Application of *in Vivo* Microdialysis to Transdermal Absorption of Methotrexate in Rats

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Received June 7, 1993; accepted December 10, 1993

Microdialysis was applied to determine the *in vivo* transdermal absorption of methotrexate (MTX) in rats with or without a new penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101). A solution composed of 2.5 m*M* MTX and 3% (w/v) HPE-101 was applied to the shaved abdomen, in which a semipermeable membrane cannula of 10-mm length was inserted intracutaneously with the use of an L-shaped needle. Intradermal microdialysis was performed at a flow rate of 1.0 μL/min for 12 hr. The concentration of MTX in the dialysate was measured by fluorescence polarization immunoassay (FPIA). HPE-101 (3%, w/v) significantly increased the dermal MTX concentration from 0.06 ± 0.04 μ*M* in the control to 56 ± 26 μ*M* in the dialysate from 8 to 12 hr. HPE-101 at concentrations of 0.75, 1.5, 2.25, and 3% (w/v) enhanced the total recovery of MTX in dermal dialysate from 0 to 10 hr by approximately 5, 18, 42, and 500 times compared with the control, respectively. The microdialysis system is useful for assessing *in vivo* transdermal drug absorption.

KEY WORDS: microdialysis; methotrexate; *in vivo* transdermal absorption; HPE-101.

INTRODUCTION

Microdialysis as an *in vivo* sampling technique permits the analysis of test substances with minimal tissue damage from the extracellular space of most body tissues. We have successfully applied microdialysis to the pharmacokinetic study for drugs capable of penetrating the blood–brain barrier with L-dopa as the model drug in rats (1). Microdialysis was demonstrated to be suitable for assessing drug distribution in specific brain regions *in vivo*.

Recently, Ault *et al.* (2) examined *in vitro* dermal transport of 5-fluorouracil (5-FU) using a Franz diffusion-cell receptor compartment, together with a microdialysis probe implanted in excised rat skin *in vitro*. They suggested the feasibility of *in vivo* dermal microdialysis. We applied microdialysis to study transdermal drug absorption *in vivo*, using methotrexate (MTX) and a new penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101) (3).

MATERIALS AND METHODS

Materials and Apparatus

MTX was supplied by Lederle Japan Co., Ltd. (Tokyo). The TDx assay reagent set for fluorescence polarization immunoassay (FPIA) of MTX was purchased from Abbott Laboratories (Abbott Park, IL). All other chemicals were of special reagent grade. HPE-101 was synthesized in our laboratories as described previously (3). The microdialysis system consisted of a CMA/100 microinjection pump (Carnegie Medicin, Stockholm) and CMA/10 microdialysis probes with a dialysing membrane length of 10 mm and an outer diameter of 0.5 mm (Carnegie Medicin). The probe was perfused continuously with Ringer's solution (pH 6.5) at a rate of 1.0 μL/min during the experimental period.

Animal Experiments

Male Wistar rats (SPF), weighing 280–320 g, were anesthetized with urethane (1.5 g/kg as 300 mg/mL, i.p.), and anesthesia was continued throughout the experimental period.

For intradermal microdialysis, abdominal fur of the rats was shaved using a Thrive Model 900 animal clipper (Daitoh Electric Co., Tokyo), taking care not to damage the skin. The skin was incised over the dermis in the shaved abdominal region, followed by intradermal insertion of an introducer assembled by inserting the L-shaped needle into the black tubing. After setting the black tubing under the skin, the L-shaped needle was withdrawn, followed by inserting the microdialysis probe. The black tubing was torn off by pulling upward and outward. After completion of the probe implantation, a semicircle glass reservoir with an inner diameter of 20 mm was placed over the shaved abdominal region. At 1 hr after probe implantation, 2 mL of MTX solution (2.5 m*M*) composed of 60% (v/v) ethanol and 40% (v/v) phosphate buffer (50 m*M*, pH 5.0) with or without 3% (w/v) HPE-101 was applied to the reservoir. The dialysate samples were collected into a small sample tube from time 0 to 1, 1 to 2, 2 to 4, 4 to 6, 6 to 8, and 8 to 12 hr.

