

CYP3A-INDUCING EFFECT OF HYPERFORIN AND DERIVATIVES IN RODENTS

Lavinia Cantoni*, Marco Rozio, Alessandra Mangolini and Silvio Caccia

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan, Italy

Running title:

CYP-3A induction by hyperforin and derivatives

*Corresponding author:

Dr. Lavinia Cantoni

Laboratory of Molecular Pathology, Head

Istituto di Ricerche Farmacologiche "Mario Negri"

Via Eritrea 62, 20157 Milan, Italy

Phone: +39 02 39014423

ABSTRACT

Objective. The search for more stable and safer analogs of hyperforin, which shares most of the antidepressive properties of the extracts of *Hypericum perforatum* (St. John's wort), has led to the synthesis and pharmacological evaluation of some derivatives. In this study we preliminarily evaluated the inductive potential of octahydrohyperforin and hyperforin trimethoxybenzoate in rodents. They were compared to hyperforin which induced the activity and expression of CYP3A protein in the mouse and is responsible for some important interactions involving St. John's wort extracts and conventional drugs.

Methods. Male CD1 mice and CD-COBS rats were given oral doses of each compound and blood samples were collected after the first and last dose for drug measurements. Livers were removed after the last dose for measurement of microsomal activity of erythromycin N-demethylase (ERND) and expression of CYP3A1/2.

Results and Conclusion. In mice, after four days of equimolar doses (in terms of hyperforin), octahydrohyperforin did not appreciably modify hepatic ERND and CYP3A1/2 whereas hyperforin significantly increased them both. In mice and rats, hyperforin trimethoxybenzoate did not induce ERND activity even after 12 days of treatment. Octahydrohyperforin rapidly reached the systemic circulation although plasma concentrations were variable and tended to be lower after the last dose, as previously observed for hyperforin in mice. Plasma concentrations of hyperforin after oral hyperforin trimethoxybenzoate were low, suggesting that this ester is not an efficient pro-drug of hyperforin in rodents.

KEY WORDS

Octahydrohyperforin; Hyperforin trimethoxybenzoate; Hyperforin; St. John's wort extract; CYP3A; Rodents

INTRODUCTION

Hyperforin shares most of the pharmacological properties of the extracts of *Hypericum perforatum* L. (St. John's wort) (1, 2), which are gaining popularity for the treatment of mild to moderate depressive disorders. This main phloroglucinol component interacts with central neurotransmitter transporters and receptors believed to be causally involved in depression (3). The pharmacological potency of different extracts correlates with their hyperforin content, further suggesting that this component plays a decisive role in the antidepressant-like activity of St. John's wort (4-6). However, hyperforin is a potent agonist of the pregnane X receptor that regulates the expression of some cytochrome P450 (CYP) enzymes and of the P-glycoprotein transmembrane pump and is very likely also responsible for clinically relevant interactions involving the extracts and conventional drugs (7-9). This and its tendency to oxidation when exposed to light and air (10) has led to the synthesis of several derivatives, including esters, salts and reduced derivatives. These are now undergoing pre-clinical studies (11-13), including an evaluation of their inductive potential.

The aim of the present study was to preliminarily evaluate in rodents the inductive potential of hyperforin trimethoxybenzoate and octahydrohyperforin compared to hyperforin which induced the activity and expression of CYP3A protein to much the same extent as a high-hyperforin hydroalcoholic St. John's wort extract (14).

MATERIALS AND METHODS

Male CD1 mice (22-25 g) and CD-COBS rats (175-200 g) (Charles River, Calco, Como, Italy) were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Animals were given oral doses of a St. John's wort extract containing 4.5% hyperforin, hyperforin (dicyclohexylammonium salt), trimethoxybenzoate ester of hyperforin or octahydrohyperforin (lithium salt) (Indena S.p.A., Milan, Italy), twice daily for three and eleven (hyperforin trimethoxybenzoate only) days while at day four and twelve, respectively, they were treated only once. Animals were killed 24 hr after the last dose. The hydroalcoholic extract was suspended in 1.6% carboxymethylcellulose sodium salt, while the other compounds were suspended in 4% Tween 80 in water. All solutions were freshly prepared immediately before use.

