Published in final edited form as:

Chem Biol Interact. 2008 January 10; 171(1): 37–44.

Cytotoxic effects of the dietary flavones chrysin and apigenin in a normal trout liver cell line

P. A. Tsuji and T. Walle*

Department of Cell and Molecular Pharmacology and Experimental Therapeutics Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425

Abstract

Many flavonoids have been shown to possess prooxidant properties, capable of causing oxidative stress, especially at larger doses. Here, we examined the potential cell toxicity caused by exposure to the hydroxylated flavones chrysin, apigenin, luteolin and quercetin in comparison to the methylated flavones 5,7-dimethoxyflavone and 3',4'-dimethoxyflavone in normal rainbow trout hepatocytes. The hydroxylated flavones, especially chrysin, demonstrated cell toxicity and inhibition of DNA synthesis at very low (2 μ M) concentrations. The cytotoxicity of chrysin may partially be due to its metabolism by myeloperoxidase, which was shown to be present in these normal trout liver cells (164 pmol/min/mg protein). In contrast, methylated flavones showed no significant metabolism by myeloperoxidase and no signs of toxicity, even at much higher concentrations. These results may be useful for further investigations of cytotoxicity of dietary flavonoids.

Keywords

Chrysin; Apigenin; Flavonoids; Fish model; Cytotoxicity; Myeloperoxidase

1. Introduction

Flavonoids are a structurally diverse class of polyphenolic compounds ubiquitously found among plants and produced as a result of plant secondary metabolism [1]. Estimated dietary flavonoid intake can reach 50–800 mg per day [2], or higher, if dietary supplements are consumed. In human and animal models, many of these flavonoids and other polyphenols have been shown to have cancer chemopreventive properties, as well as beneficial effects in cardiovascular disease [3]. These effects may be due to the antioxidant properties of the flavonoids [4], although more recent findings show that interactions with essential signal transduction pathways may be more important [5,6].

In a recent study conducted in the normal trout CRL-2301 hepatocytes [7], we noticed, when comparing effects of methylated versus unmethylated flavones, that these cells showed evidence of toxicity. This is a potential problem when ingesting larger doses of flavonoids. Indeed, it is important to realize that many flavonoids, although being antioxidants, have also been shown to have prooxidant properties [8], capable of causing oxidative stress, for example

*Corresponding author. Thomas Walle, Ph.D. Department of Cell and Molecular Pharmacology & Experimental Therapeutics Medical University of South Carolina 173 Ashley Avenue, P.O. Box 250505 Charleston, SC 29425 Tel.: 1 (843) 792–2507 Fax: 1 (843) 792–2475 E-mail address: wallet@muscc.edu (T. Walle).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

through peroxidase-induced phenoxyl radicals [9]. Also, the flavonol quercetin has been shown to undergo cellular oxidation, mediated either by peroxidases [10] or by nonenzymatic chemical reactions and covalent binding to cellular protein and DNA [11,12].

To investigate toxic and other effects of xenobiotics in general, cell culture models from a variety of hosts have been used, among which cultured fish cells have found utility in many areas, including ecotoxicity testing [13], carcinogenesis [14], signal transduction [15], oxidative stress [16], reproductive toxicity [17], organ-specific toxicity and metabolism [18]. Rainbow trout (*Oncorhynchus mykiss*) in particular is one of the more commonly used fish species in aquaculture, and has been used as a sensitive *in vivo* model [19,20], as well as a cell model [21-25].

In the present study, we examined the potential cell toxicity caused by exposure to flavonoids such as chrysin, apigenin, luteolin, quercetin, 5,7-dimethoxyflavone (5,7-DMF) and 3',4'-dimethoxyflavone (3',4'-DMF) (Fig. 1), using cultured normal rainbow trout CRL-2301 hepatocytes.

2. Materials and methods

2.1. Chemicals

5,7-Dimethoxyflavone (5,7-DMF) and 3',4'-dimethoxyflavone (3',4'-DMF) were purchased from Indofine Chemical Co. (Somerville, NJ). Chrysin, quercetin, apigenin, luteolin, myeloperoxidase from human leukocytes and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade.

