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ORIGINAL ARTICLE

Elizabeth Fox · Robert F. Murphy
Cynthia L. McCully · Peter C. Adamson

Plasma pharmacokinetics and cerebrospinal fluid penetration of hypericin in nonhuman primates

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Abstract Hypericin, a polycyclic aromatic dianthroquinone, is a natural pigment derived from the plant *Hypericum perforatum* (St John's Wort). The compound has been synthesized and shown to inhibit the growth of malignant glioma cell lines in vitro via inhibition of protein kinase C. Oral hypericin has entered clinical trials in adults with recurrent malignant glioma. *Purpose*: The present study was performed to characterize the plasma pharmacokinetics (PK) and cerebrospinal fluid (CSF) penetration of hypericin in nonhuman primates. *Methods*: Hypericin was administered as an intravenous bolus dose of 2 mg/kg ($n = 3$) or 5 mg/kg ($n = 1$). Plasma and CSF (ventricular or lumbar) were sampled prior to administration and at frequent intervals for up to 50 h after administration of the drug. Hypericin concentrations in plasma and CSF were determined using a specific reverse-phase HPLC assay. *Results*: Mean peak plasma concentration of hypericin following the 2 mg/kg dose was 142 ± 45 μM. Elimination of hypericin from plasma was biexponential, with an average alpha half-life of 2.8 ± 0.3 h and average terminal half-life of 26 ± 14 h. *Conclusions*: The 2 mg/kg dose in the nonhuman primate was sufficient to maintain plasma concentrations above 10 μM (the in vitro concentration required for growth inhibition of human glioma cell lines) for up to 12 h. No hypericin was detected in the CSF of any animal (lower limit of detection 0.1 μM); the CSF penetration is therefore less than 1%. A severe dose-limiting photosensitivity skin rash was seen at the 5 mg/kg dose level.

Key words Pharmacokinetics · CNS malignancies · Hypericin

E. Fox (✉) · R. F. Murphy · C. L. McCully · P. C. Adamson[1]
Pediatric Oncology Branch, National Cancer Institute,
Building 10, Room 13N240, 10 Center Dr.,
Bethesda, MD 20892, USA
Tel.: +1-301-4961756; Fax: +1-301-4020575

Present address:
[1]The Children's Hospital of Philadelphia, ARC 907,
34th and Civic Center Blvd., Philadelphia, PA 19104, USA

Introduction

Hypericin, 4,5,7,4′,5′,7′-hexahydroxy-2,2′-dimethyl-mesonaphthodianthrone (Fig. 1) is a natural pigment derived from the plant *Hypericum perforatum*, which is commonly called St. John's Wort. Medicinal properties have been ascribed to extracts of *Hypericum* sp. since ancient times. Recently there has been renewed interest in hypericin for its potential antidepressant, antiviral, and anticancer properties. In European studies, *Hypericum perforatum* extracts (LI160) containing hypericin and its analog pseudohypericin have been effective and well tolerated in the treatment of depression [8].

In murine models, hypericin has been found to have antiviral and antiretroviral properties. The mechanism of antiviral activity of hypericin is postulated to be secondary to inhibition of protein kinase C [12] which results in interference with viral replication and infection. Photoactivation of hypericin by visible light enhances antiviral activity in vitro [7, 9]. In phase I studies conducted by the AIDS Clinical Trials Group (ACTG 150/258), however, patients receiving hypericin experienced severe phototoxicity without significant changes in their disease status [7].

Cytotoxic effects of hypericin have been observed in vitro in glioma, epidermoid carcinoma, leukemic, and mouse mammary carcinoma cell lines. The cytotoxic effects were not dependent on visible light. Anker et al. have demonstrated a dose-dependent cytotoxic effect of hypericin on T98G human malignant glioma cells in vitro with cytotoxicity observed at concentrations ≥10 μM [2]. Based on these observations, hypericin has entered phase I clinical trials in patients with refractory CNS tumors [5].

The pharmacokinetic behavior of intravenously administered hypericin and the penetration of the agent across the blood-brain barrier have not been studied in detail. Therefore, we studied the plasma pharmacokinetics and cerebrospinal fluid (CSF) penetration of hypericin in a nonhuman primate model

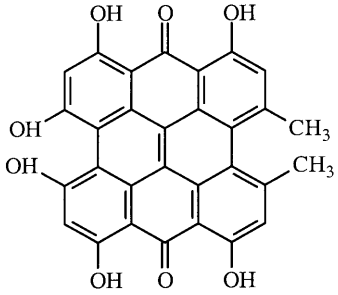


Fig. 1 Hypericin (MWT 504)

which is highly predictive of human pharmacokinetics [1, 4].

