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Effects of hypericin on the oxidative stress and modulation of cytochrome P450 (CYP1A) activity in microsomes

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ABSTRACT

Hypericin is a pigment present in the widely distributed medicinal plant *Hypericum perforatum* L. (Hypericaceae). In our research, hypericin was found to be an inhibitor of NADPH/Fe^{2+} induced microsomal lipid peroxidation and NADPH-dependent lucigenin chemiluminescence emission *in vitro*. Hypericin also inhibited the microsomal CYP1A-dependent 7-ethoxyresorufin *O*-deethylase (EROD) which participates in the metabolic activation of xenobiotics including chemical carcinogens.

Keywords: *chemiluminescence; EROD activity; Hypericumperforatum; lipid peroxidation*

INTRODUCTION

Hypericum perforatum (Hypericaceae), a perennial flowering plant, is distributed in Europe, Asia, North Africa and North America. This plant is well known in traditional medicine in Europe as well as in Traditional Chinese Medicine. It is effective especially in the treatment of mild to moderate depression (Mennini *et al.,* 2004). The drug acts as a sedative and has been known to contain a red dianthrone pigment hypericin, which has been assumed to be a primary active constituent with significant receptor affinity for GABA and benzodiazepine receptors (Cott, 1997). Hypericin is a natural photosensitizer, which possesses white light-induced antitumour activity *in vivo* (Vandenbogaerde *et al.,* 1996).

Under optimal conditions of exposure to light, hypericin exhibited a strong inhibitory activity against HSV-1 (herpes simplex virus) and HIV-1 (human immunodeficiency virus). There was a significant reduction of a light-induced antiviral activity of hypericin under hypoxic conditions. Only when the concentration of hypericin reached the cytotoxic level there was an apparent light-independent antiviral effect (Hudson*et al.,*1994; Park*et al.,*1998).

H. perforatum is one of the most commonly used herbal medications, nevertheless clinical reports indicate that *H. perforatum* increases the activity of cytochrome P450 enzyme and can reduce plasma concentrations of certain drugs. In search for compounds with chemoprotective activity we isolated hypericin from *H. perforatum* and evaluated its antioxidant and CYP1A modulating activities.

MATERIALS AND METHODS

General

Hypericum perforatum L. was collected in Medicinal Herbs Centre of Masaryk University Brno and identified by prof. Václav Suchý, Department of Natural Drugs, VFU Brno, Czech Republic. Hypericin was isolated and purified from the aerial

parts of *H. perforatum* and identified by spectral methods. Spectral data corresponded with data published previously (Piperopoulos *et al.,* 1997). All the chemicals were purchased from Sigma Chem. Co. (Czech Republic).

Isolation of Microsomal Fraction

The hepatic microsomal fraction was isolated from C57Bl/6 mouse liver tissue by homogenization and differential centrifugation. The microsomes were washed once, resuspended in 0.05 M Tris-HCl buffer, pH 7.5, containing 20% of glycerol and 0.1 mM EDTA and stored at –80 °C until used.

Determination of antioxidant activity

The inhibition of Fe^{2+}/NADPH-dependent lipid peroxidation was determined *in vitro* by the method of thiobarbituric acid reactive species (TBARs) (Uchiyama *et al.,* 1978). The reaction mixture contained microsomes, 0.1 M Tris-HCl buffer, pH 7.6, 10 µL hypericin dissolved in DMSO or DMSO alone as the control and an oxygen radicals generating system. The concentration of TBARs was measured at 532 nm. Lucigenin (1.7 mM) was used as a chemiluminescence amplifier and NADPH (0.3 mM) as a generating system in the CL method (Scholz *et al.,* 1990). The reaction mixture contained microsomes, 10 µL hypericin dissolved in DMSO or DMSO alone as the control.

Determination of cytochrome P4501A (CYP1A)-dependent EROD activity

The microsomal 7-ethoxyresorufin *O*-deethylase (EROD) was determined using direct fluorimetric method (Prough *et al.,* 1997). The reaction mixture contained microsomes, 2 µM ethoxyresorufin, 0.1 M Tris-HCl buffer, pH 7.6 and 0.3 mM NADPH. The *in vitro* inhibition of the EROD activity was assayed in the same reaction mixture with addition of 10 µL hypericin dissolved in DMSO or DMSO alone as the control.

RESULTS AND DISCUSSIONS

We evaluated the effect of hypericin on Fe^{2+}/NADPH-enhanced microsomal lipid peroxidation and on lucigenin-amplified chemiluminescence (CL) induced by NADPH *in vitro*. The effect of hypericin on cytochrome P4501A (CYP1A) activity, which is involved in a bioactivation of xenobiotics, was tested in a microsomal fraction of C57Bl/6 mouse liver. Natural antioxidant quercetin was used as a standard in the experiments.

The microsomal lipid peroxidation was induced enzymatically by NADPH and Fe^{2+}. Hypericin inhibited production of thiobarbituric acid-reacting substances (TBARs) in a concentration-dependent manner but was weaker compared to the standard in the concentration 10 µM (figure 1). The IC_{50} values were 5 µM for hypericin and 4.5 µM for quercetin. In the previous study, hypericin was found to have no cytotoxic effect in the dark while significantly stimulated lipid peroxidation after irradiation with visible light (Hadjur *et al.,* 1996).

