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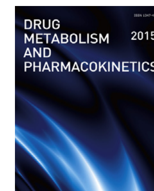

Regular article

Mechanism for transport of ivermectin to the stratum corneum in rats

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ABSTRACT

Ivermectin (IVM) is used as an oral medication for scabies, a skin infection caused by a mite, *sarcoptes scabiei*, which parasitizes in the stratum corneum. After oral administration IVM is absorbed from the intestine, and finally distributed to the stratum corneum to eliminate the mites. However its transport mechanism remains unclear. A pharmacokinetic study was performed using hairless Wistar Yagi (HWY) rats, which have no or atrophied sebaceous glands, and Wistar rats as a reference. After oral administration of IVM to both groups, the area under the concentration–time curve of IVM in the dermis and epidermis (dermis–epidermis) of HWY rats were about 60% lower than that of Wistar rats, even though the plasma concentration profiles were comparable in both groups. In addition at 12 h after the administration, IVM concentration in the outer stratum corneum, the shallower layer of the dermis–epidermis, was higher compared to that in the deeper layer. In the dermis–epidermis of the skin from various locations, the concentrations of IVM and squalene, the latter of which is secreted to the skin surface via the sebaceous gland, were positively well correlated. Those results suggest that IVM is transported to the stratum corneum via the sebaceous glands.
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1. Introduction

Scabies is a skin infection caused by a mite, *sarcoptes scabiei*, which parasitizes in the stratum corneum and generates allergic symptoms with pruritus. Ivermectin (IVM) is an extremely hydrophobic derivative of the avermectins, a family of macrocyclic lactones produced by the filamentous bacterium *Streptomyces avermitilis*. It has a broad spectrum of activity toward nematode and arthropod parasites and is used widely in humans for scabies treatment in many countries. For the treatment of scabies, IVM is given orally once or twice every a week. IVM is absorbed from the intestine, and finally distributed to the stratum corneum to eliminate the mites. Thus the IVM concentration in the stratum corneum would be an important parameter for the evaluation of its efficacy. IVM exposure levels in plasma of humans and animals have been reported by some researchers [1–3]. However, there were few reports investigating IVM concentration in the skin including stratum corneum in either humans [4,5] or animals [6,7]. The relationship between the plasma and skin concentrations remains unclear.

In general, there are four mechanisms by which drugs can distribute to the stratum corneum as follows. As two of the mechanisms, drug molecules in subcutaneous blood vessels are secreted to the surface of the skin through the sweat glands or the sebaceous glands. Which mechanism is responsible for the transport of a molecule depends on its liposolubility. It was reported that both itraconazole and terbinafine were secreted with the sebum, while micro amounts of itraconazole (log P = 5.6) were secreted also with the sweat but terbinafine (log P = 7.4) was not secreted with sweat [8]. The other two mechanisms of distribution of IVM to the surface layer are by simple diffusion and by turnover of the stratum corneum. IVM was thought to be transported to the surface of the skin via the sebaceous glands, since the concentration was reported to be higher on the surface of the skin from an oily area than that from a non-oily area in humans [5]. However, the detailed mechanism remains unclear. In addition, little information is available from preclinical studies about distribution of IVM to the stratum corneum. In the present study we investigated the pharmacokinetics of IVM in plasma and the dermis–epidermis to examine the relationship between those concentrations, and to clarify the possible mechanism by which IVM is transported to the stratum corneum in rats.

2. Materials and methods

2.1. Animals

Male Hairless Wistar Yagi (HWY) and Wistar rats (7 weeks old) were obtained from Japan SLC Inc. (Shizuoka, Japan) and used after

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more than 1 week of acclimatization. All rats were housed in a temperature (23 ± 1 °C) and humidity (55 ± 5%)-controlled room with 12-h light/dark cycle. Water was available ad libitum throughout the study. Food was also available ad libitum throughout the study except for the experiments as described below. The protocols were approved by the institutional review committee in the Tokyo University of Science as animal experiment protocols No. Y13033 and Y14032. All animals were handled in accordance with the institutional and national guidelines for the care and use of laboratory animals.

2.2. Animal studies

Three animal experiments were conducted as described below. For oral administration of IVM in the experiments, 400 μg/mL of IVM solution was prepared as follows: Ivomec® Injection (commercially available injectable formulation of IVM, Merial Japan Co., Ltd., Tokyo, Japan) was diluted with a mixture of propylene glycol:ethanol:water = 13:27:60 (v/v/v) according to our previous reports [3].

2.2.1. Experiment 1

The fur from the dorsal skin of nine Wistar rats (8 weeks) was shaved by hair clippers with extra precautions to avoid damage to the skin under isoflurane anesthesia on the day before the experiments, and then the rats were fasted overnight. They were equally divided to 3 groups ($n = 3$), and were administered IVM orally at the doses of 400, 800 and 1600 μg/kg, respectively. At 0 (pre-dose), 1, 2, 4, 6, 9, 12, 24, 48, 96 and 144 h after the administration 0.2 mL of blood was collected using heparinized syringes from the jugular vein under isoflurane anesthesia. The plasma was obtained by centrifugation of the blood at 3000 g at 4 °C for 10 min. At the same sampling time points about 0.5 mm thickness of the dermis–epidermis sample (about 5–10 mm square) was excised from the shaved area using a surgical knife according to Sobue et al. [9]. The plasma and dermis–epidermis were stored at −80 °C until the determination of IVM concentration.

