 DOI: https://doi.org/10.5114/ceji.2018.80041

Influence of trans-resveratrol on macrophage and lymphocyte activity in rainbow trout (*Oncorhynchus mykiss*) – *in vitro* study

ELŻBIETA TERECH-MAJEWSKA[1], ANDRZEJ KRZYSZTOF SIWICKI[2], SYLWIA HERMAŃSKA[2], EDYTA KACZOREK-ŁUKOWSKA[2], JOANNA PAJDAK-CZAUS[1], PATRYCJA SCHULZ[2]

[1]Department of Epizootiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland
[2]Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

Abstract

The objective of this study was to investigate the effect of trans-resveratrol, a potent antioxidant with anti-inflammatory and chemopreventive properties, naturally occurring in many fruits and plants on lymphocytes proliferation and also on macrophages metabolic and phagocytic activity. The aim of this study was to demonstrate the immunomodulatory effects of the compound on fish immunocompetent cells and determine the type of this interaction (immunosuppression or immunostimulation). Proliferative activity of lymphocytes was studied by MTT assay, and the respiratory burst was evaluated using the respiratory burst activity (RBA) test. Phagocytic killing was tested using the PKA test. The experiment have shown that trans-resveratrol suppressed blood B cells, while there was no significant influence on blood T lymphocytes. However, insignificant stimulatory effect occurred at the lowest concentration. In addition, the compound inhibited proliferation of T and B lymphocytes isolated from the organs. Importantly, trans-resveratrol caused stimulation of blood and organs macrophages phagocytic killing, and also increased the respiratory burst of macrophages isolated from organ. These results suggest a potential use of trans-resveratrol as an immunomodulator of innate immunity in fish. This is particularly important, as this kind of resistance plays leading role in protecting the body against infection. In comparison, adaptive immunity is slower and also much less precise.

Key words: *trans-resveratrol, in vitro studies, immunomodulators, cellular immunity.*

(Centr Eur J Immunol 2018; 43 (3): 241-247)

Introduction

Resveratrol (3,5,4'-trihydroxystilbene – RT) is a natural polyphenol and a member of the stilbene family. Its biological activity is associated mainly with its antioxidant properties, and it involves free radical scavenging and inhibition of free radical reactions. Resveratrol also exhibits oestrogenic activity and is classified as a phytoestrogen. Its chemical structure resembles that of the synthetic oestrogen diethylstilbestrol (*trans*-4,4'dihydroxy-α,β-diethylstilbene). Resveratrol is produced by plants as part of a protective response to external stressors, including injury, cold stress, UV radiation, drought, heavy metal ions and pathogens (microorganisms and/or their metabolites).

3,5,4'-trihydroxystilbene was first isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) in 1939 by Takaoka [1]. The compound is also present in fruit, including grapes (*Vitis vinifera*) and red grapefruits (*Citrus paradisi*) [2]. Medicinal plants such as common knotgrass (*Polygonum aviculare*) and other knotgrass species are also a rich source of resveratrol. To date, the compound has been isolated from more than 72 plant species, and the search for other natural sources of resveratrol continues.

Resveratrol exists in two isomeric forms, *cis* and *trans*, which differ in biological activity. *Trans*-resveratrol (TRT) has four natural analogues: piceatannol (3,5,3'-trihydroxy-4'-methoxy-*trans*-stilbene), pinostilbene (3,3',4'-trihydroxy-5-methoxy-*trans*-stilbene), pterostilbene (3,5-dimethoxy-4'-hydroxy-*trans*-stilbene) and rhapontigenin (3,5,3'-trihydroxy-4'-methoxy-*trans*-stilbene) [3]. It is considered to be a high biomedical activity compound [4, 5].

The metabolic activity of TRT was demonstrated in a study of rat leukocytes, which revealed that the analysed compound inhibits the production of eicosanoids responsible for inflammatory processes and thrombocyte aggregation [6]. *Trans*-resveratrol has cytoprotective, antiviral, antifungal, antibacterial and cytotoxic properties. It protects the circulatory system and prevents neurodegenerative processes. As a strong antioxidant, it delivers

Correspondence: Joanna Pajdak-Czaus, Department of Epizootiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, 13 Oczapowskiego St., 10-719 Olsztyn, Poland, e-mail: joanna.pajdak@uwm.edu.pl
Submitted: 22.06.2016; Accepted: 19.07.2016

anti-inflammatory effects, and it is a nonselective inhibitor of cyclooxygenases COX-1 and COX-2. Much attention has been given to the chemopreventive activity of TRT and its ability to inhibit carcinogenesis in all three stages of inhibition, promotion and progression [4, 7].

