

Investigation of Pharmacokinetic Data of Hypericin, Pseudohypericin, Hyperforin and the Flavonoids Quercetin and Isorhamnetin Revealed from Single and Multiple Oral Dose Studies with a Hypericum Extract Containing Tablet in Healthy Male Volunteers

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Summary

Hypericins, hyperforin and flavonoids are discussed as the main components contributing to the antidepressant action of St. John's wort (*Hypericum perforatum*). Therefore, the objective of the two open phase I clinical trials was to obtain pharmacokinetic data of these constituents from a hypericum extract containing tablet: hypericin, pseudohypericin, hyperforin, the flavonoid aglycone quercetin, and its methylated form isorhamnetin. Each trial included 18 healthy male volunteers who received the test preparation, containing 900 mg dry extract of St John's wort (STW 3-VI, Laif[®] 900), either as a single oral dose or as a multiple once daily dose over a period of 14 days. Concentration/time curves were determined for the five constituents, for 48 h after single dosing and for 24 h on day 14 at the end of 2 weeks of continuous daily dosing. After single dose intake, the key pharmacokinetic parameters were determined as follows: Hypericin: Area under the curve ($AUC_{(0-\infty)}$) = 78.33 h · ng/ml, maximum plasma concentration (C_{max}) = 3.8 ng/ml, time to reach C_{max} (t_{max}) = 7.9 h, and elimina-

tion half-life ($t_{1/2}$) = 18.71 h; pseudohypericin: $AUC_{(0-\infty)}$ = 97.28 h · ng/ml, C_{max} = 10.2 ng/ml, t_{max} = 2.7 h, $t_{1/2}$ = 17.19 h; hyperforin: $AUC_{(0-\infty)}$ = 1550.4 h · ng/ml, C_{max} = 122.0 ng/ml, t_{max} = 4.5 h, $t_{1/2}$ = 17.47 h. Quercetin and isorhamnetin showed two peaks of maximum plasma concentration separated by about 3–3.5 h. Quercetin: $AUC_{(0-\infty)}$ = 417.38 h · ng/ml, C_{max} (1) = 89.5 ng/ml, t_{max} (1) = 1.0 h, C_{max} (2) = 79.1 ng/ml, t_{max} (2) = 4.4 h, $t_{1/2}$ = 2.6 h; isorhamnetin: $AUC_{(0-\infty)}$ = 155.72 h · ng/ml, C_{max} (1) = 12.5 ng/ml, t_{max} (1) = 1.4 h, C_{max} (2) = 14.6 ng/ml, t_{max} (2) = 4.5 h, $t_{1/2}$ = 5.61 h. Under steady state conditions reached during multiple dose administration similar results were obtained. Further pharmacokinetic characteristics calculated from the obtained data were the mean residence time (MRT), the lag-time, the peak-trough fluctuation (PTF), the lowest observed plasma concentration (C_{min}), and the average plasma concentration (C_{av}). The data obtained for the five constituents generally corresponded well with values previously published. The trial preparation was well tolerated.

Key words

- Antidepressant
- Flavonoids
- Hyperforin
- Hypericin
- *Hypericum perforatum*
- Laif[®] 900
- Pseudohypericin
- STW 3-VI, pharmacokinetics

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Zusammenfassung

Ermittlung pharmakokinetischer Daten von Hypericin, Pseudohypericin, Hyperforin und den Flavonoiden Quercetin und Isorhamnetin nach oralen Einmal- und Mehrfachdosis-Studien mit einer Hypericum-Extrakt enthaltenden Tablette an gesunden männlichen Versuchspersonen

Hypericine, Hyperforin und Flavonoide werden als die Hauptkomponenten der antidepressiven Wirkung von Johanniskraut (*Hypericum perforatum*) diskutiert. Deshalb war das Ziel der beiden offenen klinischen Studien der Phase I, pharmakokinetische Daten dieser Komponenten einer Hypericum-Extrakt enthaltenden Tablette zu ermitteln: Hypericin, Pseudohypericin, Hyperforin, das Flavonoid-Aglykon Quercetin und dessen Methylderivat Isorhamnetin. An jeder Studie nahmen 18 gesunde männliche Probanden teil, die das Testpräparat mit 900 mg Johanniskraut-Trockenextrakt (STW 3-VI, Laif® 900) entweder als einzelne orale Dosis oder mehrfach als einmal tägliche

