INFLUENCE OF ETHANOL SOLUTION OF RESVERATROL ON LEUKOCYTE COUNT AND PHAGOCYTIC ACTIVITY IN PIGLETS

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Abstract

Ten six-week-old White Large piglets were administered resveratrol (res) in a daily dose of 3 mg/kg0.75 b.w. Another ten piglets were used as controls. Both groups were housed in individual pens, fed ad libitum a complete feed mixture. Blood samples were collected from v. jugularis at the beginning of weeks 1 and 2 of the experiment. The total number and different types of white blood cells (WBC) were determined. The percentage of phagocytising cells (PB values) and the phagocyte activity index (PAI) were determined in neutrophils. The administration of res caused a significant decrease in the number of WBC (P<0.01) at the end of week 1. The decrease was recorded in the number of lymphocytes (P<0.01) and almost halved in neutrophils (P<0.01), particularly in young cell band-forms (P<0.01). After two weeks of res administration no further reduction in leukocyte count was found. In the controls, a significant (P<0.01) decrease in WBC, caused by the reduced number of lymphocytes (P<0.05), was observed only at the end of the experiment and the number of neutrophils remained unchanged. The number of bands at the end of the first week was even significantly higher (P<0.05). Res lowered PB (P<0.05) and the PAI (P<0.01) values at the end of the experiment. It can be concluded that res caused a significant, although only temporary, suppression of leukopoiesis, with a consequent decrease in neutrophil phagocyte activity.

Key words: lymphocytes, phagocytosis, resveratrol.

Resveratrol (trans-3, 5, 4′-trihydroxystilbene) was first isolated in 1940 from the underground part of white hellebore, Veratrum. It has already been found in over 72 species of plants (21). It belongs to phytoalexins, i.e. secondary plant metabolites, which are produced in response to certain fungal infections (1). In the late 20th century, comprehensive information on resveratrol was obtained thanks to its presence in grapevine, Vitis vinifera (18). For research purposes, resveratrol is usually extracted from different plants, or it is produced synthetically (21). Resveratrol is one of the most potent natural antioxidants, a scavenger of free radicals (19). It belongs to tumour inhibitors (11), is mentioned in connection with anti-inflammatory effects, and has the ability to reduce blood fats and cholesterol levels (9, 17). Trans-resveratrol is a very effective phytoestrogen that modulates oestrogen metabolism (13, 15). Resveratrol experiments have so far been mainly performed on laboratory animals, i.e. on rats, rabbits, and mice (4, 7) or in vitro experiments (10).

In the study reported here, the effects of resveratrol extracted from Polygonum cuspidatum on total leukocyte, lymphocyte, and neutrophil counts in piglets were tested. The pig as a model species was selected because during the early postnatal period there are very dynamic age changes in numbers, types, and functional abilities of leukocytes. In a relatively short time, it is possible to monitor the influence of these extracts on given changes. Regarding the fact that leukocytes are an important part of the developing immune system of piglets, we expect to use the knowledge gained in veterinary medicine. Similarity of physiological functions of the chosen model with the human body also allows a wider interpretation of the results.

Material and Methods

Two litters of 26-week-old Large White breed piglets were divided equally into a control group (C) and an experimental group (E). In each group, there were six males and four females (Table 1).
Piglets were fed *ad libitum* a complete feed mix for piglet rearing and had free access to drinking water. Dry extract of resverin from *Polygonum cuspidatum* roots, standardised to 50% content of trans-resveratrol and its glycosides and 6% content of emodin, partly soluble in water and readily soluble in ethanol, were administered daily to the piglets through a gastric probe according to the pattern below (Innovation Institute, Opava).

Blood samples were collected by puncture of the *v. jugularis* at the beginning of the experiment (sampling 1), after 7-d administration of resveratrol (sampling 2), and after 14-d administration (sampling 3). The blood samples were tested for total white blood cell (WBC) count using the haematological analyser CELLTACα (Nihon Kohden, Japan), and manually for the differential leukocyte count on blood smears stained with May-Grünwald-Giemsa-Romanowski stain. The following action was the phagocyte activity test (23). After the blood samples were incubated with microspheric hydrophilic particles (2-hydroxyethyl metacrylate particles, MSHP) and the particles absorbed by neutrophils in the stained blood smears were counted, and the percentage of phagocytising cells (PB values) and the phagocyte-activity index (PAI) were determined. The health state and the weight of the piglets were monitored in the same intervals of the experiment.