As shown in figure 2, hypericin inhibited lucigenin-augmented chemiluminescence (CL) induced by reactive oxygen species (ROS), mainly by superoxide. IC_{50} for hypericin and quercetin were 1 µM and 194 µM, respectively.

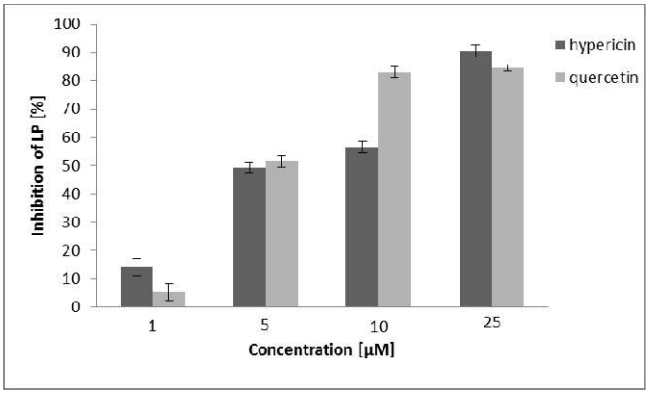

Figure 1. *In vitro* inhibition of the Fe^{2+}/NADPH-dependent lipid peroxidation.LP, lipid peroxidation in hepatic microsomes. Values are expressed as mean ± SEM, n=6.

CL emission was measured upon incubation with NADPH. As it was reported, photoactivated hypericin produces singlet oxygen and superoxide anion radical via the inhibition of mitochondrial succinoxidase and oxidative stress-initiated mitochondrial damage as a key target in hypericin phototoxicity (Johnson *et al.*, 1998). On the other hand, hypericin caused inhibition of superoxide

generation of neutrophil via a mechanism involving the inhibition of tyrosin kinase, protein kinase C and NADPH oxidase. IC_{50} for NADPH oxidase was 10 nM (Nishiuchi *et al.,* 1995). In the inhibition mechanism of lipid peroxidation and NADPH-induced lucigenin CL either scavenging reactive oxygen species (ROS) or inhibition of NADPH oxidase dependent enzymatic reactions generated by ROS can be involved.

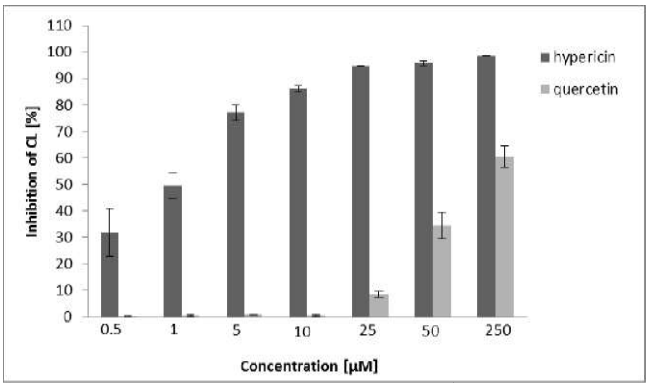

Figure 2. *In vitro* inhibition of lucigenin-amplified chemiluminescence. CL, chemiluminescence in hepatic microsomes. Values are expressed as mean ± SEM, n=6.

The potency of hypericin to inhibit the CYP1A-dependent 7-ethoxyresorufin *O*-deethylase activity (EROD) *in vitro* is given in the figure 3. Hypericin inhibited the EROD production in a concentration dependent manner in the range 5-25 µM and the potency of hypericin was lower than thst of quercetin. The IC_{50} values were 2 µM for quercetin and 13 µM for hypericin. The EROD activity was measured by the fluorescence method. The inhibition potency of hypericin can be caused by its phototoxicity because the reaction mixture was under irradiation. In human hepatocyte model hypericin had no effect on CYP enzymes but hyperforin treatment resulted in a significant increase of activity of CYP3A4 and CYP2C9 (Komoroski *et al.,*2004).

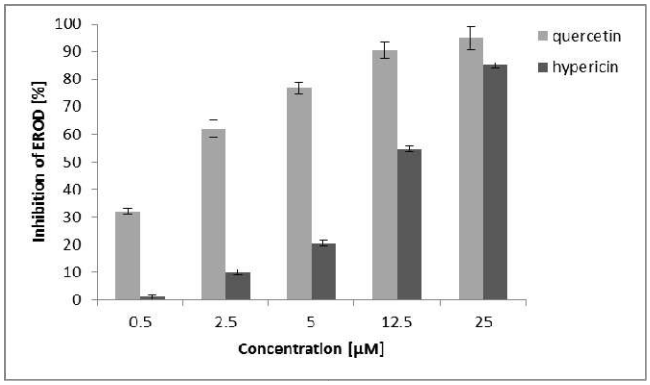

Figure 3. *In vitro* inhibition of the CYP1A-dependent EROD activity. EROD, 7-ethoxyresorufin O-deethylase activity in hepatic microsomes. Values are expressed as mean ± SEM, n=3.

CONCLUSIONS

In conlusion, hypericin represents a promising natural compound with interesting biological activities. However, its phototoxicity is a limiting factor for its use as a pharmacotherapeutic agent.

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Conflicts of Interest

The authors declare no conflict of interest.