Localizing the mechanism of action of Paracetamol in the mouse brain

Yu Ying Liew, Gavin Dawe

Department of Pharmacology, Faculty of Medicine, National University of Singapore. 10 Kent Ridge Road, Singapore.

ABSTRACT

Based on the evidence that the antinociceptive effects of paracetamol (acetaminophen) could be mediated centrally, distribution of the cellular activity of the drug after oral administration was aimed to be determined in mouse. This study comprises four groups of mice which were categorized into saline group, paracetamol group, aspirin group and paracetamol group without hot plate test. First, the mice were orally injected with drugs and 30min later they were treated in hot plate to induce pain. Two hours later, mice were perfused with 4% paraformaldehyde. The brains were removed and paraffin embedded. Each brain was sectioned into 6µm and fixed on coated slides. C-fos polyclonal antibody (with dilution 1:100 in phosphate buffer solution (PBS)) was used as a marker of pain. Brown colour was developed with the aid of Diaminobenzidine (DAB) chromagen. Stained slides were observed under light microscope and digital photographs were taken. Significant of Fos expression of paracetamol group at the posterior ventral medial region of brain was observed. Many nociceptive neurons were expressed in the periqueductal grey (PAG), cuneiform nuclei (CnF), pedunculopontine tegmental nuclei (PPTg) and parabrachial internal lateral nucleus (PBil). C-fos expressions of the above regions were not seen in aspirin group. These results suggest that aspirin and paracetamol administrated orally may be mediated by different nociceptive processing in the mice brain.

INTRODUCTION

Paracetamol and Aspirin are widely used analgesic drugs and act as inhibitor of cyclooxygenase to inhibit brain prostaglandin synthetase. Paracetamol is considered to be a nonsteroidal anti-inflammatory drug (NSAID). Two cyclooxygenase isoenzyme, COX-1 and COX-2 are known to catalyze the rate-limiting step of prostaglandin synthesis and are targets of NSAID. Recently, COX-3, derived from COX-1 was sequenced and it was found that it was inhibited by paracetamol preferentially. It was significantly more sensitive to paracetamol than either COX-1 or COX-2 at the lower substrate concentration. However, aspirin was tested to be a COX-1 preferential inhibitor. Thus, inhibition of COX-3 in brain and the spinal cord may be the long sought-after mechanism of action of paracetamol. (N.V. Chandrasekharan et al, 2002) The aim of this study is to find out the mechanism of action of paracetamol in mouse brain.

METHODS

Mice were habituate to handling for one week and to oral admistration of saline for three days. Dosage of drugs was given according to mouse weight. (With standard dose 0.1ml/20g mouse). The hot is performed after 30min after oral administration. The animals were perfused and processed for paraffin embedding. Brain were sectioned into
6µm. C-fos immunoreactive cells were stained with by DAB chromagen and slides were observed under light microscope.

RESULTS
The results shown mice that treated with paracetamol and aspirin have higher resistance to hot plate test because they have higher licking time and jump off latency than the saline group.

Saline group was a control group which showed the basic Fos expression under pain induction. (see Fig 3) Fos expression was detected at the midbrain, especially at the periaqueductal gray (PAG) and adjacent regions including the cuneiform nuclei (CnF) and extended to hypothamus area, including dentae gyrus (DG) and CA3 fields. Fos protein were expressed at the PAG, deep mesencephalic nuclei (DpMe), superior colliculus (SC) as well as the parabrachial and presubicum areas when treated with paracetamol. Meanwhile, mice treated with aspirin have relatively low expression of Fos protein at the PAG and surrounding areas, pedunculopontine tegmental nuclei (PPTg) and CnF. Paracetamol group without hot plate test has given information of Fos expression upon hot plate stimulation. It was distributed at the DpMe area and CA3 field.
Further study was to find out the $Fos$ expression upon heat stimulation. Contour graph of paracetamol was subtracted with paracetamol group without hot plate treatment. This gave the distribution of $Fos$ expression at PAG, CnF, SC, PaS and PrS.

Fig 3: Each contour graph was plotted according to the region of interested. Regions that have higher stained $Fos$ protein have been coloured light blue or pink and the least number of stained protein were dark blue or purple coloured. (i) Saline: $Fos$ expression at PAG, DpMe, CnF, DG, CA3 field. (ii) Paracetamol: $Fos$ expression at PAG, DpMe, SC, PaS and PrS. (iii) Aspirin: $Fos$ expression at PAG, CnF and PPTg. (iv) Paracetamol without hotplate: $Fos$ expression at DpMe and CA3 filed.

Fig 4: (v) Contour graph on $Fos$ expression on hot plate test by subtracting paracetamol from paracetamol without hot plate treatment. $Fos$ expression shown at PAG and extended to CnF, SC, PaS and PrS. (vi) Contour graph on paracetamol subtracting saline group showed regions that did not blocked upon paracetamol administration. $C-fos$ expression at PaS and PrS. (vii) Contour graph by subtracting paracetamol from aspirin showed the regions that have not been blocked by aspirin administration. $Fos$ expression was found at CnF, PBil, and PPTg.
By subtracting the paracetamol treated group with the saline group, results gained shown *Fos* expression at PaS and PrS, which were the regions where paracetamol could not block the pain transmission. Contour graph of *Fos* expression plotted by subtracting aspirin group with paracetamol group showed the mechanism of action of aspirin in blocking pain transmission but not in paracetamol. It was found that *c-fos* expressions were detected at cuneiform nuclei (CnF), parabrachial internal lateral nucleus (PBil), and pedunculopontine tegmental nuclei (PPTg).

**CONCLUSION**

Data stated have given support that oral administration of paracetamol and aspirin have effectively blocked the antinociceptive reaction. These were proven in the immunostaining of *Fos* antibody. The study of paracetamol distributions at the posterior ventral medial region of mice brain was highlighted. It was found that paracetamol has different mechanism of action as compare to aspirin. Paracetamol was found unable to block the neurons transmission to the subiculum while aspirin did not work in blocking CnF, PBil and PPTg. All these nucleuses have been studied to involved in pain transmission.

Besides hot plate test, this study was also performed by diffusion of noxious inhibitory controls. (Bouhassira D, 1990) They concluded that the PAG, CNF, and PB, three structures that are putatively involved in the modulation of pain. In addition to that, similar study conducted by Choi, Lee and Suh (2001) was performed in researching the antinociceptive profiles of aspirin and paracetamol in formalin, substance P and glutamate pain models. These are alternatives that may enhance the results of the present study.

**ACKNOWLEDGEMENTS**

I wish to thank Dr. Gavin Dawe for giving me a chance to involve in this project as well as his guidance all along the project. Besides, I would like to show my appreciation to Rajini, Dr Vivek Verma, Ou LianYun, Francis and all the fellow students that have been teaching me and giving me support.

**REFERENCES**

