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# Co-administration of morphine and gabapentin leads to dose dependent synergistic effects in a rat model of postoperative pain

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ABSTRACT

Despite much evidence that combination of morphine and gabapentin can be beneficial for managing postoperative pain, the nature of the pharmacological interaction of the two drugs remains unclear. The aim of this study was to assess the interaction of morphine and gabapentin in range of different dose combinations and investigate whether co-administration leads to synergistic effects in a preclinical model of postoperative pain. The pharmacodynamic effects of morphine (1, 3 and 7 mg/kg), gabapentin (10, 30 and 100 mg/kg) or their combination (9 combinations in total) were evaluated in the rat plantar incision model using an electronic von Frey device. The percentage of maximum possible effect (%MPE) and the area under the response curve (AUC) were used for evaluation of the antihyperalgesic effects of the drugs. Identification of synergistic interactions was based on Loewe additivity response surface analyses. The combination of morphine and gabapentin resulted in synergistic antihyperalgesic effects in a preclinical model of postoperative pain. The synergistic interactions were found to be dose dependent and the increase in observed response compared to the theoretical additive response ranged between 26 and 58% for the synergistic doses. The finding of dose-dependent synergistic effects highlights that choosing the right dose–dose combination is of importance in postoperative pain therapy. Our results indicate benefit of high doses of gabapentin as adjuvant to morphine. If these findings translate to humans, they might have important implications for the treatment of pain in postoperative patients.



## 1. Introduction

Annually, millions of surgeries are being performed, ranging from minimally invasive to amputation surgery and the majority leads to the development of postoperative pain (Weiser et al., 2008; Wu and Raja, 2011). Acute postoperative pain is a complex physiological response that is related to the tissue trauma occurring during surgery and results in hypersensitivity of the central nervous system and leads to irregular sensory perception of pain, such as allodynia and hyperalgesia (Brennan, 2011). Postoperative pain can increase the risk of postsurgical complications; it interferes severely with patient recovery leading to delayed discharge times and raises significantly the cost of medical healthcare (Brennan et al., 2007; Kehlet et al., 2006; Pavlin et al., 2002). Novel surgical techniques, such as laparoscopic procedures have widely been used in order to reduce the development of postoperative pain. However, the use of these innovative techniques is still limited to small operations, hence the need for rational pharmacotherapy is still necessary (Fagotti et al., 2011).

Morphine remains the "gold standard" for postoperative pain management. However its use is hampered by development of tolerance in many patients leading to a corresponding increase in dose requirements (Dumas and Pollack, 2008). Even though opioids are very effective in relieving pain, they often lead to severe adverse effects such as nausea, vomiting, ileus, respiratory depression, and sedation (Dahl et al., 2010). An often suggested hypothesis is that simultaneous treatment with opioids and non-opioid analgesics that target distinct pain transduction pathways in the body might lead to a synergy of increased analgesic effects, so that lower doses of both drugs can be used with a subsequent lower occurrence of adverse effects (White and Kehlet, 2010).

Gabapentin, a non-endogenous amino acid and a structural analogue of the neurotransmitter GABA (Honarmand et al., 2011) is a safe and well-established anticonvulsant drug (Rose and Kam, 2002). Despite the structural resemblance with GABA, gabapentin is neither active on GABA receptors nor is it transformed metabolically into GABA. Gabapentin's main mechanism of action involves high affinity binding to the α2δ protein, an auxiliary subunit of the voltage gated calcium channels in the CNS. Selective binding of gabapentin to the α2δ subunit inhibits $Ca^{2+}$ influx into the presynaptic terminal and therefore modulates the release of neurotransmitters (Taylor et al., 1998). The use of gabapentin has been well established for the treatment of neuropathic pain, through preclinical (Abdi et al., 1998) and clinical testing (Bennett and Simpson, 2004; Rose and Kam, 2002; Rosner et al., 1996;

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Singh and Kennedy, 2003). Preclinical (Field et al., 1997a) and clinical (Chang et al., 2014; Dauri et al., 2009; Kong and Irwin, 2007; Mathiesen et al., 2007) evidence also suggests that gabapentin can be of value for treatment of postoperative pain. Preclinical combination studies of morphine and gabapentin have been performed and synergistic effects have been demonstrated in models of neuropathic and acute pain in the rat (De la O-Arciniega et al., 2009; Matthews and Dickenson, 2002; Shimoyama et al., 1997; Smiley et al., 2004). Clinical studies have further strengthened the hypothesis that gabapentin may increase the antinociceptive effect of morphine and reduce morphine consumption in patients with neuropathic cancer pain (Keskinbora et al., 2007) and postoperative pain (Dirks et al., 2002; Gilron, 2007).

Despite much evidence indicating beneficial effects of gabapentin as an adjuvant analgesic for the treatment of postoperative pain, the heterogeneity of clinical trials have rendered establishment of an optimal gabapentin dose difficult and no systematic study can be found in which combinations of morphine and gabapentin lead to synergistic effects in postoperative pain (Dahl et al., 2014; Tiippana et al., 2007). It is well recognized that the presence of synergistic effects between two drugs can be dose dependent (Tallarida, 2012) meaning that the complete characterisation of drug–drug interactions can only be achieved by administering the drugs in a range of different dose combinations.