Gabe über einen Zeitraum von 14 Tagen erhielten. Für die fünf Komponenten wurden die Konzentrations-Zeit-Verläufe über 48 h nach der Einmalgabe sowie über 24 h am Tag 14 am Ende der zweiwöchigen kontinuierlichen täglichen Einnahme bestimmt. Nach der Einmalgabe wurden für die wichtigsten pharmakokinetischen Parameter folgende Werte bestimmt: Hypericin: Fläche unter der Kurve ($AUC_{(0-\infty)}$) = 78.33 h · ng/ml, maximale Plasmakonzentration (C_{max}) = 3.8 ng/ml, Zeit bis zum Erreichen von C_{max} (t_{max}) = 7.9 h und Eliminationshalbwertszeit ($t_{1/2}$) = 18.71 h; Pseudohypericin: $AUC_{(0-\infty)}$ = 97.28 h · ng/ml, C_{max} = 10.2 ng/ml, t_{max} = 2.7 h und $t_{1/2}$ = 17.19 h; Hyperforin: $AUC_{(0-\infty)}$ = 1550.4 h · ng/ml, C_{max} = 122.0 ng/ml, t_{max} = 4.5 h und $t_{1/2}$ = 17.47 h. Quercetin und Isorhamnetin wiesen jeweils zwei Peaks maximaler Plasmakonzentration auf, die um etwa 3 – 3.5 h voneinander getrennt auftraten. Quercetin: $AUC_{(0-\infty)}$ = 417.38 h · ng/ml, C_{max} (1) = 89.5 ng/ml, t_{max} (1) = 1.0 h, C_{max} (2) = 79.1 ng/ml, t_{max} (2) = 4.4 h und

$t_{1/2}$ = 2.6 h; Isorhamnetin: $AUC_{(0-\infty)}$ = 155.72 h · ng/ml, C_{max} (1) = 12.5 ng/ml, t_{max} (1) = 1.4 h, C_{max} (2) = 14.6 ng/ml, t_{max} (2) = 4.5 h und $t_{1/2}$ = 5.61 h. Unter Steady-State-Bedingungen nach wiederholter Verabreichung wurden ähnliche Ergebnisse erzielt. Weitere pharmakokinetische Kenngrößen, die aus den ermittelten Daten errechnet wurden, waren die mittlere Verweildauer (MRT), die Verzögerungszeit, die Peak-Trough-Fluktuation (PTF), die minimale Plasmakonzentration (C_{min}) und die durchschnittliche Plasmakonzentration (C_{av}). Die für die fünf Komponenten ermittelten Werte stimmten im allgemeinen gut mit den bisher publizierten überein. Das Testpräparat wies eine gute Verträglichkeit auf.

1. Introduction

St. John's Wort (*Hypericum perforatum*) has a long history as a medicinal herb for diverse symptoms. Today the most prominent are its antidepressant properties. In this indication the clinical efficacy of St. John's Wort in comparison to placebo and standard synthetic antidepressants has been confirmed in many clinical trials, conducted in compliance with modern GCP standards [1, 2]. Besides the phototoxic effects mainly known from grazing animals after excessive ingestion, St. John's wort can generally be regarded as a safe treatment option to standard synthetic antidepressants. However, like many other medicines and also some foods, St. John's wort can alter the metabolism of concomitantly administered drugs because of its influence on the cytochrome P450 system, particularly the CYP 3A4 isoenzyme [3, 4].

Today, medications with extracts of St. John's Wort are produced by many pharmaceutical companies, mostly with standardized formulations, differing primarily in the extract solution, drug-extract ratio and the amount of the extract per tablet. All these extracts contain a great number of constituents, i.e. hypericin, pseudohypericin, hyperforin and flavonoids discussed as the main constituents of the antidepressive action. According to the European Pharmacopoeia the naphthodianthrone hypericin – together with its 2'-hydroxymethyl derivative pseudohypericin – is regarded as the main substance, for which standardization of extracts is

required [5]. However, in the meantime there are indications that the phloroglucinol derivative hyperforin, structurally related to the compounds humulon and lupulon from hops, as well show antidepressant activities [6, 7]. In the past few years the hypothesis, hyperforin is too unstable to significantly contribute to the pharmacological action of hypericum extracts, could be rejected because of the detection of intact hyperforin in human blood for as long as 72 h after drug intake. In vitro and animal tests have indeed shown some antidepressant activities of hyperforin. In the last years, such activities have also been detected for the flavonoid drug ingredients [6, 8], the most prominent of which are the quercetin glycosides hyperoside, quercitrin, isoquercitrin and miquelianin.

Pharmacokinetic investigations in humans have been published for hypericin and pseudohypericin from several clinical trials, both after single drug intake of different dosages and after continuous administration three times a day [9–13] or once a day [14] over periods of 1–2 weeks. Pharmacokinetic data in humans for hyperforin have been reported from two studies, investigating both single and multiple drug administration after a once daily dosing over 1–2 weeks [14, 15]. Till now, pharmacokinetic data for flavonoids have been published once for hypericum extracts [14], but data for the pharmacokinetics of quercetin and its glycosides are complex and contradictory [16].

Although pharmacokinetic data of the important constituents of different extracts of St. John's wort showed comparable results, there is still the possibility that different formulations could result in different pharmacokinetic and bioavailability data. Therefore, the present two phase I clinical trials have been performed to investigate oral bioavailability of five constituents of a hypericum extract STW 3-VI containing tablet and to obtain basic pharmacokinetic data for hypericin, pseudohypericin, hyperforin and the flavonoid aglycone quercetin and its methylated form isorhamnetin after single dose administration and after multiple once daily intake on 14 continuous days.

