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Letter to the Editor

Efficiency of sirolimus delivery to the skin is dependent on administration route and formulation

Since sirolimus (rapamycin) was discovered as an inhibitor of mammalian/mechanistic target of rapamycin (mTOR), investigators have focused on its potential as an anti-tumor drug because mTOR signaling controls complex cellular processes, including protein synthesis, cell cycle, and cell growth, and is frequently hyperactivated in tumor tissues [1]. Tuberous sclerosis complex (TSC) is an autosomal-dominant disease that results in hamartoma formation in almost all organs, including brain, heart, kidney, lung, and skin. Loss of function of *TSC1/TSC2* genes causes constitutively activated mTOR signaling [2]. Oral administration of sirolimus and its derivative demonstrated efficacy in TSC tumors [3–5]. Although both oral and topical sirolimus therapies improved facial angiofibromas associated with TSC, topical application led to more rapid improvement than oral administration [6,7]. We hypothesize that this effect results from differences in sirolimus delivery to the skin between topical and oral administration routes. Blood sirolimus levels are typically assessed following therapy, and information about tissue drug levels from clinical or animal tests is scarce [7,8]. Thus, we evaluated skin and blood sirolimus concentrations following topical or oral sirolimus administration in mice to more thoroughly investigate the sirolimus delivery properties.

We tested drug delivery to skin and blood following a single administration of different concentrations of sirolimus gel (SG) to the back skin of hairless (HR) mice (Supplementary Materials and methods; Fig. 1a). Sirolimus was detected in the skin in all test groups within 1 h in a dose-dependent manner (median, 0 [0% SG, 0 mg dose], 0.30 [0.05%, 0.063 mg], 0.64 [0.2%, 0.25 mg], 1.3 [0.4%, 0.5 mg], and 6.9 ng/mg [0.8%, 1 mg]) but was detected in blood only in mice that received 0.8% SG (median, 0.29 ng/ml; Fig. 1b). A time-course analysis of skin and blood sirolimus concentrations after administration of topical SG (0.8%, 1 mg dose) or Oral S (1.25 mg/ml, 0.25 mg dose) revealed that skin sirolimus concentrations were essentially constant (Fig. 1c and d). Administration of 0.8% topical SG resulted in the following median skin sirolimus concentrations: 2.5, 6.3, 7.2, 8.7, and 3.6 ng/mg after 1, 3, 6, 12, and 24 h, respectively (Fig. 1c). Median blood sirolimus concentrations increased at 12–24 h after 0.8% topical SG, with levels of 0.69, 0.55, 4.5, 98, and 130 ng/ml after 1, 3, 6, 12, and 24 h, respectively (Fig. 1c). By contrast, Oral S did not significantly enhance skin sirolimus concentrations (0.058, 0.081, 0.043, 0.059, and 0.0085 ng/mg after 1, 3, 6, 12, and 24 h, respectively), although blood sirolimus levels in these animals declined with time (200, 230, 65, 19, and 3.9 ng/ml after 1, 3, 6, 12, and 24 h, respectively; Fig. 1d). We tested the effects of multiple applications of 0.8% SG (1 mg dose) administered for 1, 3, 7, and 15 d. Median skin sirolimus concentrations 1 d after the final SG treatment remained constant without a concomitant increase in blood sirolimus levels following the repeated SG applications (Fig. S1). These combined results suggest that topical application of SG more efficiently delivers the drug to the skin than Oral S administration in HR mice.