Animal studies demonstrating the anti-aging effects of RT and its ability to lower the risk of obesity have set new directions for research. *Trans*-resveratrol extended the lifespan of mice fed a high-calorie diet and led to a general improvement in their health [7, 8]. There were found specific immunomodulatory effects of RT on the Sirtuin gene expression family (SIRTs), enzymes with a very complex physiological role. The best known enzyme in this regard in this group is SIRT1, involved in many cellular processes by inhibiting the activity of transcription factors, e.g. nuclear transcription factor κB (NFκB). As a result, it shows anti-inflammatory, antitumor, and antiviral effects [9].

The analysed compound significantly increases the lifespan of simple organisms, including yeasts (*Saccharomyces cerevisiae*), roundworms (*Caenorhabditis elegans*) and fruit flies (*Drosophila melanogaster*). It prolongs the survival of short-lived fish such as *Nothobranchius furzeri* and improves their cognitive abilities and swimming efficiency. *Trans*-resveratrol is used in the production of dietary supplements and cosmetics with alleged anti-aging properties [7, 10-16].

Defence mechanisms and antioxidant potential are part of an organism's immune system. The influence of RT on the immune system has been relatively poorly investigated, and this aspect has become the focus of recent research due to the growing interest in immune-based diseases. The results reported by various authors differ considerably; therefore, the data supplied by *in vitro* and *in vivo* studies have to be validated and systematized using various experimental models. Fish constitute a good animal model for investigating the influence of various factors on the immune system. In the evolutionary process, fish were the first class of vertebrates with a fully developed immune system containing elements of both innate and adaptive immunity. They were the first vertebrates to develop fully differentiated lymphatic organs whose functions and structure continued to evolve in other species. Therefore, *in vitro* as well as *in vivo* studies on immunocompetent cells are used to evaluate the ecotoxicity and immunotoxicity of pharmacological agents [17-20].

The aim of this study was to evaluate *in vitro* the influence of different concentrations of TRT on activity of macrophages and lymphocytes isolated from the blood and blood-forming organs of rainbow trout (*Oncorhynchus mykiss*).

Material and methods

Trans-resveratrol

A stock solution of *trans*-resveratrol (Sigma-Aldrich) with a concentration of 0.001 M in 1 M dimethyl sulfoxide – DMSO (POCH SA) was diluted to final concentrations of 1.25, 2.5, 10 and 20 μM with the RPMI-1640 medium (Sigma-Aldrich) on microtitre plates.

Fish

The experiment was performed on cells isolated from whole blood and blood-forming organs of four rainbow trout (*Oncorhynchus mykiss*) weighing 1-1.5 kg each. Fish were supplied by the Salmonid Breeding Farm of the Inland Fisheries Institute in Rutki (Poland). Fish were kept in 100 litre tanks with recirculated and oxygenated water at 15°C. Rainbow trout were anesthetized with Propiscin (INF – Inland Fisheries Institute in Olsztyn, Poland) at 0.2 ml·l^{-1} of water before the collection of experimental samples [21].

Leukocyte isolation

Blood and organs (head kidney and spleen) were sampled aseptically. Blood was drawn from the tail vein into test tubes with heparin. Whole blood samples of 2 ml were combined with 2 ml of RPMI-1649 with L-glutamine and sodium carbonate (Sigma-Aldrich) and 1% antibiotic (Sigma-Aldrich). Blood-forming organs were isolated and homogenized with the addition of RPMI-1640 (Sigma-Aldrich) on sterile Petri plates. The homogenate was passed through a 25 μm mesh filter and centrifuged for 15 minutes at 2000 × g (4°C). The sediment was suspended in 4 ml of RPMI and layered on 3 ml of Gradisol G (Aqua-Medica, Łódź, Poland) or Gradisol L (Aqua-Medica, Łódź, Poland) (1 : 1 ratio) to isolate leukocytes by density gradient centrifugation. The samples were centrifuged for 40 minutes at 2000 × g (4°C), the interphase was separated, and the material was rinsed three times and centrifuged for 15 minutes at 2000 × g (4°C) each time. After the last centrifugation, the supernatant was discarded, and the sediment was suspended in 2 ml of RPMI with 10% fetal calf serum (FCS, Sigma-Aldrich). The same method was used to isolate blood cells. Tests were carried out in 96-well titre plates (Nunc, Denmark). Each well was filled with a suspension of 5 × 10^6 cells·ml^{-1}. The plates were incubated at 22°C.