2. Subjects, materials and methods

2.1. General

The two open phase I clinical trials were conducted at LAFAA Laboratory for Contract Research in Clinical Pharmacology and Biopharmaceutical Analytics GmbH, Bad Schwartau (Germany), in Juli/August 2002 (single dose) and Oktober/November 2002 (multiple dose), respectively. Trial medication was STW 3-VI¹⁾, 1 tablet containing 900 mg dry extract of St John's Wort (*Hypericum perforatum*), drug extract ratio 3–6:1, extraction solvent 80 % (v/v) ethanol. Each tablet (lot no. 010502 KP) contains about 1.74 mg total hypericin (hypericin:pseudohypericin \approx 1:2), 17.2 mg hyperforin, and 97.2 mg flavonoids. Both trials were performed in compliance with the European recommendations of Good Clinical Practice guidelines, ICH-Guidelines, the declaration of Helsinki, national regulatory requirements and approved by a local ethics committee. In both trials, the pharmacokinetic characteristics for hypericin, pseudohypericin, hyperforin, quercetin and isorhamnetin were calculated by means of the biostatistics program BIOQ V3 (Byk Gulden Pharmaceuticals, Konstanz, Germany).

2.2. Subjects

Each trial recruited 18 subjects from a pool of male volunteers of Caucasian origin and in the age range 18–45 years, who were assessed as healthy based on physical examination, medical history, and clinical laboratory tests, who were of normal weight (BMI 20–25 kg/m², not exceeding 90 kg), who had not been smoking within at least 1 month prior to the study, and who gave their written informed consent. Exclusion criteria were hypersensitivity against St John's Wort, all serious and acute diseases, chronic alcohol or drug abuse, HIV or hepatitis infection, surgery of the gastrointestinal tract, chronic medication within 4 weeks before the study, or any other medication within 10 days before the study. Participants had to refrain from the consumption of grapefruits, grapefruit juice, St John's Wort tea, methylxanthine containing beverages and food, and alcohol within 48 h prior to and during the study period.

¹⁾ Laif® 900; manufacturer: Steigerwald Arzneimittelwerk GmbH, Darmstadt (Germany).

2.3. Study protocol

2.3.1. Single dose study

Subjects were hospitalized from 8:30 p.m. on the day before study day 1 until up to 48 h post drug administration. Fasting conditions were required from 9:00 p.m. on the day before study begin until 12:00 a.m. on study day 1. The trial medication of 1 tablet containing 900 mg *Hypericum* extract was taken with 200 ml tap water at 7:00 a.m. on study day 1. Blood samples (2 × 5.6 ml) were taken pre-dose and at the following intervals post drug administration: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, and 48 h. After centrifugation, the plasma samples were kept frozen (–20 °C) until analysis for hypericin/pseudohypericin, hyperforin and quercetin/isorhamnetin. Primary pharmacokinetic characteristic to be calculated from plasma concentration/time data was AUC_(0-∞) (total area under the plasma concentration/time curve). Secondary pharmacokinetic characteristics were: AUC_(0-tz) (area under the concentration/time curve from zero to the last time point), C_{max} (maximum plasma concentration), t_{max} (time to reach maximum plasma concentration), t_{1/2} (terminal elimination half-life), MRT (mean residence time), and lag-time (time to attain the first measurable plasma concentration). Tolerability and safety of the trial medication were assessed by monitoring adverse events, vital signs, ECG parameters, clinical laboratory examinations, and urine status.

2.3.2. Multiple dose study

Subjects were ambulatory during days 1 through 13 and advised to take 1 tablet of the trial medication at 7:00 a.m. after breakfast every day. During this period, blood samples were obtained on days 1, 4, 11, 12, and 13 before drug intake in the morning. For profiling, the subjects were hospitalized from 8:30 p.m. on day 13 until up to 24 h following the last drug administration at 7:00 a.m. on study day 14. Fasting conditions were applied from 9:00 p.m. on day 13 until 12:00 a.m. on day 14. Blood samples (2 × 5.6 ml) were taken predose and after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 10, 12, 16, 20, and 24 h. The plasma samples were stored deep-frozen as described for the single dose study. Primary pharmacokinetic characteristic was AUC₍₀₋₂₄₎ (area under the concentration/time curve during the profiling day 14). Secondary characteristics were PTF (peak-trough fluctuation), C_{min} (lowest observed plasma concentration), C_{max} (highest observed plasma concentration), C_{av} (average plasma concentration), and t_{max} (time to reach maximum plasma concentration). Tolerability and safety were monitored as described for the single dose study.

2.4. Principles of the analytical methods

2.4.1. Hypericin/pseudohypericin

Hypericin and its 2'-hydroxymethyl derivative pseudohypericin were determined simultaneously. Quantification was achieved after repeated extractions of a mixture consisting of plasma (300 µl of the unknown sample, calibration standard or quality control sample), dimethylsulfoxide, acetonitrile, 2-butoxyethanol and dansylamide as internal standard. The repeated extractions were performed with ethyl acetate at 37 °C for 15 min each. The evaporated extracts were reconstituted in a methanol/tetrahydrofuran/phosphate buffer solution (pH 4). Chromatographic separation and detection was carried out by high-performance liquid chromatography using a reversed-phase system and fluorescence detection with excitation at 315 nm and emission at 598 nm.

