SUPPORTING INFORMATION

Visualizing Dermal Permeation of Sodium Channel Modulators by Mass Spectrometric Imaging

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Materials/Methods

Preparation of STX-ge: To a solution of STX–hexylamine tris-heptafluorobutyrate (1.5 mg, 1.4 μmol) in 250 μL of a 1:3 mixture of pH 9.5 buffer (0.1 M $NaHCO_3/Na_2CO_3$) and MeCN was added the glycerol ether N-hydroxysuccinimide ester (1.9 mg, 4.6 μmol, 3.3 equiv). The solution was stirred for 2.25 h at room temperature, then acidified with 1 mL of 0.1% aqueous trifluoroacetic acid (TFA). The mixture was filtered through a Fisher 0.2 μm PTFE syringe filter and the filter washed with an additional 0.5 mL of 0.1% TFA. The filtrate was purified by reverse phase HPLC (Altima C18, 10 μm, 22 x 250 mm column, eluting with a gradient flow over 40 min of 25:75 MeCN/0.1% aqueous CF_3CO_2H → 80:20 MeCN/0.1% aqueous CF_3CO_2H, 214 nm UV detection). At a flow rate of 12 mL/min, STX-ge had a retention time of 21.4–22.8 min and was isolated following lypophilization as a white powder (0.7 mg, 0.75 μmol, 54%). ^{1}H NMR (D_2O, 500 MHz) δ 4.67 (s, 1H), 4.22 (dd, 1H, *J* = 10.0, 10.0 Hz), 4.03-3.98 (m, 1H), 3.80-3.74 (m, 2H), 3.68-3.64 (m, 1H), 3.62-3.46 (m, 11H), 3.14 (dd, 2H, *J* = 6.5, 6.5 Hz), 3.12-3.06 (m, 2H), 2.43-2.26 (m, 2H), 2.21 (dd, 2H, *J* = 7.0, 7.0 Hz), 1.62-1.43 (m, 12H), 1.36-1.28 (m, 10H), 0.89 (t, 6H, *J* = 7.0, 7.0 Hz) ppm; HRMS (ES^+) calculated for $C_{33}H_{62}N_8O_8$ 698.4691 found at *m/z* 699.4760 (MH^+).

Human tissue: Human skin samples were obtained from four patients undergoing plastic surgery at Stanford Medical School. All patients gave written informed consent following approved IRB protocols. Within minutes of surgical resection, the excess fat was carefully removed from the inner layers of the

skin specimen, which was then placed in a shallow dish partially filled with synthetic interstitial fluid. The surface of the skin was carefully handled and dried prior to compound application. Confined circular treatment areas (d = 1 cm) were created by delineating the application area with ink and surrounding the site with Vaseline. For each application site, 25 nmol of compound was applied in 25 µL of vehicle. After 1, 4 or 10 hours of application, the skin was frozen in a -80° C freezer. Five different compounds were tested on human skin: lidocaine (Sigma Aldrich), aconitine, saxitoxin, STX-ge, and BTX-A derivative. The two vehicles tested were ethanol (Fisher) and DMSO (Fisher).

UV burn injury: Confined circular treatment areas of the human skin samples were exposure to UV (300 to 450 nm, 3100mJ/cm^2) irradiation before application of the compound. UV light was directly applied to skin over 25s[1] using the BlueWave® 200 Version 3.0 UV Curing Spot Lamp with Intensity Adjustment (Dymax Inc., Torrington, CT). After UV exposure, 25 nmol of compound was applied in 25 µL of vehicle to each application site. After 4 hours of application, the skin was frozen in a -80° C freezer.

Animals: Animal experiments were conducted using ~300 g male Sprague Dawley rats. The hair on the back of the rats was removed 24 h before drug application. Under urethane anesthesia, confined circular treatment areas (d = 1 cm) were created by marking the application area with ink and surrounding the application area with Vaseline. For each application site, 25 nmol of the compound was applied in 25 µL of vehicle. After 4 hours of application the animal was sacrificed and its skin removed and frozen in -80° C freezer. The following compounds were tested *in-vivo* rat skin: 1) Saxitoxin, 2) Saxitoxinol, 3) STX-ge and 4) Aconitine in the following vehicles: water, ethanol, DMSO and a cream formulation.

Tissue Sample Preparation: Frozen skin samples were sectioned at 25 µm thickness using a Leica CM1950 cryostat (Leica Mycrosystems Inc., Buffalo Grove, IL). The tissue sections were thaw mounted onto positively charged glass slides. The cryostat section holder and blade were cleaned with 70% ethanol solution in between the preparation of each tissue section to avoid tissue contamination. The slides were stored at -80° C and prior to analysis dried in a dessicator for approximately 15 minutes.