Blood samples were collected after the first and the last dose for drug measurements in plasma. Hyperforin and octahydrohyperforin were extracted from plasma by a solid-liquid extraction procedure and quantified by high-performance liquid chromatography with UV detection as recently described (15)

Livers were removed after the last dose and microsomes were prepared for measurement of the activity of erythromycin N-demethylase (ERND) and the expression of CYP3A1/2 by Western blot (14).

RESULTS

Table 1 compares the plasma maximum concentration (C_{max}) and the area under the curve (AUC_t) of octahydrohyperforin and hyperforin after the first and the last dose in mice. The dose of octahydrohyperforin (18.4 mg/kg) was equimolar to that of hyperforin (18.1 mg/kg) in terms of hyperforin and approximated the hyperforin content of the 4.5% St. John's wort extract (300 mg/kg), that induced hepatic ERND and CYP3A1/2 in the mouse (14). Like hyperforin, octahydrohyperforin rapidly reached the systemic circulation but plasma concentrations were variable and tended to be lower after the last dose of this schedule.

By contrast, plasma concentrations of hyperforin trimethoxybenzoate were low. Plasma concentrations of its active metabolite were also low, near or below the limit of hyperforin quantitation after oral dosing (data not shown).

In mice, after four days of treatment, octahydrohyperforin (18.4 mg/kg) did not appreciably modify hepatic ERND and CYP3A1/2 (Fig. 1A) whereas hyperforin (18.1 mg/kg) significantly increased these parameters (about two-fold) (Fig. 1B). This effect was comparable to that of the hydroalcoholic extract (14). Hyperforin trimethoxybenzoate (10 mg/kg) did not induce ERND activity even after 12 days of treatment in rats and mice (Fig 2).

Mice and rats apparently tolerated these treatments, as there were no real differences in body weight with either the extract, hyperforin or its trimethoxybenzoate ester or octahydrohyperforin, except for a slight decrease on the last day of treatment, mostly caused by fasting, as a similar reduction was seen in vehicle-treated mice.

Table 1. Exposure to octahydrohyperforin and hyperforin after oral doses in mice

Compound (mg/kg)	Dose	C _{max} (μ M)	AUC _t (nmol/mL.min)
Octahydrohyperforin (18.4)	First	1.18 \pm 0.16	212
	Last	0.64 \pm 1.03	85
Hyperforin (18.1)	First	0.84 \pm 0.61	111
	Last	0.38 \pm 0.61	43

Octahydrohyperforin and hyperforin were suspended in 4% Tween 80 in water.

C_{max} = peak plasma concentration.

AUC_t = area under the curve over the sampling interval: up to 6h after the first dose and up to 2 h after the last dose for hyperforin; up to 8 h regardless of the dose for octahydrohyperforin.