Table 1: Demographic characteristics of study participants.

	Single dose study	Multiple dose study
N (gender)	18 (male)	18 (male)
Median age, years (range)	28 (21–41)	31 (21–41)
Median weight, kg (range)	75 (65–88)	75 (66–88)
Median height, cm (range)	180 (172–189)	179 (172–190)

For hypericin and pseudohypericin the intra-batch and the inter-batch precision and accuracy of the method did not exceed the limits accepted by the FDA [17] (precision: CV \leq 15 %, at LOQ: CV \leq 20 %; accuracy \leq \pm 15 %, at LOQ: accuracy \leq \pm 20 %).

2.4.2. Hyperforin

Hyperforin was analyzed after extraction of the plasma (1000 μ l of the unknown sample, calibration standard or quality control sample) with an ethyl acetate/isooctane/acetonitrile mixture. The evaporated extracts were reconstituted in an acetonitrile/water/methanol mixture. Chromatographic separation and quantification was achieved by HPLC using a reversed phase system, UV-detection at 273 nm and external standardization. All operations must be carried out under protection from direct light.

The intra-batch and the inter-batch precision was characterized by coefficients of variation never exceeding 4.6 %. The intra-batch and the inter-batch accuracy did not exceed \pm 7.3 %.

2.4.3. Quercetin/isorhamnetin

Quercetin and its methylated derivative isorhamnetin were analyzed simultaneously after enzymatic hydrolysis of the plasma (300 μ l of the unknown sample, calibration standard or quality control sample) with purified β -glucuronidase solution for 2 h at 37 °C. Naringenin was used as internal standard. Borate buf-

fered (pH 8.1) hydrolysed samples were extracted with ethyl acetate. The evaporated extracts were reconstituted in acidified acetonitrile/methanol. High-performance liquid chromatographic separation worked with a reversed phase system and UV-detection at 360 nm.

For quercetin and isorhamnetin the intra-batch and the inter-batch precision and accuracy of the method did not exceed the limits accepted by the FDA [17] (precision: CV \leq 15 %, at LOQ: CV \leq 20 %; accuracy \leq \pm 15 %, at LOQ: accuracy \leq \pm 20 %).

3. Results

Demographic data of the trial participants are summarized in Table 1. All subjects completed the study according to the protocol. The concentration/time curves for hypericin, pseudohypericin, hyperforin, quercetin and isorhamnetin are shown in Fig. 1A (single dose) and Fig. 1B (multiple dose). The pharmacokinetic data obtained after single and multiple dosing are summarized in Tables 2 and 3, respectively.

3.1. Hypericin

After single dose administration, the mean value curve of plasma hypericin concentrations was characterized by a very slow increase followed by a slow decline. Of all investigated compounds, hypericin showed the lowest mean value of C_{max} (3.8 ng/ml) and mean AUC_(0- ∞) value (78.33 h · ng/ml), but the longest for mean t_{max} (7.9 h), mean lag-time (1.92 h), mean $t_{1/2}$ (18.71 h) and MRT (28.67 h).

In the multiple dosing study, trough levels were determined on study days 1, 4, 11, 12, 13, and 14 in the morning prior to drug intake. The results showed that

Table 2: Pharmacokinetic parameters after single dose administration. Values are given as means \pm SD (range).

	Hypericin	Pseudohypericin	Hyperforin	Quercetin	Isorhamnetin
AUC _(0-∞) [h · ng/ml]	78.33 \pm 28.09 (30.42–120.13)	97.28 \pm 39.69 (34.95–204.81)	1550.4 \pm 370.5 (816.8–2230.5)	417.38 \pm 254.06 (34.49–1168.30)	155.72 \pm 133.37 (10.31–421.65)
AUC _(0-tz) [h · ng/ml]	62.71 \pm 22.27 (27.94–99.01)	84.34 \pm 35.12 (32.17–184.30)	1339.1 \pm 328.9 (680.1–1887.8)	393.75 \pm 243.73 (27.57–1100.73)	124.89 \pm 118.45 (6.89–385.96)
C_{max} [ng/ml]	3.8 \pm 1.4 (1.7–6.3)	10.2 \pm 3.9 (3.5–16.6)	122.0 \pm 45.5 (49.8–237.6)	1) 89.5 \pm 78.3 (24.6–354.4) 2) 79.1 \pm 38.6 (16.1–155.0)	1) 12.5 \pm 7.5 (4.5–33.0) 2) 14.6 \pm 10.0 (3.8–42.9)
t_{max} [h]	7.9 \pm 1.3 (5.0–10.0)	2.7 \pm 0.7 (1.5–4.0)	4.5 \pm 1.2 (3.0–8.0)	1) 1.0 \pm 0.4 (0.5–2.0) 2) 4.4 \pm 1.1 (2.5–6.0)	1) 1.4 \pm 0.5 (1.0–2.5) 2) 4.5 \pm 1.5 (2.0–8.0)
$t_{1/2}$ [h]	18.71 \pm 4.76 (11.71–27.54)	17.19 \pm 8.44 (4.92–38.44)	17.47 \pm 4.53 (9.97–27.58)	2.60 \pm 1.64 (0.79–7.04)	5.61 \pm 8.14 (0.91–37.41)
MRT [h]	28.67 \pm 6.25 (18.17–41.00)	20.21 \pm 8.50 (8.94–44.47)	20.88 \pm 6.25 (13.71–39.32)	4.68 \pm 1.43 (1.77–7.13)	10.29 \pm 12.08 (2.26–56.86)
lag-time [h]	1.92 \pm 0.39 (1.50–3.00)	0.58 \pm 0.12 (0.50–0.75)	1.32 \pm 0.51 (0.75–2.50)	0.54 \pm 0.18 (0.25–1.00)	0.71 \pm 0.17 (0.50–1.00)