Respiratory burst activity test

The metabolic activity of monocytes and macrophages was determined in the respiratory burst activity (RBA) test after stimulation with phorbol myristate acetate (PMA, Sigma-Aldrich) according to the method described by Chung & Secombes [22] and modified by Chettri *et al.* [23]. Each well in a 96-well plate was filled with 100 μl of the cell suspension (from blood or organs) and incubated for 2 h at 22°C. Non-adherent cells were discarded by rinsing with RMPI-1640. *Trans*-resveratrol solutions were added, and the plates were incubated for 24 h. After incubation, 100 μl of PMA (1 μg ml^{-1}) with 0.1% nitro blue tetrazolium (NBT, Sigma-Aldrich) was added to

each well, and the mixture was incubated for 30 minutes at 24°C. Absolute alcohol was added to stop the reaction, and the plates were twice rinsed with 70% ethanol. The plates were dried at room temperature, and 120 µl of KOH and 140 µl of DMSO were added to each well to dissolve the produced formazan. Absorbance was measured at 620 nm with the Sunrice absorbance reader (Tecan, Austria). The results were expressed as mean extinction of PMA-stimulated cells relative to the control (non-stimulated) sample.

Potential killing activity test

The phagocytic activity of monocytes and macrophages was determined in the potential killing activity test (PKA) where NBT is reduced to insoluble formazan by phagocytic cells. The PKA test was conducted based on the procedure proposed by Rook *et al*. [24] and modified by Małaczewska *et al*. [25]. Each well in a 96-well titre plate was filled with 100 µl of cell suspensions (from blood or organs) and incubated for 2 h at 22°C. Non-adherent cells were discarded by rinsing with RMPI-1640. *Trans*-resveratrol solutions were added and the plates were incubated for 24 h. Suspensions of live *Aeromonas hydrophila* (1×10^8 cells) in the amount of 100 µl were added with 0.1% NBT to each well containing 100 µl of cell suspension. The plates were incubated for 30 minutes at 24°C, and the contents were poured out. Absolute alcohol was added to stop the reaction, and the plates were twice rinsed with 70% ethanol. The plates were dried at room temperature, and 120 µl of KOH and 140 µl of DMSO were added to each well to dissolve the produced formazan. Absorbance was measured at 620 nm with the Sunrice absorbance reader (Tecan, Austria). The results were expressed as mean extinction of phagocytic cells relative to a control (non-stimulated) sample.

Mitogenic titrate test

The proliferative capacity of lymphocytes was determined in a mitogenic titrate test (MTT) according to the protocol described by Mosmann [26]. Mitogens were added to cell suspensions to stimulate lymphocyte proliferation: concanavalin A (Con A, Sigma-Aldrich) in the amount of 50 µg ml^{-1} for T cells and *Serratia marcescens* lipopolysaccharide (LPS, Sigma-Aldrich) in the amount of 25 µg ml^{-1} for B cells. Cell suspensions were incubated with different concentrations of *trans*-resveratrol for 72 h. After incubation, 25 µl of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma-Aldrich) dissolved in physiological buffer solution (PBS, Biomedica, Poland) was added to the wells and incubated for 4 h. The supernatant was discarded, and 100 µl of DMSO (dimethyl sulfoxide, POCh, Gliwice, Poland) was added to each well. The reaction was measured at a wavelength of 570 nm in the Sunrice absorbance reader (Tecan, Austria). All samples were analysed in three replicates relative to the control sample without mitogens. The results were used to calculate mean optical density (OD) relative to the control sample.

Statistical analysis

The results were processed statistically in the GraphPad Prism 6 application. Data were subjected to one-way ANOVA or its non-parametric equivalent. Statistical significance at $p < 0.05$ was estimated using Dunnett's test or Dunn's test (non-parametric analysis). The significance of differences are marked on charts with stars, according to

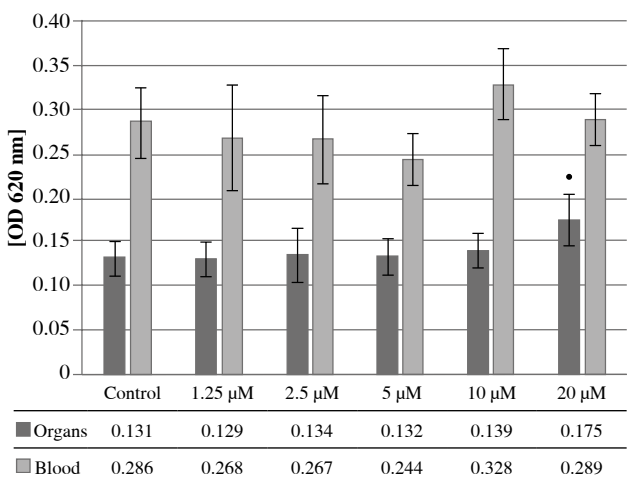


Fig. 1. The influence of TRT on the metabolic activity of macrophages isolated from the blood and blood-forming organs of rainbow trout

