

Nitroparacetamol (NCX-701) and Pain: First in a Series of Novel Analgesics

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ABSTRACT

The combination of numerous classic drugs with nitric oxide donors has led to the development of new compounds with promising therapeutic activities in a great variety of situations, including cardiovascular and respiratory systems, ocular pressure, inflammation, and pain. One of the first compounds developed was NCX-701 or nitroparacetamol, resulting from the combination of paracetamol, a classic and popular analgesic used in a great number of over-the-counter medications because of its antipyretic and analgesic properties, and a nitrooxybutyryl moiety, which releases nitric oxide at a low but steady level. Although paracetamol is devoid of most of the gastrointestinal toxicity associated with aspirin-like drugs, this type of compounds was first designed to take advantage of the cytoprotective properties of nitric oxide when released at low concentrations. However, the combination of these molecules also resulted in an unexpected enhancement of the analgesic activity of paracetamol. In fact, NCX-701 has been shown to be effective in acute nociception as well as in neuropathic pain, situations in which paracetamol and other COX inhibitors are devoid of any effect. In addition, NCX-701 is more potent and, in some circumstances, more effective than its parent compound in different models of inflammatory pain. Furthermore, whereas paracetamol lacks any effective antiinflammatory action, NCX-701 might reduce inflammation. All these results taken together imply that the mechanism of action

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of NCX-701 is different from that of paracetamol, although it is not yet established for either molecule. NCX-701 appears to be a promising compound in the treatment of different types of pain, with a likely better profile of side effects than its parent molecule, paracetamol. Although recent clinical trials provided data consistent with the preclinical profile of NCX-701, further studies are needed to support its clinical use.

INTRODUCTION

New generations of antiinflammatory drugs have been developed in an attempt to enhance the analgesic and antiinflammatory activities of classic nonsteroidal antiinflammatory drugs (NSAIDs), as well as to reduce the adverse effects caused by these agents. A first attempt was the synthesis of selective cyclooxygenase-2 (COX-2) inhibitors, which initially appeared to generate less adverse effects than selective COX-1 or than nonselective COX inhibitors, when given at therapeutic dosage. Unfortunately, the first generation of selective COX-2 inhibitors did not seem to show a better pharmacological profile than nonselective COX inhibitors and had unexpectedly severe adverse effects. All these problems lowered expectations for these new analgesics (Bejarano and Herrero 2003; Herrero et al. 2003 and references within). A different strategy was the development of drugs that combined classic and well-known NSAIDs with other antinociceptive compounds. The rationale for this approach was based on the synergistic, or supraadditive, enhancement of the analgesic effects observed with the combined administration of COX inhibitors and other antinociceptive agents. A good example of this research has been the synthesis of nitric oxide (NO)-releasing derivatives of nonsteroidal antiinflammatory drugs (NO-NSAIDs).

NO was originally known as an endothelial-derived relaxing factor, owing to its vasodilatory effects. Today we know that NO participates in several physiological functions and is a regulator of inflammatory responses (Wallace 2005). NO, at low concentrations, is involved in the maintenance of the integrity of the gastrointestinal mucosa, with an important role in the homeostatic maintenance of gastric mucosal blood flow. At low doses NO has actions similar to those of prostaglandins, and this led to the idea that NO donors might be useful as protectors of the gastrointestinal mucosa. On the other hand, one of the major adverse effects associated with the administration of classic NSAIDs is gastrointestinal toxicity (Brown et al. 1992; Whittle 1993; Elliott et al. 1995; del Soldato et al. 1999). As a result, the development of NO-NSAIDs was first based on the hypothesis that NO might compensate for the gastrointestinal toxicity resulting from the inhibition of COX enzymes. The introduction of a NO-releasing moiety to the backbone of COX inhibitors led to the development of stable, high-potency, long-lasting NO donor drugs with an inhibitory action on COX enzymes (Fiorucci et al. 2001; Keeble et al. 2001; Keeble and Moore 2002). The resulting NO-NSAIDs seemed to be not only safer for gastrointestinal mucosa but also more effective analgesics than their parent compounds (Fiorucci et al. 1999; Fiorucci et al. 2000; Keeble and Moore 2002; Romero-Sandoval et al. 2002; Chiroli et al. 2003; Fiorucci and Antonelli 2006).