DESI Imaging: A lab-built DESI-MS imaging ion source coupled to a LTQ-Orbitrap mass spectrometer was used for tissue imaging. Mass spectra were acquired in the positive ion mode using Orbitrap as the mass analyzer at 60,000 resolving power in the range *m/z* 100 - 1100. Pure acetonitrile (Fisher) was used as the histologically compatible solvent system for DESI-MSI at a flow rate of 2 µL/min, and

nitrogen gas pressure of 175 psi. The tissue sections were scanned using a 2D moving stage with spatial resolution of 100 µm or 150 µm. The software ImgGenerator (freeware, http://www.msimaging.net/) was used for converting raw files into 2D images. Spatially accurate ion images were assembled using BioMap software (freeware, http://www.maldi-msi.org/).

Histology: After DESI-MS imaging, the same tissue sections were stained using standard hematoxylin and eosin (H&E). H&E stained tissue sections were evaluated by veterinary pathologist Dr. Richard Luong. Optical images of the H&E stained tissue sections were obtained using a microscope Axioskop 2 Plus (Carl Zeiss International, Thornwood, NY) and digitally overlaid with DESI-MS ion images for compounds depth penetration calculation.

Supporting Tables

Table S1. Tentative identification of ions detected from human skin using high mass resolution/high mass accuracy and tandem mass spectrometry analyses.

Ion detected (*m/z*)	Tentative Attribution[a]	Molecular Formula[b]	Mass error (ppm)[c]
140.0679	4 aminopentanoic acid	$C_5H_{11}NO_2Na$	2.40
156.0417	4 aminopentanoic acid	$C_5H_{11}NO_2K$	2.60
184.0729	Phosphocholine	$C_5H_{15}NO_4P$	2.18
518.3205	Lyso-PC(16:0)	$C_{24}H_{50}NO_7PNa$	1.99
534.2945	Lyso-PC(16:0)	$C_{24}H_{50}NO_7PK$	1.36
615.4940	DG(34:2)	$C_{37}H_{68}O_5$	2.90
639.4947	DG(36:4)	$C_{39}H_{68}O_5Na$	1.88
641.5092	DG(36:3)	$C_{39}H_{70}O_5Na$	3.60
672.4198	PC(25:0)	$C_{33}H_{64}NO_9PNa$	1.80
688.3937	PC(25:0)	$C_{33}H_{64}NO_9PK$	1.80
715.5622	DG(40:2)	$C_{43}H_{80}O_5K$	2.10
717.5781	DG(40:1)	$C_{43}H_{82}O_5K$	1.70
719.5941	DG(40:0)	$C_{43}H_{84}O_5K$	1.30
725.5557	SM(34:1)	$C_{39}H_{79}N_2O_6PNa$	0.88
741.5302	SM(34:1)	$C_{39}H_{79}N_2O_6PK$	0.70
756.5502	PC(32:0)	$C_{40}H_{80}NO_8PNa$	0.98

772.5240	PE(38:5)	$C_{43}H_{76}NO_7PNa$	1.10
772.5241	PC(32:0)	$C_{40}H_{80}NO_8PK$	0.91
780.5505	PC(34:2)	$C_{42}H_{80}NO_8PNa$	0.90
782.5681	PC(34:1)	$C_{42}H_{82}NO_8PNa$	0.02
785.5681	PG(36:0)	$C_{42}H_{83}O_9PNa$	1.80
787.5839	PG(36:0)	$C_{42}H_{85}O_9NaP$	2.10
796.5240	PC(34:2)	$C_{42}H_{80}NO_8PK$	1.22
798.5406	PC(34:1)	$C_{42}H_{82}NO_8PK$	0.11
804.5502	PC(36:4)	$C_{44}H_{80}NO_8PNa$	1.10
806.5673	PC(36:3)	$C_{44}H_{82}NO_8PNa$	0.79
808.5819	PC(36:2)	$C_{44}H_{84}NO_8PNa$	0.72
811.5840	PG(38:2)	$C_{44}H_{85}O_9NaP$	2.20
813.5999	PG(38:2)	$C_{44}H_{87}O_9NaP$	2.60
824.5556	PC(36:2)	$C_{44}H_{84}NO_8PK$	0.72
825.6926	TG(48:2)	$C_{51}H_{94}O_6Na$	2.00
828.5358	PC(38:6)	$C_{46}H_{80}NO_7PK$	7.50
832.5816	PC(38:4)	$C_{46}H_{84}NO_8PNa$	1.18
848.5555	PC(38:4)	$C_{46}H_{84}NO_8PK$	1.37
849.6932	TG(50:4)	$C_{53}H_{94}O_6Na$	1.27
851.7086	TG(50:3)	$C_{53}H_{96}O_6Na$	2.02
851.7091	TG(50:3)	$C_{53}H_{96}O_6Na$	0.94
853.7252	TG(50:2)	$C_{53}H_{98}O_6Na$	0.50
855.7417	TG(50:1)	$C_{53}H_{100}O_6Na$	0.57
867.6822	TG(50:3)	$C_{53}H_{96}O_6K$	1.90
869.6984	TG(50:2)	$C_{53}H_{98}O_6K$	1.30
875.7086	TG(50:5)	$C_{55}H_{96}O_6Na$	1.54
877.7244	TG(50:4)	$C_{55}H_{98}O_6Na$	1.36
879.7406	TG(54:6)	$C_{55}H_{100}O_6Na$	0.57
881.7582	TG(54:6)	$C_{55}H_{102}O_6Na$	2.25
891.6826	TG(52:5)	$C_{55}H_{96}O_6K$	1.20
893.6984	TG(52:4)	$C_{55}H_{98}O_6K$	1.20
895.7150	TG(52:3)	$C_{55}H_{100}O_6K$	0.09
897.7321	TG(54:5)	$C_{55}H_{102}O_6K$	1.70

