blood Leucocytes) to TNFα induction did not develop. Only after several 21-day cycles of TPP administration, the level of TNFα may decline moderately (Inglot et al., 1993a,b). To reach the optimal condition for the production of TNFα, continuous treatment with a low dose of TPP for several weeks may be applied (Inglot et al., 1993b).

Our results suggest the immunomodulative or angiomodulative action of peat extract in rats in LIA-test, which basically determinates the level of mononuclear cells activation (Skopińska-Różewska et al., 1993). Whether angiogenesis stimulation occurs with participation of the inhibitory or stimulatory factors inducing the production of inhibitor(s) through rat’s spleen lymphocytes remains unclear. We found that the angiogenic activity was significantly higher in treated rats at the 28th day after MI in comparison to control (WAA; 0.77 ± 0.044 vs. 1.073 ± 0.075, Mann-Whitney test, p < 0.039). However, it should be added that a significant difference was also found between two control groups (WAA; 1.12 ± 0.068 vs. 0.77 ± 0.044, Mann-Whitney test, p < 0.008) as well as between intact animals. This was to be expected to some extent, while the compensatory angiogenesis developed immediately after MI reaching maximum at the 11th day with subsequent decrease at the 28th day, whereas the time accurate treatment with TPP (just four days, see experimental protocol) effectively prolonged this process up to the 28th day.

Similarly PCNA immunostaining of the treated group at 28 days after MI showed moderately stimulated endothelial cells and fibroblasts—the signs of stimulated revascularization of an infarct scar.
Generally, the differentiated small vessels, found mainly in the hearts of treated rats, suggest that the process of cica
tisation and revascularization in damaged tissue took place
during and after TPP treatment (three days after discontinuance of administration), while on the 11th day after MI mainly young granulation tissue enriched in young vessels was visible, whereas on the 28th day after MI—only good vascularized scar consisted of partly young and partly new vessels. On the other hand, it would be interesting to study whether the use of peat extracts promotes revascular-
isation via arterioles or venules, and which could be benefi
cial for the human model; this effect was par
elly observed using different and a prior independent methods.

In summary, it can be concluded that the short and appro
priate (in dosage) TPP administration after experi-
mental myocardial infarction in rats in vivo acts as an
angioimmunomodulatory substance, preventing ischemic
cardiomyopathy with proangiogenic activity.

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similar in mice and rats after myocardial infarction but differences occur

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