Pharmacologic Inhibition of TRPA1 Counteract CS Tear Gas Agent-induced Cutaneous Injuries

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We optimized the solvent for dissolving CS tear gas agent for application on mouse ears. We tested common solvents such as dimethyl sulfoxide (DMSO) and dichloromethane (DCM). CS dissolved in DCM remained like a powder on the ears after application. This might be explained by the higher vapor pressure of DCM (350 Torr) compared to the lower vapor pressure of DMSO (0.6 Torr). Application of CS tear gas agent dissolved in DMSO gave robust inflammation response in mouse models compared to DCM (Figure S1). Therefore, we chose DMSO as a solvent for CS tear gas skin injury studies. Further, DCM has relatively more toxic effects compared to DMSO.

Figure S1

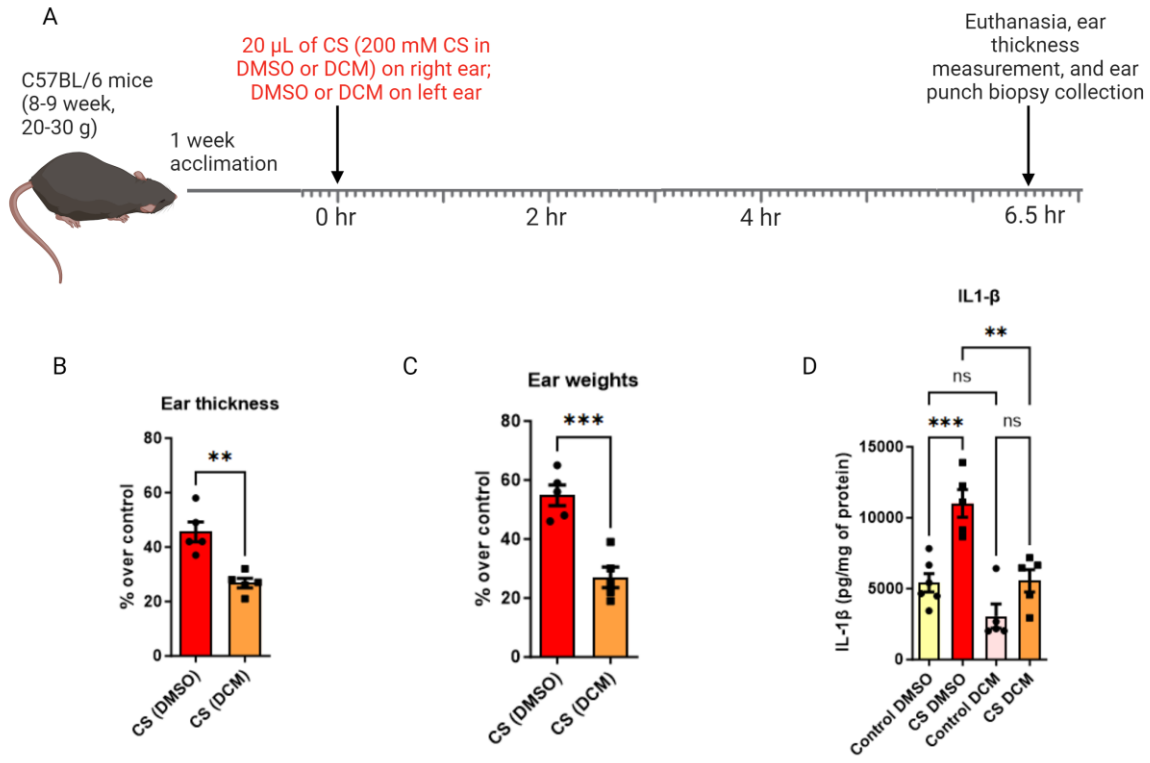

Figure S1. Effects of solvents on CS tear gas agent-induced cutaneous inflammation. (A) Study paradigm. Right ears of C57BL/6 male mice were exposed to 20 µL of CS (200 mM, dissolved in either DMSO or DCM) and left ears to DMSO or DCM (vehicle, 20 µL). At 6.5 hours post-CS exposure, mice were euthanized, ear thickness was measured, and ear punch biopsies were collected. (B-D) Ear thickness, ear punch biopsy weights, and pro-inflammatory cytokine (IL-1β) assessment. Data were analyzed by either Student's t-test or one-way ANOVA with Tukey's post-hoc multiple comparison test. Data are presented as mean ± SEM, n=5 per group. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = non-significant.

