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Combined carbamazepine and pregabalin therapy in a rat model of neuropathic pain

T. S. Hahm, H. J. Ahn*, S. Ryu, M. S. Gwak, S. J. Choi, J. K. Kim and J. M. Yu

Department of Anaesthesiology and Pain Medicine, Samsung Medical Centre, Sungkyunkwan University School of Medicine, 50, Ilwon-Dong, Kangnam-Gu, Seoul, Republic of Korea

* Corresponding author. E-mail: hyunjooahn@skku.edu

Editor's key points

- Optimal therapy for neuropathic pain has not been established.
- There is a need for exploration of combination therapies with different modes of action.
- A variety of dose combinations of pregabalin and carbamazepine were assessed using behavioural and functional measures.
- Synergism was detected mainly in the higher dose range.

Background. Carbamazepine and pregabalin have proven effects against neuropathic pain. Carbamazepine blocks voltage-dependent Na^+ channels, whereas pregabalin blocks voltage-dependent Ca^{2+} channels. The authors hypothesized that the co-administration of these drugs would synergistically reduce neuropathic pain.

Methods. Neuropathic pain was induced by L5 nerve ligation in Sprague–Dawley rats. To determine their ED_{50} values, carbamazepine and pregabalin were orally administered at 0.3, 3, 10, or 30 mg kg^{-1}. The drugs were then co-administered at 0, $1/4 \times ED_{50}$, $1/2 \times ED_{50}$, $1.5 \times ED_{50}$, and $2 \times ED_{50}$ to determine the ED_{50} and ED_{75} values of the drugs in combination. Allodynia was determined using the von Frey hair test and dose–effect curves and isobolograms were used to investigate drug interactions. Levels of the acute reactive protein c-Fos in the dorsal horn were evaluated as an indicator of pathological nerve excitation.

Results. At ED_{50} levels, carbamazepine and pregabalin did not exhibit synergism, but doses higher than ED_{75} were found to be synergistic. The combination index was 0.18 (strong synergy) and dose reductions were 35.7-fold for carbamazepine and 6.8-fold for pregabalin when co-administered when compared with a single administration at ED_{75}. The percentage allodynia relief was only 60% for carbamazepine and 80% for pregabalin by single administration, whereas their co-administration relieved allodynia by 100%. Furthermore, treatment decreased c-Fos expression in the dorsal horn, but expressional differences between animals treated with carbamazepine plus pregabalin were not significantly different from those treated with single drug.

Conclusions. Carbamazepine and pregabalin ameliorate neuropathic pain synergistically at higher doses.

Keywords: carbamazepine; ion channels; neuralgia; pregabalin

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Neuropathic pain is a chronic condition arising from injury or disease of the peripheral or central nervous system. It is a common problem and may affect around 8% of the population.[1] Furthermore, neuropathic pain is difficult to treat and the therapeutic efficacies of drugs are only about 50%, which makes the management of neuropathic pain challenging for physicians.[2]

The mechanisms responsible for neuropathic pain are not well established, and therefore, treatment largely depends on empirical measures, previous drug efficacies, and trial-and-error.[3] In clinical practice, physicians choose a drug and increase its dosage until side-effects are intolerable, and often, these side-effects prevent further administration.[4]

Recently, changes in ion channels have attracted attention as a possible aetiology of neuropathic pain. Treatments modulating targeted ion channels are based on the known reorganizations of ion channels in neuromas, dorsal root ganglia, the spinal cord, and the brain after nerve injury. Na^+ and Ca^{2+} channels are known to be abnormally activated, up-regulated, or both, and thus, blocking these channels offers the possibility of reducing neuropathic pain.[5]

Anti-epileptic drugs that target neuronal excitability by modulating ion channels, receptors, and intracellular signalling pathways[6 7] have been shown to reduce neuropathic pain.[8] Carbamazepine, the first anti-convulsant studied in clinical trials, probably alleviates pain by decreasing Na^+ channel conductance, whereas pregabalin acts on Ca^{2+} channels to inhibit pre-synaptic glutamate release.[7 9 10] In general, when drugs with different mechanisms of action are combined, analgesic efficacy is achieved using smaller doses and side-effects are reduced.[11] In patients with refractory partial seizures, the co-administration of carbamazepine and pregabalin was found to be highly effective.[12] However,

few studies have been conducted on neuropathic pain, although in one study on co-administered carbamazepine and gabapentin, it was found that they had a synergistic effect on trigeminal neuralgia.[13] Therefore, we hypothesized that the co-administration of carbamazepine and pregabalin would have a synergistic effect on neuropathic pain. In this study using a nerve ligation neuropathic pain model, behavioural measures were used to assess the effects of drug interaction on mechanical allodynia. In addition, c-Fos levels (a well-known indicator of rapid and transient neuronal activity in the central nervous system) were measured in the dorsal horn neurones,[14 15] to determine the extent to which different treatments suppress neuronal activity.[16]

Methods

Experiments were approved by our Institutional Animal Care Committee and were performed in accordance with the ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines.

