and 4-item conditions in the verbal WM task. Alternatively, the observed negative correlation between participants' negative moods and the PFC activity might not be attributed to the pure WM-related activity, but rather might be derived from other verbal-related cognitive process shared across both low and high WM-load conditions of the verbal WM task. In addition, as we found the mood states were correlated with activation values for the first peak (a 5-s period starting 5 s after S1 onset) not for the second peak (a 5-s period starting 5 s after S2 onset; see Supplementary Table S3), the activity of our main finding might reflect rehearsal process in the maintenance period and not the central executive. Thus, it is not clear if the WM alone reflects the PFC activity, and future studies are necessary to clarify the cognitive components responsible for the relationship between the PFC activity and mood states.

In addition, it might be pointed out that the changes in brightness of the fixation cross and/or the auditory cues in the WM tasks could influence the PFC activity. Although we did not test the influence of the slight changes in the visual and auditory stimuli on the brain signals, one characteristic of our study was a selective correlation with negative mood in the verbal WM task, which was not found in the spatial WM task. As these two WM tasks share the changes in brightness of the fixation cross and the auditory cues, it is clear that these visual/auditory stimuli themselves did not intervene in our main finding.

4.5. Conclusion

In spite of some limitations described above, we demonstrated that individuals experiencing higher levels of negative moods during the past week (as assessed with the POMS) showed lower levels of PFC activity during a verbal WM task, which replicated the results of our previous study based on an independent sample (Aoki et al., 2011). Moreover, this relationship was not explained by individual differences in personality traits or by age, gender, handedness, or task performance. The results extend our previous work by controlling for personality differences among individuals and provide valuable insight into the neurobiological substrates of natural mood, which should be distinguished from those of personality.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.pscychresns.2012.10.009.

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Transdermal delivery of adriamycin to transplanted Ehrlich ascites tumor in mice

Masataka Shiozuka, Yoshiaki Nonomura, and Ryoichi Matsuda*

Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan; cmuscle@mail.ecc.u-tokyo.ac.jp (M.S.); cnono@mail.ecc.u-tokyo.ac.jp (Y.N.); cmatsuda@mail.ecc.u-tokyo.ac.jp (R.M.)

*Corresponding author: cmatsuda@mail.ecc.u-tokyo.ac.jp (R.M.); Tel.:+81-3-5454-6637

Abstract:

The transdermal delivery of anti-cancer drugs is considered to be unfeasible due to the low permeability of drugs through the dermis. However, we previously showed that a thioglycolate-based depilatory agent increases the drug permeability of mouse skin. In the present report, we investigated the skin permeability and efficacy of the anti-cancer drug adriamycin increased when administered transdermally to mice in combination with a thioglycolate-based depilatory agent. Adriamycin in combination with depilatory treatment significantly reduced Ehrlich tumor growth in hairless mice as compared to that of non-depilatory-treated hairless mice. In addition, our transdermal delivery method for adriamycin increased the therapeutic effectiveness of this agent by decreasing toxicity. Moreover, measurement of adriamycin autofluorescence revealed that transdermally applied adriamycin penetrate the dermis after depilatory agent treatment. These results indicate that the transdermal delivery of anti-cancer drugs is feasible by pretreating skin with a thioglycolate-based depilatory agent.

Keywords: Trandermal drug delivery; Thioglycolate; Adriamycin

1. Introduction

Oral and intravenous administration are the two main drug delivery routes for anti-cancer drugs. Transdermal delivery is considered to be unfeasible for cancer treatment due to the low permeability of drugs through the dermis. However, the skin is the largest organ in the body and an obvious route for both local and systemic drug delivery. Thus, the transdermal delivery of anti-cancer drugs may be useful in the clinical settings if the skin permeability of drugs can be increased.

We previously showed that the skin permeability of gentamicin increased when combined with a thioglycolate-based depilatory agent [1]. Ultrastructural studies revealed that alteration and expansion of intracellular spaces in the epidermis and dermis were responsible for the increase of drug permeability of depilatory agent-treated skin. Transdermal drug delivery possesses several advantages over oral and intravenous drug administration [2], in that it: 1) bypasses gastrointestinal incompatibility and the hepatic 'first-pass' effect; 2) reduces side-effects through optimization of blood concentration-time profiles; 3) involves patient-activated/patient-modulated delivery, which enhances patient compliance; 4) enhances target specificity; and 5) reduces medical treatment costs.