We optimized the concentration of CS tear gas for the mouse ear inflammation model. We dissolved CS in DMSO at various molar concentrations (50, 100, and 200 mM) and applied to mouse ears. Across studied parameters (ear thickness, ear punch biopsy weights, extravasation of inflammatory exudate, and IL-1β pro-inflammatory cytokine), there was no statistically significant difference among the tested CS concentrations (Figure S2). We chose 200 mM concentration as this concentration gave a robust injury phenotype. Further, we wanted to test potential therapeutic compounds in a model that represents severe injury phenotype in humans.

Figure S2

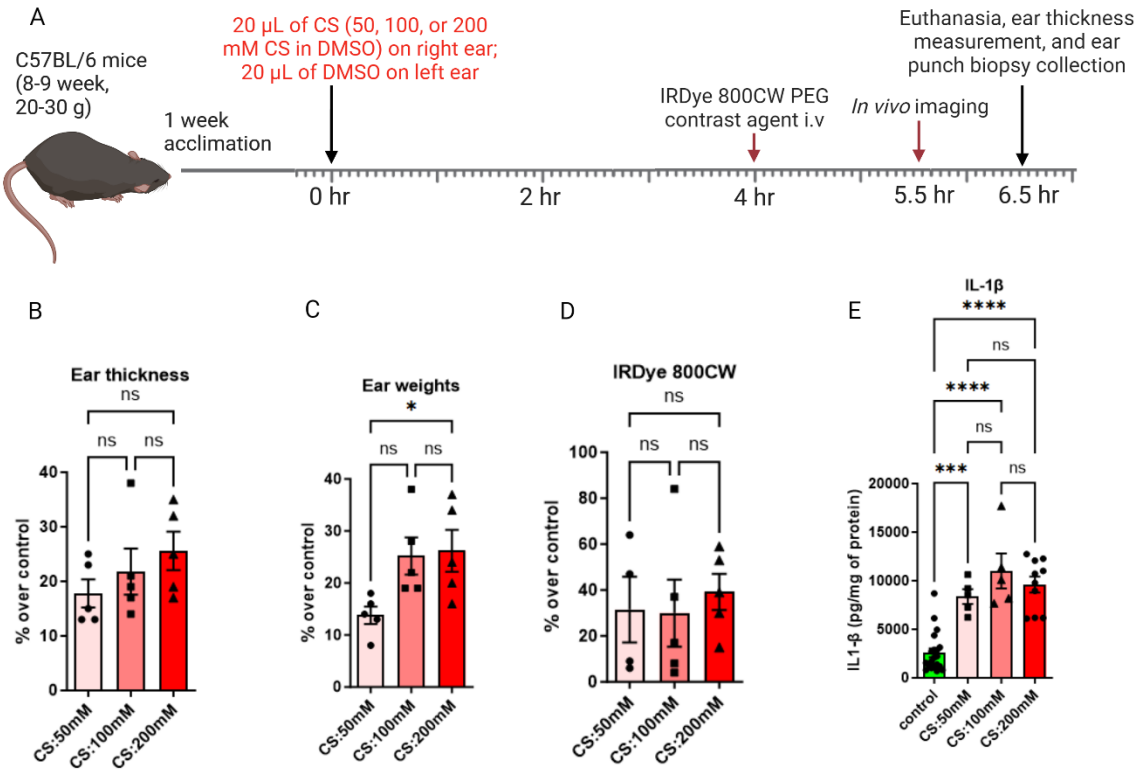

Figure S2. Titration of CS tear gas concentration for optimization of mouse ear skin injury model. (A) Study paradigm. Right ears of C57BL/6 male mice were exposed to 20 µL of CS at various molar contrations (50, 100, and 200 mM, dissolved in DMSO) and left ears to DMSO (vehicle, 20 µL). At 4 hours post-CS exposure, mice were injected with IRDye 800CW contrast agent intravenously (i.v) and *in vivo* imaging was performed at 5.5 hours post-CS exposure. At 6.5 hours post-CS exposure, mice were euthanized, ear thickness was measured, and ear punch biopsies were collected. (B-F) Ear thickness, ear punch biopsy weights, extravasation of pro-inflammatory exudate, and pro-inflammatory cytokine (IL-1β) assessments. Data were analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test. Data are presented as mean ± SEM, n=5 per group. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, ns = non-significant.