The possible enhancement of safety by NO is explained, as already mentioned, by its cytoprotective properties. The enhancement of analgesia was, however, an unexpected observation, the mechanism of which is not yet well understood. NO is released not only in peripheral tissues but also in the nervous system, where it acts as a neurotransmitter and/or neuromodulator (Kiss and Vizi 2001) and plays complex roles in the processing of nociceptive information. In fact, it has been shown that NO may either induce

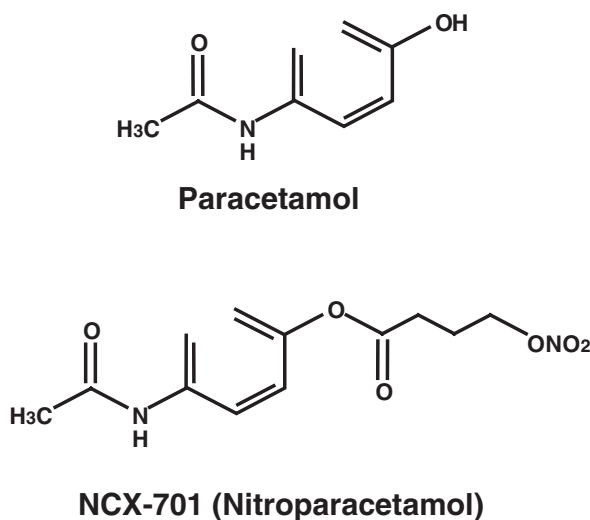


FIG. 1. Chemical structure of paracetamol and NCX-701 (nitroparacetamol).

hyperexcitability and, therefore, hyperalgesia, or have an inhibitory action on the nociceptive system, causing analgesia (Meller and Gebhart 1993; Kurihara and Yoshioka 1996; Chiari et al. 2000). Disparate effects seem to be related to the levels of NO, so that at low levels NO is more likely to have antinociceptive action (Kurihara and Yoshioka 1996; Sousa and Prado 2001).

NCX-701, also known as NO-paracetamol, NO-acetaminophen, nitroparacetamol, and nitroacetaminophen (Fig. 1), is one of the first NO-releasing derivatives with analgesic properties developed following this strategy (Moore and Marshall 2003; Joshi 2004). NCX-701 is a combination of a nitrooxybutyryl moiety with paracetamol (acetaminophen) linked through an ester linkage. Its chemical name is butanoic acid, 4-(nitrooxy)-4-(acetylamino)phenyl ester.

Paracetamol is an acylated aromatic amide used as antipyretic and analgesic in adults and children instead of aspirin-like drugs or COX inhibitors, but it lacks antiinflammatory effects (Glenn et al. 1977; Seegers et al. 1981) and, although its mechanism of action is not yet fully understood, it is likely to be different from that of COX inhibitors and may involve an indirect activation of cannabinoid CB1 receptors (Bertolini et al. 2006, for review). It is a major ingredient in a high number of cold and flu medications and possesses a remarkably safe profile when administered at therapeutic doses (see, for review, Eccles 2006). Nevertheless, its wide availability makes overdose very common. Ingestion of large doses of paracetamol may cause acute liver toxicity owing to massive centrilobular necrosis (Poulin 2000), which is its main clinical limitation. Paracetamol is one of the most widely used analgesic-antipyretic drugs and is a first-line choice for mild to moderate pain management, including chronic pain conditions such as osteoarthritis (Prescott 2000; Bannwarth 2006). Therefore, any improvement in its effectiveness and/or safety would impact significantly on the treatment of pain. NCX-701 might be the result of this improvement, although it seems important to clarify whether NCX-701 has real advantages over its parent compound, paracetamol, in the treatment of pain. The purpose of the present article is to review the efficacy of NCX-701 in acute nociception and in inflammatory and neuropathic

pain, as well as its possible mechanisms of action and the benefits of its use compared to paracetamol.