Adriamycin (doxorubicin hydrochloride) is an anthracycline antibiotic that is commonly used in the treatment of a wide range of cancers, carcinomas and soft tissue sarcomas. Here, we investigated whether thioglycolate-based depilatory agent-treatment increases the skin permeability of adriamycin and its anti-tumor activity for cancer cells grown underneath the skin. To determine the efficacy of our depilatory method, the anti-tumor effect and distribution of adriamycin applied as a cream to Ehrlich solid tumor-bearing hairless mice were examined by measuring tumor size and adriamycin autofluorescence, respectively.

2. Experimental Section

Adriamycin cream was prepared by mixing Adriacin (Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) with White Ointment (Nikko Pharmaceutical Co., Ltd., Gifu, Japan) using a planetary centrifugal mixer, AR-100 (Thinky INC., Tokyo, Japan). The final concentration of adriamycin was 0.2 or 0.6 mg/g of cream. Five-week-old female hairless mice (HR1; body weight, approximately 20 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan) and were housed individually under controlled temperature and humidity conditions, and had free access to water and food. The present study was approved by Animal Ethics Committee of the University of Tokyo.

Ehrlich carcinoma cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37 ˚C in a humidified 5% CO_2 atmosphere. To prepare cells for transplantation into mice, exponentially growing cells were harvested, washed, and then resuspended in RPMI 1640. Ten million of Ehrlich carcinoma cells were transplanted subcutaneously into the backs of hairless mice. Three days after the transplantation, mice were randomly divided into 4 groups containing 6 animals per group.

Depilatory cream was obtained from Reckitt Benckiser Co., Ltd. (Tokyo, Japan) and was applied once every 3 days for 1 min to the mouse skin, which was then rinsed with warm water to remove the cream. Adriamycin cream (0.5 g) was then gently applied by rubbing onto the skin of the transplantation site daily for 21 days (a total of 7 treatments with depilatory cream were performed during this time). Treatment started 3 days after tumor cell transplantation when tumors reached a diameter of approximately 1 cm. At the end of the treatment, the mice were euthanized with an overdose of ether. Euthanasia and carcass disposal were performed in accordance with the institutional guidelines for animal care of the University of Tokyo. Solid tumors were washed with saline after being excised, and then weighed.

To examine the distribution of adriamycin, 10-μm cryosections of the tumor specimens were prepared and then examined under a fluorescence microscope (Axioplan, Carl Zeiss GmbH, Oberkochen, Germany) to visualize adriamycin, which has excitation and emission peaks of 488 nm and 556/582 nm, respectively. The obtained images were optimized for contrast and brightness using Photoshop CS5 software (Adobe Inc., San Jose, CA, US).

3. Results and Discussion

Adriamycin is a potent anti-cancer agent that is clinically useful for the treatment of acute leukemias, malignant lymphomas, and carcinomas [3]. In the present study, we have shown that the use of depilatory treatment allows for the potential transdermal delivery of this anti-cancer drug. We examined the anti-tumor effect of adriamycin administered transdermally to hairless mice bearing solid tumors that were induced by transplantation of Ehrlich's carcinoma cells, which are widely used to form xenografts. Ehrlich carcinoma is a transplantable, poorly differentiated malignancy and grows in both solid and ascitic forms [4]. The anti-cancer effects of adriamycin with the depilatory treatment were evaluated by the inhibition of tumor growth, which was determined by comparing the weight and size of harvested tumors from untreated control and treated mice. As shown in Fig. 1, transdermal treatment with adriamycin cream led to a reduction in tumor size compared to the untreated control group. The effect of adriamycin in solution was also examined by applying the solution (0.1 mg/0.2 mL saline/ day) directly onto skin, but no reduction in tumor size was observed (data not shown). Moreover, subcutaneous injection of the adriamycin solution (0.1 mg/0.2 mL saline/day) into the transplantation site resulted in the death of all mice by the 15th consecutive day of treatment, whereas all mice administered adriamycin transdermally lived until the end of experiment.

