PHARMACOKINETIC PROFILES IN RATS AFTER INTRAVENOUS, ORAL, OR DERMAL ADMINISTRATION OF DAPSONE

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ABSTRACT:

Dapsone is a potent anti-inflammatory and antibacterial agent that has been used extensively in the oral treatment of leprosy and dermatitis herpetiformis. This study compared the pharmacokinetic profile of dapsone in rats given a single oral or i.v. 12 mg/kg dose (n = 8/group) or a single dermal application of 12 or 60 mg/kg (n = 12/group) in an aqueous gel application medium containing 10 or 25% diethylene glycol monoethyl ether (DGME). Blood samples (200 μl) were collected via tail vein from each rat and pooled at intervals up to the 24-h period. A terminal blood sample was collected by cardiac puncture from each animal. Plasma concentrations of dapsone were determined by liquid chromatography atmospheric pressure ionization tandem mass spectroscopy. There was no treatment-related overt toxicity observed in any of the animals. Peak levels were reached 1 h after oral dosing (4890 ng/ml), and 6 to 8 h after dermal application, with Cmax values of 1.62, 5.56, and 12.8 ng/ml, for 12 mg/kg at 10 or 25% DGME, and for 60 mg/kg at 25% DGME, respectively. Bioavailability was calculated at 78% after oral dosing and <1% after dermal application. Apparent elimination half-lives (t1/2) were similar after i.v. and oral dosing. Both the calculated area under the plasma concentration versus time curve up to 24 h and Cmax values were 3- to 4-fold higher in the dermal application group administered 12 mg/kg dapsone in 25 versus 10% DGME gel, whereas the calculated area under the plasma concentration versus time curve up to 24 h and Cmax values for the 60 mg/kg group were only 3.3- and 2.3-fold greater than those obtained after application of 12 mg/kg in 25% DGME. These results show that both systemic exposure and peak plasma concentrations of dapsone are minimized by dermal versus oral administration of the compound.

Orally administered dapsone (diaminodiphenylsulphone) is a potent anti-inflammatory and antibacterial compound and has been used clinically for many years in the treatment of leprosy and dermatitis herpetiformis (Pieters and Zuidema, 1987). The daily oral dose of dapsone for the treatment of leprosy is typically reported to be 50 to 100 mg, but ranges from 50 to 400 mg (Zuidema et al., 1986). Dosages for the treatment of dermatologic disorders can range from between 50 mg weekly to 700 mg daily (Swain et al., 1983). Dapsone is highly protein-bound and extensively metabolized, and the parent drug, its monoacetyl metabolite, and their hydroxylated metabolites are found in urine, partly conjugated as N-glucuronides and N-sulfates (Zuidema et al., 1986). Dapsone is metabolized, in part, by the 3A family of isoenzymes of cytochrome P-450 to hydroxylamines (Watkins, 1994). These metabolites have been reported to cause the methemoglobinemia seen in all patients treated orally with dapsone, as well as hemolysis (Coleman, 1995; Chang et al., 1996). The life span of erythrocytes is decreased in patients taking oral dapsone (Zuidema et al., 1986). Profound hemolysis can occur in patients having glucose-6-phosphate dehydrogenase (G6PD) deficiency when treated with sulfones, including dapsone (Katz, 1999). However, daily oral dapsone doses of 50 mg or less in healthy G6PD-deficient persons will not produce detectable hemolysis (DeGowin, 1967). Less common side effects of oral dapsone treatment include idiosyncratic reactions such as agranulocytosis, cutaneous eruptions, leukopenia, peripheral neuropathy, psychosis, and minor neurological and gastrointestinal complaints (Chang et al., 1996).

The dapsone therapeutic serum concentration range has been estimated to be 0.5 to 5 mg/ml (Zuidema et al., 1986), although there is significant variability in oral dose administered, pharmacokinetics, effective therapeutic response, and side effects in humans. Concentrations determined in skin biopsies of patients treated orally with dapsone suggest that skin and plasma concentrations are similar (Swain et al., 1983). Attempts to increase tolerance of patients to dapsone have included coadministration of antioxidants, such as vitamins C and E (Prussick et al., 1992), or metabolic inhibitors of cytochrome P-450, such as cinetidine (Coleman, 1993). Another approach to reducing the side effects would be to apply dapsone directly to the affected area. Dermal application might provide effective concentrations of dapsone at the target tissue, but reduce systemic exposure and thus decrease the frequency and/or severity of side effects. Although topical dosing would not be suitable for treating leprosy or as a singular therapy for dermatitis herpetiformis, other skin conditions known to respond to dapsone could be ideal for topical therapy.