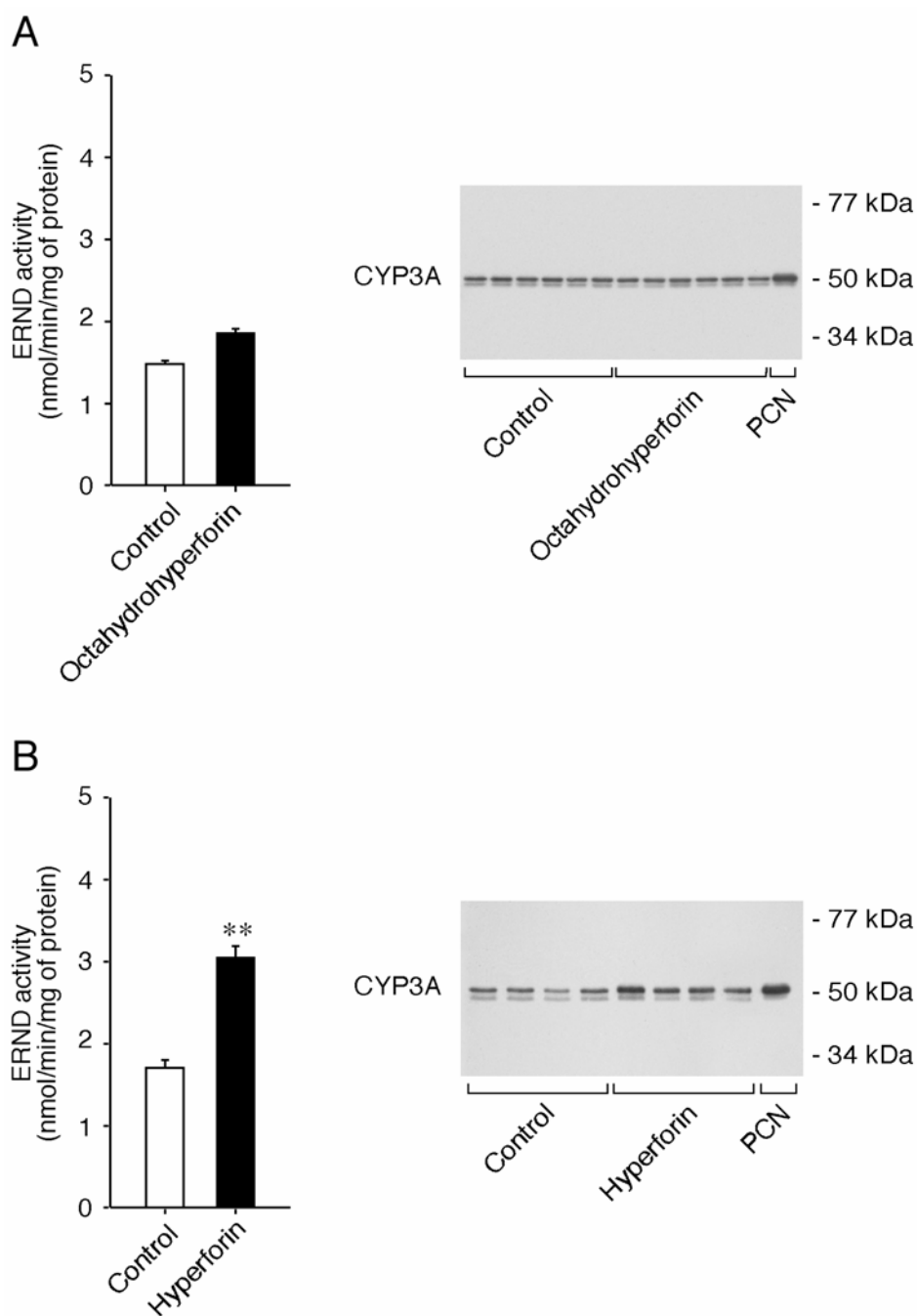


Figure 1: Effect of octahydrohyperforin and hyperforin on activity of hepatic erythromycin N-demethylase and expression of CYP3A1/2 in mice

Mice were treated orally with the vehicle, octahydrohyperforin (18.4 mg/kg) (Panel A) or hyperforin (18.1 mg/kg) (Panel B) twice daily for three days and once at day four. They were then fasted and killed after 24 hr. Values are mean \pm SE of six mice.