We next compared the anti-tumor effects of adriamycin of two different doses and intervals during a 14-day treatment period. The daily transdermal administration (0.05 mg/0.5 g) and intermittent (once every 3 days) subcutaneous injection of adriamycin (0.05 mg/0.2 mL) had equivalent anti-cancer effects on tumor weight (Fig. 2). In contrast, the transdermal administration of adriamycin resulted in statistically higher increase in the body weight gain rate ($p<0.05$), which was used as a measure of toxicity, compared to subcutaneous injection, and was statistically similar to the control. These data suggest that the transdermal delivery of adriamycin can increase its therapeutic effectiveness by diminishing toxicity without affecting its anti-tumor activity.

We also examined the distribution of adriamycin following its application in cream form to the back area of hairless mice pre-treated with and without a depilatory agent. Adriamycin was detected in thin sections of the treatment area by its autofluorescence [5]. As shown in Fig. 3, only transdermally administered adriamycin with the depilatory agent was observed in the epidermis to dermis and reached the underlying muscle layer.

We previously reported that liquid chromatography-tandem mass spectrometry (LC-MS/MS) can be used to validate the effect of a depilatory agent on the *in-vivo* permeation of gentamicin [1]. LC-MS/MS analysis of the sera and muscle tissue extracts of hairless mice confirmed that the treatment drug was not detectable in the non-depilatory-treated group, but was present in the depilatory-treated-group. Using electron microscopy, we previously observed a large expansion of the intercellular gaps and extraordinary spaces in the basal and prickle-cell layers in depilatory agent-treated mice [1]. These results indicate that alteration and expansion of the intracellular spaces in the basal and prickle-cell layers of dermis may be due to the shrinkage of cells in those layers, which in turn leads to reduced resistance. Lee *et al.* [6] have shown that depilatory agents enhance transepidermal drug delivery by reducing the resistance of both the transcellular and intercellular

routes of the stratum corneum. These findings, together with our present results for adriamycin, suggest that the combination of a skin impermeable drug with a depilatory agent increases drug penetration into the epidermis, where it produces a loco-regional of systemic effect through the vascular network.

Transdermal drug delivery systems offer many advantages over conventional administration routes [7]. However, given the low permeability of external molecules through the skin, it remains a minor portal of entry for drugs in the clinical setting [8]. Therefore, various approaches aimed at decreasing the resistance of skin to drug penetration have been investigated [9]. For example, Herai *et al.* [10] found that the penetration enhancer monoolein significantly increased the *in-vitro* skin permeation and retention of adriamycin in the stratum corneum. In addition, Han *et al.* [11] reported that although transdermal adriamycin delivery is enhanced by liposomal formulations, topical applications have a few limitations with regard to delivery capacity and speed. The liposome-mediated delivery of adriamycin proceeds through follicular routes and has a significant synergistic effect in combination with iontophoresis.

Figure 1. Effect of transdermally administered adriamycin on tumor growth in hairless mice.
In mice treated with adriamycin cream (0.1 or 0.3 mg/day), the tumor size and weight (middle and bottom sections) were significantly smaller than those in the untreated control group (upper sections). A higher dose of adriamycin (bottom section) slightly improved the inhibition of tumor growth. Scale bar = 1 cm