For blood microdialysis, the midline neck region was incised to expose the jugular vein, followed by insertion of a guide cannula (Carnegie Medicin) into the vein through the pectoral muscle. The microdialysis probe was then inserted into the vein via the guide cannula. The dialysate samples were collected from the jugular vein as for the intradermal microdialysis.

To determine the effect of varying the concentration of HPE-101 on the transdermal absorption of MTX, the total recovery of MTX in dermal dialysate from 0 to 10 hr was measured at HPE-101 concentrations of 0, 0.75, 1.5, 2.25, and 3.0% (w/v).

Analysis of MTX in the Dialysate

Free MTX was analyzed by the Abbott TDx automated fluorescence polarization analyzer (Abbott Laboratories, Irving, TX) based on FPIA technology (4). Data were analyzed with the Mann–Whitney *U* test.

RESULTS AND DISCUSSION

The use of a new penetration enhancer, HPE-101, gave rise to a marked increase in MTX concentrations in both dermal dialysate and blood dialysate as shown in Fig. 1. The

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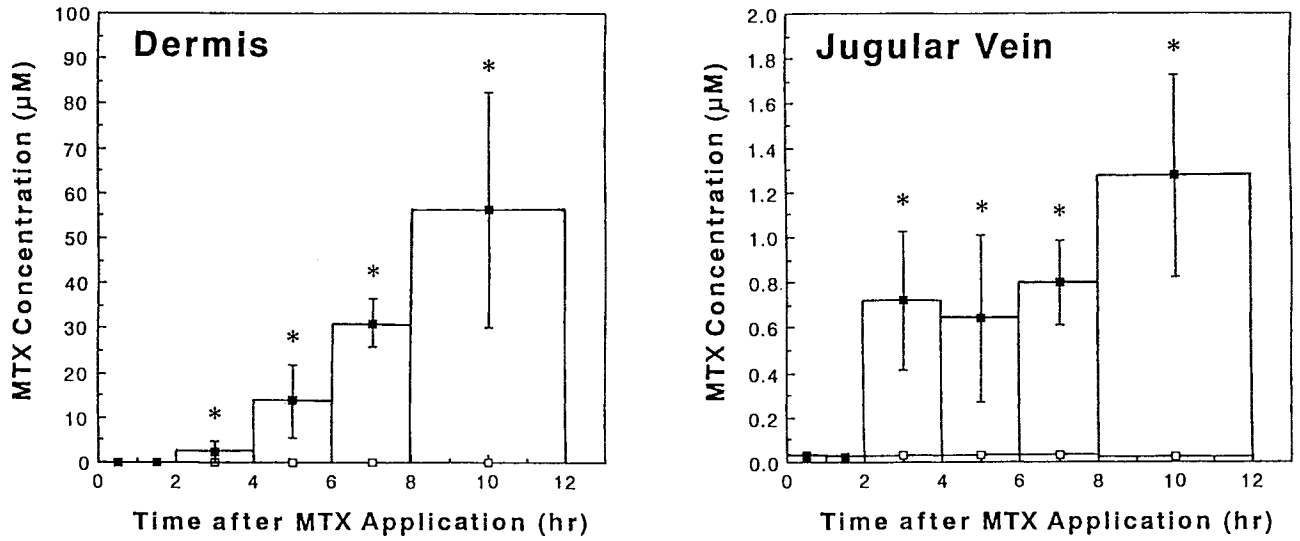

Fig. 1. MTX concentration–time profiles in the dialysates collected from the dermis and the jugular vein with (■) or without (□) 3% HPE-101. Data represent the mean ± SD of four or five rats. (*) Significantly different from the control at $P < 0.02$ by Mann–Whitney U test.

concentration of MTX in dermal dialysate with 3% HPE-101 increased gradually for 4 hr, followed by a rapid increase until 12 hr. The highest concentration of MTX in dermal dialysate was observed in the fraction from 8 to 12 hr. It increased from 0.06 ± 0.04 μM in the control to 56 ± 26 μM by coapplication of 3% HPE-101 ($P < 0.02$). Similarly, the MTX concentration in the fraction from 8 to 12 hr in blood dialysate was markedly increased, from 0.03 ± 0.01 μM in the control to 1.3 ± 0.4 μM by 3% HPE-101 ($P < 0.02$). Therefore, a therapeutic plasma level of MTX (near 1 μM) can be attained by topical MTX, when applied with HPE-101.