1) first maximum, 2) second maximum.

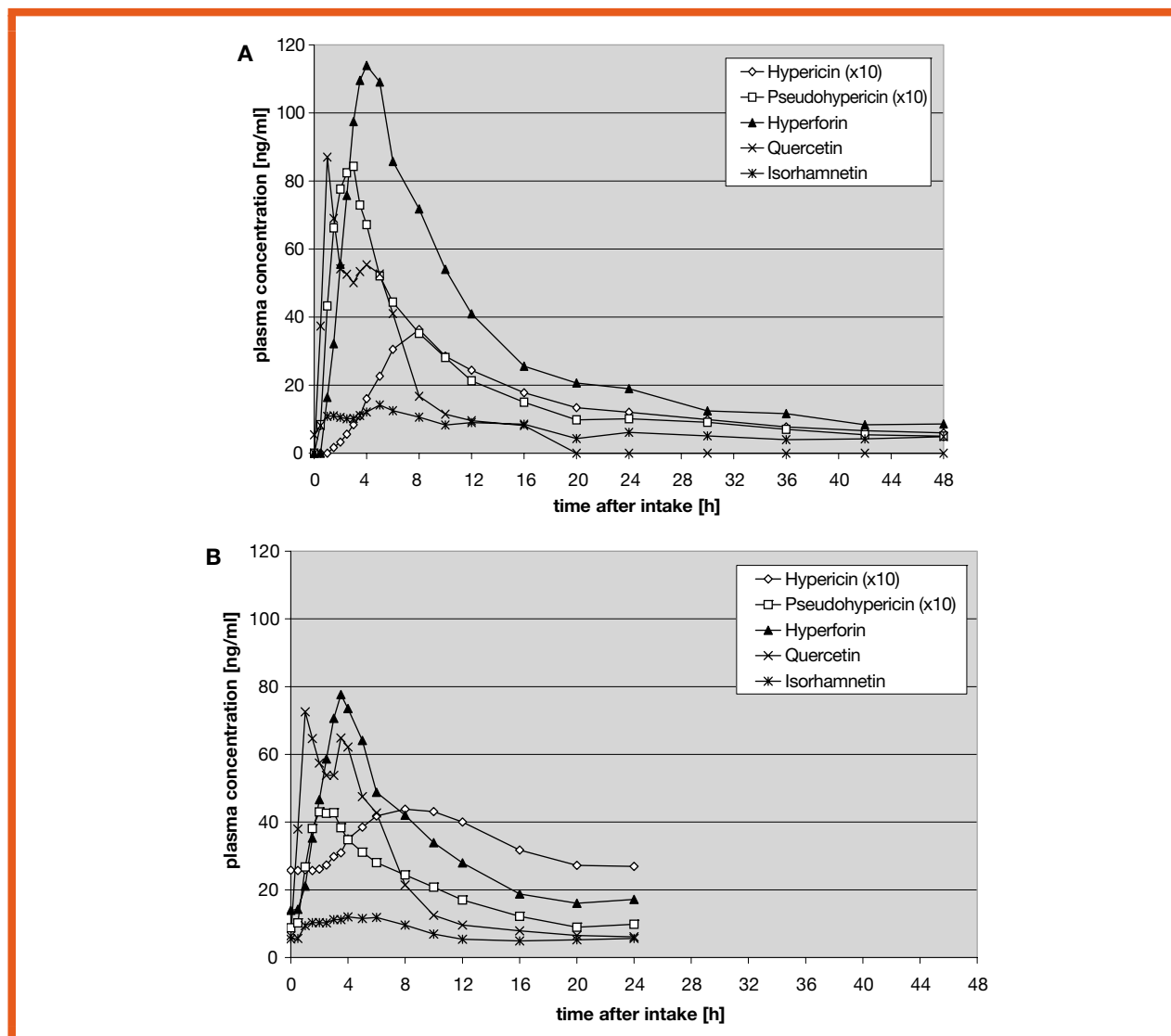


Fig. 1: Pharmacokinetics of constituents of *Hypericum* extract in plasma after intake of *Hypericum* extract STW 3-VI once daily. (A) After single dosing, (B) after multiple dosing (14 days). Data points are expressed as arithmetic means. Note that concentrations of hypericin and pseudohypericin are multiplied by 10.

