Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Phytochemistry 67 (2006) 2550–2560
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Hyperforin and its analogues inhibit CYP3A4 enzyme activity

Ju-young Lee [a], Rujee K. Duke [b], Van H. Tran [a], James M. Hook [c], Colin C. Duke [a,*]

[a] Pharmaceutical Chemistry, Faculty of Pharmacy, University of Sydney, Building A15, Science Road, Sydney, NSW 2006, Australia
[b] Department of Pharmacology, University of Sydney, NSW, Australia
[c] NMR Facility, School of Chemistry, University of New South Wales, NSW, Australia

Received 11 July 2006; received in revised form 13 September 2006

Abstract

Literature indicates that herb–drug interaction of St. John's wort is largely due to increased metabolism of the co-administered drugs that are the substrates of cytochrome P450 (CYP) 3A4 enzyme, alteration of the activity and/or expression of the enzyme. The major St. John's wort constituents, acylphloroglucinols, were evaluated for their effects on CYP3A4 enzyme activity to investigate their roles in herb–drug interaction. Hyperforin and four oxidized analogues were isolated from the plant and fully characterized by mass spectral and NMR analysis. These acylphloroglucinols inhibited activity of CYP3A4 enzyme potently in the fluorometric assay using the recombinant enzyme. Furoadhyperforin (IC_{50} 0.072 μM) was found to be the most potent inhibitor of CYP3A4 enzyme activity, followed by furohyperforin isomer 1 (IC_{50} 0.079 μM), furohyperforin isomer 2 (IC_{50} 0.23 μM), hyperforin (IC_{50} 0.63 μM) and furohyperforin (IC_{50} 1.3 μM). As the acylphloroglucinols are potent inhibitors of the CYP3A4 enzyme, their modulation of the enzyme activity is unlikely to be involved in increased drug metabolism by St. John's wort.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: St. John's wort; Acylphloroglucinols; Hyperforin; Furohyperforin; Furoadhyperforin; NMR; MS and CYP3A4 enzyme

1. Introduction

St. John's wort preparation is the most widely used herbal medicine for the treatment of mild-to-moderate depression (Josey and Tackett, 1999). The preparation has become an alternative to synthetic antidepressants because of comparable clinical efficacy while lacking major side effects (Schrader, 2000; Woelk, 2000; Lecrubier et al., 2002; Schulz, 2006; Lawvere and Mahoney, 2005). In 2001, St. John's wort was the leading treatment for depression, outselling fluoxetine (Prozac®) by a factor of four in Germany (Di Carlo et al., 2001). Since reports of adverse herb–drug interaction, the sale of the St. John's wort preparations has dropped sharply. In USA, sales peaked at $300 million in 1998 and decreased to $9 million in 2004–2005 (Blumenthal, 2005).

St. John's wort preparation is an over-the-counter product for the treatment of mild-to-moderate depression. However, multiple cases of interactions with conventional medications have limited the safe use of St. John's wort (Ernst, 1999). St. John's wort has been shown to lower the blood concentrations of a large number of concomitant medications including cyclosporine, indinavir and oral contraceptives (Breidenbach et al., 2000; Mai et al., 2000; Piscitelli et al., 2000; Hall et al., 2003).

CYP3A4 enzyme metabolises the majority of the drugs used clinically (∼60%) and therefore is frequently implicated in herb–drug interactions either by alteration of the activity and/or expression of the enzyme (Guengerich, 1997). Evidence in the literature indicates that clinical herb–drug interaction of St. John's wort is mainly due to induction of CYP3A4 enzyme (Mueller et al., 2006; Markowitz et al., 2003; Wang et al., 2001; Roby et al., 2000; Mannel, 2004).

St. John's wort preparations consist of the leaves and flowering tops of *Hypericum perforatum* L. The prepara-

* Corresponding author. Tel.: +61 2 9351 2321; fax: +61 2 9351 4391.
E-mail address: colind@pharm.usyd.edu.au (C.C. Duke).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.09.018

tion contains several classes of compounds including naphthodianthrones (hypericin, pseudohypericin), acylphloroglucinols (hyperforin, adhyperforin), flavonols (quercetin, kaempferol), flavonol glycosides (quercetrin, rutin), biflavones (amentoflavone, biapigenin), and xanthones (1,3,6,7-tetrahydroxy-xanthone) (Nahrstedt and Butterweck, 1997). Hypericin, flavonoids and hyperforin have been suggested as the constituents contributing to the antidepressant activity of St. John's wort (Cott, 1997; Butterweck et al., 2003; Calapai et al., 2001; Müller, 2003).

Hyperforin was shown to potently block serotonin reuptake into the presynaptic nerve endings in rat synaptosomal preparations with an IC_{50} value of 0.2 μM (Müller, 2003; Chatterjee et al., 1998; Keller et al., 2003), whereas the oxidized hyperforin analogues were much less potent (IC_{50} values >20 μM) (Verotta et al., 2002; Verotta et al., 2000). These findings suggest that hyperforin has higher efficacy as an antidepressant than the oxidized analogues.