Experiments were carried out under an institutionally-approved procedure in accordance with ethical principles and the Protection of Animals against Cruelty Act and follow-up rule. Student’s *t*-test was used for statistical processing of all data by the programme Microsoft Excel (mean values and standard deviation).

### Results and Discussion

Resveratrol administered to growing piglets at 3 mg/kg*0.75* b.w. did not significantly influence somatic development of the piglets. The body weight of the resveratrol group slightly exceeded the weight of the control group at the end of experiment but the growth of both groups was relatively the same.

However, it influenced the dynamics of changes in the total WBC count as well as the representation of individual types of cells in the WBC count (Fig. 1).

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**Table 1**

<table>
<thead>
<tr>
<th>Experiment design</th>
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<tbody>
<tr>
<td>Control group (n=10)</td>
<td>Experimental group (n=10)</td>
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<tr>
<td><strong>Day 0</strong></td>
<td><strong>Blood sampling 1</strong></td>
</tr>
<tr>
<td><strong>Day 1-6</strong></td>
<td>20 ml of 15% alcohol per piglet per day</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td><strong>Blood sampling 2</strong></td>
</tr>
<tr>
<td><strong>Day 8–13</strong></td>
<td>30 ml of 15% alcohol per piglet per day</td>
</tr>
<tr>
<td><strong>Day 14</strong></td>
<td><strong>Blood sampling 3</strong></td>
</tr>
</tbody>
</table>

**Fig. 1.** Total WBC counts. E 1 vs 2 (P<0.01); C 1 vs 3 (P<0.01).  **Fig. 2.** Number of neutrophils. E 1 vs 2 (P<0.01).
Fig. 3. Number of bands. E 1 vs 2 (P<0.01); C 1 vs 3 (P<0.01). Fig. 4. Number of lymphocytes. E 1 vs 2 (P<0.01); C 1 vs 3 (P<0.01); E 2 vs 3 (P<0.05); C 2 vs E 2 (P<0.05).

Table 2

<table>
<thead>
<tr>
<th>Day/Sampling</th>
<th>Control group</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/1</td>
<td>81.9 ± 14.89</td>
<td>86.9 ± 6.67</td>
</tr>
<tr>
<td>7/2</td>
<td>80.8 ± 8.09</td>
<td>77.1 ± 14.9</td>
</tr>
<tr>
<td>14/3</td>
<td>94.0 ± 4.9</td>
<td>84.4 ± 13.7</td>
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C 1 vs 3 (P<0.01); C 2 vs 3 (P<0.01); C 3 vs E 3 (P<0.05).

Table 3

<table>
<thead>
<tr>
<th>Day/Sampling</th>
<th>Control group</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/1</td>
<td>10.46 ± 3.40</td>
<td>10.39 ± 2.62</td>
</tr>
<tr>
<td>7/2</td>
<td>12.83 ± 2.53</td>
<td>13.92 ± 3.88</td>
</tr>
<tr>
<td>14/3</td>
<td>20.68 ± 1.77</td>
<td>16.57 ± 3.95</td>
</tr>
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</table>

C 1 vs 3 (P<0.01); E 1 vs 3 (P<0.01); C 3 vs E 3 (P<0.01).

Compared with the group C, where the number of WBC dropped markedly only at the end of the experiment (P<0.01), the drop in the E occurred after one week administration of resveratrol. That change was mainly due to a sharp decrease (P<0.01) in the number of neutrophils (Fig. 2) and a significant reduction (P<0.01) in the number of lymphocytes (Fig. 4).

It is well known that in piglets the number of neutrophils decreases in the first weeks of their life in favour of lymphocytes (28). However, we do not believe that the reduction in the number of neutrophils in the group E can be explained by this ontogenetic development only. In this case the same sharp drop would also appear in the group C. Because in the group E we found a significant decrease in both the total number (Fig. 3) and percentage of representation of young-bands forms of neutrophils, the role of resveratrol should be assumed. Fig. 3 also documents the different development of the band number of both groups. While in the group C this parameter increased in connection with age, in the group E this development trend was interrupted after 1 week of resveratrol application and the number of bands reached a significantly lower level in comparison with controls (P<0.05).

