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Efficacy and safety of combined low doses of either diclofenac or celecoxib with gabapentin versus their single high dose in treatment of neuropathic pain in rats

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ABSTRACT

Neuropathic pain is a worldwide health problem with no consensus regarding its optimal therapy. This study compared the analgesic effect and gastric, hepatic, and renal safety of combined low doses of diclofenac and celecoxib with gabapentin versus their individual high doses in the treatment of neuropathic pain in rats. Left sciatic nerve ligation was used as neuropathic pain model. Rats were allocated into 7 groups (7 rats for each): sham control; model group (received vehicle); Gaba-group (received gabapentin (100 mg/kg /day); Diclo 10-group (received diclofenac (10 mg/kg); Cele 10-group (received celecoxib (10 mg/kg/day); Gaba + Diclo 5 (receivedgabapentin(100 mg/kg /day) plus diclofenac (5 mg/kg); Gaba + Cele 5 (received gabapentin (100 mg/kg/day) plus celecoxib (5 mg/kg)). The analgesic effect was assessed using both hot plate and acetone tests. The impact of the used drugs on peptic ulcer index, liver enzymes, and serum urea and creatinine was evaluated, along with histopathological examination and oxidative stress parameters. Combination therapy of low dose of either diclofenac or celecoxib, with gabapentin showed higher analgesic effect compared with their individual high doses as indicated by prolonged response time in hot plate test and decreased frequency of paw withdrawal in acetone test. Their effect was associated with gentle effect on gastric mucosa, renal and hepatic integrity and oxidative stress parameters. In conclusion, the use of combined low doses of either diclofenac or celecoxib with gabapentin is better than high dose monotherapy regarding both the efficacy and safety.

1. Introduction

Neuropathic pain is a worldwide health problem that affects 6.9% to 10% in general population [1]. It results from dysfunction or lesions in nervous system and is associated with several disease conditions including diabetes and disc prolapse. It is manifested by hyperalgesia (exaggerated response to painful stimuli) allodynia (painful response to normal stimuli), and spontaneous pain sensation. Treatment of neuropathic pain with traditional analgesics achieves non satisfactory effect. Thus, combination of different pain modulators including non-steroidal anti-inflammatory drugs (NSAIDs) and antiepileptic's (e.g. gabapentin) is recommended in recent guidelines [2,3].

NSAIDs are widely used in treatment of neuropathic pain. The main mechanism of action of NSAIDs is via inhibition of cyclooxygenase (COX) enzyme. NSAIDs are classified into either non selective COX-inhibitors (inhibit both COX-1 and COX-2) or selective COX-2 inhibitors (Preferentially inhibit COX-2). Diclofenac, a non-selective COX-inhibitor, and celecoxib, a selective COX-2 inhibitor, are among the most commonly prescribed NSAIDs [4].

NSAIDs are known to produce several adverse effects including gastrointestinal, renal and hepatic insults. Consequently, various strategies have been postulated to alleviate adverse effects of NSAIDs including use of small doses, short duration of therapy, combination with other pain modulators such as antidepressant and antiepileptic agents.

Gabapentin is an antiepileptic drug that commonly used in treatment of neuropathic pain. Its mechanism of action is not fully explored. Inhibition of voltage-gated calcium channels and disruption of N-methyl-D-aspartate (NMDA) activated pathways are involved in its mechanism of action [5].

The current study was tailored to compare the efficacy and safety of

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combined low doses of either diclofenac or celecoxib, with gabapentin versus high doses of individual drugs in treatment of neuropathic pain in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 150–200 g were used in the present study. Rats were purchased from the National Research Center, Giza, Egypt. Rats were housed in stainless steel cages. They were left freely wandering in their cages for two weeks with 12 h dark/light cycles for acclimatization before starting the experiment. They were allowed free access to tap water and normal rats' diet (El-Nile Company, Egypt). All experimental protocols were approved by the faculty board and local ethical committee and coincide with international guidelines.

2.2. Chemicals

Gabapentin was purchased from Eva Pharma, Cairo, Egypt. Diclofenac was purchased from Novartis, Cairo, Egypt and celecoxib was purchased from Pfizer, Cairo, Egypt. Gabapentin, diclofenac and celecoxib powders were suspended in 1% aqueous solution of carboxymethyl cellulose.

2.3. Sciatic nerve ligation

The model of sciatic nerve injury was done according to previous method [6]. Briefly, animals were anesthetized with ketamine hydrochloride (80 mg/kg, IP) and xylazine (10 mg/kg). An incision (about 2 cm) was made at the left mid-thigh level. Then, sciatic nerve was explored after muscles dissection. A sterile chromic gut suture was gently ligated around the isolated sciatic nerve proximal to its trifurcation. The muscles overlying the nerve were sutured with chromic gut ligature. Development of hyperalgesia resulted in a guarding behavior of the ipsilateral hind paw.