Hyperforin inhibited the activity of CYP3A4 enzyme with an IC_{50} of 2.3 μM in the testosterone 6β-hydroxylase assay (Obach, 2000). However, little is known about its oxidized analogues in the modulation of CYP3A4 enzyme activity. In this study, hyperforin and its oxidized analogues (Fig. 1) were isolated and fully characterized by mass spectra and NMR (600 MHz) and examined for the modulation of CYP3A4 enzyme activity.

2. Results and discussion

2.1. Characterization of hyperforins

Hyperforin and its analogues were characterized based on their mass spectral, ^1H and ^{13}C NMR data. Homonuclear and heteronuclear 2D-NMR including COSY, TOCSY, ^1H–^{13}C HSQC and ^1H–^{13}C HMBC were also employed to assign the chemical shifts of these compounds. The NMR and mass spectra of the isolated hyperforin and its analogue furohyperforin were consistent with the published data (Cui et al., 2004; Orth et al., 1999; Verotta et al., 1999; Wang et al., 2004; Fuzzati et al., 2001). The spectra of other hyperforin analogues including furoadhyperforin, furohyperforin isomer 1 and furohyperforin isomer 2 exhibited resonances consistent with the published partial NMR spectra (Vugdelija et al., 2000; Wolfender et al., 2003).

2.2. Mass spectra of hyperforin and its oxidized analogues

The electrospray ionization (ESI) spectrum of hyperforin (Table 1) showed a protonated molecular ion at *m/z* 537 and a base peak at *m/z* 505 corresponding to a loss of dimethylketene [O=C=C(CH_3)$_2$] from the K^+ adduct of hyperforin. Hyperforin exhibited moderate intensity ions at *m/z* 491 and 469 corresponding to the loss of an isoprene unit (2-methyl-1,3-butadiene, −68 amu) from $[M+Na]^+$ and $[M+H]^+$, respectively, and *m/z* 477 and 411 corresponding to the loss of 2-methyl-2,4-pentadiene (−82 amu) from $[M+Na]^+$ and the loss of isobutene plus dimethylketene (−126 amu) from $[M+H]^+$, respectively. The ion at *m/z* 413 which corresponded to a loss of an isobutene unit from *m/z* 469 was also observed.

Furohyperforin gave a much simpler ESI spectrum than its parent compound hyperforin. Furohyperforin exhibited the protonated molecular ion at *m/z* 553 as the base peak and a weak peak at *m/z* 535 corresponding to the loss of H_2O from $[M+H]^+$as well as two moderate intensity ions at *m/z* 349 $[M+H-204]^+$ and *m/z* 293 $[M+H-204-56]^+$. The ion at *m/z* 349 corresponded to the elimination of the olefin $C_{15}H_{24}$ from the cyclohexanone part of the bicyclic structure (Fuzzati et al., 2001) from protonation at the C-9 carbonyl, and *m/z* 293 a loss of isobutene from the isoprene unit at C-8. $[M+H-204]^+$ and $[M+H-204-56]^+$ appear to be characteristic of oxidized hyperforin with a free C-8 isoprene unit and C-9 carbonyl including furohyperforin (*m/z* 349 and 293) and furoadhyperforin (*m/z* 363 and 307).

The spectra of furohyperforin isomers 1 and 2 were similar. Both showed a protonated molecular ion at *m/z* 553 as the base peak and a prominent Na^+ adduct at *m/z* 575, and moderate intensity ions at *m/z* 485 and 465 corresponding to the respective loss of isoprene (−68 amu) and dimethylketene plus H_2O (−88 amu) from $[M+H]^+$. The only significant difference in the spectra of the two isomers was the intensity of the peak at *m/z* 485 which was approximately doubled in isomer 2. In contrast, the spectrum of furoadhyperforin was distinctly different from the other furohyperforins in this study exhibiting Na^+ adduct ion at *m/z* 589 as the base peak and a moderate intensity protonated molecular ion at *m/z* 567. The spectrum also showed a weak K^+ adduct at *m/z* 605 and a weak ion at *m/z* 521 corresponding to the loss of the isoprene unit from $[M+Na]^+$.

2.3. NMR of hyperforin and its oxidized analogues

Hyperforin and its homologue adhyperforin exhibited broad peaks in the ^1H NMR spectra and poor resolution of ^{13}C NMR signals (Verotta, 2003; Maisenbacher and Kovar, 1992) due to slow equilibration between the tautomers of the β-dicarbonyl system. The β-dicarbonyl moiety, which is stabilized in MeOH (Cui et al., 2004), is also responsible for the reactivity of hyperforin and adhyperforin. We also found that the ^1H NMR signals of hyperforin were sharper in CD_3OD than in the other solvents and the ^{13}C NMR had to be carried out in CD_3OD to avoid decomposition. Hyperforin analogues lacking the β-dicarbonyl moiety have greater stability than hyperforin, consistent with the findings of others (Verotta et al., 2002; Verotta et al., 1999; Vugdelija et al., 2000; Wolfender et al., 2003). The analogues exhibited better resolution of spectra in $CDCl_3$ and did not require stabilization by a protic solvent such as CD_3OD. These observations strongly indicate that the β-dicarbonyl system in

Download English Version:

https://daneshyari.com/en/article/5167517

Download Persian Version:

https://daneshyari.com/article/5167517

Daneshyari.com