PHARMACOLOGY

NCX-701 in Acute Pain

NCX-701 has been shown to be effective in rats as an antinociceptive in acute pain conditions. Paracetamol, like many COX inhibitors, is usually not effective in experiments carried out in animals without inflammation. This is due to the fact that the synthesis of prostaglandins is not augmented and, therefore, there is no clear target to modulate. However, in experiments performed in rats, using the single motor unit (SMU) technique, NCX-701 effectively reduced neuronal responses to noxious mechanical stimulation with an ED₅₀ of 147.1 $\mu\text{mol/kg}$ (41.5 mg/kg) and a minimum effective dose (MED) of 120 $\mu\text{mol/kg}$ (Fig. 2; Romero-Sandoval et al. 2002). Using the same technique and experimental protocol, the systemic administration of paracetamol (Romero-Sandoval et al. 2002), as well as COX inhibitors (Herrero et al. 1996; Mazario et al. 2001), did not reduce significantly the nociceptive responses evoked by natural stimulation.

The SMU technique allows the recording of direct spinal cord neuronal responses, activated by noxious stimuli, using a noninvasive experimental approach. The technique is especially useful for pharmacological experiments because data are very reproducible in the whole animal (Mazario et al. 1999; Romero-Sandoval et al. 2002; Gaitan et al. 2004;

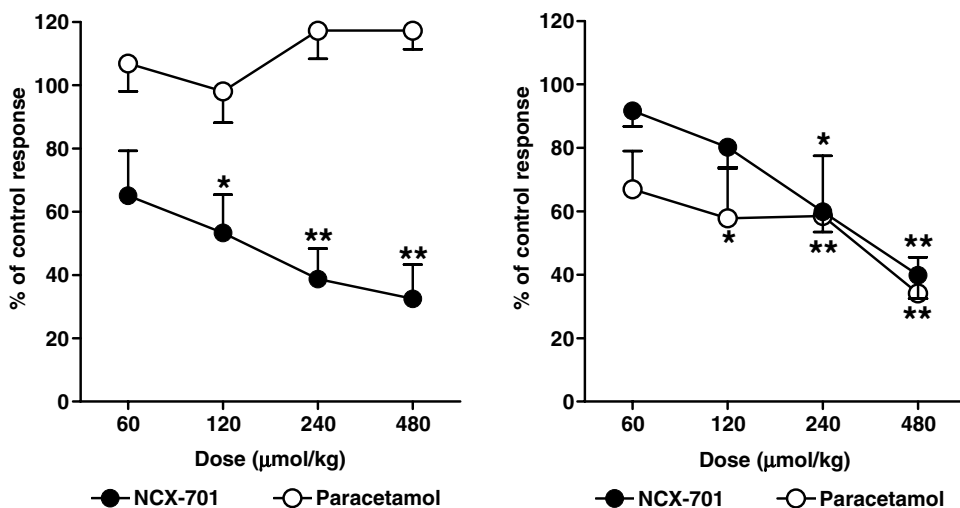


FIG. 2. Antinociceptive effect of NCX-701 and paracetamol in normal rats (left panel) and in rats with inflammation (right panel). The i.v. administration of NCX-701, but not paracetamol, was very effective in the reduction of spinal cord neuronal responses activated by noxious mechanical stimulation in normal animals (modified from Romero-Sandoval et al. 2002). In animals with monoarthritis, however, NCX-701 and paracetamol i.v. were equipotent and equieffective in the reduction of nociceptive responses evoked by noxious mechanical stimulation (modified from Romero-Sandoval et al. 2003). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, comparison versus control response with the one-way ANOVA, with the post hoc Dunnett test.