Table 3: Pharmacokinetic parameters after multiple dose administration obtained on day 14. Values are given as means \pm SD (range).

	Hypericin	Pseudohypericin	Hyperforin	Quercetin	Isorhamnetin
AUC ₍₀₋₂₄₎ [h · ng/ml]	81.41 \pm 18.56 (52.77–120.87)	45.94 \pm 21.95 (16.40–91.16)	769.4 \pm 234.6 (398.6–1233.6)	459.43 \pm 201.64 (160.60–873.88)	120.72 \pm 94.32 (35.80–429.00)
PTF [%]	72.27 \pm 25.07 (41.92–120.69)	247.01 \pm 70.64 (103.91–367.10)	229.9 \pm 61.8 (128.9–338.9)	n.a.	n.a.
C _{min} [ng/ml]	2.33 \pm 0.65 (1.27–3.70)	0.77 \pm 0.48 (0.30–2.05)	12.6 \pm 4.6 (7.2–21.8)	n.a.	n.a.
C _{max} [ng/ml]	4.79 \pm 1.20 (3.23–7.41)	5.22 \pm 2.90 (2.23–14.53)	87.1 \pm 36.5 (38.7–190.5)	1) 77.8 \pm 34.1 (41.0–167.3) 2) 89.2 \pm 43.9 (28.2–181.3)	1) 12.5 \pm 6.0 (4.7–28.1) 2) 17.1 \pm 9.4 (6.6–44.1)
C _{av} [ng/ml]	3.39 \pm 0.77 (2.20–5.04)	1.91 \pm 0.91 (0.68–3.80)	32.1 \pm 9.8 (16.6–51.4)	19.1 \pm 8.4 (6.7–36.4)	5.0 \pm 3.9 (1.5–17.9)
t _{max} [h]	7.3 \pm 1.7 (5.0–12.0)	2.5 \pm 0.9 (1.5–5.5)	3.9 \pm 1.3 (1.5–7.0)	1) 1.25 \pm 0.35 (1.00–2.00) 2) 4.67 \pm 1.49 (2.50–7.50)	1) 1.66 \pm 0.54 (1.00–3.00) 2) 5.10 \pm 1.40 (3.50–7.50)

n.a.: not available. 1) first maximum, 2) second maximum.

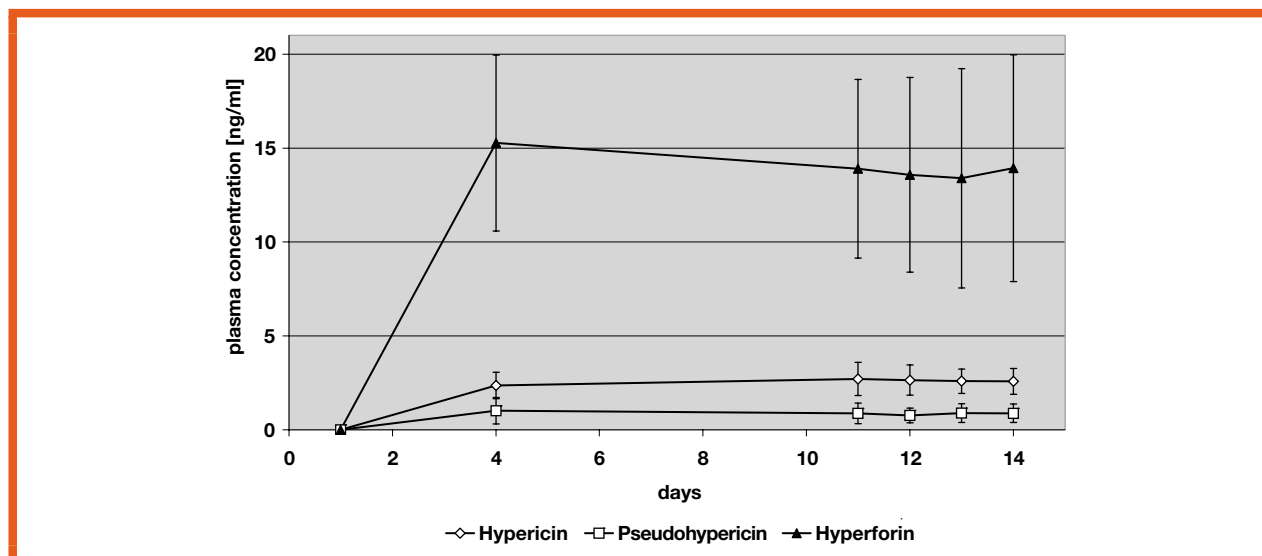


Fig. 2: Trough plasma concentrations of the hypericins and hyperforin during 14 days of multiple dosing. Data points are expressed as arithmetic mean (\pm SD).

steady state was reached before the profiling day 14 (Fig. 2). The mean plasma concentration/time curve closely resembled the one after single dose administration, as did the pharmacokinetic data. The mean maximum plasma concentration C_{\max} was increased (4.79 ng/ml) but still the lowest of all. Although C_{\max} is lower compared to pseudohypericin, C_{\min} value of 2.33 ng/ml and C_{av} value of 3.39 ng/ml are higher. Hypericin, again, had the longest mean t_{\max} value of all substances (7.3 h) which was nevertheless shorter than after single dose intake. Hypericin also showed the lowest mean PTF (72.27%). The mean $AUC_{(0-24\text{ h})}$ value of 81.41 h · ng/ml was very similar to the mean $AUC_{(0-\infty)}$ value in the single dose study.

