Selegiline Percutaneous Absorption in Various Species and Metabolism by Human Skin

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Purpose. A Selegiline Transdermal System (STS) is under development for indications which may not be optimally or safely treated with oral selegiline. Studies were conducted to evaluate the in vitro penetration and skin metabolism of selegiline in order to better understand the toxicological findings and the observed plasma levels of selegiline and its metabolites in animals and man.

Methods. In vitro penetration studies were conducted in four different species (male hairless mice, male and female rats, female dog and male Micropig®) and compared to human skin. In another study, viable human skin was used to estimate the extent of metabolism of selegiline by human skin using Franz diffusion cells.

Results. Results indicated that female dog and male Micropig® skin were reasonable animal models for 24 hour in vitro selegeline penetration through human skin. Penetration of selegeline through rat skin and hairless mouse skin was 2-fold and 3-fold higher than through human skin, respectively. Metabolism was negligible in human skin. Selegeline metabolites (L-methamphetamine and N-desmethylselegiline but not L-amphetamine) were detected at all time points but the extent of selegeline metabolism was negligible. The cumulative 24 hour in vitro selegeline permeation from a 1.83 mg/cm² STS through human skin was 5.0 mg. This was similar to the in vivo permeation in humans as assessed by residual patch analysis.

Conclusions. The similarity of dog and human skin permeation results support the use of the dog as a species for evaluating the toxicology of transdermally-administered selegeline. Selegeline is not metabolized cutaneously and hence the metabolic profile following STS administration is likely due to hepatic metabolism only.

KEY WORDS: skin permeation; metabolism; selegeline; transdermal.

INTRODUCTION

Selegeline (SEL) is a selective inhibitor of MAO-B which is administered orally in the treatment of Parkinson's disease as an adjunct to L-DOPA. SEL undergoes extensive first-pass metabolism after oral administration which can be bypassed by transdermal delivery (1). The levels of selegeline after oral administration are highly variable (2). The recommended oral dose regimen of 5 mg bid has been defined on the basis of likely dose limiting toxicities, namely the potential for hypertensive crisis or 'cheese effect' and insomnia due to the selegeline's amphetamine metabolites. Non-MAO-B related pharmacology of selegeline suggests that the antagonistic actions of selegeline's amphetamine metabolites may be responsible for the lack of conclusive evidence for the neuroprotective effects of orally-administered selegeline HCl (3–7). Higher selegeline doses would be desirable in depressive states where the loss of central MAO-A selectivity (and expression of MAO-A inhibition) would be beneficial (3,8). These doses are currently precluded orally due to the potentiation of hemodynamic effects (also known as the 'cheese-effect'), which may be ultimately expressed clinically as hypertension, headache, palpitation, electrocardiographic abnormalities and arrhythmias (3,8). One rationale for the STS is that higher systemic selegeline levels may be attained at the expense of amphetamine metabolite formation without potentiation of tyramine sensitivity. A single administration of the STS has shown no increase in tyramine sensitivity at doses up to 200 mg of oral tyramine (9). The percutaneous absorption/permeation of most marketed transdermal systems (e.g. nitroglycerin and nicotine) has been established in animal and human skin. It is well known that skin has metabolic capabilities. Both phase I and phase II metabolism can take place in the skin (10). Phase I reactions such as oxidation (e.g. cortisol, norepinephrine), reduction (e.g. testosterone, progesterone), hydrolysis (e.g. glucocorticoids), and phase II reactions such as glucuronidation (e.g. benzopyrene), sulfation (e.g. aminophenol), methylation (e.g. norepinephrine) and glutathione conjugation (e.g. styrene oxide) by the skin have been reported (10,11). Several enzymes such as NADPH-cytochrome c reductase and glutathione transferase are present in the skin. As shown in Figure 1, SEL is converted to N-desmethylselegeline (DES) and L-Methamphetamine (MET), and subsequently, both MET and DES are converted to L-amphetamine (AMP) (2,12). This conversion takes place in the gut as well as liver and leads to highly variable and low plasma levels of parent SEL after oral administration (2). These conversions are cytochrome P450-mediated (most likely CYP 2D6 and CYP 3A4) (2,13). Given the density of cytochrome P450 enzymes in human skin, it is possible that there is a dermal component to the metabolism of SEL after transdermal administration similar to topical testosterone (10).