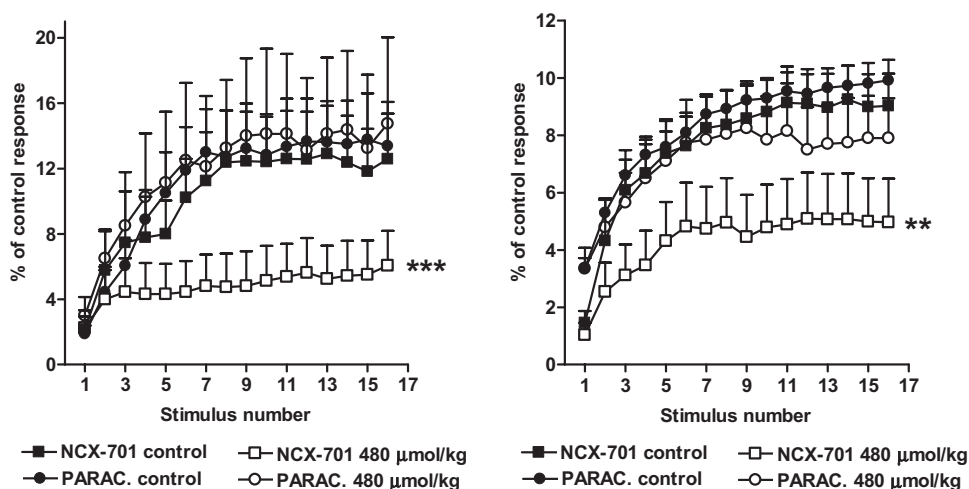


FIG. 3. Depression of the wind-up phenomenon by NCX-701 and paracetamol in normal rats (left panel) and in rats with inflammation (right panel). NCX-701 i.v. reduced wind-up in normal rats (modified from Romero-Sandoval et al. 2002) and in rats with monoarthritis (modified from Romero-Sandoval et al. 2003). Paracetamol (PARAC.) was not efficacious in any of the situations. Statistical comparison and layout are the same as in Fig. 2.

Ramos-Zepeda et al. 2004; Curros-Criado and Herrero 2005). In addition, it allows the combination of natural and electrical stimuli, something especially useful in the study of the “wind-up” phenomenon (see Herrero et al. 2000 for review). In these experiments, NCX-701, but not paracetamol (Fig. 3), was very effective in the reduction of the spinal cord-mediated “wind-up” phenomenon, a phenomenon triggered by spinal cord neurons and evoked by high-intensity repetitive electrical stimulation. Because the generation of wind-up depends mainly on spinal cord circuitry and is mediated by NMDA (Davies and Lodge 1987; Dickenson and Sullivan 1987) and NK1 receptors (De Felipe et al. 1998), NCX-701 is likely to modulate this effect, acting directly or indirectly at the spinal cord level, rather than at the periphery (i.e., nociceptors or inflammatory tissue). These experiments also showed that the antinociceptive effects of NCX-701 were not reversed by high doses of the nonselective opioid antagonist naloxone, suggesting that an action at opioid receptors is unlikely.

In a rodent model of visceral pain, NCX-701, administered orally, reduced dose-dependently the acetic acid-induced abdominal constriction. Paracetamol was, however, poorly or not effective in this model of acute nociception. Only at very high doses it reduced the nociceptive responses significantly (al-Swayeh et al. 2000). The potency ratio of NCX-701 was ca. 20-fold higher than that of its parent compound (ED_{50} : 24.8 vs. 506 µmol/kg, respectively; al-Swayeh et al. 2000).

NCX-701 in Inflammatory Pain

NCX-701 has been demonstrated to be a better analgesic than paracetamol in inflammation-induced pain. In experiments carried out in rodents, nociceptive responses, evoked in animals by intraplantar carrageenan-induced inflammation, were more strongly

reduced by NCX-701, i.p., than by paracetamol. NCX-701 was about threefold more potent than paracetamol (ED_{50} : 156 vs. 411.6 $\mu\text{mol/kg}$) in preventing inflammation-induced mechanical hypersensitivity (al-Swayeh et al. 2000).

NCX-701 is also an efficacious analgesic agent in experimental monoarthritis. Electrophysiological experiments conducted in our laboratory showed that NCX-701 administered i.v. at doses of 15–960 $\mu\text{mol/kg}$ in monoarthritic rats was effective and equipotent to paracetamol (ED_{50} : 320 vs. 305 $\mu\text{mol/kg}$) in reducing neuronal nociceptive responses evoked by noxious mechanical stimulation (Fig. 2; Romero-Sandoval et al. 2003). In addition, NCX-701 inhibited the wind-up phenomenon in a dose-dependent manner with a minimum effective dose of 240 $\mu\text{mol/kg}$ and a maximum effect of $59 \pm 18\%$ of control response, whereas paracetamol lacked any effect in this model. Fig. 3 shows the effect of NCX-701 on the wind-up phenomenon in monoarthritic rats compared to that of paracetamol (data taken and modified from Romero-Sandoval et al. 2003). This suggests not only that NCX-701 is an effective analgesic in arthritis, but also that NCX-701 has a different mechanism of action than its parent compound. It is worth noting that in this study, NCX-701 reduced neuronal nociceptive responses either in spinalized or in spinally intact rats, indicating that its effects are mainly located in the spinal cord (Romero-Sandoval et al. 2003; see also the section on mechanisms of action for further discussion of this subject).