$p < 0.01$ by Student's t test

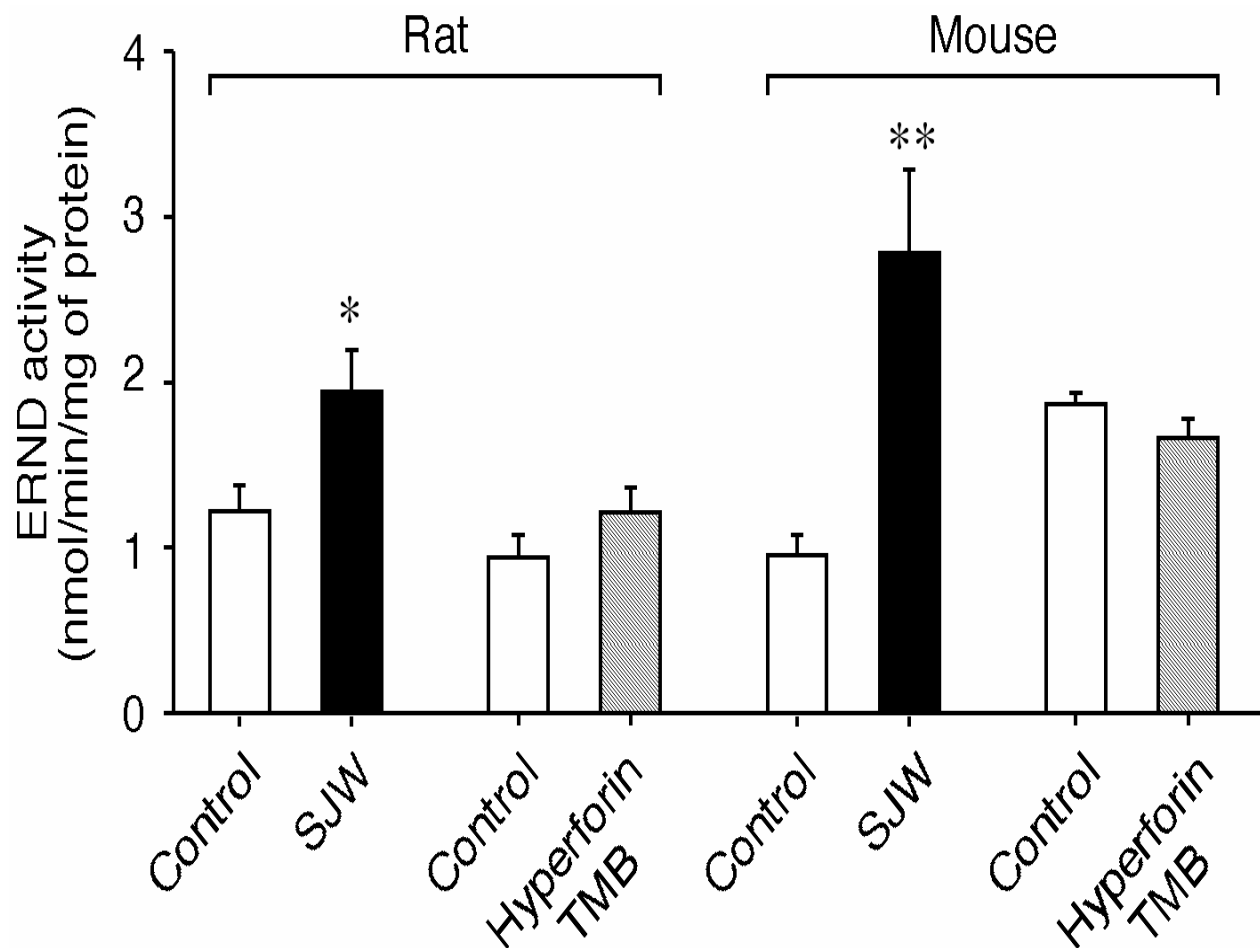


Figure 2: Effect of hyperforin trimethoxybenzoate and St. John's wort extract on activity of hepatic erythromycin N-demethylase in rats and mice

Rats and mice were treated orally with the vehicles or St. John's wort extract (SJW) (300 mg/kg) or hyperforin trimethoxybenzoate (hyperforin TMB) (10 mg/kg) twice daily for eleven days and once at day twelve. They were then fasted and killed after 24 hr. Values are mean \pm SE of six mice.