Materials and methods

Animals

Adult male rhesus monkeys (*Macaca mulatta*), ranging in weight from 8.0 to 11.6 kg, were fed Purina Monkey Chow twice daily and were group-housed in accordance with the Guide for the Care and Use of Laboratory Animals [16]. For blood sampling, catheters were surgically placed into the saphenous or femoral vein and attached to a subcutaneously implanted vascular access port (Access Technology, Skokie, Ill.). Similar access ports inserted into the jugular vein were used for drug infusion. For CSF sampling, two animals (R838A and B9884) had silicone Pudenz catheters surgically placed into the fourth ventricle and attached to a subcutaneously implanted Ommaya reservoir, as previously described [14]. In one animal (D28), lumbar CSF samples were obtained from a temporary lumbar catheter.

Drug formulation and administration

Hypericin (molecular weight 504 Da) for injection was provided by VIMRx Pharmaceuticals (Wilmington, Del.). Sterile hypericin (40 mg) was reconstituted with 20 ml 2% benzyl alcohol for a 2 mg/ml sterile solution. Further dilution with D5W or normal saline yielded a 1 mg/ml final drug concentration. Hypericin (2 mg/kg or 5 mg/kg) was administered over 10 minutes through a jugular venous port.

Chemicals

2-Butoxyethanol, dimethyl sulfoxide, sodium phosphate dibasic, and triethylamine were obtained from Sigma (St. Louis, Mo.). HPLC grade acetonitrile, methanol and water were obtained from Fisher Scientific (Fair Lawn, N.J.). Ethyl acetate and sodium phosphate monobasic were obtained from Mallinckrodt (Paris, Ky.) and JT Baker (Phillipsburg, N.J.), respectively.

Experiments

The pharmacokinetics of hypericin were studied after administration of an intravenous (i.v.) dose of 2 mg/kg (animals D28, R838A, and B9884) or 5 mg/kg (animal D28). Blood samples, which were drawn through a venous port into heparinized tubes, were obtained prior to and 10, 15, and 30 min and 1, 2, 3, 4, 6, 8, 10, 24, 26, 48, and 50 h after drug administration. Plasma was immediately separated by centrifugation. CSF samples were collected prior to and 1, 2, 3, 4, 6, 8, 10, 24, and 48 h after drug administration. A lumbar catheter was used for CSF collection in animal D28, and Ommaya reservoirs were utilized for animals R838A and B9884. All samples were stored at –70 °C until assayed.

Sample analysis

The concentration of hypericin was determined by a previously described reversed-phase high-performance liquid chromatography (HPLC) assay with modification [13, 15]. Briefly, to each 500 µl plasma sample, 200 µl dimethyl sulfoxide, 200 µl 90:10 (v/v) acetonitrile/2-butoxyethanol, and 250 µl ethyl acetate were sequentially added. The samples were then incubated at 37 °C for 15 min, centrifuged (3000 *g* for 3 min at room temperature), and the organic layer removed and analyzed by HPLC. CSF samples were not extracted.

The HPLC system consisted of an RP-18 5 µm 4.6 × 30 mm column (Applied Biosystems, Foster City, Calif.) coupled with a Nova-Pak phenyl 4 µm 3.9 × 150 mm column (Waters, Milford, Mass.), two Waters Model 510 pumps and a WISP Model 712 autosampler (Waters). Mobile phase A consisted of 69% methanol/30% 0.05 *M* sodium phosphate (pH 7.0)/1% triethylamine, and mobile phase B consisted of 69% methanol/30% HPLC grade water/1% triethylamine. Hypericin was eluted using a linear gradient from 100% A to 100% B over 3 min at a flow rate of 1.5 ml/min, followed by 13 min of 100% B. Equilibration of the column was achieved by a linear change from 100% B to 100% A over the next 2 min followed by 12 min of isocratic flow of mobile phase A. This resulted in a complete analysis cycle time of 30 min. Eluant was monitored with a Waters 490 multiwavelength detector at 590 nm. The retention time of hypericin was approximately 7 min.

In an aqueous matrix and CSF, the lower limit of detection (LOD) was 0.1 µ*M* and the lower limit of quantification (LOQ) was 0.2 µ*M*. In plasma, the LOD and LOQ were 0.1 and 0.3 µ*M*, respectively. Recovery of hypericin from extracted plasma was 90%. The intraday coefficient of variation was $<15\%$ and $<8\%$ for 0.5 µ*M* and 10 µ*M* hypericin, respectively. The interday coefficient of variation was 13% for 0.5 µ*M* and 8% for 10 µ*M* hypericin concentrations. Hypericin was stable in plasma stored at –70 °C.

