

The rat formalin test: Can it predict neuropathic pain treatments?

A. Ellis, N. Benson, I. Machin, and L. Corradini

Pfizer Global Research and Development, Sandwich, UK, amanda.ellis2@pfizer.com

Widely used, the formalin test is a tonic model of continuous pain resulting from formalin-induced tissue injury. It is a useful model, particularly for the screening of novel compounds, since it encompasses inflammatory, neurogenic, and central mechanisms of nociception [5,6]. Reports can vary widely with regard to concentration of formalin (itself a 37% dilute solution of formaldehyde) as well as the recording and characterisation of resultant pain behaviours in rats [4,5,6]. Therefore the effect of 1.25, 2.5, and 5.0% formalin on nociceptive behaviours was evaluated by measuring two commonly reported pain-like behaviours – flinching and licking/biting [1,2,3], associated with formalin intraplantar injection. Three clinically effective neuropathic pain treatments were tested (Gabapentin, Duloxetine, and Mexiletine) [1,2,3] to determine if these could reduce formalin-induced pain-like behaviours. An electronic recording method (Noldus Observer 5.0) was explored as an alternative to manual methods using observation and stop clocks. This allowed simultaneous recording of licking/biting and flinching behaviours.

Methods

All procedures were carried out under the Animals (Scientific Procedures) Act, 1986. Male Sprague Dawley rats (200-300g, Charles River, UK) were housed in groups of 4 under a 12 hour light/dark cycle with food and water *ad libitum*. All experiments were carried out by an investigator blind to drug treatments.

Formalin test

Rats were placed singly in perspex boxes (30 x 30 x 30cm, with a mirror placed on the back panel to aid observation) for approximately 15 minutes before the start of the test for habituation. Animals were pre-treated with standard analgesics at different time points according to existing pharmacokinetic/pharmacodynamic (PKPD) data (data not shown). Rats then received intraplantar injection into the right hindpaw of a 50ul solution of either 1.25, 2.5, or 5.0% formalin solution (saline vehicle) using a 29G needle, and placed immediately back in the boxes. Pain-like behaviours (licking/biting and flinching) were recorded in 5 minute time bins using the Pocket Observer (Noldus) for 45 minutes. Data were also considered in either the Early Phase (0-10mins) or the Late Phase (11-45mins). Animals were euthanased immediately at the end of the study. All experiments were recorded using a video camera as back up information.

Noldus Observer PDA

The PDA was pre-programmed to record two rats simultaneously for each investigator, and the behaviours of interest – licking/biting, flinching, and “rest” of the ipsilateral paw. To record a behaviour the appropriate subject and then behaviour, was selected on the screen as the behaviour began (ie Rat 1, Lick). At the end of the experiment the data were uploaded to a PC which was programmed to calculate the durations of each occurrence of recorded behaviour within user-defined intervals (in this case 5 minute time bins). This information was then exported to a Microsoft Excel spreadsheet for final analysis of this raw data.

PKPD and Statistical Analysis

Each treatment group was compared at each time point, as well as the Early and Late phases, to vehicle treated groups using one-way ANOVA and post-hoc student's t-test. Results were expressed as mean \pm S.E.M.

PKPD data analyses were carried using NONMEM V5 and S-Plus V 6.1 (Insightful corp, US).

Results

Unilateral intraplantar injection of formalin in the rat generates a biphasic pain-like behaviour characterised by licking/biting/ and flinching. A comparison with the effect of higher (5%) and lower (1.25%) doses of formalin, suggested 2.5% solution generates a robust effect and acceptable levels of variability. Therefore this dose was chosen for the pharmacological characterisation of the model. Data obtained using stop clocks were found to be consistent with those obtained using the Pocket Observer, and not significantly different at any time point (data not shown). Therefore further data capture was performed using the Pocket Observer allowing simultaneous comparison of both licking/biting and flinching end-points.

Three clinically effective neuropathic pain treatments (Gabapentin 100mg/kg p.o., Duloxetine 1, 3, 10mg/kg i.p., and Mexiletine 10 and 30mg/kg p.o.) [1,2,3] were tested. Although no significant effect was seen on flinching behaviour, all three compounds reduced the duration of licking/biting behaviour in the second phase of the formalin test. Gabapentin, Duloxetine, and Mexiletine significantly ($p < 0.05$) reduced pain-like behaviour at 100 mg/kg, 10 mg/kg and 30 mg/kg respectively. Further PK-PD analysis is ongoing in order to establish whether this model can be used to predict dose translation from rat to human. The results of the rat model PKPD analyses will be presented in the context of clinically used exposures.

In conclusion the rat formalin test, using a 2.5% solution with licking/biting as the measured end-point, represents a suitable model to explore new targets and establish their potential as treatments in neuropathic pain. This data supports similar findings previously demonstrated by Vissers et al. showing pharmacological correlation between the formalin test and neuropathic pain behaviours [7]. Ongoing PKPD analysis will help to establish whether this model can also be a suitable tool to help dose prediction from rat to man.

Finally, the Observer represents a useful tool for electronic data storage which is more temporally detailed than traditional methods. Advantages of this method included the electronic storage of raw data (detailing timing of recorded events), as well as accuracy and objectivity of recordings. Additionally it allows the observation of multiple behaviours in multiple subjects, using just one PDA. Although convenient, further development of the Observer software would be useful to speed up the process of transferring data into a suitable format for graph and statistical analysis.

References

1. Dixit, R., Bhargava, V.K., Kaur, N. (1999). Antinociceptive effect of gabapentin in the formalin test. *Methods and Findings in Experimental and Clinical Pharmacology*, **21**, 481-482.
2. Iyengar, S., Webster, A.A., Hemricke-Luecke, S.K., Yu Xu, J., Simmons, R.M.A. (2004). Efficacy of Duloxetine, a Potent and Balanced Serotonin-Norepinephrine Reuptake Inhibitor in Persistent Pain Models in Rats. *The Journal of Pharmacology and Experimental Therapeutics*, **311**, 576-584.
3. Jett, M.F., McGuirk, J., Waligora, D., Hunter, J.C. (1997). The effects of mexiletine, desipramine, and fluoxetine in rat models involving central sensitisation. *Pain*, **69**, 161-169.
4. Kaneko, M., Hammond, D.L. (1997). Role of Spinal γ -Aminobutyric Acid_A Receptors in Formalin-Induced Nociception in the Rat. *The Journal of Pharmacology and Experimental Therapeutics*, **282**, 928-938.
5. Lee, I.O., Kong, M.H., Kim, N.S., Choi, Y.S., Lim, S.H., Lee, M.K. (2000). Effect of different concentrations and volumes of formalin on pain response in rats. *Acta Anaesthesiologica Sinica*, **38**, 59-64.
6. Tjølsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H., Hole, K. (1992). The formalin test: an evaluation of the method. *Pain*, **51**, 5-17.
7. Vissers, K.C.P., Geenen, F., Biermans, R., Meert, T.F. (2006). Pharmacological correlation between the formalin test and the neuropathic pain behaviour in different species with chronic constriction injury. *Pharmacology, Biochemistry and Behaviour*, **84**, 479-486.