$p < 0.05$ by Student's t test

DISCUSSION

The search for more stable and safer analogs of hyperforin has led to the synthesis and pharmacological evaluation of some of its salts, esters and reduced derivatives. The aim is to identify “antidepressant-like” derivatives without hyperforin’s ability to induce the expression of intestinal P-glycoprotein and intestinal and hepatic CYP3A, since these effects are responsible for some clinically relevant interactions between St. John's wort extracts and conventional drugs (7-9). As a first step we evaluated octahydrohyperforin and hyperforin trimethoxybenzoate since these compounds had antidepressant-like activity comparable to or even higher than hyperforin in rodents (11-13). Hyperforin trimethoxybenzoate did not appreciably modify hepatic ERND activity, possibly because it is not an efficient pro-drug of hyperforin as previously found in rats given intraperitoneal doses of this ester (12). Octahydrohyperforin rapidly reached the systemic circulation (15) and in mice exposure tended to be lower after the last dose of a twice-daily regimen, suggesting that, like hyperforin (10, 14), its metabolism involves oxidative reactions which may be autoinduced after repeated dosing. However, unlike hyperforin, the activity of the CYP3A-dependent ERND did not rise significantly in the mouse after doses equimolar in terms of hyperforin, suggesting that the two compounds behave differently with regard to the pattern and potential induction of CYP enzymes. Because there are large species differences in the response of inducible enzymes to xenobiotics, a definitive risk assessment of the induction potential of octahydrohyperforin must await in vitro and in vivo human studies.

ACKNOWLEDGEMENTS

Hyperforin, octahydrohyperforin and hyperforin trimethoxybenzoate were kindly provided by Indena S.p.A., Milan, Italy.

REFERENCES

1. Chatterjee SS, Noldner M, Koch E, Erdelmeier C. Antidepressant activity of *Hypericum perforatum* and hyperforin: the neglected possibility. *Pharmacopsychiatry* 1998; 31, Suppl 1:7-15.
2. Gambarana C, Tolu PL, Masi F, et al. A study of the antidepressant activity of *Hypericum perforatum* on animal models. *Pharmacopsychiatry* 2001; 34 Suppl 1: S42-44.
3. Butterweck V, Nahrstedt A, Evans J, et al. In vitro receptor screening of pure constituents of St. John's wort reveals novel interactions with a number of GPCRs. *Psychopharmacology (Berl)* 2002;162: 193-202.
4. Erdelmeier CA. Hyperforin, possibly the major non-nitrogenous secondary metabolite of *Hypericum perforatum* L. *Pharmacopsychiatry* 1998; 31 Suppl 1: 2-6.
5. Laakmann G, Schule C, Baghai T, Kieser M. St. John's wort in mild to moderate depression: the relevance of hyperforin for the clinical efficacy. *Pharmacopsychiatry* 1998; 31 Suppl 1: 54-59.
6. Cervo L, Rozio M, Ekalle-Soppo CB, Guiso G, Morazzoni P, Caccia S. Role of hyperforin in the antidepressant-like activity of *Hypericum perforatum* extracts. *Psychopharmacology (Berl)*. 2002; 164: 423-428.
7. Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL, Kliewer SA. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *PNAS* 2000, 97:7500-7502.
8. Ioannides C. Pharmacokinetic interactions between herbal remedies and medicinal drugs. *Xenobiotica* 2002; 32: 451-478.
9. Zhou S, Lim LY, Chowbay B. Herbal modulation of P-glycoprotein. *Drug Metab Rev* 2004; 36:57-104.
10. Caccia S. Antidepressant-like components of *Hypericum perforatum* extracts: an overview of their pharmacokinetics and metabolism. *Curr Drug Met* 2005; 6: 531-543
11. Bombardelli E, Morazzoni P, Riva A. IDN 5491 (Hyperforin trimethoxybenzoate): A new antidepressive drug. *Eur. Neuropsychopharmacol.* 2002; 12 Suppl. 3: S240.

12. Cervo L, Mennini T, Rozio M, et al. Potential antidepressant properties of IDN 5491 (hyperforin-trimethoxybenzoate), a semisynthetic ester of hyperforin. *Eur Neuropsychopharmacol* 2005; 15: 211-218
13. International Publication Number: WO 03/091194, International Application Number: PCT/EP03/04100, International Publication Date: 6 November 2003.
14. Cantoni L, Rozio M, Mangolini A, Hauri L, Caccia S. Hyperforin contributes to the hepatic CYP3A-inducing effect of *Hypericum perforatum* extract in the mouse. *Toxicol Sci* 2003; 75: 25-30.
15. Rozio M, Fracasso C, Riva A, Morazzoni P, Caccia S. High-performance liquid chromatography measurement of hyperforin and its reduced derivatives in rodent plasma. *J Chromatogr B* 2005; 816: 21-27