Pharmacokinetic analysis

A two-compartment open model consisting of central and peripheral compartments with a first-order elimination from the central compartment (Fig. 2) was fitted to the plasma concentration-time data from the individual hypericin i.v. bolus experiments. Model parameters were estimated with a weighted (EWT function) fit using MLAB (Civilized Software, Bethesda, Md.). Clearance, volume of distribution, and half-lives were derived from the estimates of the model parameters. Area under the concentration-time curves (AUCs) for CSF were derived using the linear trapezoidal method [6, 18]. The fraction of hypericin penetrating the CSF was calculated from the ratio of the CSF $AUC_{0-\infty}$ to plasma $AUC_{0-\infty}$.

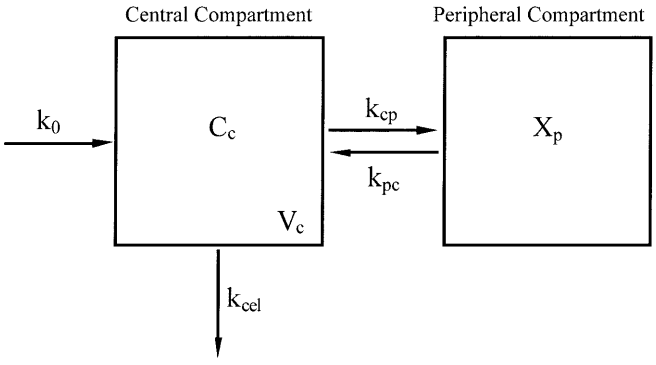
Results

Plasma pharmacokinetics

The plasma hypericin concentration-time profiles after the 2 mg/kg i.v. bolus dose are shown in Fig. 3, and the two-compartment fitted model parameters are summarized in Table 1. The mean peak plasma concentration after the 2 mg/kg bolus was 142 ± 45 µ*M*, and the elimination of hypericin was biexponential, with an average alpha half-life of 2.8 ± 0.3 h and terminal half-life of 26 ± 14 h. The mean (± SD) clearance was 6 ± 2 ml/kg per h and the mean (± SD) AUC was 646 ± 146 µ*M* · h. The pharmacokinetic parameters derived from the fitted model parameters are shown in Table 2.

CSF penetration

Hypericin was not detected in the CSF of any animal during the study (LOD 0.1 μ*M*). Based on the LOD, the penetration of hypericin into CSF was less than 1%.



$$\frac{dC_c}{dt} = \frac{k_0}{V_c} - k_{cel} \bullet C_c - k_{cp} \bullet C_c + \frac{k_{pc} \bullet X_p}{V_c}$$

$$\frac{dX_p}{dt} = k_{cp} \bullet C_c \bullet V_c - k_{pc} \bullet X_p$$

Fig. 2 Two-compartment open model with first-order elimination of hypericin from the central compartment. C_c is the concentration of hypericin in the central compartment, X_p is the amount of drug in the peripheral compartment, and V_c is the volume of the central compartment. The intercompartmental rate constants are k_{pc} and k_{cp}. The drug infusion rate is k_0, and k_{cel} is the elimination rate constant. The equations describe the hypericin concentration in the central compartment and the amount of hypericin in the peripheral compartments as a function of time

Fig. 3 Hypericin plasma concentration-time curves following an i.v. bolus dose of 2 mg/kg in three nonhuman primates (D28, R838A, and B9884)

Toxicity

The 2 mg/kg i.v. bolus dose of hypericin was well tolerated in three nonhuman primates during this study. Transient, severe photosensitivity rash occurred in the single animal at the 5 mg/kg dose level. Edema and a pruritic, erythematous rash with evolution to eschar was observed on the face and light-exposed skin areas of the animal. Mild anorexia and transient elevation in hepatic transaminases (peak AST 82 U/l, ALT 85 U/l) occurred. All abnormalities appeared within 24 h of hypericin administration and resolved within 12 days. Due to the adverse reaction, only limited plasma and CSF samples were obtained at the 5 mg/kg dose level, and therefore, no pharmacokinetic modeling could be performed.

Discussion

Signal transduction pathways that are critical for tumor growth and survival is an area of intense research for the development of novel anticancer agents (for review see reference 17). Hypericin, an inhibitor of protein kinase C, is one such agent that has undergone phase I testing in adult cancer patients and phase II testing in patients with HIV infection. Preclinical studies have suggested that hypericin may have activity in gliomas, and therefore we studied the central nervous system pharmacology of this agent.