One skin condition known to respond to dapsone therapy is acne.
a double-blind placebo-controlled study, 12 of 23 patients improved, with nine greatly improving on receiving up to 200 mg oral dapsone daily (Ross, 1961). A 484 patient-uncontrolled study using 300 mg of oral dapsone weekly showed an 80% remission of lesions in severe acne after a 3-month treatment period (Kaminsky et al., 1974). Geniaux et al. (1981) reported a 75% improvement in 9 of 11 patients within 8 weeks using 300 mg of oral dapsone per week. A well controlled acne study evaluated oral dapsone versus oral 13-cis retinoic acid (Prendiville et al., 1988). Although 100 mg of oral dapsone daily was shown inferior to oral 13-cis retinoic acid in a 16-week study of nodular cystic acne, oral dapsone did demonstrate statistically significant improvement at weeks 8 (P < .05) and 16 (P < .01). When considering the anti-inflammatory properties of dapsone and the potency of this antibacterial with regard to Propionibacterium acnes (MIC = 1 µg/ml; Godowski and Franklin, 2000) it would be expected that topical dapsone should be even more effective than oral dapsone in the treatment of acne. Because acne is a medical condition of otherwise healthy adolescents with an expected full life span ahead, a dapsone product would need to show sufficiently low systemic uptake to assure avoidance of side effects. Therefore, this study compared the pharmacokinetics of dapsone after a single i.v., oral, or dermal administration of the drug to male rats to determine whether topical dosing would dramatically reduce systemic uptake compared with oral dapsone therapy.

Materials and Methods

Animals. A total of 52 male Sprague-Dawley rats were obtained from Harlan Sprague-Dawley (San Diego, CA). The rats were 8 to 9 weeks old at the time of testing. All animals were quarantined for 7 days and examined to assure that they were healthy before release from quarantine. Rats were randomly assigned to one of five experimental groups, three groups containing 12 rats each and two groups containing eight rats. Randomization was based on a modified Latin square. Animals were housed individually in stainless steel, wire-bottomed cages in an environmentally controlled room. The animals received food ad libitum except for approximately 16 to 18 h before treatment, and approximately 4 h after dapsone administration.

Chemicals. Dapsone, USP grade, was manufactured by VIS Farmaceutici S.P.A. (Padova, Italy). Dapsone was prepared in a vehicle of 5% ethanol/5% Tween 80/90% sterile water (w/w/v) for oral administration, and in a vehicle of 5% ethanol/5% Tween 80/90% sodium chloride (w/w/v) for i.v. administration in a final concentration of 4 mg of dapsone/ml. For dermal application, dapsone in a diethylene glycol monooethyl ether (DGME) aqueous gel medium was supplied by Atrix Laboratories, Inc. (Fort Collins, CO). Three formulations of the test article were used: 1) 1% dapsone in a 10% DGME gel; 2) 1% dapsone in a 25% DGME gel; and 3) 5% dapsone in a 25% DGME gel.

Intravenous Dosing. Each rat was anesthetized with isoflurane before surgical exposure of the jugular vein. The dapsone solution was administered at a volume of 3 ml/kg, for a dose of 12 mg/kg, as a slow bolus injection into the exposed jugular vein. Immediately after dosing, the wound was closed with staples and the animal positioned for initial blood sample collection.

Oral Dosing. The dapsone solution was administered at a volume of 3 ml/kg, for a dose of 12 mg/kg, by stomach intubation using a 16-gauge feeding needle.

