Gabapentin and the Neurokinin$_1$, Receptor Antagonist CI-1021 Act Synergistically in Two Rat Models of Neuropathic Pain

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ABSTRACT

The present study examines the effect of combinations of gabapentin (Neurontin) and a selective neurokinin (NK), receptor antagonist, 1-(1H-indol-3-ylmethyl)-1-methyl-2-oxo-2-[[1-phenylethyl]amino]ethyl]-2-benzoturanylmethyl ester (CI-1021), in two models of neuropathic pain. Dose responses to both gabapentin and CI-1021 were performed against static alldynia induced in the streptozocin and chronic constriction injury (CCI) models. Theoretical additive lines were calculated from these data. Dose responses to various fixed dose ratios of a gabapentin/CI-1021 combination were then examined in both models. In the streptozocin model, administration of gabapentin/CI-1021 combinations at fixed dose ratios of 1:1 and 60:1 resulted in an additive effect with dose response similar to the theoretical additive line. However, a synergistic interaction was seen after fixed dose ratios of 10:1, 20:1, and 40:1 with static alldynia completely blocked and the dose responses shifted approximately 8-, 30-, and 10-fold leftward, respectively, from the theoretical additive values. In the CCI model, after fixed dose ratios of 2:1 and 20:1, combinations of gabapentin and CI-1021 produced an additive response. At the fixed dose ratio of 10:1 static alldynia was completely blocked with an approximate 10-fold leftward shift of the dose response from the theoretical additive value, indicating synergy. The combination of gabapentin with a structurally unrelated NK receptor antagonist, (2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994), also produced synergy, at a fixed dose ratio of 20:1. This ratio completely blocked streptozocin-induced static alldynia and was approximately shifted leftward 5-fold from the theoretical additive value. These data suggest a synergistic interaction between gabapentin and NK receptor antagonists in animal models of neuropathic pain.

Neuropathic pain is a consequence of disease or trauma to peripheral nerves or the central nervous system. Thus, this type of pain affects many people with a wide range of ailments (e.g., trauma, diabetes, stroke, postherpetic neuralgia, and cancer). In the clinic, neuropathic pain patients can present with a number of different pain subtypes, with allodynic sensations (pain to previously innocuous stimuli) being among the most common and debilitating. Conventional analgesics, such as opioids and nonsteroidal anti-inflammatory drugs have limited therapeutic value in the management of neuropathic pain. This has led to the use of adjuvant analgesics, such as antidepressants and anticonvulsants, for the management of these types of pains. However, no agent is fully effective in all patients and undesirable side effects are common (James and Page, 1994; Galer, 1995).

In 1996, we described studies suggesting that gabapentin may be useful in the treatment of pain and anxiety (Singh et al., 1996). A number of preclinical reports followed demonstrating that gabapentin, unlike opiates, had no effect on transient physiological pain responses (Field et al., 1997a,b; Hunter et al., 1997; Shimoyama et al., 1997) but possessed antihyperalgesic and antiallodynic properties in animal models of inflammatory, surgical, and neuropathic pain (Xiao and Bennett, 1995; Singh et al., 1996; Field et al., 1997a,b, 1999a,b; Hunter et al., 1997; Shimoyama et al., 1997; Hwang and Yaksh, 1997). In 1998, the first randomized, double blind, placebo-controlled clinical trials were reported that demonstrated that gabapentin showed some efficacy in approximately 50% of patients with postherpetic neuralgia or diabetes-induced neuropathy (Backonja et al., 1998; Row-

ABBREVIATIONS: NK, neurokinin; CCI, chronic constriction injury; PWT, paw withdrawal threshold; MED, minimum effective dose; CI-1021, 1-(1H-indol-3-ylmethyl)-1-methyl-2-oxo-2-[[1-phenylethylamino]ethyl]-2-benzoturanylmethyl ester; CP-99994, (2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine.
botham et al., 1998). These data confirmed the preclinical studies and also the growing body of anecdotal clinical evidence.

For many years, it has been hypothesized that the peptide substance P plays an important role in pain processing due to its localization in and release from primary afferent fibers after noxious stimuli (Otsuka and Yoshioka, 1993). Substance P selectively interacts with the endogenous neurokinin (NK₁) receptor. The development of selective, nonpeptide NK₁ receptor antagonists has been critical in elucidating the role of substance P in pain. A large amount of preclinical evidence suggests that these compounds possess antihyperalgesic and antiallodynic properties in models of pain (Ma and Hill, 1999). CI-1021 is a selective NK₁ receptor antagonist (Singh et al., 1997) that has been shown to block the maintenance of static allodynia in models of neuropathic pain (Field et al., 1998; Gonzalez et al., 2000). There have been a limited number of clinical trials of NK₁ receptor antagonists in pain (Dionne, 1999), but the clinical utility of these compounds has yet to be fully determined.