NCX-701 as an Antiinflammatory Drug

It has been reported in the literature that paracetamol does not reduce inflammation and, therefore, cannot be considered an antiinflammatory drug (Glenn et al. 1977; Seegers et al. 1981). However, NCX-701 i.p. has been observed to have antiinflammatory properties at relatively low doses (ED_{50} : 169.4 $\mu\text{mol/kg}$, 25 mg/kg) when administered to rats with carrageenan-induced soft tissue inflammation (al-Swayeh et al. 2000). In this study, paracetamol did not reduce the level of inflammation even at a very high dose (300 mg/kg). This finding supports a mechanism of action of NCX-701 that is different from that of paracetamol. However, we were not able to observe a similar action in our laboratory when studying the antiinflammatory properties of NCX-701 in monoarthritis (Romero-Sandoval et al. 2003).

In vitro experiments demonstrated that NCX-701 inhibits production of proinflammatory cytokines (Marshall and Moore 2004). In this study NCX-701 (10–100 μM) reduced human blood interleukin (IL)- 1β and tumor necrosis factor α (TNF- α) production induced by lipopolysaccharide (LPS). These effects were blocked by the NO-scavenging agent carboxy-PTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1*H*-imidazol-1-yloxy-3-oxide potassium). Interestingly, neither paracetamol nor the NO donor NOC-5 (1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-isopropyl-1-triazene) affected these cytokines after LPS challenging (see Mechanism of Action section for further discussion).

In a model of endotoxemia caused by the i.v. administration of LPS, NCX-701 prevented, but did not reverse, LPS-induced hypotension, and increased plasma nitrate/nitrite levels and expression of COX-2 and iNOS in liver and lungs. These effects were not observed after paracetamol (except COX-2 expression in lung; Marshall et al. 2006). NCX-701 did not affect LPS-induced plasma markers of organ dysfunction or increased heart rate. In light of these findings, the addition of an NO donor molecule seems to enhance the pharmacological

profile of paracetamol, suggesting that the combination of the two molecules makes this compound a more effective analgesic and confers antiinflammatory activity, which it otherwise lacks.

NCX-701 in Neuropathic Pain

There are not many data available on a possible use of NCX-701 in the treatment of neuropathic pain, especially if considering that NSAIDs and COX inhibitors, in general terms, are not effective against this type of pain. However, the NO-releasing derivative of gabapentin, NCX-8001 (nitro-gabapentin; [1-(aminomethyl)cyclohexane acetic acid 3-(nitroxymethyl)phenyl ester]), has been shown to be a more efficacious antinociceptive agent in neuropathic pain than its parent compound gabapentin (Wu et al. 2004). Experiments carried out recently in our laboratory have shown that NCX-701, but not paracetamol, is an effective antinociceptive agent in neuropathic rats (Curros-Criado and Herrero 2006). Neuropathic hyperalgesia was developed in rats following partial ligation of the sciatic nerve (Seltzer et al. 1990), hyperalgesia was assessed by behavioral experiments, studying withdrawal reflex responses to mechanical and thermal stimulation. The antinociceptive activity of NCX-701 was compared to that of paracetamol after intravenous or intrathecal administration. In this study, NCX-701 induced a dose-dependent reduction of responses to noxious mechanical stimulation, either after i.v. or intrathecal administration, with an intensity and efficacy similar to that seen with gabapentin under similar experimental conditions. It is premature to say whether or not NCX-701 will be useful in the treatment of neuropathic pain, but these preliminary experiments look promising.