Our previous study showed that HPE-101 produced a continuous increase in enhancing activity for dermal penetration of indomethacin at concentrations up to 3% (w/v), above which the enhancing activity reached a plateau (3). Therefore, we limited the concentration range of HPE-101 to 3% (w/v) in this study. The effect of HPE-101 on dermal MTX permeability was also examined at concentrations less than 3% (w/v). As shown in Fig. 2, HPE-101 at concentrations of 0.75, 1.5, 2.25, and 3% (w/v) enhanced the total recovery of MTX in the dermal dialysate from 0 to 10 hr by approximately 5, 18, 42, and 500 times compared with the control, respectively ($P < 0.02$). The activity of enhancer is generally found to increase gradually and then attain a plateau with increasing enhancer concentration (5,6). In this study, the effect of HPE-101 on the dermal permeability of MTX was demonstrated to increase rapidly the total recovery of MTX in the dermal dialysate with increasing HPE-101 concentration from 0.75 to 3% (w/v). It might be expected that the abdominal skin is damaged proportionately with the increase in the enhancing activity of HPE-101. However, there were no morphological changes on the abdominal surface within the limits of macroscopic observation.

MTX is an effective agent in the treatment of psoriasis, although its use is limited by toxic effects on other rapidly proliferating tissue such as bone marrow, gut epithelium, and liver. Therefore, topical application of MTX has been tested. Fry and McMinn (7) reported partial or complete clearing in seven of nine cases with blistering and purpuric reactions in surrounding normal skin, indicating considerable pharmacological activity. On the other hand, Van Scott and Reinertson (8) could find no detectable effect using MTX under occlusive dressings. This discrepancy must be reconsidered in light of the low permeability of MTX without any enhancer, e.g., HPE-101, with little absorption expected. The use of HPE-101 may permit the topical use of MTX.

We successfully demonstrated intradermal implantation

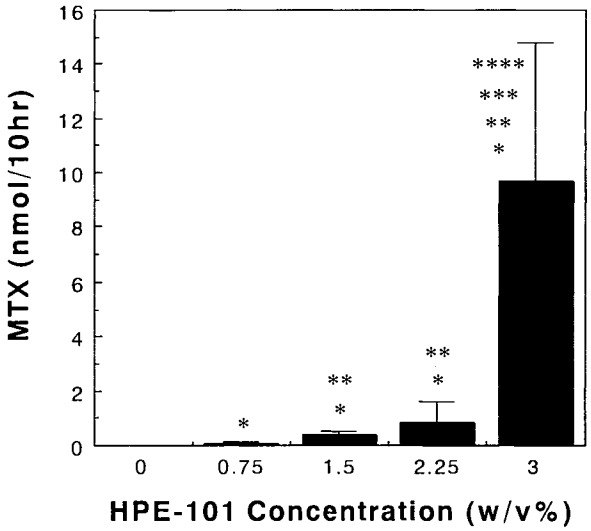

Fig. 2. Effect of HPE-101 concentration on the total recovery of MTX in the dermal dialysate from 0 to 10 hr after the application of MTX with HPE-101 at varying concentrations, 0, 0.75, 1.5, 2.25, and 3%. Data represent the mean ± SD of four rats. Data analysis was performed by Mann–Whitney U test. (*) Significantly different from 0% (the control), $P < 0.02$. (**) Significantly different from 0.75%, $P < 0.02$. (***) Significantly different from 1.5%, $P < 0.02$. (****) Significantly different from 2.25%, $P < 0.02$.

of a microdialysis probe *in vivo* which can assess changes in drug concentrations following coapplication of an enhancer.

ACKNOWLEDGMENT

This study was performed at the laboratory of the Animal Center for Biomedical Research, Nagasaki University School of Medicine.

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