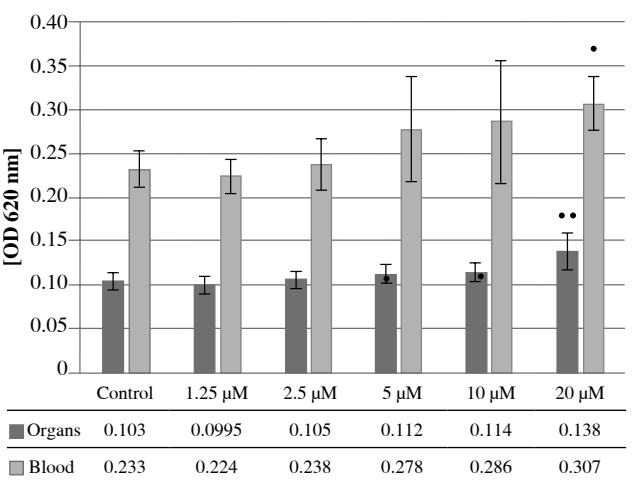
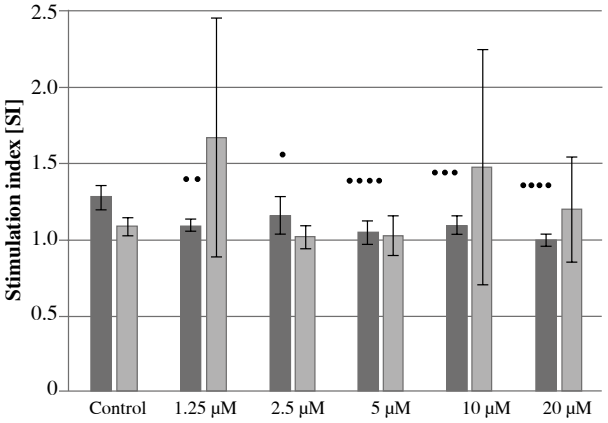
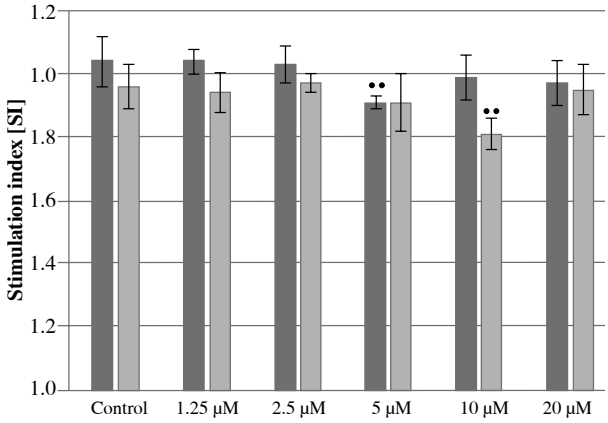


Fig. 2. The influence of TRT on the phagocytic activity of macrophages isolated from the blood and blood-forming organs of rainbow trout



	Control	1.25 µM	2.5 µM	5 µM	10 µM	20 µM
Organs	1.28	1.10	1.16	1.05	1.10	1.00
Blood	1.09	1.67	1.02	1.03	1.48	1.20

Fig. 3. The influence of TRT on the proliferation of ConA-stimulated lymphocytes isolated from the blood and blood-forming organs of rainbow trout



	Control	1.25 µM	2.5 µM	5 µM	10 µM	20 µM
Organs	1.04	1.04	1.03	0.91	0.99	0.97
Blood	0.96	0.94	0.97	0.91	0.81	0.95

Fig. 4. The influence of TRT on the proliferation of LPS-stimulated lymphocytes isolated from the blood and blood-forming organs of rainbow trout

the rule: $^{*}p < 0.05$; $^{**}p < 0.1$; $^{***}p < 0.01$ $^{****}p < 0.001$. Empirical distribution was compared with normal distribution of data in the Kolmogorov-Smirnov test. The results were presented as means ±standard deviation.

Results

In the RBA test, the metabolic activity of PMA-stimulated blood monocytes was not statistically significant (Fig. 1). An analysis of the PKA activity test of blood monocytes incubated with different doses of TRT and suspensions of *Aeromonas hydrophila* in NBT demonstrated a significant increase in killing activity at 20 µM ($p < 0.05$) (Fig. 2).

In this concentration, TRT stimulated the RBA of macrophages from fish immunological organs ($p < 0.05$) (Fig. 1). The PKA of macrophages was stimulated within a broader range of TRT concentrations at 5 µM ($p < 0.05$), 10 µM ($p < 0.05$) and 20 µM ($p < 0.01$) (Fig. 2).