899.7084	TG(54:5)	$C_{55}H_{102}O_6K$	2.00
901.7244	TG(54:6)	$C_{57}H_{98}O_6Na$	1.38
903.7415	TG(54:5)	$C_{57}H_{100}O_6Na$	0.98
905.7570	TG(56:7)	$C_{59}H_{100}O_6H$	2.50
907.7733	TG(54:3)	$C_{57}H_{104}O_6Na$	1.56
907.7735	TG(56:8)	$C_{59}H_{103}O_6$	1.70
917.6985	TG(56:7)	$C_{57}H_{98}O_6K$	1.10
919.7145	TG(54:5)	$C_{57}H_{100}O_6K$	0.76
921.7309	TG(54:4)	$C_{57}H_{102}O_6K$	0.11
923.7472	TG(54:3)	$C_{57}H_{104}O_6K$	0.99
929.7557	TG(56:6)	$C_{59}H_{102}O_6Na$	0.40
943.7137	TG(56:5)	$C_{59}H_{100}O_6K$	0.70
945.7298	TG(56:6)	$C_{59}H_{102}O_6K$	0.22
947.7459	TG(56:7)	$C_{59}H_{104}O_6K$	0.22

[a] Tentative assignments were based on data obtained from tandem mass spectrometry experiments and high mass accuracy measurements. TG = triacylglycerol; PC = glycerophoscholine; DG = diacylglycerol; SM = sphingomyelins; PG = glycerophosphoglycerols; PE = glycerophosphoethanolamines; (X:Y) denotes the total number of carbons and double bonds in the fatty acid chains.

[b] Molecular formulas of the protonated or sodium/potassium adducts of the assigned molecules.

[c] Mass errors were calculated based on the exact monoisotopic *m/z* of the protonated or sodium/potassium adducts of the assigned molecules.

Table S2. Average penetration depth in human and rat skins for each compound studied using different vehicles.

Human Skin				
Vehicle	Aconitine	Lidocaine	STX-ge	BTX-A
Ethanol	3.05 ± 0.91 (n = 6)	1.24 ± 0.38 (n = 5)	*No penetration* (n = 7)	2.00 ± 0.61 (n = 4)
DMSO	N/A	N/A	0.62 ± 0.03 (n = 3)	N/A
EMLA	2.49 ± 0.84 (n = 4)	N/A	0.45 ± 0.10 (n = 4)	1.10 ± 0.20 (n = 4)

Rat Skin		
Vehicle	Aconitine	STX-ge
Ethanol	1.90 ± 0.40 (n = 7)	0.90 ± 0.14 (n=5)
DMSO	2.60 ± 0.06 (n=3)	1.12 ± 0.42 (n=7)