Dermal Application. One day before treatment, rats were anesthetized with ketamine/xylazine (7:1). The back and shoulders of each rat were shaved and then washed with acetone. Care was taken not to abrade the skin. After shaving, a glass-contoured rectangular enclosure (2.5 cm × 5 cm; maximum height, 1.3 cm) was glued onto the middle of the back of each animal with a cyanacrylate-based glue. Silicone medical adhesive, type A, was applied as a seal around the outside of each enclosure. A target amount of 300 mg of gel medium containing one of the dapsone concentrations was applied evenly by spatula to the enclosed area on the back of each rat, resulting in a dose of 12 or 60 mg/kg. A nonocclusive plastic cover was then glued to the top of the rectangular enclosure to prevent the animal from disturbing the application site, and the enclosure was further secured with surgical tape.

Sample Collection and Processing. Blood samples (200 µl) were collected via the tail vein of each rat at 1, 2, 4, 6, 8, and 24 h after treatment. Additionally, samples were collected at 5, 15, and 30 min for rats receiving i.v. or oral doses. To obtain sufficient plasma volumes for repeat analysis, samples within each group were pooled by time interval into heparinized tubes that were cooled on ice. After the 24-h blood collection via tail vein, a terminal blood sample was collected via cardiac puncture after anesthesia with ketamine/xylazine (7:1). Terminal samples were not pooled. Blood samples collected after i.v. or oral dosing were centrifuged at 2100 × 100 rpm for 10 min, and samples collected after dermal applications were centrifuged at 2600 × 100 rpm for 10 min. The plasma was removed and stored frozen. Plasma samples were shipped on dry ice to Alta Analytical Laboratory (El Dorado Hills, CA) for analysis.

Analytical Method. Plasma (0.1 ml) was extracted with methyl-t-butyl ether after the addition of internal standard (meta-dapsone, 3-aminophenyl sulfone from Aldrich, Milwaukee, WI) and 0.1 ml of 0.5 M Na2CO3. The organic phase was evaporated to dryness under nitrogen, the samples were reconstituted with 0.2 ml of 30:70 acetonitrile/water and analyzed by HPLC TurbolonSpray liquid chromatography (LC) with tandem mass spectroscopy detection. A Shimadzu LC-10AD LC system (Shimadzu Corp., Columbia, MD) was connected to a HTS PAL autoinjector (LEAP Technology, Research Triangle Park, NC). The extracts were injected (15 µl) onto a 100 × 2 mm (5 µm) Keystone BT 1 column (Keystone Scientific, State College, PA). Analytes were eluted isocratically using a mobile phase of 65:35 water/acetonitrile with 5 mM ammonium acetate with 0.1% formic acid at 0.2 ml/min. Using these conditions, dapsone was baseline-resolved from all other isomers including the internal standard. The retention times were approximately 3.5 and 4.8 min, for dapsone and meta-dapsone, respectively.

Detection was by positive ionization tandem mass spectrometry using a Perkin Elmer SCIEX API 3000 triple quadrupole mass spectrometer (Concord, Ontario, Canada) equipped with a TurbolonSpray atmospheric pressure ionization interface. The instrument was operated in the multiple reaction monitoring mode (MRM) using the same molecular precursor and daughter ions (m/z 248.9 → ml/z; 155.9) for both dapsone and meta-dapsone. Curtain and nebulizer gas flow settings were 12 and 5, respectively, and auxiliary gas flow was 8 l/min. Ionspray voltage and TurbolonSpray temperature settings were 5000 V and ~375°C, respectively. The dynamic range of the assay was 0.5 to 100 ng/ml.

Peaks were integrated using Perkin Elmer SCIEX MacQuan, version 1.5 software. Calibration curves were derived from peak area ratios (analyte/internal standard) using a least-squares regression of the ratio versus the nominal concentration of the calibration curve standard. A weighting of 1/x2 was found to be optimal. Accuracy and precision for the assay was assessed by analyzing quality control samples (n = 5) at three concentrations (1.5, 40, and 80 ng/ml) before sample analysis. Intra-assay accuracy and precision ranged from 95.5 to 100.8 and 2.5 to 3.0%, respectively.

Calculations. Mean and S.E. values were determined by Microsoft Excel, version 5. Area under the plasma concentration versus time curve (AUC) up to 24 h was determined by the trapezoidal (log/linear) rule (Stewart, 1995) using Microsoft Excel, version 5.0 to perform the calculation and then checked using WinNonlin version 3.0 (Pharsight Corporation, Mountain View, CA). The apparent terminal-phase half-life (t1/2) was calculated as ln2/AUC. Clearance (CL) was calculated as Dosed/AUCt.ine and volume of distribution at steady state (Vss) as (AUMCt.ine/AUCt.ine). Bioavailability was calculated as (AUCp.o.)/(Doseiv × AUCi.v.) × (Dosep.o.).