An increase in blood ConA-stimulated T cell proliferation was noted in response to the lowest dose of *trans*-resveratrol (1.25 µM), but not at the remaining concentrations (2.5 µM, 5 µM, 10 µM, 20 µM) (Fig. 3). The proliferative capacity of LPS-stimulated B cells from the blood of rainbow trout was significantly lowered only in response to a *trans*-resveratrol dose of 10 µM) ($p < 0.01$) (Fig. 4).

Mitogenic titrate test of Con A-stimulated lymphocytes from the spleen and head kidney of rainbow trout decreased significantly in response to all five concentrations of TRT. A particular drop in proliferative capacity was noted at a concentrations of 5 µM ($p < 0.0001$) and 20 µM ($p < 0.0001$), followed by 1.25 µM ($p < 0.01$) and 10 µM ($p < 0.001$). Proliferation was less suppressed at a dose of 2.5 µM ($p < 0.05$) (Fig. 3).

A statistical analysis of TRT's effect on the proliferation of fish organ cells stimulated with LPS demonstrated a significant decrease only at a concentration of 5 µM ($p < 0.01$) (Fig. 4).

Discussion

Research has demonstrated that when TRT is applied to LPS-stimulated macrophages, it significantly inhibits the secretion of interferon γ (IFNγ), interleukins IL-1, IL-4 and IL-6, and tumour necrosis factor α (TNF-α), but significantly increases the production of IL-10. TRT decreases expression of the molecules CD28 and CD80, which play an important role in the immune response, and suppresses the T-helper cells Th1 and Th2 and cytotoxic T cells (Tc). According to some reports, low concentrations of TRT have a stimulatory effect on the immune system. The immunomodulatory (immunosuppressive or immunostimulatory) properties of TRT have practical applications in the prevention and treatment of various human and animal diseases [27-30].

The results of this *in vitro* study into TRT's influence on lymphocyte proliferation and the phagocytic and metabolic activity of macrophages isolated from rainbow trout contribute to our limited knowledge of the above. Our study also offers a new insight into the analysed compound's biological effect on immunocompetent cells in fish in comparison with mammals. The results contribute valuable information about TRT's influence on cells isolated from blood and organs.

Metabolic activity of macrophages isolated from blood and organs

Trans-resveratrol did not exert a significant influence on phagocytes isolated from blood. At lower concentrations (1.25, 2.5 and 5 μM), the studied compound had a somewhat inhibitory effect on the respiratory burst, but it acted as a stimulant at higher doses (10 and 20 μM). The metabolic activity of macrophages isolated from blood-forming organs was significantly stimulated only at a concentration of 20 μM, whereas the remaining concentrations produced results similar to those noted in the control sample. The inhibitory influence of TRT on the respiratory burst could be attributed to its antioxidant properties and the ability to scavenge free radicals. However, the predominant immunostimulatory effect of *trans*-resveratrol observed in this study is not consistent with published findings. Already at a dose of 1 μM, the analysed compound inhibited the respiratory burst of human leukaemia U937 cells differentiated with retinoic acid and vitamin D_3 [31]. Similar observations were made when THP-1 cells infected with *Chlamydia pneumoniae* were exposed to TRT concentrations of 1 and 10 μM [32]. A study of mouse peritoneal macrophages treated with TRT doses of 3 and 30 μM delivered similar results [33]. The stimulation of the respiratory burst in rainbow trout macrophages could be attributed to the fact that free radicals also play a positive role by controlling various immune responses, stimulating T cells and inducing the adhesion of leukocytes to vascular endothelium, thus promoting leukocyte transport from the circulatory system to the site of inflammation.

Phagocytic activity of macrophages isolated from blood and organs

This experiment demonstrated that at concentrations of 2.5-20 μM, TRT stimulated the phagocytic activity of macrophages isolated from blood and immunological organs, but its stimulatory effect was statistically significant only at 20 μM for blood and 5, 10 and 20 μM for organs. Our results differ from the findings reported in other studies where the stimulatory influence of TRT was noted only at concentrations of 1-10 μM, whereas concentrations of 10 μM and higher had an inhibitory effect on phagocytic activity. The stimulatory influence of low concentrations of TRT was reported in a study on the intracellular killing of *Candida albicans* by human macrophage-like cells. Phagocytosis was inhibited when TRT was applied at a dose of 10 μM. The phagocytic activity of peritoneal exudate macrophages directed against *Kluyveromyces lactis* was inhibited by concentrations of 10-100 μM [34]. The phagocytosis of *Escherichia coli* and *Staphylococcus aureus* was inhibited by THP-1 cells of the human monocytic leukaemia cell line when exposed to a *trans*-resveratrol dose of 10 μM [35]. The results of our study suggest that in fish, the tested concentrations of TRT do not exert an immunosuppressive influence on macrophages. This is a very important observation because the immune system of fish relies mainly on non-specific responses and the phagocyte system. TRT could stimulate Toll-like receptor (TLR) signalling pathways through pathogen-associated molecular patterns (PAMPs) or by increasing TLR expression. The analysed compound could also regulate the expression of other families of pathogen recognition receptors (PRRs) such as scavenger receptors (SRs) and C-type lectin receptors (CLRs) C [35]. The examined polyphenol could also intensify chemotaxis by stimulating the release of chemotactic factors.