There are many in vitro experiments, which demonstrated the inhibitory effect of resveratrol on blood cell proliferation. For instance, $10^{-4}$ M concentration of resveratrol (23 mg.l$^{-1}$) in the incubation medium inhibited human peripheral blood mononuclear cell proliferation (2). At the lower concentration ($10^{-5}$ M)–2.3 mg.l$^{-1}$, the inhibitory effect was not observed. The dose-dependent inhibitory effect of resveratrol on the growth cycle of acute myeloid leukaemia cells was also described by Estrov (8).

In this respect, King et al. (13) postulated that future research should focus on transfer of in vitro findings into in vivo models. In experiments on laboratory animals, synthetic resveratrol was added to diets at various concentrations to test its effects, including its possible toxicity. Juan et al. (12) administered 20 mg of resveratrol per kg body weight daily to rats for a month. They found a slight increase in
liver enzymes in the experimental animals but no symptoms of its toxicity. Toxicity symptoms were observed at a concentration of 3,000 mg/kg of body weight (6), which demonstrates that resveratrol is well metabolised in the organism. The daily dose of 37.5 mg per piglet in our experiment was far from these toxic doses. However, it was high in comparison with the beneficial intake of resveratrol recommended for human organism. This beneficial effect related to the wine consumption – the so-called French paradox - was observed during long-term administration of about 1-2 mg of resveratrol a day (16). By comparison, one litre of red wine contains 5-10 mg of total stilbene transformable to resveratrol.

Previous experiments on rats showed that 4 mg/kg$^{0.75}$ of resveratrol administered to rats for only a week significantly decreased the number and functional activity of thrombocytes (7). In our study, comparable doses of resveratrol at 3 mg/kg$^{0.75}$ to piglets after one week’s administration caused suppression of the majority of examined values. In the second week of the experiment, we found no significant change, although resveratrol administration per unit of metabolic body weight remained unchanged.

Apart from the temporary suppression of leukopoiesis, resveratrol also significantly affected phagocyte activity. This activity in the group E showed no increase during the experiment compared with the group C (Table 2). It reached the minimum level after one-week’s administration of resveratrol. At the end of week 2 of the experiment, the resveratrol-supplemented piglets had a significantly lower percentage of PB and also PAI (P<0.05 and P<0.01, respectively). We believe that these findings coincided with the lower number of more active phagocytic bands (Tables 2 and 3).

In order to confirm this assumption, other data about immunology should be collected. However, our results are in agreement with the former work by Cavallaro et al. (3). In their study, resveratrol showed a significant dose-dependent inhibitory effect on some activities of polymorphonuclear leukocytes. Recently, Kohnen et al. (14) also reported the inhibitory effect of resveratrol on equine neutrophil myeloperoxidase and Tou and Urbizo (22) demonstrated the inhibitory influence of structurally similar diethylstilbestrol and resveratrol in the presence of ethanol on the degranulation of neutrophils, hence its anti-inflammatory effect. Phagocyte activity is linked with activation of reactive oxygen species and nitric oxide by phagocytes. Ciz et al. (5) documented that resveratrol in wine, like other polyphenols, decreased the release of this product. The scavenging activity of this compound is important in the protection of cells and tissues against oxidative damage but may be also related to attenuation of phagocyte responses.

It is clear from the results that one week administration of resveratrol at a daily dose of 3 mg/kg$^{0.75}$ body weight has no significant effect on the growth of piglets but causes temporarily suppression of their leukopoiesis. This resulted in drop of the total WBC count and mainly in the occurrence of young forms of neutrophils. We suppose that like in vitro experiments, resveratrol administered per os in relatively high doses can inhibit the growth cycle of leukocytes, including their activity. On the one hand, the effects on leukocyte count were not noticeable after the second week of administration of resveratrol but the phagocyte activity of neutrophils at the end of the experiment was still significantly lower.

References