2.4. Experimental design

A total of 49 rats were allocated into 7 groups (7 rats per group). Model group (received vehicle); Gaba-group (received gabapentin (100 mg/kg /day)) [7]; Diclo10-group (received diclofenac (10 mg/kg)) [8]; Cele10-group (received celecoxib (10 mg/kg/day)) [9]; Gaba + Diclo5 (received gabapentin (100 mg/kg/day) plus diclofenac (5 mg/kg)) [10]; Gaba + Cele5 (received gabapentin (100 mg/kg/day) plus celecoxib (5 mg/kg)) [11]. In addition to sham group which subjected to surgical exploration of left sciatic nerve without nerve ligation and received vehicle.

All drugs were administered orally by gavage for 4 weeks. To assess of nociception, rats were subjected to behavioral tests at the end of the study.

The doses of diclofenac and celecoxib were justified according to previous studies [8–11] and calculated from human equivalent doses using the following formula: Human equivalent dose (mg/kg) = Animal dose (mg/kg) × Animal K_m / Human K_m where K_m is a correction factor reflecting the relationship between body weight and body surface area. For a typical 60 kg adult human, the K_m is 37, whereas for rat the average K_m is 6 [12]. The dose of 5 mg/kg of either diclofenac or celecoxib is equivalent to about 50 mg/day (the minimal clinically used dose).

2.5. Behavioral tests

2.5.1. Hot plate test

Hot plate is a test of thermal pain. Before performing the test, each rat was habituated twice to the hot plate. Each rat was placed on the hot plate with a fixed temperature of 55 ± 0.5 °C to observe the pain responses manifested by hind-paw-licking or jumping. The response latency was recognized as the recorded time (in seconds) between the platform and reaction. The test was performed at the end of the 4thweek [13,14].

2.5.2. Acetone test

Acetone test was used to assess the cold allodynia.After adaptation for 15 min, one drop (50 µL) of absolute acetone was applied to the plantar surface of the left foot using a syringe connected to polyethylene tube [15]. The number of foot withdrawal responses after application of acetone was recorded for one minute. The test was repeated 10 times with an interval of 5 min between each test. The frequency of response to acetone was expressed as a paw withdrawal frequency according to the following formula [number of paw withdrawals/number of trails] × 100) [16].

2.6. Collection of blood and tissue samples

At the end of experiment, the rats were anaesthetized with ether, blood samples were collected from abdominal aorta. Sera were separated and stored in aliquots at −80 °C till used for estimation of liver transaminases and renal functions. Liver, kidney and stomach were dissected. Tissue samples were stored at −80 °C for biochemical studies.

2.7. Assessment of gastric, hepatic and renal injuries

Gastric mucosal lesions were expressed in terms of the ulcer index (U.I.). According to Till et al [17], the severity factor is graded as follows; 0 for no lesions; 1 for petechae; 2 for erosions less than 1 mm; 3 for erosions of 1 to less than 2 mm; 4 for erosions of 2–4 mm and 5 for erosions greater than 4 mm in length. The partial scores were then summated to obtain the ulcer index of the animal examined. The U.I. for each group was taken as the mean lesion score of all the rats in that group.

Liver and renal injuries were assessed biochemically by measurement of liver enzymes and renal function, respectively as well as by histopathological examination.

Alanine transaminase (ALT) and Aspartate transaminase (AST) activities in the serum were assessed by enzymatic colorimetric methods using commercial kits (spectrum diagnostic, Egypt).

Serum creatinine and urea levels were determined using commercial kits from Spectrum Diagnostics (Cairo, Egypt).

Small pieces of stomach, liver and kidney from each rat were fixed in 10% formalin, dehydrated in a graded alcohol series, cleared with xylene and embedded in paraffin wax. Hematoxylin and Eosin stain (H &E) was used in staining the sections (5 µm thick) to study the general histological structure, using an Olympus (U.TV0.5XC-3) light microscopy. Slides were photographed using an Olympus digital camera. Images were processed using Adobe Photoshop [21].

2.8. Assessment of oxidative stress in tissue homogenates

Specimens from liver, kidney and stomach were weighed and homogenized separately in potassium phosphate buffer 10 mM pH (7.4). The homogenates were centrifuged at 4000*g* for 15 min. The supernatant was used for determination of malondialdehyde (MDA) level, total nitrite/nitrate (NOx) and superoxide dismutase (SOD) activity.

MDA, an index of lipid peroxidation, was determined by using 1,1,3,3-tetramethoxypropane as standard [18]. Total NOx, the stable oxidation end products of nitric oxide, served as an index of nitric oxide level and was measured by reduction of nitrate into nitrite using activated cadmium granules, followed by color development with Griess reagent in acidic medium [19].