

Supplementary Materials for

CT-guided injection of a TRPV1 agonist around dorsal root ganglia decreases pain transmission in swine

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This PDF file includes:

Fig. S1. Axial CT at the L5 level showing drug/contrast mixture in the ventral epidural space from bilateral periganglionic injections.

Table S1. Criteria for disability scoring before and after CT-guided injections.

Table S2. Clinical gait scoring before and after RTX injection.

Table S3. Withdrawal latency to C-fiber activation with laser stimulus—all animals.

Legends for movies S1 and S2

Other Supplementary Material for this manuscript includes the following:

(available at

www.sciencetranslationalmedicine.org/cgi/content/full/7/305/305ra145/DC1)

Movie S1 (.mp4 format). Withdrawal behavior from infrared laser heat stimulus. Movie S2 (.mov format). Withdrawal behavior from noxious mechanical (pinprick) stimulus.

Supplementary Materials

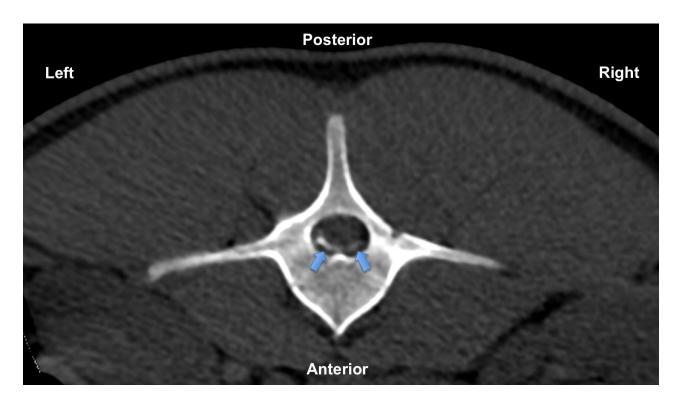


Figure S1. Axial CT at the L5 level showing drug/contrast mixture in the ventral epidural space from bilateral periganglionic injections. In a small number of injected levels (4/56 injections, n=7 animals), we observed direct extension of a detectable, small volume of contrast material (blue arrows) from periganglionic injections of RTX (right) or vehicle (left) into the dependent, ventral epidural space (animal lying prone on CT table). A thin layer of radiopaque (white) contrast medium can be seen continuously layering within the ventral epidural space, providing evidence of the proposed mechanism for drug diffusion and a potential mechanism for the mild, but statistically significant increase in withdrawal latencies we observe in vehicle injected hindlimbs.

	Day 1	Day 2	Day 3	Wk 2	Wk 3	Wk 4
0 - pig freely able to stand from a lying position and move freely in pen with balanced weight on all four limbs	7	7	7	7	7	7
1 - pig raises immediately to standing position but demonstrates an occasional reluctant movement of the limb innervated by either the RTX or vehicle treated DRGs and/or uneven distribution of body weight with regard to treatment status of affected limbs	0	0	0	0	0	0
2 - pig moves slowly with short steps with extended periods of time in order to reduce weight on an injected limb or refuse to bear weight	0	0	0	0	0	0
3 - pig unable to rise from the ground.	0	0	0	0	0	0

Table S1. Criteria for disability scoring before and after CT-guided injections. Disability scores were assessed while animals remained in primary enclosures at days 1,2 and 3 post procedure and thereafter once weekly at weeks 2, 3, and 4. No lameness was observed in any of the animals.

	Before injection	After Injection
1 – Walks normally, normal gait	7	7
2 - Slightly lame when walking	0	0
3 - Moderately lame when walking	0	0
4 – Severely lame when walking	0	0
5 - Reluctant to rise and will not walk more than five paces	0	0

Table S2: Clinical gait scoring before and after RTX injection. 7/7 animals were given normal clinical gait scores by two independent observers reviewing pigs walking a predetermined circuit before injection and during week four after injection. Scoring was based on gait distance, walking speed, limb support and symmetry of movement.

Table S3. Withdrawal latency to C-fiber activation with laser stimulus—all animals. Individual response times (in seconds) to laser stimulation in each cohort. Each response time represents a discrete laser stimulus.