We next prepared different topical sirolimus formulations to test whether the drug delivery properties depend on the delivery material. We performed a time-course study of the delivery efficiencies of sirolimus cream (SC) and sirolimus liniment (SLN). Median sirolimus concentrations at 1, 3, 6, 12, and 24 h after a single application of 0.4% SC were 15, 25, 29, 25, and 31 ng/mg, respectively, in the skin and 0, 0, 1.2, 23, and 34 ng/ml, respectively, in the blood (Fig. 2a). Median sirolimus concentrations at 1, 3, 6, 12, and 24 h after a single application of 0.4% SLN were 1.1, 1.5, 1.3, 1.2, and 0 ng/mg, respectively, in the skin and 0, 130, 590, 540, and 100 ng/ml, respectively, in the blood (Fig. 2b). Skin sirolimus levels were essentially constant, whereas blood sirolimus levels increased after 12 and 24 h in SC- and SG-treated mice (Figs. 1c, 2a, and c). In contrast, skin sirolimus levels in SLN-treated mice were constant at 1, 3, 6, and 12 h and then declined to essentially undetectable levels at 24 h. Blood sirolimus levels in SLN-treated mice increased after 3 h, then reached higher levels than those in blood of SC- or SG-treated mice at 6 and 12 h and then declined at 24 h (Fig. 2b and c). Estimated sirolimus delivery (% dose) to skin was higher following topical administration than following oral administration, demonstrating more efficient skin penetration and retention by SG and SC and more rapid permeation to blood by SLN (Fig. 2d). Blood sirolimus delivery via topical SLN was comparable to that of Oral S according to the estimated % dose in blood at 1, 3, 6, 12, and 24 h (Oral S, 0.16, 0.18, 0.052, 0.015, and 0.0031%, respectively; topical SLN, 0, 0.053, 0.24, 0.22, and 0.042%, respectively [Fig. 2d]). These results indicate efficient sirolimus delivery is dependent not only on dosage but also on the delivery route and vehicle. Sirolimus solvents, including ethanol, diethylene glycol monoethyl ether, and some fatty acids that were included in the base material of the liniment, are known to modulate skin penetration and retention [9]. These findings suggest that topical sirolimus formulation should be selected based on their optimized delivery to the intended therapeutic target of the skin.

This study had several limitations. Because mouse skin is much thinner than human skin and applied sirolimus doses were higher than for clinical use, the topical sirolimus used in this study may penetrate mouse skin more easily than human skin. At least, we have confirmed penetrated sirolimus in neurofibroma type 1 tumors after 6 months of SG treatment (0.2% and 0.4%) by LC–MS/MS (unpublished data) in a clinical trial. Further studies are required to understand percutaneous sirolimus penetration

