A randomized, double-blind study has been carried out, and the results are presented below. This proprietary study was performed at the Indiana State University School of Medicine, Department of Microbiology and Immunology, under the direction of Professor Mary T. Johnson, PhD.

Carrageenan-Induced Inflammation in the Rat Hind Paw

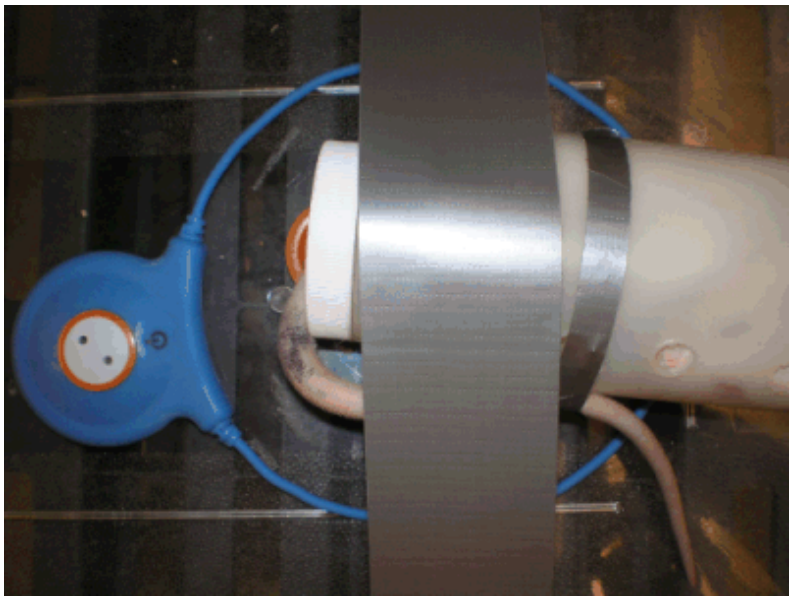
This is a validated model of inflammation wherein an inflammatory response is produced by intraplantar injection of carrageenan in a rat hind paw[1,2]. Localized edema and pain are produced, both of which may be measured using validated methodologies. This rat hind paw inflammation model is routinely employed for assessment of anti-inflammatory pharmacological agents and physical modalities[3].

Methods

Male Sprague–Dawley rats (200-250g instead of 175-200g) were employed. Paw edema was induced by intraplantar injection of 0.1 ml of 2% carrageenan in saline[3]. This concentration of carrageenan causes a rapid rise of edema over approximately 200 minutes post-injection, followed by a more gradual increase over the next several hours[3]. In this study, edema and pain were followed for 225 minutes, i.e., over the most virulent inflammatory phase, overlapping slightly into the plateau phase[3]. Edema was measured with a water displacement plethysmometer[1] (Stoelting, Wood Dale, Ill). Hyperalgesia was quantified as the pressure at which paw withdrawal occurs using a paw pressure analgesia instrument[2] (Stoelting, Wood Dale, Ill).

32 rats were randomly divided into two groups of 16: **Torino, Torino Sham**. All cohorts were treated and measurements made according to the following regimen. Baseline pain and edema were obtained pre-injection. The first treatment was within 15 minutes post-injection, followed by pain and edema measurement at 45 min. The second treatment was at 105 min, followed by measurements at 135 min and the third at 195 min, followed by measurements at 225 min post-injection.

All active animals received 15 min treatments with a pulse modulated radio frequency signal. The Torino (Torino model) signal was a 2 msec burst of 27.12 MHz sinusoidal waves repeating at 2/sec. The Torino signals were delivered with a single turn 15 cm diameter circular antenna (coil). Treatment was directed to the hind paw by placing the rat in a plastic restraining cylinder and locating the cylinder such that only the rear quarter of the rat was within the coil. An example, a Torino is shown in the photo. As may be seen, the tail has been further restrained in order to prevent the animal from turning or rotating in the cylinder. In this manner, the rat remains supine and the injected hind paw is maintained parallel to the coil surface and within the central region of uniform treatment field distribution during the entire duration of treatment. Animals in both sham cohorts were also placed in plastic restraining cylinders, tails were further restrained, and they were



identically positioned in a sham coil as in the photo. The rat hind paw is approximately 4 cm in length with a mean diameter of approximately 0.75 cm for the 200-250 gm animals used in this study. The B-field at the hind paw position for the Torino was measured with a NIST traceable calibrated loop probe (model FCC-301-1-MR1, Fischer Custom Communications, Torrance, CA) connected to a calibrated 100 MHz oscilloscope (model 2358, Tektronix, Beaverton, OR). The mean B-field throughout the volume of the hind limb was 60 ± 7 mG for the Torino. The loop probe also allowed signal consistency to be verified throughout the study by an unblinded investigator having no contact with the carrageenan injection, or Torino, or pain and edema evaluations. Torino exposure systems were coded and equally divided into sham or active units. Device codes were randomized and maintained off site by Ivivi. Once activated, both active and sham devices appeared identical to treatment personnel. This allowed both active and sham animals to be treated identically, while allowing all investigators who handled rats or evaluated pain and edema to remain blinded until all data were collected.