Hairless Wistar Yagi (HWY) rats (8 weeks, $n = 3$), on which the dorsal skin had been shaved to remove vellus hair completely, were orally administered IVM at a dose of 800 μg/kg, and then blood and dermis–epidermis were collected at 0 (pre-dose), 1, 2, 4, 6, 9, 12, 24, 48, 96 and 144 h after the administration as described above. The plasma was obtained by centrifugation of the blood. The plasma and dermis–epidermis were stored at −80 °C until the determination of IVM concentration.

2.2.2. Experiment 2

Wistar rats (8 weeks, $n = 4$), on which the dorsal skin had been shaved, were fasted overnight and then 800 μg/kg IVM was administered orally to them, and 0.2 mL of blood was collected at 0 (pre-dose), 1, 2, 4, 6, 9 and 12 h after the administration as described above. The plasma was obtained by centrifugation of the blood. Twelve hours after the administration stratum corneum was collected from the shaved area by a tape stripping method using D-squame® Skin Sampling Discs (CuDerm Corporation, Dallas, TX, USA) according to Thiele et al. [10] as follows. The disc was affixed to the shaved area of the back, and then peeled off to collect the stratum corneum. A disc was affixed and peeled a total of 20 times at the same spot using a new disc for each sample. The weight of the disc was measured before and after use to determine the collected amount of stratum corneum. The first 10 samples and the second 10 samples were defined as outer and inner stratum corneum, respectively. The disc samples were stored at −20 °C until the measurements of the IVM concentration. After the sampling of stratum corneum was completed, the remaining dermis–epidermis in the same shaved area was collected as described above. Those samples were stored at −80 °C until the determination of IVM concentration.

2.2.3. Experiment 3

Ten Wistar rats (8 weeks), on which the abdominal and dorsal skin had been shaved, were fasted overnight. Then the skin surface of the foot pads of one forelimb and one hindlimb, the abdomen and the back were swabbed with ether to remove sebum immediately before the administration of IVM according to Archibald et al.[11]. After that, 800 μg/kg IVM was administered orally to them and they were placed under isoflurane anesthesia for 6 or 12 h (five rats for each time duration) to prevent them from removing IVM from the surface of the skin by licking or walking. In the group observed for 6 h, 0.2 mL of blood was collected at 1, 2, 4 and 6 h after the administration and dermis–epidermis were collected from the foot pads of one forelimb and one hindlimb at 6 h in the same manner as in experiment 1. In the group observed for 12 h, blood was collected at 1, 2, 4, 6, 9 and 12 h, and dermis–epidermis were collected at 12 h after administration as described above. The plasma was separated from blood by centrifugation as described above and was used for the determination of IVM concentration. The dermis–epidermis were used for the determination of IVM and squalene (SQ) concentrations. Since SQ is a unique component of sebum and secreted to the surface via the sebaceous glands [12,13], its concentration was used an index of the sebaceous gland activity.

2.3. Determination of IVM concentration in plasma and skin samples

IVM concentration in plasma was determined as described previously [14]. Briefly, plasma was mixed with acetonitrile containing acetic acid and abamectin (ABM; internal standard: Hayashi Pure Chemical Industries, Ltd., Osaka, Japan). The mixture was centrifuged to obtain the supernatant for the measurement. The supernatant was analyzed by a high performance liquid chromatography (HPLC) system and tandem mass spectrometer (API-3200 LC–MS/MS system, HPLC; Shimadzu, Kyoto, Japan, MS/MS; AB Sciex, Framingham, MA), which was equipped with Symmetry C_{18} column (2.1 × 150 mm, 5 μm, Waters, Milford, MA). Mass spectrometry was performed using electrospray ionization in the positive ion mode. The transitions m/z 897.5 to 329.3 for IVM and m/z 895.5 to 327.1 for ABM were detected in the multiple reaction monitoring mode.

The amount of IVM in the stratum corneum on the discs used for sample collection was determined by the following procedure. Each set of 10 discs (for outer or inner stratum corneum) was immersed in 150 μL of methanol containing 300 ng/mL of ABM, and it was shaken well to extract the IVM into the methanol. Then, the extracted IVM solution was mixed with 850 μL of ultra-pure water and was cleaned up by solid-phase extraction using Sep-Pak® cartridge (Vac 1 cc/50 mg C_{18} Cartridges, Waters Corporation, Milford, MA, USA) according to Asbakk et al. [15], with slight modification as follows. The cartridge was pre-conditioned by 1 mL of methanol and 2 mL of ultra-pure water. Then the sample was applied to the cartridge at a flow rate of about 2 mL/min. Then unbound materials and water were washed out with 500 μL of ultra-pure water containing 20% (v/v) methanol. The column was eluted with 500 μL of acetonitrile. Then the eluate was evaporated and the residue was reconstituted with 150 μL of acetonitrile. The reconstituted sample was analyzed by LC–MS/MS system as described above.

The dermis–epidermis were homogenized with 60% methanol and centrifuged. The supernatant was mixed with acetic acid and ABM, and was centrifuged to obtain the supernatant in the same manner as that for pretreatment of plasma. The supernatant was analyzed by LC–MS/MS system as described above.

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