Combination therapy is often used to increase the clinical utility of analgesic agents (Eisenach and Gebhart, 1999). The coadministration of two such compounds often achieves analgesia at lower doses than required for either compound alone, leading to enhanced pain relief and a reduction of side effects (Eisenach and Gebhart, 1999). Herein, we describe the effect of coadministration of gabapentin and CI-1021 in two models of neuropathic pain. Part of this work has been published previously in abstract form (Field et al., 2000).

Materials and Methods

Animals. Male Sprague-Dawley rats (200–250 g), obtained from Charles River (Margate, Kent, UK), were housed in groups of six. All animals were kept under a 12-h light/dark cycle (lights on at 7:00 AM) with food and water ad libitum. All experiments were carried out by an observer who was unaware of drug treatments.

Development of Diabetes in Rat. Diabetes was induced in rats by a single i.p. injection of streptozocin (50 mg/kg) as described previously (Field et al., 1999b). Control animals received a similar administration of isotonic saline.

CCI Surgery in Rat. CCI surgery was performed as described previously (Field et al., 1999a).

Effect of Combinations on the Maintenance of Streptozocin or CCI-Induced Static Alldynia. Dose responses to gabapentin, CI-1021, and CP-99,994 were first performed alone in the CCI and streptozocin models. The dose-response data for both compounds in the combination were used to determine theoretical additive lines using the method described by Tallarida (2000). Combinations were examined after a fixed ratio design. A dose response to each fixed dose ratio of the combination was performed and compared with the theoretical additive line. On each test day, baseline paw withdrawal thresholds (PWTs) to von Frey hairs were determined before drug treatment. All compounds were administered p.o. and PWT reexamined for up to 4 h. CI-1021 or CP-99,994 was administered directly after gabapentin. The data are expressed at the 1-h time point in the streptozocin model and at the 2-h time point in the CCI model because these times represent the peak antiallodynic effects.

Evaluation of Static Alldynia. Static allodynia was measured using Semmes-Weinstein von Frey hairs (Stoelting, Wood Dale, IL). Animals were placed into wire bottom cages allowing access to the underside of their paws. Animals were habituated to this environment before the start of the experiment. Static allodynia was tested by touching the plantar surface of the animals right hind paw with von Frey hairs in ascending order of force (0.7, 1.2, 1.5, 2, 3.6, 5.5, 8.5, 11.8, 15.1, and 29 g) for up to 6 s. Once a withdrawal response was established, the paw was retested, starting with the next descending von Frey hair until no response occurred. The highest force of 29 g lifted the paw as well as eliciting a response, thus representing the cutoff point. The lowest amount of force required to elicit a response was recorded as the PWT in grams.

Drugs. CI-1021 and CP-99,994 were synthesized at Pfizer Global Research and Development (Cambridge, UK), and gabapentin was synthesized at Pfizer Global Research and Development (Ann Arbor, MI). CI-1021 was dissolved in gelucre at a temperature of 65°C and once dissolved maintained at 45°C. Gabapentin was dissolved in water. CP-99,994 and streptozocin (Aldrich, Dorset, UK) were dissolved in 0.9% (w/v) NaCl. Drug administrations were made in a volume of 1 ml/kg. Gabapentin was administered orally directly followed by the oral administration of CI-1021.

Data Analysis. Data for dose responses were subjected to a Mann-Whitney U test to compare drug values with controls. The dose-effect data for the individual compounds (acting alone) were used to calculate the theoretical (regression) line of additivity for each fixed ratio combination (Tallarida et al., 1997; Tallarida, 2000). The calculated potency value from the line of additivity was then compared with the corresponding value obtained experimentally to assess significance using the software package PharmToolsPro (The McCary Group, Elkins Park, PA).

Results

Effect of Gabapentin and CI-1021 Alone on Streptozocin-Induced Static Alldynia. CI-1021 dose dependently (1–30 mg/kg p.o.) blocked the maintenance of static allodynia with an MED of 3 mg/kg (Fig. 1). The antiallodynic action of CI-1021 lasted for over 3 h at the doses of 10 and 30 mg/kg (Fig. 1). Similar administration of gabapentin also dose dependently (10–100 mg/kg p.o.) blocked the maintenance of static allodynia with an MED of 10 mg/kg (Fig. 1).

Effect of Gabapentin and CI-1021 Alone on CCI-Induced Static Alldynia. The oral administration of either CI-1021 (10–100 mg/kg) or gabapentin (10–100 mg/kg) dose dependently blocked the maintenance of static allodynia with MEDs of 30 mg/kg (Fig. 2). The highest dose (100 mg/kg) of gabapentin, but not of CI-1021, completely blocked static allodynia (Fig. 2).