The plasma pharmacokinetics of hypericin after an i.v. bolus dose in our nonhuman primate model were described by a two-compartment model, with a terminal elimination half-life of 26 h. A dose of 2 mg/kg (about 40 mg/m^2) was sufficient to maintain plasma hypericin concentrations above the in vitro cytotoxic threshold of 10 μ*M* [2, 5] for up to 12 h after bolus administration.

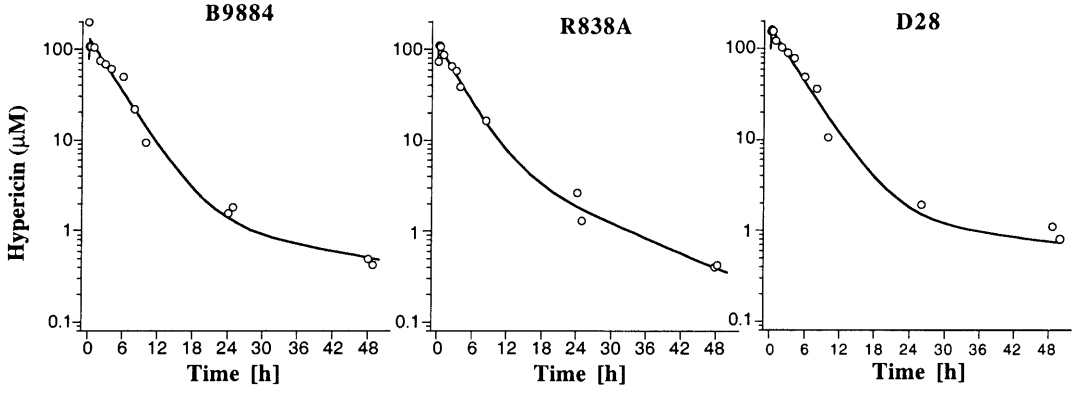


Table 1 Model parameters

	V_c (l/kg)	k_{cel} (h^{-1})	k_{cp} (h^{-1})	k_{pc} (h^{-1})
D28	0.024	0.21	0.023	0.02
R838A	0.036	0.23	0.037	0.076
B9884	0.031	0.22	0.020	0.028
Mean ± SD	0.030 ± 0.006	0.22 ± 0.01	0.027 ± 0.009	0.041 ± 0.030

Table 2 Plasma AUC, clearance, and half-life

	AUC ($\mu M \cdot$ h)	CL (l/kg/h)	$T_{1/2}\alpha$ (h)	$T_{1/2}\beta$ (h)
D28	803	0.0049	2.9	39.1
R838A	514	0.008	2.5	11.2
B9884	620	0.0065	2.9	27.6
Mean ± SD	646 ± 146	0.006 ± 0.002	2.8 ± 0.3	26.0 ± 14.0

The pharmacokinetics of hypericin in this nonhuman primate model are consistent with the pharmacokinetics of the drug in humans in whom the terminal half-life is 40 h and the peak plasma concentration was 0.06 μM following an i.v. dose of 0.001 mg/kg (about 0.04 mg/m^2) [11].

In the nonhuman primate, CSF penetration of hypericin was limited. CSF drug exposure was estimated to be $<1\%$ of the plasma drug exposure, and CSF concentrations were below the LOD (0.1 μM) at all time-points after the 2 mg/kg i.v. bolus dose. There was also no detectable hypericin in the CSF after the 5 mg/kg dose, but the significant phototoxicity observed precluded a more detailed plasma pharmacokinetic analysis.

The dose-limiting skin reaction was observed in the single animal at the 5 mg/kg dose. This reaction was consistent with ''hypericinism'' previously described in livestock grazing on *Hypericum* sp. This symptom complex consists of anorexia, elevated liver transaminases and the evolution of a skin rash in light-exposed areas of skin [3, 10]. More recently, the AIDS Clinical Trials Group have reported results of a phase I study of oral and i.v. hypericin in HIV-infected adults in which photosensitivity was the dose-limiting toxicity. In this trial, significant phototoxicity occurred following administration by both the i.v. and oral routes, and at all dose levels (0.25 and 0.5 mg/kg) and schedules evaluated [7].

Metabolism of hypericin is poorly characterized. Kerb et al. examined hypericin metabolism in humans and were unable to detect hypericin in urine before or after the addition of glucuronidase and sulfatase. Based on the chemical structure and molecular size, they speculate that conjugation with glucuronic acid and subsequent biliary excretion occurs [11].

The CSF penetration of hypericin is minimal, and it appears unlikely that cytotoxic concentrations of the drug could be achieved at tolerable doses. Additional preclinical studies with hypericin therefore appear warranted prior to further clinical evaluation of this drug as a potential treatment agent for tumors of the central nervous system. However, other agents which target critical signal transduction pathways remain an important area of drug development for the treatment of brain tumors.

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