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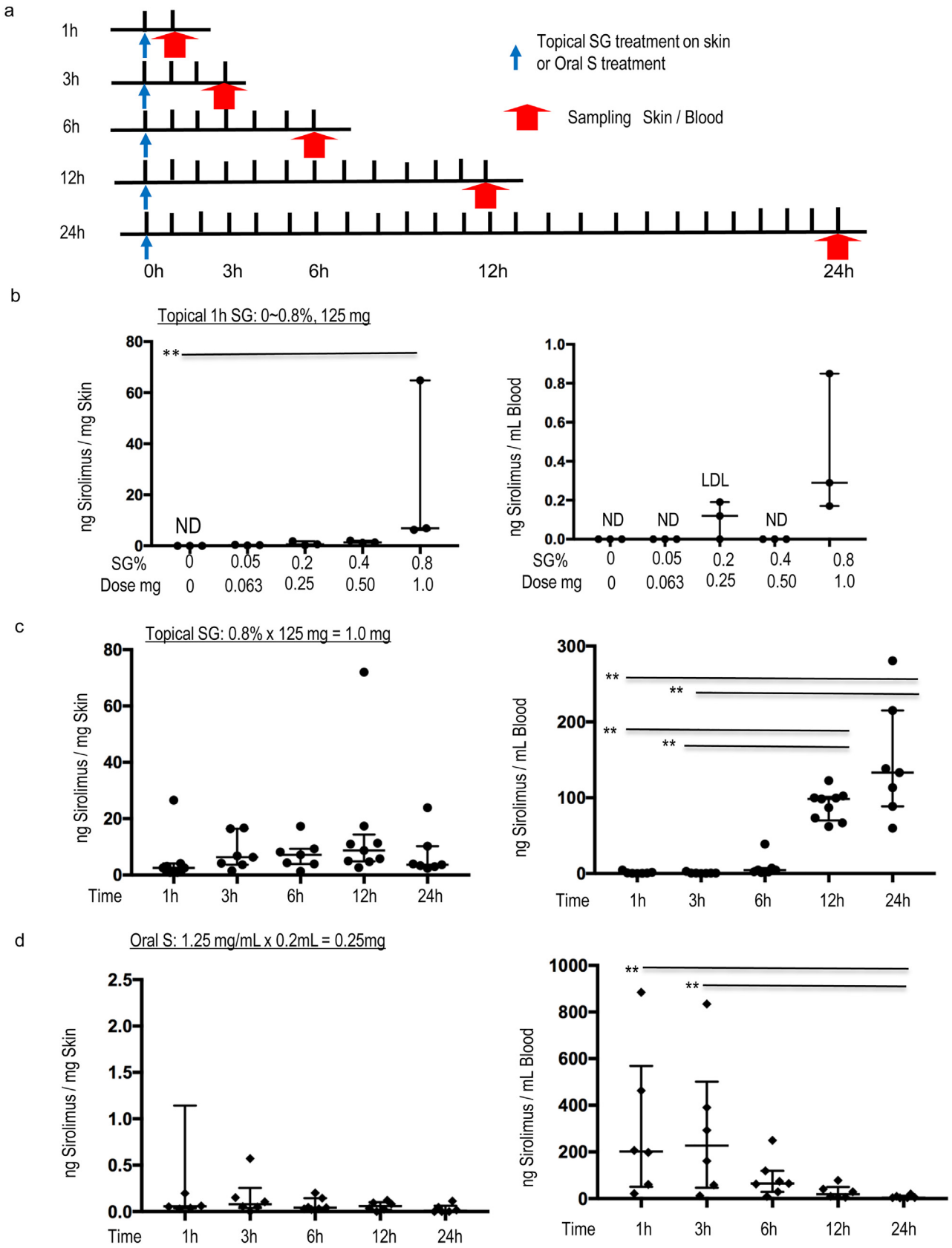

Fig. 1. Evaluation of skin and blood sirolimus levels by liquid-chromatography and tandem mass spectrometry (LC–MS/MS) after a single administration of oral or topical sirolimus in hairless mice. (a) Schematic of animal experiment. (b) Skin and blood sirolimus concentrations 1 h after topical applications of indicated concentrations and final sirolimus doses of SG formulations. $n = 3$. (c) Skin and blood sirolimus concentrations at the indicated time after application of SG (0.8%, 1 mg dose). $n = 6–9$. (d) Skin and blood sirolimus concentrations at the indicated time after Oral S (1.25 mg/ml, dose 0.25 mg) administration. $n = 6$ or 7. SG, sirolimus gel; Oral S, oral sirolimus. ND, not detected; LDL, lower detection limit. Bars show medians with interquartile ranges. $^{**}p < 0.01$ by Kruskal Wallis test with Dunn's multiple comparison tests.