Effect of Combinations of Gabapentin and CI-1021 on Streptozocin-Induced Static Alldynia. Gabapentin and CI-1021 had peak antiallodynic actions at 1-h postadministration in the streptozocin model. For clarity, only combination data for this time point are shown. The dose-response data for gabapentin and CI-1021 alone at 1 h were used to calculate theoretical additive lines (Fig. 3a) Gabapentin and CI-1021 were administered at fixed dose ratios of 1:1, 10:1, 20:1, 40:1, and 60:1. After a fixed dose ratio of 1:1 and 60:1, combinations of gabapentin and CI-1021 produced effects close to the theoretical additive line, indicating an additive response (Fig. 3). However, after fixed dose ratios of 10:1 20:1, and 40:1 static allodynia was completely blocked by respective total doses of 11, 3.15, and 10.25 mg/kg compared with respective theoretical additive total doses of 82.5, 89.9, and 94.6 mg/kg (Fig. 3). This confirms a synergistic interaction between the two compounds with the 10:1, 20:1, and 40:1 ratios shifted approximately 8-, 30-, and 10-fold from the theoretical additive lines (Fig. 3). Both gabapentin and CI-1021 had similar durations of action when administered alone (Fig. 1) and in combination in this model (data not shown).
Effect of Combinations of Gabapentin and CI-1021 on CCI-Induced Static Allodynia. Gabapentin and CI-1021 had peak antiallodynic actions at 2 h postadministration. All compounds were administered p.o., and PWTs were reexamined up to 4 h postdrug. Results are expressed as median force (grams) required to induce paw withdrawal in 8 to 10 animals/group (vertical bars represent first and third quartiles). *P < 0.05; **P < 0.01; ***P < 0.005 significantly different (Mann-Whitney U test) from vehicle-treated group at each time point.

Effect of Combinations of Gabapentin and CI-1021 on CCI-Induced Static Allodynia. Gabapentin and CI-1021 had peak antiallodynic actions at 2 h postadministration in streptozocin-treated animals before drug administration. All compounds were administered p.o., and PWTs were reexamined up to 4 h postdrug. Results are expressed as median force (grams) required to induce paw withdrawal in 8 to 10 animals/group (vertical bars represent first and third quartiles). *P < 0.05; **P < 0.01; ***P < 0.005 significantly different (Mann-Whitney U test) from vehicle-treated group at each time point.

Effect of Combinations of Gabapentin and CP-99,994 on Streptozocin-Induced Static Alldodynia. A dose-response analysis with CP-99,994 showed that its peak antiallodynic effect occurs at 1 h post-treatment, similar to gabapentin. Thus, the combination study was expressed at this time point. The dose-response data for gabapentin and CI-1021 alone at 1 h were used to calculate theoretical additive lines (Fig. 4). Gabapentin and CI-1021 were administered at fixed dose ratios of 5:1, 10:1, and 20:1. After fixed dose ratios of 5:1 and 20:1, combinations of gabapentin and CI-1021 produced an additive interaction (Fig. 4). However, the fixed dose ratio of 10:1 demonstrated synergy with static allodynia completely blocked by a total dose of 11 mg/kg compared with a theoretical additive total dose of 110 mg/kg (Fig. 4). Both gabapentin and CI-1021 had similar durations of action when administered alone (Fig. 2) and in combination in this model (data not shown).

Effect of Combinations of Gabapentin and CP-99,994 on Streptozocin-Induced Static Allodynia. Gabapentin and CP-99,994 alone at 1 h were used to calculate theoretical additive line (Fig. 5a). Gabapentin and CP-99,994 were administered at a fixed dose ratio of 20:1. Static allodynia was completely blocked by a total dose of 21 mg/kg compared with a theoretical additive total dose of 105 mg/kg (Fig. 5b). This represents a synergistic interaction with an approximate 5-fold leftward shift in the dose response compared with the theoretical additive line (Fig. 5b).

Discussion

We have previously reported that both gabapentin and CI-1021 can block the maintenance of streptozocin-induced static allodynia (Field et al., 1998, 1999b). The present study confirms and expands on these findings by demonstrating that both compounds can also block the maintenance of CCI-induced static allodynia. The major finding of the present study was the synergistic interaction between the two compounds when coadministered in both the streptozocin and CCI models of neuropathic pain. It is known that CI-1021 shows species differences with respect to its affinity for the NK1 receptor. Thus, it possesses at least 2 orders of magnitude higher affinity for the human and guinea pig than the rodent NK1 receptor (Singh et al., 1997). This difference is apparent in neuropathic pain models where 100-fold higher doses are required to block pain responses in the rat compared with the guinea pig (Field et al., 1998; Gonzalez et al., 2000). It could be suggested that CI-1021 might lose selectivity at high doses and that the antiallodynic action might not involve the NK1 receptor. However, results from a number of studies seem to argue against this and support the
involvement of the NK₁ receptor. Radioligand binding studies have shown that CI-1021 is a highly selective NK₁ receptor antagonist (Singh et al., 1997), and we have demonstrated that CI-1021 dose dependently blocks the tactile and thermal hypersensitivity induced by activation of central NK₁ receptors in the rat (Field et al., 1998; Gonzalez et al., 1998). Moreover, this antagonism was complete and required the same doses as those that blocked allodynia/hyperalgesia in models of neuropathic pain. Furthermore, at these doses CI-1021 had no effect on the NK₂ receptor-mediated tactile hypersensitivity (Field et al., 1998) and only a partial and transient effect on the NK₂ receptor-mediated thermal hypersensitivity (Gonzalez et al., 1998).