The aim of this study was to assess the interaction of morphine and gabapentin in a range of different dose combinations and investigate whether co-administration of morphine and gabapentin can lead to synergistic effects in the plantar incision model of postoperative pain in the rat. Identification of synergistic interactions was based on three-dimensional response surface analyses in accordance with the concept of Loewe additivity (Greco et al., 1995; Tallarida and Raffa, 2010).

## 2. Materials and methods

### 2.1. Study design

Data was obtained from two separate studies (studies A and B). Study A consisted of 16 treatment arms, where the experimental animals were dosed with either saline or one of three predefined doses of morphine (1, 3 and 7 mg/kg), gabapentin (10, 30 and 100 mg/kg) or their combination following incision surgery. Von Frey measurements were taken at predefined time points. In study B the experimental animals were dosed with either saline or three predefined doses of morphine (1.5, 5 and 10 mg/kg) after incision surgery and von Frey measurements were taken at predefined time points. Both studies were blinded and randomized. The number of animals used were $n = 6$ per group ($n = 96$ in total) for study A and $n = 4$ per group ($n = 24$ in total) for study B.

### 2.2. Animals

All experiments were approved by the Danish Animal Experiments Inspectorate (Dyreforsøgstilsynet). The animals were treated according to the Ethical Guidelines for Investigation of Experimental Pain in Conscious animals, as issued by the International Association for the Study of Pain (Zimmermann, 1986). The experiments were conducted on male Sprague-Dawley rats (Taconic A/S, Denmark). The animal weight varied between 300–350 g. The animals were housed in groups of six with food and water available ad libitum. Care was taken to maintain constant environmental conditions. The room temperature was maintained at 20–23 °C and room illumination was on a 12/12 h light–dark cycle (lights on: 06:00–18:00 h). The animals were allowed to acclimatize to the laboratory environment for at least ten days before entering the study. Animals (study A) were used as part of a pharmacokinetic study prior to the present study. Here the animals were dosed with the same amount of drug or combination of drugs as the ones used in the present study and blood samples were taken in predefined time points. After a full recovery period of one week the animals were used for the present study.

### 2.3. Surgical procedure

Incisional surgery was performed as previously described by Brennan et al. (1996) with slight modifications. Surgery was performed under 2 % isoflurane anaesthesia (Univentor™ 1200 Anaesthesia Unit). A 1-cm longitudinal incision was made through the skin and fascia with a number 10 blade (Swann Morton™), starting 0.5 cm from the proximal edge of the heel and extending towards the paw. The plantar muscle was elevated and incised longitudinally leaving the muscle origin and insertion points intact. After haemostasis with gentle pressure, the skin was sutured with two mattress sutures of 5-0 nylon on a FS-2 needle (Ethicon™). After surgery, the animals were placed in their original cages, where they were allowed to recover for a period of approximately 1 h. After the surgical procedure and during the study, the paw incisions were frequently checked and signs of suture tearing would lead to exclusion of the study. No animals suffered from suture tearing, thus no animals were excluded from the study. At the end of the study (maximum 8 h postoperatively), all animals were euthanized, using a combination of a mixture of $O_2/CO_2$ for anesthetization and the technique of cervical dislocation for the finalization of the procedure.

### 2.4. Von Frey testing

All animals were placed in individual Plexiglas™ cages on an elevated mesh and were allowed to acclimatize for at least 30 min before behavioural testing commenced. Withdrawal thresholds in response to mechanical stimulation (tactile allodynia) following the incision was assessed using an automated electronic von Frey Anesthesiometer (IITC™ 2390 Series with a 800 g test probe, CA, U.S.A.) as described by Whiteside et al. (2004), fitted with a single nonflexible monofilament of stable diameter (0.8 mm). Pressure was applied at the lateral, proximal side of the incision as described by Brennan et al. (1996) with an increasing and steadily escalating force (approximately 5 g/s) (Ängeby Möller et al., 1998). Rapid withdrawal of the hindpaw was considered a positive reaction to a noxious stimulus. Three measurements were taken per predefined time point and the average of the measurements was used in the data analysis. A cut-off value of 60 g was chosen, above which the rat paw would be lifted by the probe. All measurements that exceeded the cut-off value were treated as equal to 60 g (5.1% of the full dataset). Baseline responses of naïve animals were obtained prior to the surgery, postoperative responses were obtained approximately 1 h after surgery and prior to dosing and drug responses were obtained for a period of 300 min post dosing.

### 2.5. Drugs and administration

Morphine hydrochloride (20 mg/mL; Morphine DAK) was purchased from community pharmacies and diluted to the appropriate concentrations (1.2, 1.8, 3.6, 6, 8.4 and 12 mg/mL) for the six different dosing groups (1, 1.5, 3, 5, 7 and 10 mg/kg) with sterile isotonic saline. Gabapentin (Teva Pharmaceuticals, BeerSheva, Israel) was dissolved in sterile isotonic saline towards the appropriate concentrations (12, 36 and 120 mg/mL) for the three dosing groups (10, 30 and 100 mg/kg). The drugs were administered subcutaneously in dosing volumes of 0.8–1.0 mL/kg. All animals received two s.c. injections with an interval of 1–2 min at separate injection sites in order to minimize the risk of physicochemical interactions of the two formulations. The monotherapy arms received saline as the second injection. Drug solutions were prepared by the investigator who performed the behavioural experiments and were blinded by an