Supporting Figures

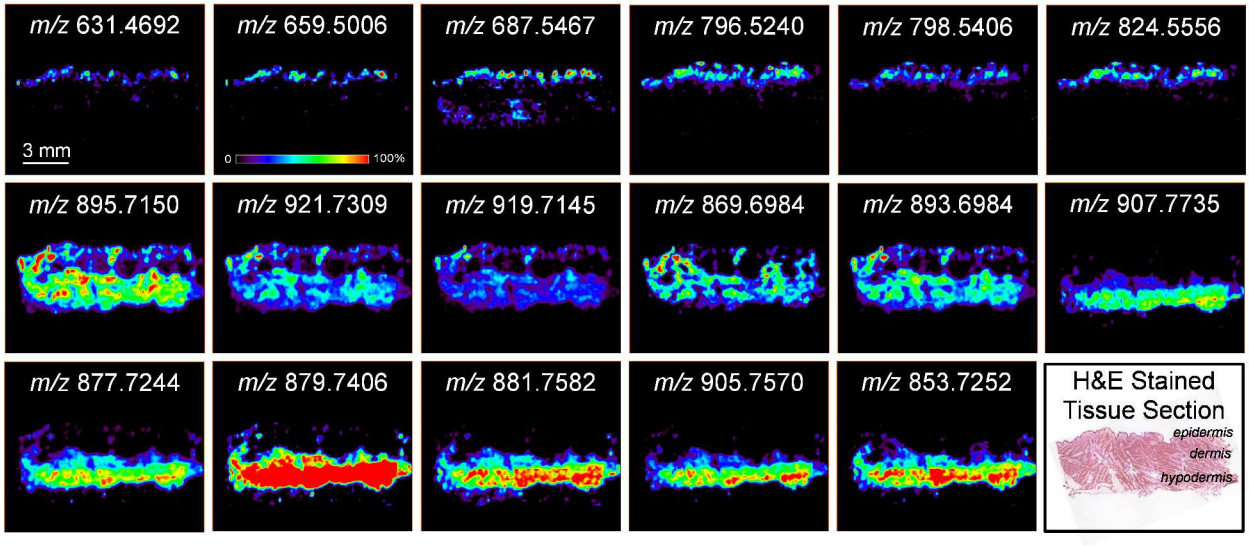

Figure S1. Selected positive ion mode DESI-MS ion images of human skin sample and optical image of the same tissue section images by DESI after H&E stain are shown.

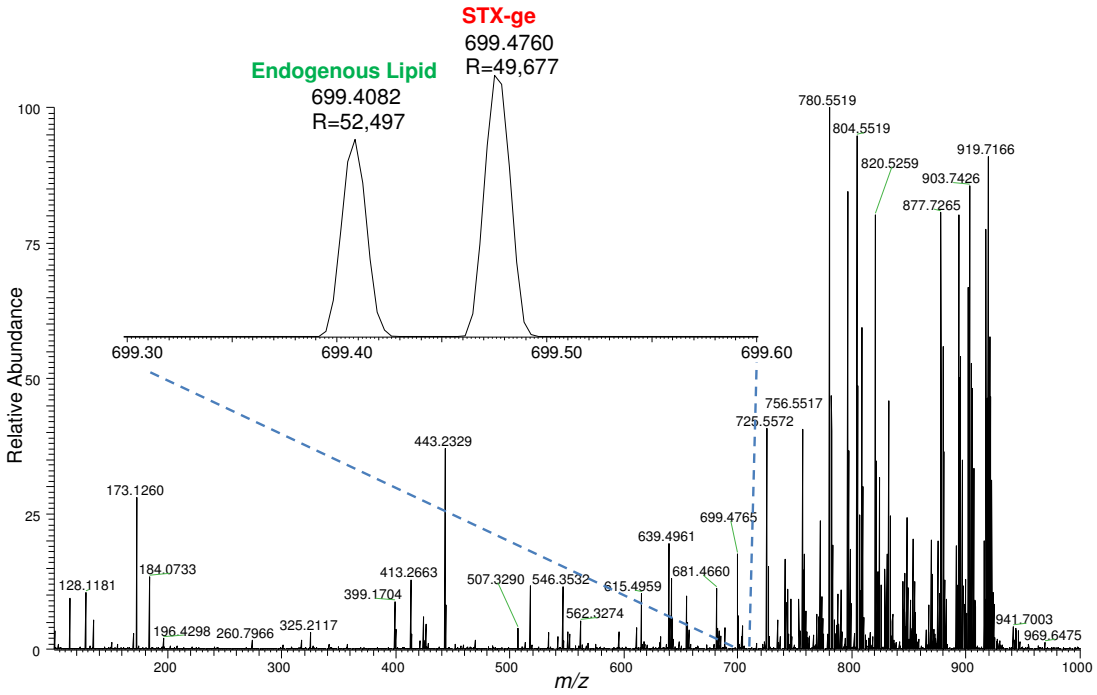

Figure S2. Positive ion mode DESI-MS mass spectrum of human skin from *m/z* 100 - 1000. Inset shows the range m/z 699.30 - 699.60 in which peaks of the compound STX-ge and an endogenous lipid (detected at the same nominal *m/z*) are resolved using high mass resolution DESI-MSI. (R = Orbitrap resolving power at the *m/z* shown).