2.2. Cell culture and treatment

Normal rainbow trout hepatocytes (CRL-2301), originally isolated and characterized by Ostrander *et al.* [26], were obtained from the American Type Culture Collection (Rockville, MD) and were grown in Minimum Essential Medium (MEM) with 10% heat-inactivated fetal bovine serum, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 1.32 g/L sodium chloride and 1% penicillin-streptomycin in uncoated flasks in a humidified atmosphere with 5% carbon dioxide at 18°C. At 80–90% confluency, the cells were treated with varying concentrations of flavonoids. Vehicle dimethyl sulfoxide (DMSO, $\leq 0.1\%$ of final volume) was used as a control in all experiments. For treatments of 48 h, the cells were exposed to fresh medium containing polyphenol/DMSO every 24 h. The cells were used at passages 9–15.

2.3. Microscopy

Cells at various stages during treatment were observed regularly with a transmission light microscope (Nikon TMS #212586) at 100x magnification. Digital photographs were taken through the ocular with a Canon Powershot S400 camera using 3x magnification, taking a snap shot of a representative area of the well at a final magnification of 300x. Image J 1.37 v, a public domain Java image processing program (Wayne Rasband, NIH, <http://rsb.info.nih.gov/ij/>), was used to estimate the culture dish area covered with cells after treatment with 25 μ M flavonoids for 24 h. Using photographic images obtained as described above, areas lacking cells were highlighted with the free-hand tool and subtracted from the total area of a confluent cell monolayer. Three or more cultures were analyzed for each treatment. Cell coverage was expressed as percent of the DMSO-treated controls.

2.4. Cell proliferation assay

The proliferation rate of cells was determined as the rate of incorporation of bromodeoxyuridine (BrdU) into cellular DNA, using a kit from Calbiochem® (EMD Biosciences, La Jolla, CA). The cells were seeded in 96-well plates and grown to 50–60%

confluency. They were treated with flavonoids overnight and subsequently labeled with BrdU for four hours, followed by a 30-min incubation with fixative/denaturing solution and a one-hour incubation with anti-BrdU antibody. Unbound antibodies were washed away and the cells were incubated with horseradish-peroxidase-conjugated anti-mouse IgG for 30 min. After another wash step, tetra-methylbenzidine solution was added as substrate to each well and incubated for 15 min at room temperature in the dark. Sulfuric acid (stop solution) was added and the absorbance was read immediately at 450 nm with 600 nm background subtraction.

2.5. Myeloperoxidase activity in cells

A modified method after van der Woude *et al.* [12] and Kagan *et al.* [27] was employed. Trout cells as well as human leukemia HL-60 cells (positive control) were grown in uncoated T-75 flasks. Upon reaching 80% confluency, the trout cells were trypsinized and aliquots of $1 - 2 \times 10^6$ trout and HL-60 cells were spun down, washed in Hanks' buffer, centrifuged and resuspended in complete assay buffer (0.1 M potassium phosphate buffer, pH 7.0, with 0.1% Triton X-100, 0.1 mM PMSF, 0.02% cetyltrimethylammonium bromide) and sonicated for 15 s on ice. The resulting homogenates were centrifuged ($1100 \times g$), and the supernatants used for the peroxidase assay and protein determination [28]. Guaiacol (15 mM, final concentration) was added, and the reaction was started by the addition of H_2O_2 to 0.26 mM. Guaiacol oxidation to tetraguaiacol by peroxidase was monitored for 10 min at 25°C using spectrophotometric analyses at 470 nm absorbance. The enzyme activity was calculated as nmol tetraguaiacol formed/min/mg protein from the change in A_{470} over time: $4 \text{ guaiacol} + 2 H_2O_2 \rightarrow \text{tetraguaiacol} + 4 H_2O$ with $\epsilon(\text{tetraguaiacol})_{470} = 26600 \text{ mM}^{-1} \text{ cm}^{-1}$ [12]. Reaction mixtures without H_2O_2 or without cell homogenates were used as negative controls.