The objectives of these studies were to evaluate the penetration of SEL through the skin of various species (i.e. hairless mice, rats, female dog, Micropig®) to determine the appropriate animal model for future pharmacokinetic and toxicokinetic studies with the STS, as well as to determine the extent of metabolism of SEL by viable human skin. The proposed duration of application of the STS is 24 hours, with the skin being the rate-limiting step for selegeline penetration since the patch contains no rate-controlling membrane or reservoir.

METHODS

Skin Penetration Studies

An in vitro study was conducted involving the application of the STS (1.83 mg/cm²) to excised male hairless mouse, male and female rat, female dog, male Micropig® and human male abdominal skin preparations using Franz diffusion cells. Male
hairless HRS/J mice (7–8 week old) were obtained from Jackson Laboratory, Bar Harbor, ME. Male and female CRL CDBR VAF/ + rats (8–9 weeks) were obtained from Charles River Laboratories, Inc., Portage, MI. All animals were healthy and acclimatized before sacrifice to extract the skin. A sample of fresh skin from a 39 week old female beagle dog was obtained from Hazelton Wisconsin (Madison W1) and sample of fresh skin from a 17-week old male Yucatan Micropig® was obtained from Charles River Laboratories, Inc., Windham ME. Fresh samples of human abdominal skin were obtained from the International Institute for Advancement of Medicine (IIAM), Exton, PA. The dog (shaved skin), Micropig®, and human skin samples were shipped on wet ice and maintained in saline until initiation of the study.

Skin discs were prepared by modification of the method described by Kao et al. (14) for full-thickness excised skin preparations. The rats and mice were given an anesthetic overdose of carbon dioxide, just prior to removal of skin. The fur of the rats were clipped from the dorsal trunk in the thoracic region. A piece of clipped skin was removed and placed on a petri dish. Skin samples from dog, Micropig® and man were processed in the same way. The fat and connective tissue were removed from skin pieces with a spatula. Discs (one inch in diameter) were cut from various skin samples. The thickness of the skin samples was also measured using a vernier caliper.

The Franz-type diffusion cells consisted of 10 mL flange diffusion cells mounted on a variable speed mounting console, which was set at 600 rpm. The cell itself was a water-jacketed, magnetically stirred chamber with a flat flange joint and cap for fastening the skin. The cell was filled with 0.9% sodium chloride solution (pH 7.0) and kept at 37°C. The excised skin was mounted such that the viscera was bathed in the fluid. In the case of human skin studies, the skin was chilled just before applying to the cell to improve the adhesion of the patch to the skin. In general, 3 skin samples of each type of species were used. The receptor medium was withdrawn at 4, 8, 12, 18 h for all the species except for Micropig® and human studies, in which 24 and 30 hour samples were also collected.

The amount of SEL that penetrated the skin was reported over the collection interval and described as cumulative amount/sq. area absorbed. The mean steady-state rate of penetration was calculated as the slope of the linear segment of the curve after plotting the cumulative amount/sq. area absorbed per interval versus time. The time to reach steady-state was variable but was usually about 8 h. The lag time between the application of dose and attainment of a steady-state rate of penetration was calculated by dividing the negative of y-intercept by the slope. With a zero (or positive) y-intercept, the lag-time is necessarily zero. Interspecies comparisons were performed using 2-tailed t-tests and a t-test adjusted for multiple comparison at a α-level of 0.05.

**Penetration and Metabolism of Selegiline in Human Skin**

Human skin samples were obtained within 24 h of surgery or death and stored at −70°C until initiation of study. The skin samples were from one caucasian male and two caucasian