Animal preparation

Male Sprague–Dawley rats, weighing 130–180 g, were housed in separate cages under a 12/12 h day/night cycle and provided food and water *ad libitum*. Rats were acclimatized for 5–7 days before the experiments.

Model of neuropathic pain

Neuropathic pain was induced using the procedure described by Kim and Chung.[17] Briefly, rats were anaesthetized with 2.5% enflurane in O_2. A dorsal midline incision was then made from L3 to S2, and under microscopic guidance, the left L6 transverse process was partly resected to visualize the L4 and L5 spinal nerves. The left L5 spinal nerve immediately distal to the dorsal root ganglion was then isolated and ligated tightly with 6-0 black silk.

One week later, rats showing any sign of motor dysfunction, including abnormal ambulation or placing/stepping reflex, were excluded. Animals with a paw withdrawal threshold (PWT) of >5 g (no allodynia) for the operated hindpaw were also excluded.[18]

Test for tactile allodynia

To avoid circadian cycle effects, all studies were performed at 8 a.m. An observer unaware of the drugs/doses used performed the tactile allodynia testing, which was performed using the von Frey hair test (vFT). Tactile allodynia peaks at 1–5 weeks after surgery and recovers 10–18 weeks later.[19] In a previous study, the maximum anti-allodynic effect of oral doses of carbamazepine and pregabalin was observed at 2 h post-dosing.[10] Therefore, the vFT was performed before and 2 h after drug administration at 1 week after surgery.

For vFT, a rat was placed in a clear plastic cage ($24\times13\times13$ cm) with a 4×4 mm wire-mesh grid floor and allowed to acclimatize for at least 15 min. A series of von Frey hairs (numbers: 4.17, 4.31, 4.56, 4.74, 4.93, 5.07, and 5.18; Stoelting, Wood Dale, IL, USA) starting with number 4.31 were applied in sequence through the grid floor to the ventral surface of the operated hindpaw with sufficient pressure to cause the filament to buckle.

Brisk paw lifting within 5 s was defined as a positive response and this was followed by the application of the next weakest filament. The absence of a paw withdrawal response after five trials prompted the use of the next strongest filament. This was continued until five additional measurements had been made after recording the initial change in the paw withdrawal response. PWT results were calculated using the following formula.

$$50\%\text{g PWT} = \frac{10^{(Xf+\kappa\times0.22)}}{10\,000}$$

where Xf is the number of the final von Frey filament used, κ the statistic from the tabular value for the pattern of positive/negative responses.[20]

We also recorded side-effects such as sedation and motor dysfunction at the time of allodynia testing.

Carbamazepine and pregabalin administration

Carbamazepine was purchased from Sigma Chemical Company (St Louis, MO, USA), and pregabalin from Pfizer (Ann Arbor, MI, USA). Drugs were randomly administered 1 week after nerve ligation, that is, 0.3, 3, 10, or 30 mg kg^{-1} of carbamazepine ($n=15$ in each dose) or 0.3, 3, 10, or 30 mg kg^{-1} of pregabalin ($n=15$ in each dose) via an oral gavage tube. Carbamazepine was prepared in 100% DMSO and pregabalin in 0.9% saline. Stock drugs were prepared at 20 mg ml^{-1} and all doses were delivered as 1 ml solutions, which was achieved by adding normal saline. vFT was performed before drug administration and 2 h afterwards.

The PWT at each concentration was converted to degrees of anti-allodynic effect using the following formula

$$\text{Anti - allodynic effect} = \frac{a}{8.81}$$

where 8.81=[post-treatment PWT–pre-treatment PWT] at maximal anti-allodynia and a=[post-treatment PWT–pre-treatment PWT] at each dose.

Determining ED_{50} values

ED_{50} values and dose–effect curves were obtained using the Calcusyn program (BIOSOFT, Cambridge, UK). The ED_{50} of carbamazepine was found to be 10.3 mg (95% range: 2.6–40.2 mg) and that of pregabalin was 3.3 mg (95% range: 1.56–7.6 mg).

These ED_{50} values were used to determine doses for co-administration. Doses of $0\times ED_{50}$, $1/4\times ED_{50}$ $1/2\times ED_{50}$, $1.5\times ED_{50}$, and $2\times ED_{50}$ of each drug were co-administered, that is, carbamazepine 0 mg, pregabalin 0 mg; carbamazepine 2.6 mg, pregabalin 0.8 mg; carbamazepine 5.2 mg, pregabalin 1.7 mg; carbamazepine 15.5 mg, pregabalin 5.0; and carbamazepine 20.6, pregabalin 6.6 mg, respectively ($n=15$ per dose combination). Methods of drug