2.6. Chrysin oxidation by human myeloperoxidase

Triplicate reaction mixtures (100 μ l) containing myeloperoxidase (0.6 U), hydrogen peroxide (1.3 mM) and chrysin (2.5 μ M) in potassium phosphate buffer (pH 7.0) were incubated in the absence or presence of reduced glutathione (GSH, 5 mM) for 60 min at 37°C. The reaction was stopped by adding equal volumes of ice-cold methanol. Samples were vortexed, centrifuged and the supernatant analyzed by reverse-phase HPLC with a Symmetry® C₁₈ 5 μ m, 3.9×150 mm column (Waters Corporation, Milford, MA), a flow-rate of 0.9 ml/min and photo-diode array UV-detection at 268 nm. Samples without hydrogen peroxide or without myeloperoxidase were used as negative controls.

2.8. Statistical analyses

Results were expressed as means $\pm$ SEM of at least 3 experiments as applicable. Comparisons among means were made using two-tailed unpaired ANOVA followed by Dunnett's Multiple Comparison Test (InStat, v. 2.00). The level of significance for all experiments was set at $\alpha = 0.05$.

3. Results

3.1. Exposure of trout cells to flavones

Growth and health of the trout hepatocytes were monitored by microscopy from seeding to harvesting, with and without treatment with vehicle (DMSO) or flavonoids dissolved in DMSO. These normal trout cells were maintained at 18°C and expectedly grew more slowly than other cells maintained at higher temperatures. Nonetheless, these cells, as established and described by Ostrander [26], grow in their spindle-like form until reaching ~50% confluency, after which they form a uniform monolayer. Treatment with DMSO (<0.1%) had no visible impact on growth patterns of these cells even up to 48 h (Fig. 2).

Flavonoids at a 25 μM concentration affected cell growth and health differently. At 24 h, all flavonoids showed some inhibition of cell growth, as observed by monitoring confluency levels and verified by protein measurements. Two of the compounds with free hydroxyl groups, *i.e.*, chrysin and apigenin, dramatically reduced cell numbers, even below the number of cells present before treatment had started. Large gaps were seen in the previously semi-confluent monolayers (Fig. 2). Additionally, cells were observed lifting off and floating in the medium. Chrysin produced a clear concentration dependency in toxicity (Fig. 3). Thus, a 2 μM concentration showed no visual adverse effects, whereas 10 μM was clearly toxic, and 25 μM even more toxic. Luteolin showed a similar response as quercetin, both being considerably less toxic than chrysin, without significant differences from DMSO-controls (Fig. 4). In contrast to the hydroxylated flavones, the methylated chrysin analogue 5,7-DMF, as well as another methoxylated flavone, *i.e.* 3',4'-DMF, showed no toxicity at a 25 μM concentration (Figs. 2 and 4).

3.2. Effects of flavones on cell proliferation

Trout cell proliferation was measured as BrdU incorporation into newly synthesized DNA of actively proliferating cells (Fig 5.). The methoxylated flavone 5,7-DMF showed inhibition with an IC_{50} value of $\approx 50\text{--}100\text{ }\mu\text{M}$. This is similar to the response of two normal human cell lines, the lung BEAS-2B cells and the esophageal HET-1A cells, to 5,7-DMF [6]. In contrast, chrysin was exceedingly potent, producing an IC_{50} value as low as 2 μM (Fig. 5).

3.3. Myeloperoxidase activity in trout cells

One potential explanation for the toxicity of a molecule such as the flavone chrysin may be the presence of peroxidase-like activity in trout liver cells, which may activate chrysin to a toxic species. Peroxidase-like activity is absent in human hepatic Hep G2 cells, but present in human hepatic Kupffer cells [29], and also in the human leukemia HL-60 cells [12]. Surprisingly, normal rainbow trout liver cells showed myeloperoxidase activity (average 164 pmol/min/mg protein) that was at least as high as in the myeloperoxidase-rich HL-60 cells (average, 131 pmol/min/mg protein) (Fig. 6).

3.4. Metabolism of flavones by myeloperoxidase

Recombinant human myeloperoxidase metabolized chrysin in the presence of hydrogen peroxide (Fig. 7). Also, several apparent polar metabolites with short HPLC retention times were observed. The reaction depended on the amounts of both myeloperoxidase and hydrogen peroxide used. This reaction was abolished by the addition of 5 mM GSH (Fig. 7). In contrast, the methylated analogue 5,7-DMF was not significantly metabolized by myeloperoxidase or affected by subsequent addition of GSH (Fig. 7).