Data were analyzed using SigmaStat 3.0 (SPSS, Chicago, IL). Data which passed the Kolmogorov-Smirnov normality test were analyzed either using the Student's paired t test, or one way repeated measures, as appropriate, for intra-cohort comparisons, or using the Student's unpaired t test, or one way ANOVA, as appropriate, for inter-cohort comparisons. Differences were also compared using the Mann-Whitney or Wilcoxon rank tests, as necessary, in addition to the original analyses. Significance was accepted at $P \leq 0.05$.

Results

Evaluation of Pain

The results showed mean pre-injection pain threshold for both cohorts was not significantly different ($P = 0.551$). There was a non-significant 8 ± 0.8% decrease in mean pain threshold at 225 min versus baseline for Torino treated animals ($P = 0.311$). In contrast, mean pain threshold decreased by 26 ± 2% in the Torino sham cohorts versus their respective baseline values ($P<0.001$) at 225 minutes. Indeed, there was no observed physiologically significant pain in the Torino treated animals over the entire 225 min period. The final result was mean pain threshold was reduced approximately 3X more in the sham cohorts than in the active cohorts during the most virulent inflammatory phase in this accelerated inflammation model. The effects of Torino treatment on pain is summarized in Table I.

Table I

Comparative Effects of Torino on Pain: Rat Carrageenan Model

	Pain Threshold Baseline (gm ± SD)	Pain Threshold 225 min (gm ± SD)	Percent Decrease (vs Baseline)	P
Torino Sham	**216 ± 12**	**156 ± 40**	**28 ± 4.2%**	**< 0.001**
Torino Active	**214 ± 17**	**196 ± 19**	**8 ± 0.9%**	**NS**

It is also of interest to compare these results with those obtained with the Torino (Torino model) in the published human clinical study (Heden and Pilla, 2008), wherein approximately 3X greater post-operative pain reduction versus that in the sham cohort by POD 3 was reported.

Evaluation of Edema

The results also showed there was no significant difference in mean pre-injection hind paw volume ($P = 0.427$). All cohorts had significant increases in mean edema by 225 minutes ($P < 0.001$). The mean edema increase in the Torino sham cohort was approximately 1.7X the edema increase in the respective active cohort at 225 min ($P < 0.001$). In other words, Torino inhibited edema formation by 66 ± 7% at 225 minutes.

Table II

Comparative Effects of Torino on Edema: Rat Carrageenan Model

	Paw Volume Baseline (cc ± SD)	Paw Volume 225 min (cc ± SD)	Percent Increase (vs Baseline)	P
Torino Sham	**1.26 ± 0.05**	**2.18 ± 0.18**	**73 ± 0.14%**	**< 0.001**
Torino Active	**1.29 ± 0.05**	**1.85 ± 0.13**	**43 ± 0.10%**	**< 0.001**

Comparison with Pharmacological Analgesics

The observed Torino effects on pain and edema in the carrageenan rat hind paw model was compared to the effect of aspirin and nitroaspirin administered orally in the same model. That study[3] reported, at the highest dose used (100 mg kg^{-1}), and at 360 min, that orally administered nitroaspirin and aspirin inhibited edema formation by 46.9 ± 1.6% and 47.2 ± 3.8%, respectively ($P < 0.05$). This is to be compared with the effect of MRT/Torino in the present study, wherein edema was inhibited by 66 ± 7% within 225 minutes ($P < 0.001$). At the same dose of aspirin and nitrosaspirin, pain threshold was reduced by approximately two-fold at 360 min, compared with approximately three-fold for Torino at 225 minutes.

Discussion and Conclusions

This randomized, double-blind study was designed to demonstrate the effects of the Torino on pain and edema in a validated rat model of inflammation. Pain and edema were provoked by intraplantar injection of carrageenan. The results show that the Torino had a significant effect on pain and edema in an animal model which is routinely employed as an accurate predictor of the effect of anti-inflammatory drugs and physical modalities on humans. Further support is provided by the favorable comparison of the Torino effects from this study with those reported for aspirin and nitroaspirin in the same animal model.

References

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