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; Hypericum perforatum; 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; Parket 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...
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 \(\mu\)M (figure 1). The IC\(_{50}\) values were 5 \(\mu\)M for hypericin and 4.5 \(\mu\)M 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 \(\mu\)M and 194 \(\mu\)M, 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.](image)

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 production.
generation of neutrophil via a mechanism involving the inhibition of tyrosin kinase, protein kinase C and NADPH oxidase. IC₅₀ 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.

Hypericin inhibited the EROD production in a concentration dependent manner in the range 5-25 μM and the potency of hypericin was lower than that of quercetin. The IC₅₀ 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).

The potency of hypericin to inhibit the CYP1A-dependent 7-ethoxyresorufin O-deethylase activity (EROD) in vitro is given in the figure 3.

In conclusion, 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.

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