To assess the decontamination efficacy of water washing after skin exposure to CS tear gas agent, 30 minutes after CS exposure, we washed both surfaces of ears three times with fresh cotton applicators moistened in water. Decontamination of CS-exposed mouse ear skin with water washing did not improve the studied parameters such as ear thickness, ear punch biopsy weights, and IL-1β pro-inflammatory cytokine measured in ear punch biopsy homogenate

Figure S3

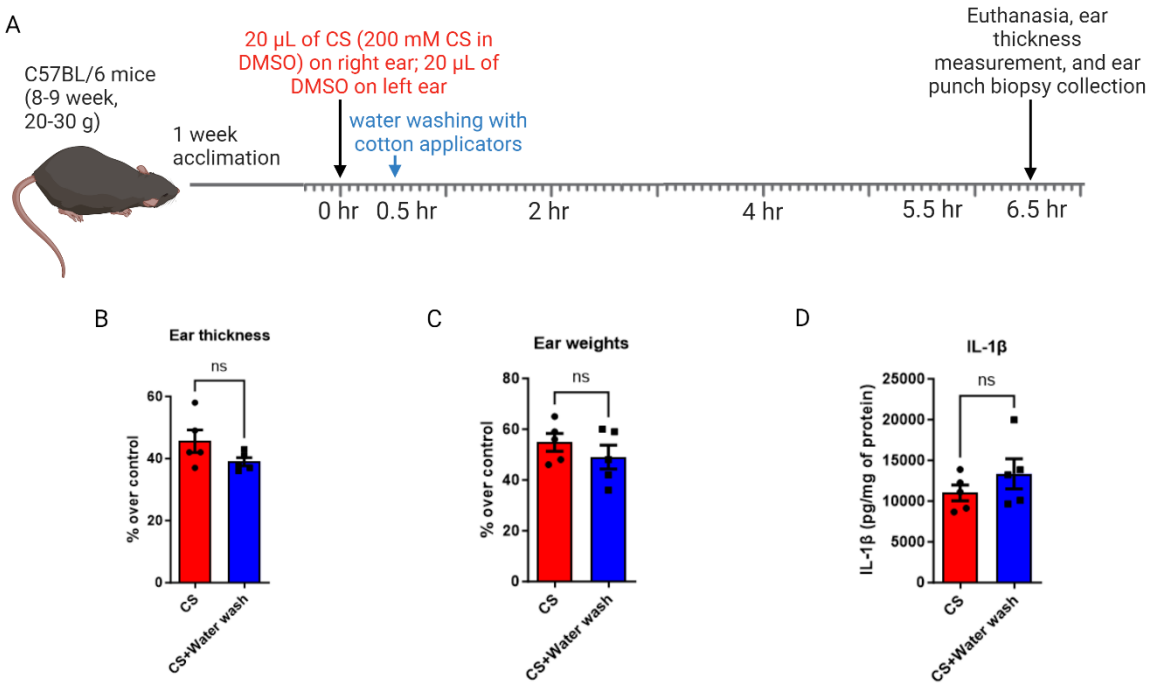

Figure S3. Decontamination of CS tear gas exposure with water washing. (A) Study paradigm. Right ears of C57BL/6 male mice were exposed to 20 µL of 200 mM CS (dissolved in DMSO) and left ears to DMSO (vehicle, 20 µL). At 6.5 hours post-CS exposure, mice were euthanized, ear thickness was measured, and ear punch biopsies were collected. (B-D) Ear thickness, ear punch biopsy weights, and pro-inflammatory cytokine (IL-1β) assessments. Data were analyzed by Student's t-test. Data are presented as mean ± SEM, n=5 per group. ns = non-significant.

samples (Figure S3).

Table S1. Development of TRPA1 inhibitor pipeline, species activity, and efficacy in human TRPA1

Name	**Species activity**	**Human IC_{50} values (nM)**	**Developed by**	**Ref**
HC-030031	Mouse, rat, and human	6200	Hydra Biosciences	(McNamara et al., 2007)
AP18	Mouse, rat, and human	3100	Novartis	(Petrus et al., 2007)
ChemBridge-5861528	Rat, and human	4900	Orion Pharma	(Wei et al., 2009)
A-967079	Mouse, rat, rabbit, pig and human	67	Abbott	(McGaraughty et al., 2010)
GRC17536	Guinea pig and human	<10	Glenmark	(India, 2014)
AMG0902	Mouse, rat, rabbit, pig, and human	131	Amgen	(Lehto et al., 2016)
BI01305834	Guinea pig and human	40	Boehringer Ingelheim	(van den Berg et al., 2021)
BAY-390	Rat and human	16	Bayer	(Mesch et al., 2023)
GDC-0334	Mouse, rat, guinea pig, and human	1.7	Genentech	(Balestrini et al., 2021)
GDC-6599 (compound 20)	Mouse, rat, guinea pig, rabbit, pig, and human	0.9	Genentech	(Terrett et al., 2021)

Additional investigational TRPA1 antagonists have been disclosed and discussed elsewhere (Achanta and Jordt, 2020; Chen and Terrett, 2020; Talavera et al., 2020).

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