The most compelling evidence that the synergy between CI-1021 and gabapentin reported herein is related to an interaction with the NK₁ receptor is provided by our findings that the structurally unrelated NK₁ receptor antagonist CP-99,994 also synergizes with gabapentin.

The mechanisms responsible for synergistic interactions are poorly understood, but a number of hypotheses have been
developed to explain these effects (Yaksh and Malmberg, 1994). These include the interaction being pharmacokinetic, where one drug increases the active levels of the other by reducing its rate of clearance or altering its metabolism. The interaction may also be pharmacodynamic where concurrent activation of distinct systems may modulate a common pathway or one compound may enhance the affinity or coupling of the other. It may also be due to physiological interactions where a third endogenous substance is released by the combination. Although a number of analgesic drug combinations have been demonstrated to produce a synergistic interaction, the mechanism(s) for their synergism are not generally known (Tallarida, 2001). In fact, synergism is actually difficult to demonstrate precisely. This demonstration requires an experimental design and a statistical analysis that distinguishes between simple additivity and superadditivity. Indeed, many claims of synergism lack the precision that is required in this demonstration (Gebhart, 1992a,b; Tallarida, 1992). Our results and experiments demonstrate that the NK1 receptor (and substance P) is involved, because we showed that two structurally different NK1 antagonists produced the synergism. Equally important is our demonstration that the enhanced response is not merely a property of gabapentin and the NK1 antagonists. The enhancement also depends on the ratio of the constituents in the combination and on the model used. Further work is required to elucidate the exact mechanism of synergy between gabapentin and NK1 receptor antagonists. However, the lack of change in duration of action of each active fixed dose ratio suggests a pharmacodynamic rather than a pharmacokinetic interaction. Further studies examining plasma and central nervous system levels of each compound after administration of the combinations are required to fully evaluate this. Previous studies have suggested that both compounds work via central mechanisms of action but involve distinct mechanisms (Field et al., 1998, 1999b), thus it is likely that this synergy is mediated centrally.

The present data demonstrate a synergistic interaction between gabapentin and NK1 receptor antagonists in animal models of neuropathic pain. Preclinical data clearly demonstrate an important role for substance P in the mediation of pain (Ma and Hill, 1999). However, the role of substance P in the human remains to be clearly determined. The limited clinical studies to date with NK1 receptor antagonists have been disappointing (Hill, 2000), but it has been suggested that the compounds used as well as the design of the trials may not have been optimal (Urban and Fox, 2000). It is possible that substance P plays more of a modulatory role in the human compared with the rat. If this is the case then monotherapy with NK1 receptor antagonists may not demonstrate any clinical efficacy. Gabapentin is effective in the treatment of neuropathic pain (Backonja et al., 1998; Rowbotham et al., 1998). These studies have demonstrated that gabapentin has a good side effect profile compared with other

![Figure 4](https://jpet.aspetjournals.org/doi/abs/10.1124/jpet.107.108407)
Synergy between Gabapentin and NK₁ Receptor Antagonists


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References


Fig. 5. Effect of a fixed dose ratio of gabapentin and CP-99,994 on the maintenance of diabetes-induced static allodynia. All data are expressed as median force (grams) required to induce paw withdrawal in six to eight animals per group (vertical bars represent first and third quartiles).

existing agents used to treat neuropathic pain with somnolence and sedation being the main side effects. It is interesting to note that in these controlled clinical trials gabapentin was effective in approximately 50% of patients. This represents a good response rate for neuropathic pain treatment but suggests there are still patients refractory to treatment. In conclusion, the present data demonstrate a synergistic interaction between gabapentin and NK₁ receptor antagonists in animal models of neuropathic pain. It is suggested that the clinical utility of gabapentin may be enhanced if it is coadministered with an NK₁ receptor antagonist such as CI-1021.

a.

![Graph showing the effect of gabapentin and CP-99,994 on paw withdrawal force.]

b.

![Graph showing the effect of a fixed dose ratio of gabapentin and CP-99,994 on paw withdrawal force.]

20:1 gabapentin:CP-99,994

Total dose (mg/kg)