Proliferative activity of lymphocytes isolated from blood and organs

An analysis of TRT's effect on the proliferation of rainbow trout lymphocytes revealed that the examined compound is a weak immunomodulator for T cells isolated from blood. Despite an absence of statistically significant results, TRT's influence on this subpopulation of lymphocytes cannot be completely ruled out. This lack of statistical significance could be attributed to the fact that specific immune responses in fish are slower and less targeted than in mammals. In a study by Boscolo *et al*. [27], *trans*-resveratrol doses of 0.1 and 10 μM had only a minor stimulatory effect on human T cells. In our study, the lowest concentration of TRT (1.25 μM) weakly stimulated lymphocytes isolated from the blood of rainbow trout, and it exerted a minor suppressive effect at the remaining concentrations. The immunostimulatory effect of the analysed compound could be attributed to the release of Th1 cytokines such as IL-2 and IFN-γ, which intensifies cell proliferation [28], as well as enhancing production of IL-1, which also stimulates T cell proliferation. The inhibitory influence of TRT could result from its ability to suppress ribonucleotide reductase and DNA polymerase, the key enzymes involved in DNA synthesis, as well as its ability to arrest the cell cycle in phase S/G2 due to the accumulation of cyclins E and A [36-38]. The anti-proliferative properties of TRT could also be attributed to its antioxidant activity because reactive oxygen species participate in cell responses to cytokines and growth factors [29]. The inhibitory influence of the analysed compound could result from its ability to suppress the expression of NF-κB, a compound that controls the transcription of genes encoding the immune response, in stimulated T cells [39].

Trans-resveratrol did not stimulate the proliferation of LPS-stimulated B cells isolated from blood. The analysed compound significantly inhibited cell proliferation at a concentration of 10 μM. Similar results were reported in another study where the proliferation of B cells from the blood of healthy volunteers was inhibited at the same concentration of *trans*-resveratrol. The above could be attributed to an increase in the activity of caspase 3, an

enzyme involved in cell apoptosis [40] and responsible for the degradation of numerous substrates (e.g. topoisomerase I) and DNA.

Trans-resveratrol had a much stronger immunomodulatory effect on lymphocytes isolated from organs. In fish, the head kidney and the spleen are the major lymphatic organs. Younger individuals are protected mainly by blood cells and macrophages from external integuments, whereas in adults, the head kidney and the spleen constitute the backbone of the immune system. In the present study, all concentrations of TRT significantly inhibited the proliferation of T cells isolated from organs. Similar results were reported by Sharma *et al*. [41], who found that TRT at concentrations of 1, 5, 10 and 20 μM had an inhibitory effect on splenocyte proliferation in mice. Those findings could suggest that TRT suppresses the immune response by stimulating macrophages to produce IL-10 (suppressor cytokine), which prevents lymphocyte activation. The cited authors also demonstrated that TRT can reduce the expression of the molecule CD28 on the surface of Th cells. The said molecule stimulates Th cells and initiates the second activation signal by interacting with the molecule CD80 on an antigen-presenting cell (APC) whose expression was also reduced by TRT. This is an important consideration since when the second activation signal is not generated, lymphocytes become anergic and they can no longer be stimulated by antigens. According to some reports, mouse splenocytes are stimulated by low doses of TRT in the range of 0.75-6 μM [28], 6.25 μM and 12.5 μM [29], but the above effect was not confirmed in our study of rainbow trout.

Trans-resveratrol suppressed the proliferation of B lymphocytes isolated from fish organs and blood. The above effect was observed at all concentrations, but it was statistically significant only at a dose of 5 μM. In the work of Sharma *et al*. [41], TRT inhibited the proliferation of mouse splenocytes at concentrations of 1, 5, 10 and 20 μM. Similarly to T cells, this reduction in proliferative activity could be attributed to enhanced production of IL-10. The tested compound could also lower the expression of costimulatory molecules CD40 that participate in the activation process, as demonstrated in mouse macrophages. Therefore, the same mechanism of action could be expected in B cells from fish [41]. Similarly to B cells isolated from blood, TRT could also enhance the activity of caspase 3 [40].