Results

There were no signs of overt toxicity in any of the animals treated with dapsone. Dapsone was detected in pooled plasma from rats for up to 24 h after a single oral or i.v. 12 mg/kg dose or single dermal applications of 12 mg/kg in 10 or 25% DGME or 60 mg/kg in 25% DGME (see Figs. 1 and 2), although levels were below quantifiable limits at 1 h in the 10% DGME group, and in 4 of 12 animals in this group at 24 h.

Noncompartmental pharmacokinetic parameters are presented in Table 1. Peak plasma concentrations of dapsone were reached 1 h
after oral dosing, and 6 to 8 h after dermal application. Bioavailability after oral dosing was calculated at approximately 78%. Bioavailability after dermal application in all vehicles was very low and ranged from 0.16 to 0.79%. Both the calculated $AUC_{0-24}$ and $C_{\text{max}}$ values were 3- to 4-fold higher in the group administered 12 mg/kg dapsone in 25 versus 10% DGME gel, whereas the calculated $AUC_{0-24}$ and $C_{\text{max}}$ values for the 60 mg/kg group were only 3.3- and 2.3-fold greater than obtained after application of 12 mg/kg in 25% DGME.

After dermal application of 12 mg/kg, terminal 24-h dapsone concentrations measured within treatment groups varied by approximately 10-fold in the samples collected by cardiac puncture (0.5–5.04 ng/ml in the 10% DGME group; 0.63–5.24 ng/ml in the 25% DGME group). Larger variability (100-fold) was seen in the 60 mg/kg group, due to an anomalously high concentration of dapsone (60.7 ng/ml) in a sample collected from a single animal. Excluding this animal gave a mean 24-h plasma concentration for the 11 remaining animals of 2.93 ng/ml compared with the value 7.75 ng/ml obtained when data from all 12 animals is considered. Because dapsone concentrations in plasma were not determined individually during the 24-h sampling period, it cannot be determined if relatively high levels also were present in this animal at the earlier collection time points. If such occurred, the kinetic parameters calculated from the results obtained in pooled samples for this group may overestimate the expected systemic exposure at this target dose. Less variability was seen after parenteral treatment, as individual terminal dapsone concentrations varied by only 2- to 3-fold in those samples collected by cardiac puncture (i.v.: 58.2–111 ng/ml; oral: 72.6–200 ng/ml). Additionally, the mean concentration calculated for each group was comparable with the respective 24-h value determined from pooled blood

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**Fig. 1.** Dapsone concentrations (nanograms per milliliter) in pooled plasma from male Sprague-Dawley rats after a single i.v. or oral dose of 12 mg/kg ($n = 8$/group).

**Fig. 2.** Dapsone concentrations (nanograms per milliliter) in pooled plasma from male Sprague-Dawley rats after a single dermal application of 12 or 60 mg/kg ($n = 12$/group).

The 1-h dapsone concentration for animals in the 10% DGME group was below the quantification limit (<0.5 ng/ml).
samples collected via tail vein; mean individual and pooled 24-h values were 89.7 versus 94.4 ng/ml, respectively, after i.v. administration, and 118.6 versus 103 ng/ml, respectively, after oral dosing.

**Discussion**

The ideal delivery of dapsone after topical application for the treatment of acne is rapid partitioning into the hair follicle/sebaceous gland. Followed by slow diffusion through the epithelial lining of the pilosebaceous unit and, finally, minimal uptake into the systemic circulation. This would maintain high concentrations of dapsone (above the MIC90) in the portion of the skin in which *P. acnes* resides, while providing low but steady levels of anti-inflammatory dapsone to the dermis. Slow partitioning out of the sebaceous unit will keep circulating plasma levels of dapsone below the concentration that causes hemolytic and other adverse events. The inherent lipophilicity of dapsone helps maximize partitioning into the sebum and should likewise minimize the diffusion of dapsone into the vascularized dermis.