Uninjected - Left	Uninjected - Right	2 wks Vehicle Only	2 wks 500ng RTX	2 wks Vehicle Only	2 wks 2000ng RTX	4 wks Vehicle Only	4 wks 500ng RTX	4 wks Vehicle Only	4 wks 2000ng RTX
6.7	7.2	7.6	10.5	8.5	10.5	6.7	9.6	6.8	9.6
5.6	4.8	5.8	10.5	10.5	10.5	4.4	13.2	7.6	15.5
5.8	4.1	5.0	10.5	8.3	10.5	5.9	12.1	5.9	10.5
9.8	6.4	10.5	10.5	5.2	10.5	5.2	7.9	4.5	6.7
13.5	6.4	9.3	8.3	8.0	8.8	3.5	15.5	6.8	15.5
8.2	6.6	3.6	9.4	6.8	10.5	4.5	15.5	7.3	15.5
7.6	6.5	10.5	10.5	8.4	9.7	3.6	15.5	6.3	15.5
5.9	7.0	5.2	7.5	7.2	9.7	10.6	5.6	6.6	13.1
9.8	6.5	6.9	10.5	6.4	9.3	6.2	5.5	6.3	9.9
8.8	4.2	4.0	10.5	10.5	10.5	5.2	9.0	5.3	15.5
7.0	4.6	6.4	8.5	10.5	10.5	8.6	9.7	4.4	4.8
12.3 7.3	8.2 5.8	7.9 4.8	10.5 9.3	6.8 10.5	10.5 10.5	9.1 3.8	11.5 13.9	7.9	10.9
5.8	6.9	6.0	7.3	10.5	10.5	4.6	15.5	7.8 6.2	9.6 15.5
5.4	3.0	4.7	7.3	9.7	10.5	4.0	15.5	11.5	7.2
7.8	8.4	8.8	9.3	10.5	10.5	3.3	15.5	5.3	15.5
6.7	8.5	6.3	10.5	10.5	10.5	11.8	15.5	7.4	7.5
8.0	3.5	7.7	10.5	9.8	9.8	14.1	15.5	4.7	15.5
4.2	8.9	7.4	10.5	6.0	10.5	13.0	15.5	6.1	6.5
5.6	9.4	4.7	10.5	8.6	10.5	7.1	15.5	7.4	9.7
7.6	8.6	8.2	5.3	6.9	10.5	10.7	15.5	5.6	11.1
4.6	8.3	6.2	10.5	8.7	10.5	12.3	15.5	5.8	15.5
4.4	6.6	7.5	7.6	5.8	10.5	15.5	14.9	7.6	5.5
7.3	4.2	7.0	8.9	8.3	10.5	5.3	15.5	12.3	15.5
6.7	6.4	7.6	10.5	5.7	5.8	10.0	15.5	7.6	5.4
4.9	4.2	6.4	6.2	9.1	8.1	12.2	15.5	5.5	4.8
7.1	3.3		7.5	4.9	10.5	15.5	15.5	4.9	15.5
4.9	13.2		10.5 10.3	10.5 10.5	3.1 10.5	9.4	15.5 15.5	5.7 15.5	15.5 15.5
4.5			5.1	10.5	6.2	8.6	15.5	6.0	15.5
3.8			5.1	7.5	5.8	6.7	15.5	4.3	6.2
6.0				8.1	10.5	10.8	15.5	9.8	7.4
4.9				4.3	10.5	6.8	12.2	6.5	12.3
3.5				10.5	10.5	5.6		12.6	15.5
3.3				4.7	10.5	15.5		15.5	8.2
7.8				5.6	7.9	6.8		4.8	15.5
5.2				3.0	10.5	15.5		8.2	5.4
4.5				3.3	7.8	7.2		8.2	8.8
4.1				8.3	6.4	12.9		12.2	6.3
3.7				5.5	7.4	15.5		8.7	15.5
4.2				5.6		5.8		9.6	15.5
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				8.3	

Video S1: Withdrawal behavior from infrared laser heat stimulus. C-fiber stimulation (red laser focus) to dermatomes innervated by DRG injected with vehicle (left) with withdrawal time (limb lift) of 6.3 seconds. In comparison, no behavioral response is seen when stimulating dermatomes innervated by DRG injected with 2000 ng RTX (right) with time reaching automatic laser shutoff (15.5 sec cutoff).

Video S2: Withdrawal behavior from noxious mechanical (pinprick) stimulus. Typical responses observed when testing vehicle and RTX treated dermatomes in two different treated animals. The first response is a definitely positive response, with near-immediate limb twitch or withdrawal to pinprick stimulus. The rate of definite positive response was very low overall (6/32, 18.8%, 4 from RTX injected dermatomes, 2 from vehicle injected dermatomes). A similarly low response rate was observed when testing areas outside treated dermatomes. The second clip shows a non-response to pinprick stimulus. The third clip is of an indeterminate response (the majority of responses were of this type), where slight limb movement is observed during or just after stimulus application but interpretation is difficult to interpret due to the possibility that the animal movement may be: a) spontaneous and unrelated to stimulus; b) reactionary due to imbalance from the induced lateral force; or c) a reaction to the light touch and/or movement of the experimental staff during stimulus application.