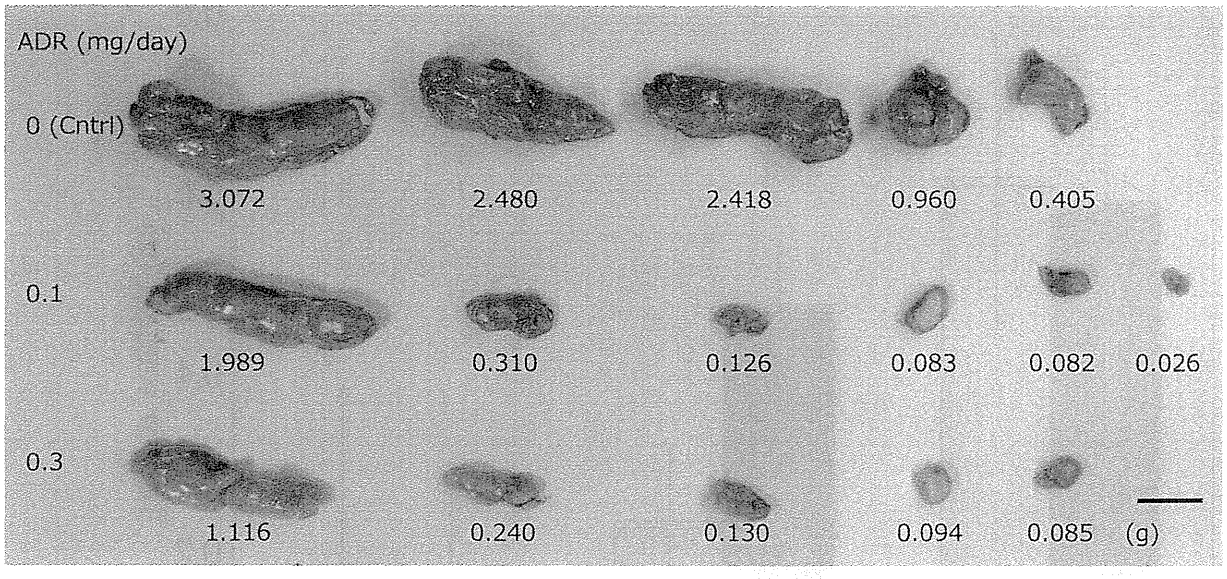


Figure 2. Comparison of transdermal and subcutaneous administration of adriamycin by weight gain rate and tumor weight.

The body weight gain rate (black bars, left axis) and tumor weight (striped bars, right axis) of tumor-bearing mice transdermally administered (TD) and subcutaneously injected (SC) with adriamycin were compared. The daily transdermal administration and the once every three days subcutaneous injectiond of adriamycin resulted in similar anti-cancer effects, as estimated by the tumor weight. However, transdermal adriamycin administration led to a higher weight gain rate in mice than subcutaneous injection ($p<0.05$, Welch's t-test), and was statistically similar to the level in the untreated control. Data are shown as the mean ± S.D.