4. Discussion

Low doses of flavonoids, such as those present in our daily diet may be safe for human health. However, that may not be the case after ingestion of high doses of these compounds commonly present in dietary food supplements. One such flavonoid, the simple flavone chrysin, can be found in health food stores and through the Internet at “recommended daily doses” of 0.5 to 3 grams. This is a dosage claimed effective in antagonizing the enzyme aromatase, thus preventing the conversion of testosterone to estradiol, a desirable effect for body builders to increase their muscle mass. Whether this is a true effect of chrysin *in vivo* in humans has been seriously challenged in a number of investigations [30-32].

However, chrysin, as shown in the present study, was the compound most toxic to the trout liver cells. This was demonstrated by light microscopy as dramatically reduced cell numbers and large gaps in the semi-confluent monolayers and also with cells lifting off. This was also

shown by chrysin effectively shutting down the *de novo* DNA synthesis. Both effects demonstrated IC₅₀ values as low as about 2 µM, which is a concentration that is 25 times less than that producing antiproliferative effects in a number of cell lines. In addition, one other flavone, *i.e.*, apigenin, appeared to be equipotent for this toxic effect, whereas luteolin and in particular quercetin were much less toxic.

One possible explanation for the toxic effects in the trout hepatocytes may be the presence of peroxidase-like activity in these cells, capable of oxidizing chrysin and other flavones to toxic products. Thus, myeloperoxidase activity was detected in these cells at a level higher than in the human myeloid HL-60 cells used as a positive control [12]. Using pure human myeloperoxidase, we could show that chrysin, but not 5,7-DMF was indeed oxidized to products with increased polarity, as detected by HPLC. In addition, GSH inhibited this oxidative process. Even though these experiments did not reveal the detailed mechanism of the cytotoxic effects, they may form the basis for further in-depth investigations.

Myeloperoxidase in neutrophils has been implicated previously in drug-induced toxicities, such as agranulocytosis and lupus by carbamazepine [33] and clozapine [34,35]. Myeloperoxidase also has been implicated in secondary acute myeloid leukemia caused by the topoisomerase II poison etoposide through its conversion to a phenoxy radical [27]. This enzyme may also be responsible for the suggestion that dietary flavonoids induce cleavage in the MLL gene and may contribute to infant leukemia [36].

In summary, we have shown that several flavones are toxic to a fish liver cell line. This occurs at low concentrations. The mechanism of this toxicity may be related to the finding that these cells express relatively high levels of myeloperoxidase, oxidizing some flavones to toxic species. The findings in the present study might be useful in the further investigation of cytotoxic and DNA-damaging effects of dietary flavonoids.

Acknowledgements

We would like to thank Kristina Walle for critical review of the manuscript, and Dr. Supriti Samanta Ray for assistance with data analyses.

This study was supported by an Environmental Protection Agency STAR Fellowship (P.A.T.) and in part by the American Association for Cancer Research grant 02A095 and the National Institutes of Health grant GM55561.

References

1. Kühnau, J. The flavonoids. A class of semi-essential food components: Their role in human nutrition.. In: Bourne, GH., editor. World Rev Nutr Diet. S. Karger, Basel; Switzerland.: 1976. p. 117-120.
2. Pietta PG. Flavonoids as antioxidants. J. Natur. Prod 2000;63:1035–42. [PubMed: 10924197]
3. Middleton EJ, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacol. Rev 2000;52:673–751. [PubMed: 11121513]
4. Rice-Evans CA. Flavonoid antioxidants. Curr Med Chem 2001;8:797–807. [PubMed: 11375750]
5. Williams RJ, Spencer JPE, Rice-Evans CA. Flavonoids: antioxidants and signalling molecules? Free Rad. Biol. Med 2004;36:838–849. [PubMed: 15019969]
6. Walle T, Ta N, Kawamori T, Wen X, Tsuji PA, Walle UK. Cancer chemopreventive properties of orally bioavailable flavonoids - methylated versus unmethylated flavones. Biochem. Pharmacol 2007;79:1288–1296. [PubMed: 17250812]
7. Tsuji PA, Walle T. Benzo[a]pyrene-induced cytochrome P450 1A and DNA binding in cultured trout hepatocytes - inhibition by plant polyphenols. Chem Biol Interact in press. 2007
8. Galati G, O'Brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Rad. Biol. Med 2004;37:287–303. [PubMed: 15223063]