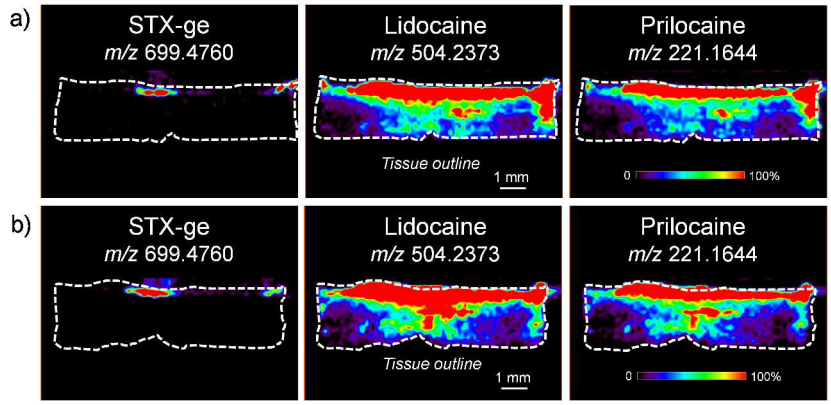

Figure S3. DESI-MS ion images showing the penetration of STX-ge using EMLA cream as vehicle in two human skin tissue sections. Ion images showing the penetration of lidocaine and prilocaine, compounds with are both present in the EMLA cream formulation, are also presented for the two sections analyzed.

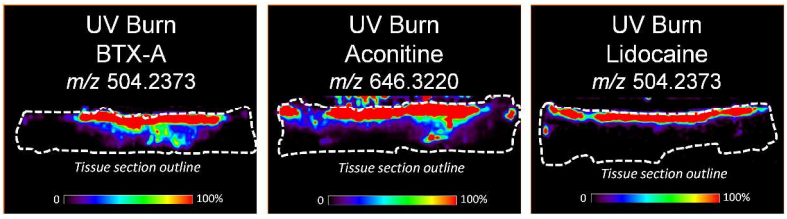

Figure S4. DESI-MS ion images showing the penetration of BTX-A, aconitine and lidocaine using ethanol as vehicle applied to human skin after UV burn.

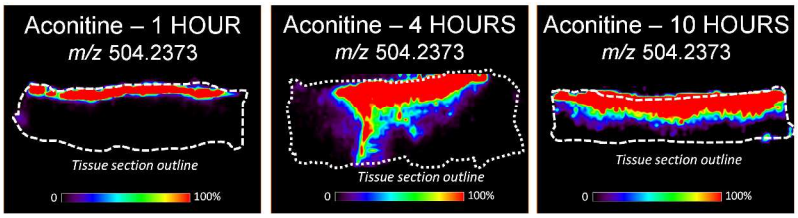

Figure S5. DESI-MS ion images showing the penetration of aconitine using ethanol as vehicle after 1, 4 and 10 hours of topical application.

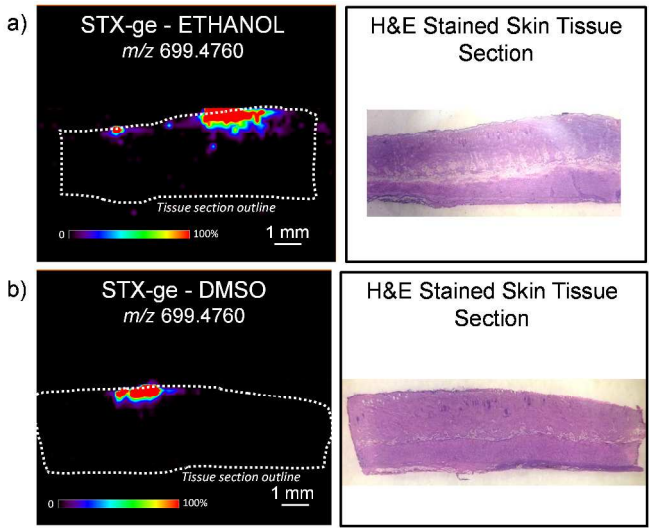

Figure S6. DESI-MS ion images showing the penetration of STX-ge tested in *in vivo* rat skin using a) ethanol and b) DMSO as vehicles. Optical images of the same tissue sections imaged by DESI after H&E stain are also shown.

References:

[1] F. T. M. C. Vicentini, Y. M. Fonseca, D. L. Pitol, M. M. Iyomasa, M. V. L. B. Bentley, M. J. V. Fonseca, *Journal of Pharmacy and Pharmaceutical Sciences* **2010**, *13*, 274-285.