3.2. Pseudohypericin

After single dose intake, the mean concentration/time curve of pseudohypericin was characterized by a rapid increase followed by an exponential decline. The mean C_{\max} of 10.2 ng/ml was reached after mean t_{\max} of 2.7 h. Pseudohypericin was already measurable after half an hour (mean lag-time 0.58 h). The mean values of $t_{1/2}$ and MRT were 17.19 h and 20.21 h, respectively, and therefore shorter as for Hypericin but with a higher individual variability. Mean $AUC_{(0-\infty)}$ was determined as 97.28 h · ng/ml.

As for hypericin, in the multiple dosing study, steady state was reached before profiling day 14. Multiple dosing resulted in a similar pharmacokinetic profile with a similar mean t_{\max} of 2.5 h but a lower mean C_{\max} of 5.22 ng/ml. The mean C_{\min} value of 0.77 ng/ml and C_{av} of 1.91 ng/ml were the lowest of all substances measurable; the mean PTF (247%) the highest of all substances. The mean $AUC_{(0-24\text{ h})}$ value (45.94 h · ng/ml) was decreased compared to the mean $AUC_{(0-\infty)}$ in the single dose study.

3.3. Hyperforin

Out of all investigated compounds, in the single dose study hyperforin showed the highest mean C_{\max} value of 122.0 ng/ml and in accordance with this high value, a mean $AUC_{(0-\infty)}$ of 1550.4 h · ng/ml. The mean t_{\max} value of 4.5 h was in between the mean values observed for the hypericins, as was the mean lag-time of 1.32 h and the mean $t_{1/2} = 17.47$ h. The MRT of 20.88 h was almost similar to that for pseudohypericin.

Again in the multiple dosing study, steady state was reached before profiling day 14. The mean concentration/time curve after multiple dosing was similar to single dosing, with a mean C_{\max} of 87.1 ng/ml and a mean t_{\max} value of 3.9 h, both lower compared to the single dose study. The mean C_{\min} of 12.6 ng/ml was by far the highest of all substances, and the mean PTF was 229.9%. The mean $AUC_{(0-24\text{ h})}$ value (769.4 h · ng/ml) was below the mean $AUC_{(0-\infty)}$ in the single dose study.

3.4. Flavonoids

Following single and multiple dose administration, the flavonoid concentrations in the blood increased rapidly, to decline after a first maximum and rose again to give a second maximum. Thus, in individual concentration/time curves two C_{\max} and t_{\max} values could be observed. During multiple dosing, no trough levels could be determined for the flavonoids as the majority of the trough concentrations were below the limits of determination. In both trials, the mean C_{\max} of quercetin were about 5- to 7-fold higher than the mean C_{\max} of isorhamnetin.

3.4.1. Quercetin

Following single dose administration, the mean quercetin concentration in the blood fell continuously after

the second maximum. 20 h post dosing the most individual quercetin concentrations were below the limit of quantitation, only 1 of the 18 was measurable until 24 h p.a. The first mean C_{\max} value [C_{\max} (1) = 89.5 ng/ml] was reached very early [t_{\max} (1) = 1.0 h] and the second one [C_{\max} (2) = 79.1 ng/ml] after 4.4 h. The mean lag-time was low (0.54 h) and comparable to pseudohypericin. The mean $t_{1/2}$ value (2.6 h) and the MRT (4.68 h) were very short compared to the other constituents. The $AUC_{(0-\infty)}$ value of 417.38 h · ng/ml was considerably higher than that of the other substances, except hyperforin.

In the multiple dose study the first mean value [C_{\max} (1) = 77.8 ng/ml] was reached at t_{\max} (1) = 1.25 h, the second one [C_{\max} (2) = 89.2 ng/ml] at t_{\max} (2) = 4.67 h. In contrast to the single dosing the second mean C_{\max} value was higher as the first mean C_{\max} value, even higher compared to the hyperforin concentration. The mean $AUC_{(0-24\text{ h})}$ value of 459.43 h · ng/ml was within the same order of magnitude as the mean $AUC_{(0-\infty)}$ value in the single dose study.