The objective of this study was to characterize the profile of systemic exposure to dapsone in rats after a single dermal application or a single oral or i.v. dose. Dapsone was detected in pooled plasma from rats for up to 24 h after treatment, with peak levels reached 1 h after oral dosing, and 6 to 8 h after dermal application. The high bioavailability observed after oral administration is consistent with that observed in other species. Pharmacokinetics in dogs and healthy human volunteers has been reported after a single oral or i.v. dose of 100 mg (Pieters and Zuidema, 1986, 1987). Nearly complete oral bioavailability and high systemic exposures were demonstrated, with AUC values ranging from 56.1 to 99.2 ng·h/ml in dogs and from 24.0 to 75.4 mg·h × liter⁻¹ in humans. Elimination half-life values ranged from 5.8 to 10.2 h in dogs, and from 15.6 to 30.4 h in humans (Pieters and Zuidema, 1987). Because the dose used in these studies was not identical with the dose level of dapsone used in the current rat study, no direct species comparisons of specific exposures may be made. Nevertheless, it is evident that dapsone has high oral bioavailability in all three species, and a lower apparent elimination half-life in rats compared with dogs or humans.

In this study, systemic exposure to dapsone after topical application was related directly to both the dose of dapsone applied (12 versus 60 mg/kg) and the concentration of DGME (10 versus 25%) in the gel application medium. In the dermal application groups, both the calculated AUCmax and Cmax values were 3- to 4-fold higher in the group administered 12 mg/kg dapsone in 25 versus 10% DGME gel. However, the highest values seen after dermal administration of 12 mg/kg were markedly lower than the values obtained after an oral dose (<1%). These results indicate that both systemic exposure and peak plasma concentrations of dapsone are minimized by dermal versus oral administration of the compound.

Exposure was not dose-proportional in the 25% DGME vehicle groups. For a 5-fold difference in dose (12–60 mg/kg), there was only a 3.3-fold increase in AUCmax and a 2.3-fold increase in Cmax. This lower than expected increase in systemic uptake is likely due to the ability of DGME to prolong the time dapsone remains in the skin before becoming systemic, i.e., provide for an intracutaneous depot (Panchagnula and Ritschel, 1991; Pavliv et al., 1994). These plasma concentrations observed after dermal administration confirm the expected outcome that applying dapsone gel to the skin will result in reduced systemic exposure. More importantly, the magnitude of the systemic uptake reduction (over a 500-fold reduction for 1% dapsone in 10% DGME) for dermal versus oral administration suggests that dose-dependent untoward effects could be completely avoided for these dapsone topical gels. Thus, these results are in agreement with the plasma profiles expected for ideal delivery of dapsone for the treatment of acne. Because even a 4-fold reduction in oral dose results in healthy G6PD-deficient patients no longer experiencing hemolysis (DeGowin, 1967), testing for this genetic trait in Asians, Blacks, or persons of Mediterranean descent would likely not be required before initiating topical dapsone therapy.

The longer time required to reach peak levels for dermal dosing (6–8 h) compared with the 1 h for oral dosing is typical for the dermal route of administration. The stratum corneum provides an effective barrier that retards penetration of topically applied actives. This results in a lag time, followed by a broad peak plasma profile. Drug may continue to diffuse into the skin, becoming systemic, even after peak plasma levels are obtained. Thus, longer than expected elimination half-lives may be found if these values are based on the first 24 h after the first single dose. The length of time that delivery continues beyond the time of peak plasma concentration could be highly formulation-dependent. The apparent elimination half-life values found in this dapsone topical gel study appear to primarily reflect the ability of the formulation to continue delivering drug for hours after gel application. It is not anticipated that the ability of the animal to clear the drug after it becomes systemic is in any way impaired by dermally applying dapsone. The single rat in the 60 mg/kg dermal group that had an anomalously high plasma concentration at 24 h also makes the calculated 77.8-h half-life value for this group suspect. If this animal is excluded, the calculated half-life is reduced to approximately 8 h.

In summary, these results indicate that both systemic exposure and peak plasma concentrations of dapsone are minimized by dermal versus oral administration of the compound. Dermal application of dapsone reduces systemic exposure, and thus should decrease the frequency and/or severity of side effects associated with oral dapsone therapy.
References