9. Chan TS, Galati G, Pannala AS, Rice-Evans CA, O'Brien PJ. Simultaneous detection of the antioxidant and pro-oxidant activity of dietary polyphenolics in a peroxidase system. *Free. Rad. Res* 2003;37:787–794.
10. Awad HM, Boersma MG, Boeren S, Van Bladeren PJ, Vervoort J, Rietjens IMCM. Quenching of quercetin quinone/quinone methides by different thiolate scavengers: stability and reversibility of conjugate formation. *Chem. Res. Toxicol* 2003;16:822–831. [PubMed: 12870884]
11. Walle T, Vincent TS, Walle UK. Evidence of covalent binding of the dietary flavonoid quercetin to DNA and protein in human intestinal and hepatic cells. *Biochem. Pharmacol* 2003;65:1603–1610. [PubMed: 12754096]
12. van der Woude H, Alink GM, van Rossum BEJ, Walle UK, van Steeg H, Walle T, Rietjens IMCM. Formation of transient covalent protein and DNA adducts by quercetin in cells with and without oxidative enzyme activity. *Chem. Res. Toxicol* 2005;18:1907–1916. [PubMed: 16359181]
13. Babich H, Borenfreund E. Cultured fish cells for the ecotoxicity testing of aquatic pollutants. *Toxicity Assessment: An International Quarterly* 1987;2:119–133.
14. Bols NC, Schirmer K, Joyce EM, Dixon DG, Greenberg BM, Whyte JJ. Ability of polycyclic aromatic hydrocarbons to induce 7-ethoxyresorufin-O-deethylase activity in a trout liver cell line. *Ecotoxicol. Environ. Safety* 1999;44:118–128. [PubMed: 10499998]
15. Pollenz RS, Necela B, Marks-Sojka K. Analysis of rainbow trout Ah receptor protein isoforms in cell culture reveals conservation of function in Ah receptor-mediated signal transduction. *Biochem. Pharmacol* 2002;64:49–60. [PubMed: 12106605]
16. George E, Riley C, McEvoy J, Wright J. Development of a fish in vitro cell culture model to investigate oxidative stress and its modulation by dietary vitamin E. *Mar. Environ. Res* 2000;50:541–544. [PubMed: 11460747]
17. Fent K. Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. *Toxicol. in vitro* 2001;15:477–488. [PubMed: 11566581]
18. van den Hurk P, Faisal M, Roberts MH. Interaction of Cadmium and benzo[a]pyrene in mummichog (*Fundulus heteroclitus*): effects on acute mortality. *Mar. Environ. Res* 1998;46:525–528.
19. Sinnhuber RO, Hendricks J, Wales JH, Putnam GB. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. *Ann. N.Y. Acad. Sci* 1978;298:389–408. [PubMed: 280189]
20. Bailey GS, Williams DE, Hendricks JD. Fish models for environmental carcinogenesis: the rainbow trout. *Environ. Health Persp. Suppl* 1996;104
21. Ahne W. Studies on the use of fish tissue cultures for toxicity tests in order to reduce and replace the fish tests. *Zbl. Bakt. Hyg* 1985;180:480–504.
22. Goss LB, Sabourin TD. Utilization of alternative species for toxicity testing: an overview. *J. Appl. Toxicol* 1985;5:193–219. [PubMed: 3900184]
23. Sadar MD, Blomstrand F, Andersson TB. Phenobarbital induction of cytochrome P4501A1 is regulated by cAMP-dependent protein kinase-mediated signaling pathways in Rainbow trout hepatocytes. *Biochem. Biophys. Res. Commun* 1996;225:455–461. [PubMed: 8753783]
24. Schmieder PK, Tapper MA, Kolanczyk RC, Hammermeister DE, Sheedy BR, Denny JS. Discriminating redox cycling and arylation pathways of reactive chemical toxicity in trout hepatocytes. *Toxicol. Sci* 2003;72:66–76. [PubMed: 12604835]
25. Gagne F, Blaise C. Acute cytotoxicity assessment of liquid samples using Rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Tech. Meth. Section* 1999:104–109.
26. Ostrander GK, Blair JB, Stark BA, Marley GM, Bales WD, Veltri RW, Hinton DE, Okihiro M, Ortego S, Hawkins WE. Long-term primary culture of epithelial cells from Rainbow trout (*Oncorhynchus mykiss*) liver. *In Vitro Cell. Develop. Biol* 1995;31:367–378.
27. Kagan VE, Kuzmenko AI, Tyurina YY, Shvedova AA, Matsura T, Yalowich JC. Pro-oxidant and antioxidant mechanisms of etoposide in HL-60 cells: role of myeloperoxidase. *Cancer Res* 2001;61:7777–7784. [PubMed: 11691792]
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol agent. *Biol. Chem* 1951;193:265–275.