3.4.2. Isorhamnetin

The shape of the mean isorhamnetin concentration/time curve shows only less distinct concentration peaks compared with the mean quercetin curve, although most individual concentration/time curves contained two distinctable peaks. The concentration level and the $AUC_{(0-\infty)}$ value (155.72 h · ng/ml) was low compared to quercetin. Comparable to quercetin most individual isorhamnetin concentrations fell below the limits of quantitation within 24 h, but after 48 h still 5 of the 18 were measurable. The first mean C_{\max} (1) = 12.5 ng/ml was reached at t_{\max} (1) = 1.4 h and the second mean C_{\max} (2) = 14.6 ng/ml at t_{\max} (2) = 4.5 h. Similar to quercetin, the mean lag-time (0.71 h) and mean $t_{1/2}$ (5.61 h) of isorhamnetin were short compared to the other substances, but mean $t_{1/2}$ and MRT = 10.29 h were higher than the respective values of quercetin.

After multiple dosing the shape of the mean concentration/time curve shows one distinctable peak, but again, most individual concentration/time curves showed two distinct peaks. Therefore, two concentration peaks – mean C_{\max} (1) = 12.5 ng/ml at mean t_{\max} (1) = 1.66 h and mean C_{\max} (2) = 17.1 ng/ml at mean t_{\max} (2) = 5.1 h – were measurable. The mean $AUC_{(0-24\text{ h})}$ value of 120.72 h · ng/ml lay within the same order of magnitude as the ones after single dosing.

3.5. Tolerability

In the single dose trial and in the multiple dose trial, no adverse events occurred. No clinically significant changes in the vital signs or the laboratory values were observed in any of the subjects.

4. Discussion

St. John's Wort contains a great number of constituents, but only a few of them – hypericins, hyperforin and flavonoids – are discussed as constituents contributing to its antidepressive properties. For the clinical efficacy of hypericum extracts the bioavailability of these constituents is of great interest. Pharmacokinetic properties of hypericins and hyperforin are well investigated with different formulations, but mostly investigated in separate studies [9–13, 15]. Till now, there exists only one publication investigating all five constituents in the same single and multiple dose study [14]. Therefore, for a good comparability, the design of the present two studies with the hypericum extract STW3-VI was equal to the previous two studies with the hypericum extract STW3 [14].

The shape of the concentration/time curves are in approximate accordance with those published earlier for the hypericins [9, 10, 11], for hyperforin [15] and for all five constituents [14].

The absorption of hypericin judged by both C_{\max} and $AUC_{(0-\infty)}$ appears to be lower in these studies compared with the earlier publications [9, 10, 11], but similar to STW3 [14]. Time course gave very similar values for lag-time and t_{\max} , whereas $t_{1/2}$ seemed to be slightly reduced. The results obtained for pseudohypericin in the present studies in general are in good agreement with previously published results. The kinetic variables of hyperforin, on the other hand, differed considerably from those reported with the hypericum extract WS 5572 [15], but were similar to those with the hypericum extract STW3 [14]. The data for $t_{1/2}$ (17.5 h), MRT (20.9 h) and t_{\max} support the results with STW3. According to these results, elimination half-life time ($t_{1/2}$) of the three constituents were in the same range between 17–19 h, thus allowing a single daily dosing. All three constituents reached steady-state conditions after multiple daily intake of one tablet.

Pharmacokinetic data of the flavonoid components confirmed with the data observed in the previous studies [14], with increased $AUC_{(0-\infty)}$ and C_{\max} values. This corresponds well to the higher flavonoid-concentration in the tablet containing STW3-VI. The shape of the concentration/time curves of quercetin and isorhamnetin were similar to those of STW3, with two peaks of maximum concentration. Interestingly, lag-time and t_{\max} (1) were similar with both preparations, but the second peak in the present studies appeared already after 4.5–5 h. Whereas in the previous studies $t_{1/2}$ and MRT were similar for quercetin and isorhamnetin, these parameters were shortened for quercetin ($t_{1/2}$: 2.6 h vs. 4.2 h, MRT: 4.7 h vs. 7.4 h) and prolonged for isorhamnetin ($t_{1/2}$: 5.6 h vs. 4.5 h, MRT: 10.3 h vs. 8.9 h). In general, these findings confirmed with concentration/time curves of quercetin published after oral intake of quercetin aglycone or rutin [16, 18]. However, the mechanism and sites of absorption of quercetin, isorhamnetin and their glycosides are still unclear [16, 18, 19]. Accord-

ing to these publications it seemed as if quercetin absorption may be influenced by the position and nature of the glycoside as well as compounds present in complex foodstuff. The fast absorption of quercetin and isorhamnetin shown in the present studies (lag time < 45 min, $t_{\max}(I) = 1.0\text{--}1.4$ h) supports that absorption of both flavonoids could take place in the upper part of the intestine, however, the second peak after 4.4–4.5 h indicates that absorption could take place in the distal part of the small intestine or the colon as well.

In conclusion, the results of the two studies with the hypericum extract STW3-VI are comparable to those obtained with other hypericum extracts, indicating similar bioavailability of the five constituents. The data demonstrate again that for hypericin, pseudohypericin and hyperforin a once daily multiple oral administration is sufficient to reach steady state conditions without accumulation of the ingredients. Over a period of 14 days medication was well tolerated by all subjects.

5. References

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