Conclusions

The results of our *in vitro* study to evaluate the influence of TRT on lymphocytes and phagocytes isolated from the blood, spleen and head kidney of rainbow trout (*Oncorhynchus mykiss*) lead to the following conclusions:

Trans-resveratrol did not induce a dose-dependent response in the immunocompetent cells of rainbow trout.

Trans-resveratrol suppressed *in vitro* the proliferation of B cells isolated from the blood of rainbow trout and the proliferation of T and B cells isolated from the blood-forming organs of rainbow trout.

The analysed compound did not exert a significant influence on T lymphocytes isolated from fish blood. It had a minor stimulatory effect on T cells at lower concentrations and a somewhat inhibitory effect in the remaining doses. It did not significantly enhance the respiratory burst of blood phagocytes, but it exerted a somewhat stimulatory effect at higher concentrations.

This compound stimulated the killing activity of phagocytes isolated from blood and organs and the metabolic activity of macrophages isolated from blood-forming organs.

Our results pave the way for further research into the influence of TRT on the immune system of fish. It is the first comprehensive study examining the activity of immunocompetent cells from blood and organs relative to the immune system of fish. *Trans*-resveratrol influences both non-specific (stimulation) and specific (suppression) immune responses, and it could stimulate non-specific defence mechanisms that play a key role in fish immunity. The adaptive immune system is less prominent due to slow lymphocyte proliferation, limited immunological memory, inhibited affinity maturation and immunoglobulin secretion in fish. The results of this study suggest that fish constitute a good model for research into the efficacy of TRT in the prevention and treatment of immune deficiencies. The immunomodulatory properties of the analysed compound could be used to stimulate disease resistance in both fish and mammals.

The authors declare no conflict of interest.

References

1. Takaoka MJ (1940): The phenolic substances of white Hellebore (Veratrum grandiflorum Loes.fil). J Faculty Sci Hokkaido Imperial University 3: 1-16.
2. Langcake P, Pryce RJ (1976): The production of reveratrol by Vitis vinifera and Rother members of the Vitaceae as a response to infection or injury. Physiol Plant Pathol 9: 77-86.
3. King RE, Bomser JA, Min DB (2006): Bioactivity of Resveratrol. Compr Rev Food Sci Food Saf 5: 65-70.
4. Fremont L (2000): Biological effects of resveratrol. Life Sci 66: 663-673.
5. Tome-Carneiro J, Larrosa M, Gonzalez-Sarrias A, et al. (2013): Resveratrol and clinical trials: The crossroad in vitro studies to human evidence. Curr Pharm Design 19: 6064-6093.
6. Kimura Y, Okuda H, Arichi S (1985): Effects of stilbeneson arachidonate metabolism in leukocytes. Biochim Biophys Acta 834: 275-278.
7. Baur JA, Sinclair DA (2006): Therapeutic potential of resveratrol: the in vivo evidence, Nat Rev Drug Discov 5: 493-506.