29. Tafazoli S, O'Brien PJ. Peroxidases: a role in the metabolism and side effects of drugs. *Drug Discov. Today* 2005;10:617–625. [PubMed: 15894226]
30. Walle T, Otake Y, Brubaker J, Walle U, Halushka P. Disposition and metabolism of the flavonoid chrysin in normal volunteers. *Br. J. Clin. Pharmacol* 2001;51:143–146. [PubMed: 11259985]
31. Saarinen N, Joshi SC, Ahotupa M, Li X, Ammala J, Makela S, Santti R. No evidence for the in vivo activity of aromatase-inhibiting flavonoids. *J. Steroid Biochem. Mol. Biol* 2001;78:213–239.
32. Gambelunghe C, Rossi R, Somnavilla M, Ferranti C, Rossi R, Ciculi C, Gizzi S, Micheletti A, Rufini S. J. Med. Food 2003;Effects of chrysin on urinary testosterone levels in human males6:387–390. [PubMed: 14977449]
33. Furst SM, Uetrecht JP. Carbamazepine metabolism to a reactive intermediate by the myeloperoxidase system of activated neutrophils. *Biochem. Pharmacol* 1993;45:1267–1275. [PubMed: 8385460]
34. Gardner I, Leeder JS, Chin T, Zahid N, Uetrecht P. A comparison of the covalent binding of clozapine and olanzapine to human neutrophils in vitro and in vivo. *Mol. Pharmacol* 1998;53:999–1008. [PubMed: 9614201]
35. Gardner I, Zahid N, MacCrimmon D, Uetrecht JP. A comparison of the oxidation of clozapine and olanzapine to reactive metabolites and the toxicity of these metabolites to human leukocytes. *Mol. Pharmacol* 1998;53:991–998. [PubMed: 9614200]
36. Strick R, Strissel PL, Borgers S, Smith SL, Rowley JD. Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. *PNAS* 2000;97:4790–4795. [PubMed: 10758153]

Fig. 1.
Chemical structures of the flavonoids studied

Fig. 2. Trout cells after 24-h exposure to medium alone, vehicle control (0.1% DMSO), or 25 μ M flavonoids in medium with 0.1% DMSO. Dead or dying cells are indicated by arrows. Magnification = 300x.

Fig. 3. Trout cells treated with various concentrations of chrysin. Cells (96% confluent) were treated for 48 hours. Magnification 300x.

Fig. 4. Quantitative analysis of cell coverage after treatment with 25 μ M flavonoids for 24 h. Cell coverage was quantified with Image J software and expressed as percent of DMSO-control. Mean $\pm$ SEM of 3–5 cultures are shown. *** and **, significantly different from DMSO-control ($p < 0.001$ & $p < 0.01$, respectively)

Fig. 5. Trout cell proliferation in the presence of varying concentrations of chrysin (squares) and its methylated analogue 5,7-DMF (triangles), as measured by BrdU incorporation into cellular DNA. Means $\pm$ SEM are shown (N = 12 from 2 independent experiments).

Fig. 6. Trout liver cells (◇) were compared with human leukemia HL-60 cells (■) for myeloperoxidase activity using guaiacol as the substrate. The average catalytic activity in trout hepatocytes was 164 pmol/min/mg protein (N = 4) and in HL-60 cells 131 pmol/min/mg protein (N = 3).

Fig. 7.

Human myeloperoxidase (6 U/ml) metabolism of 2.5 μ M chrysin (A) or 5,7-DMF (B) with hydrogen peroxide (1.3 mM) as cofactor with and without the presence of 5 mM GSH. (N = 4). *** significantly different from control ($p < 0.001$); ### significantly different from Chrysin +MPO ($p < 0.001$).