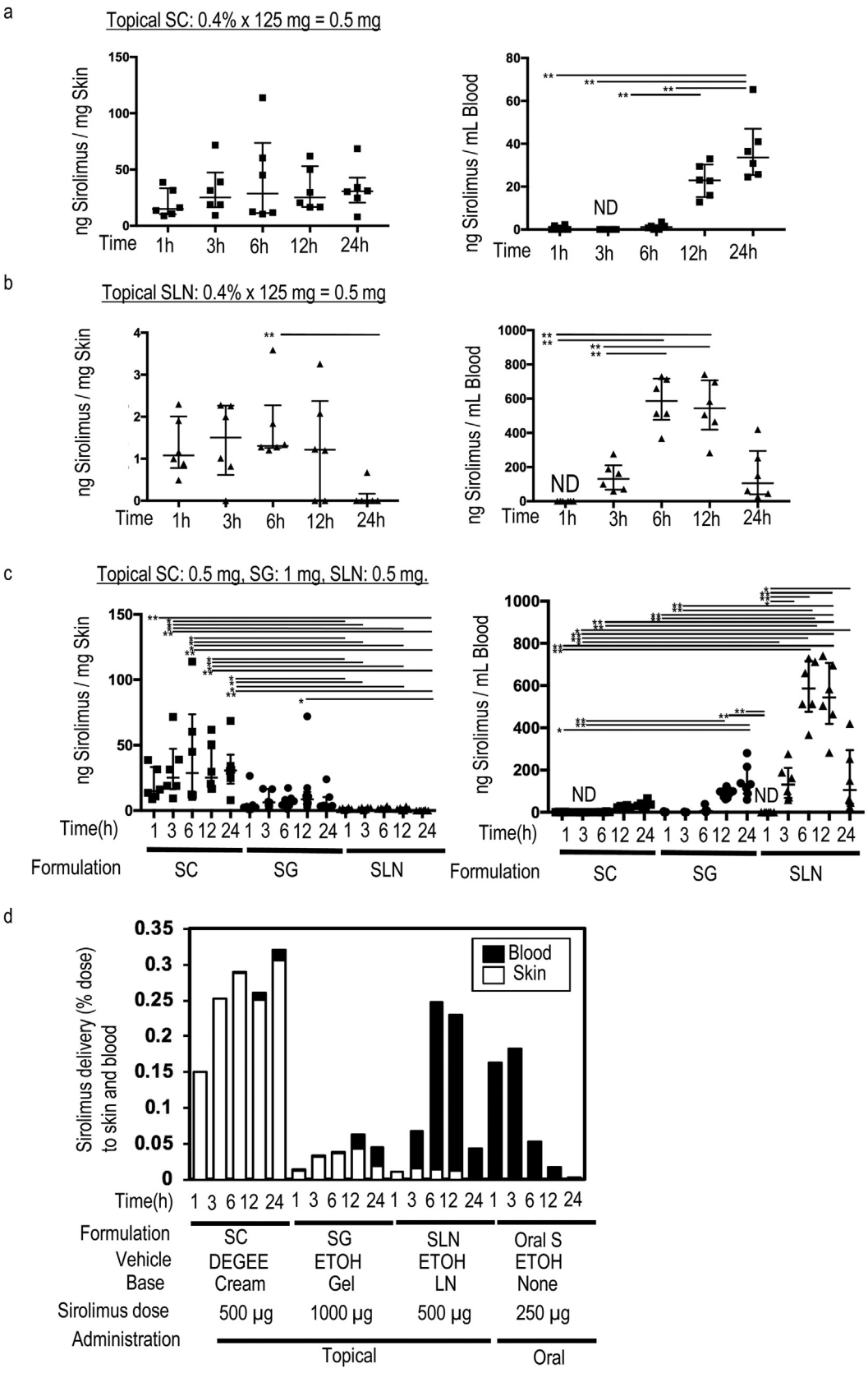

Fig. 2. Efficiency of sirolimus delivery to skin and blood depends on sirolimus formulation. Time-course study of skin and blood sirolimus concentrations after a single topical treatment with (a) SC (0.4%, 0.5 mg dose; $n = 6$) and (b) SLN (0.4%, 0.5 mg dose; $n = 6$). (c) Comparison of skin and blood sirolimus concentrations after topical application of SC, SG, or SLN treatments indicated in Figs. 1c, 2a, and b. $n = 6$–9. (d) Estimates of sirolimus deliveries (% dose) in skin and blood after a single administration of the selected sirolimus formulation at the indicated timepoint. Bars indicate median with interquartile range. $^{**}p < 0.01$, $^{*}p < 0.05$ by Kruskal–Wallis with Dunn's multiple comparisons test. ND, not detected; SG, sirolimus gel; SC, sirolimus cream; SLN, sirolimus liniment; Oral S, oral sirolimus; DEGEE, diethylene glycol monoethyl ether; ETOH, ethanol; LN, liniment.

mechanisms as well as to optimize topical sirolimus formulations for specific therapies.

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Conflicts of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.05.002.

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