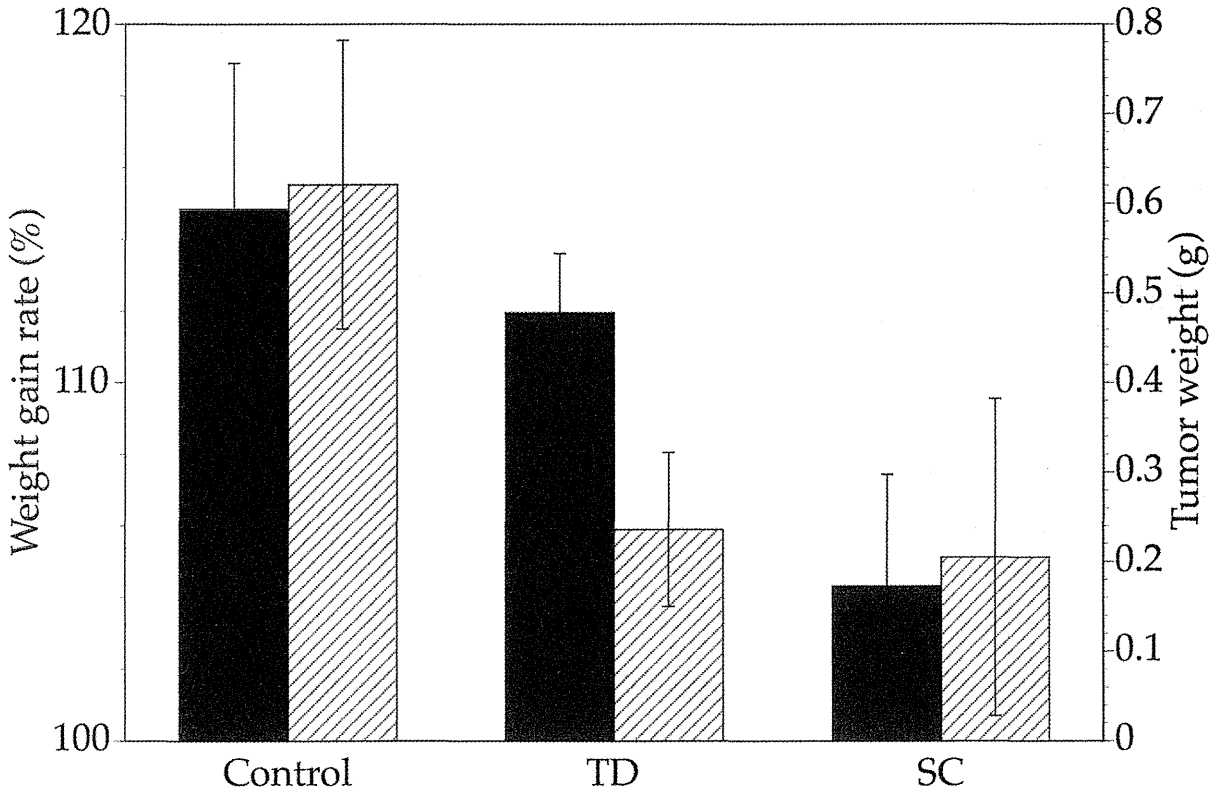
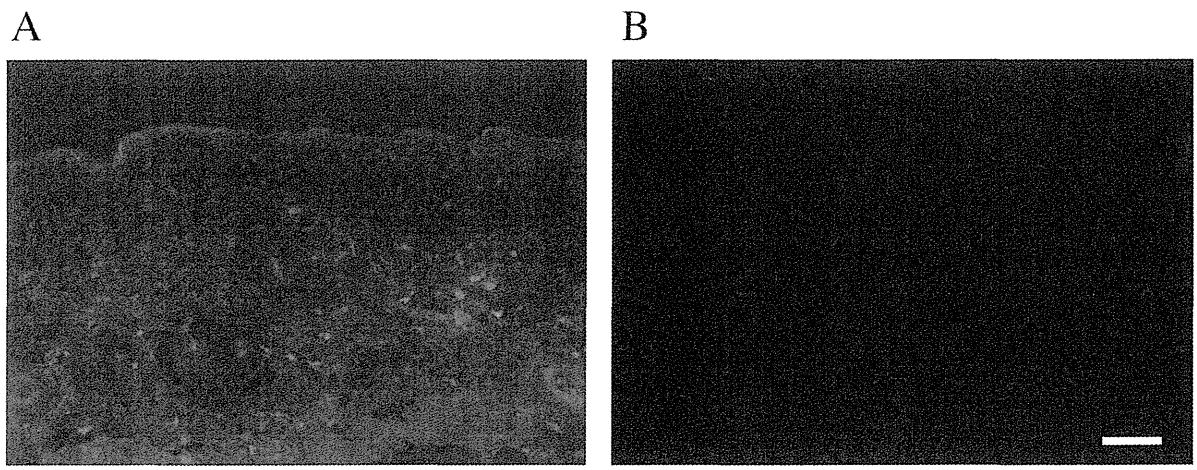


Figure 3. Distribution of transdermally administered adriamycin.

Adriamycin cream was transdermally applied to the skin of hairless mice for three consecutive days with (A) or without (B) pretreatment with a depilatory agent. The distribution of adriamycin was then detected based on its autofluorescence. Adriamycin was observed in the dermis/fascia only after pretreatment with the depilatory agent. Scale bar = 10 μm.



4. Conclusions

In conclusion, our results suggest that the pretreatment of skin with a depilatory agent increases the anti-tumor effect against Ehrlich solid tumor. Our present cancer treatment method involves the use of a thioglycolate-based depilatory agent to increase the permeability of the dermal surface and has proven to be more convenient and effective than injection. Thus, depilatory agent-treatment may be useful for the local application and systemic delivery of anti-cancer drugs.

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Conflict of Interest

The authors declare no conflict of interest.

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