8. Szkudelska K, Szkudelski T (2010): Resveratrol, obesity and diabetes. Eur J Pharmacol 635: 1-8.
9. Schirmer H, Pereira TC, Rico EP, et al. (2012): Modulatory effect of resveratrol on SIRT1, SIRT3, SIRT4, PGCLα and NAMPT gene expression profiles in wild-type adult zebrafish liver. Mol Biol Rep 39: 3281-3289.
10. Aggarwal BB, Bhardwaj A, Aggarwal RS, et al. (2004): Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res 24: 2783-2840.
11. Baur JA, Pearson KJ, Price NL, et al. (2006): Resveratrol improves health and survival of mice on high-calorie diet. Nature 444: 337-342.
12. Howitz KT, Bitterman KJ, Cohen HY, et al. (2003): Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425: 191-196.
13. Ratan HL, Steward WP, Gescher AJ, Mellon JK (2002): Resveratrol – a prostate cancer chemopreventive agent? Urol Oncol 7: 223-227.
14. Timmers S, Auwerx J, Schrauwen P (2012): The journey of resveratrol from yeast to human. Aging 4: 146-158.
15. Valenzano DR, Terzibasi E, Genade T, et al. (2006): Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr Biol 16: 296-300.
16. Wood JG, Rogina B, Lavu S, et al. (2004): Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430: 686-689.
17. Sakai M, Kobayashi M, Kawauchi H (1996): In vitro activation of fish phagocytic cells by GH, prolactin and somatolactin. J Endocrinol 151: 113-118.
18. Siwicki AK, Anderson DP, Dunier M (1996): *In vitro* immunotoxicology-alternative models for environmental toxicology. In: Biologiczne Monitorowanie Skażenia Środowiska. IRS Olsztyn; 157-170.
19. Siwicki AK, Morand M, Klein P, et al. (1998): Modulation of nonspecific mechanisms and protection against diseases in Fish. Acta Vet Brno 67: 323-328.
20. Studnicka M, Siwicki AK, Morand M, et al. (2000): Modulation of nonspecific Demence mechanisms and specific immune responses after suppression induced by xenobiotics. J Appl Ichthyol 16: 1-7.
21. Kazuń K, Siwicki AK (2001): Propiscin – a safe new anaesthetic for fish. Arch Pol Fish 9: 183-190.
22. Chung S, Secombes S (1988): Analysis of events occurring within teleost macrophages during respiratory burst. Comp Biochem Physiol 89: 539-544.
23. Chettri JK, Holten-Andersen L, Buchmann K (2010): Factors influencing *in vitro* respiratory burst assays with head kidney leucocytes from rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish Dis 33: 593-602.
24. Rook GA, Steele J, Umar S, Dockrell HM (1985): A simple method for the solubilisation of reduced NBT and its use as a colorimetric assay for activation of human macrophages by gamma-interferon. J Immunol Methods 82: 161-167.
25. Małaczewska J, Siwicki AK, Wójcik R, et al. (2014): The in vitro effect of kynurenic acid on the rainbow trout (Oncorhynchus mykiss) leukocyte and splenocyte activity. Pol J Veter Scin 17: 453-458.
26. Mosmann T (1983): Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.
27. Boscolo P, Del Signpre A, Sabbioni E, et al. (2003): Effects of resveratrol on lymphocyte proliferation and cytokine release. Ann Clin Lab Sci 33: 226-231.
28. Feng YH, Zhou WL, Wu QL, et al. (2002): Low dose of resveratrol enhanced immune response of mice. Acta Pharmacol Sin 23: 893-897.
29. Gao H, Deeb D, Media J, et al. (2003): Immunomodultaory activity of resveratrol: discrepant *in vitro* and *in vivo* immunological effects. Biochem Pharmacol 66: 2427-2435.
30. Sharma S, Chopra K, Kulkarni S (2007): Resveratrol and curcumin suppress immune responses through CD28/CTLA-4 and CD80 co-stimulatory pathway. Clon Exp Immunol 147: 155-163.
31. Poolman TM, Ng LL, Farmer PB, Manson MM (2005): Inhibition of the respiratory burst by resveratrol in human mocytes: Correlation with inhibition of PI3K signaling. Free Radic Biol Med 39: 118-132.
32. Deby-Dupont G, Mouithys-Mickalad A, Serteyn D, et al. (2005): Resveratrol and curcumin reduce the respiratory burst of Chlamydia-primed THP-1 cells. Biochem Biophys Res Commun 333: 21-37.
33. Martinez J, Moreno JJ (200): Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. Biochem Pharmacol 59: 865-870.
34. Leiro J, Alvarez E, García D, Orallo F (2002): Resveratrol modulates rat macrophage functions. Int Immunopharmacol 2: 767-774.
35. Iyori M, Kataoka H, Shamsul HM, et al. (2008): Resveratrol modulates phagocytosis of bacteria through an NF-κB-dependent gene program. Antimicrob Agents Chemother 52: 121-127.
36. Ragione FD, Cucciolla V, Borriello A, et al. (1998): Resveratrol arrests the cell division cycle at S/G2 phase transition. Biochem Biophys Res Commun 250: 53-58.
37. Fontecave M, Lepoivre M, Elleingand E, et al. (1997): Resveratrol, a remarkable inhibitor of ribonucleitide reductase. FEBS Lett 421: 277-279.
38. Sun NJ, Woo SH, Cassady JM, Snapka RM (1998): DNA polymerase and topoisomerase II inhibitors from Psoralea corylifolia. J Nat Prod 61: 362-366.
39. Gao X, Xu YX, Janakiraman N, et al. (2001): Immunomodulatory activity of resveratrol: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production. Biochem Pharmacol 62: 1299-1308.
40. Zunino SJ, Storms DH (2009): Resveratrol alters proliferative responses and apoptosis in human activated B lymphocytes in vitro. J Nutr 139: 1603-1608.
41. Sharma S, Anjaneyulu M, Kulkarni SK, Chopra K (2006): Resveratrol, a polyphenolic phytoalexin, attenuates diabetic nephropathy in rats. Pharmacology 76: 69-75.