Enhancement of the Analgesic Effect of Fentanyl by NCX-701

The combined administration of COX inhibitors with opiates has been used either in animal research or in clinical trials in an attempt to enhance the antinociceptive activity of these drugs, but also to reduce the adverse effects produced by opiates, including the development of tolerance (Burns et al. 1991; Sutters et al. 1999; Gaitan et al. 2003; Miranda et al. 2005). It was of special interest to study the possible enhancement of analgesic activity of opiates combined with NO-donor drugs, because it has been reported that a possible mechanism of action of the μ -opioid agonist morphine involves stimulation of the cGMP system via NO release (Ferreira et al. 1991). Furthermore, there is evidence that NO precursors enhance morphine-induced analgesia and diminish tolerance in mice (Pataki and Telegdy 1998). We studied, therefore, possible benefits of combining NCX-701 and the μ -opioid agonist fentanyl in the treatment of acute and inflammatory pain.

NCX-701 was shown to be not only effective as an analgesic by itself, but it appeared also to enhance the effectiveness of other drugs, such as the μ -opioid agonist fentanyl, an opioid with potent and short lasting antinociceptive effects (Gaitan et al. 2003). Fentanyl i.v. is effective in reducing spinal cord neuronal responses activated by noxious mechanical and high-intensity electrical stimulation under normal nonhypersensitive conditions. We observed that in animals with noninflammatory pain NCX-701, at subeffective doses of 4.25 to 17 mg/kg i.v., enhanced potency, effectiveness, and duration of antinociceptive action of i.v. fentanyl (Fig. 4). In the noxious mechanical stimulation model, the ED₅₀,

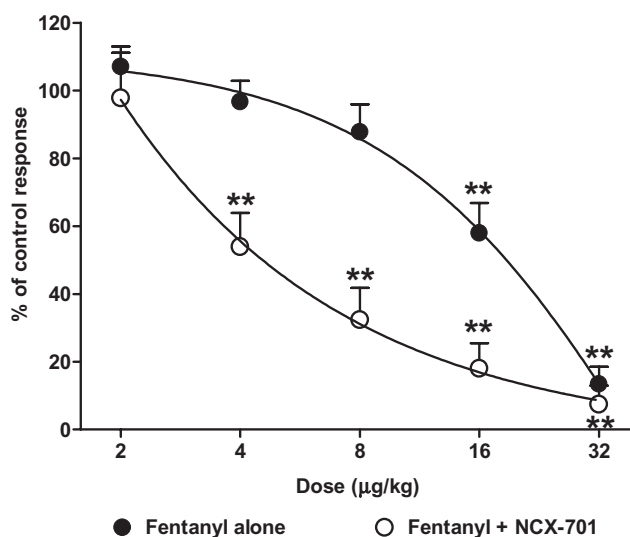


FIG. 4. Enhancement of the antinociceptive activity of fentanyl by NCX-701. The antinociceptive activity of the μ -opioid agonist fentanyl was studied either alone (fentanyl alone) or in the presence of subeffective doses of NCX-701 (fentanyl + NCX-701) in spinal cord neuronal responses activated by noxious mechanical stimulation. The administration of low doses of NCX-701, which were not capable of reducing the nociceptive responses on their own (data not shown), enhanced the potency of fentanyl (modified from Gaitan et al. 2003). In addition, the duration of the effect of fentanyl was greatly enhanced by the presence of NCX-701 (data not shown). Statistical comparison and layout are the same as in Figure 2.

the MED, and the duration of antinociceptive effects of fentanyl alone versus fentanyl plus NCX-701 were, respectively, 17 versus 5.1 $\mu\text{g/kg}$, 16 versus 4 $\mu\text{g/kg}$, and 15 min versus at least 45 min. At the subeffective doses, NCX-701 enhanced the inhibitory effect and the duration of action of fentanyl twofold. These results indicate that the combination of NCX-701 and fentanyl might be useful as a potential pain therapy.

The enhancement of the effect of fentanyl by low doses of NCX-701 has also been observed in rats with monoarthritis (Gaitan et al. 2005). In these experiments the investigators observed a significant reduction of fentanyl ED_{50} required to inhibit responses evoked by noxious mechanical stimulation: 29 versus 11.6 $\mu\text{g/kg}$. The minimal effective dose was lowered from 32 to 16 $\mu\text{g/kg}$ and the effect of fentanyl, which usually fully recovers in 15–20 min, lasted for at least 45 min.

The continuous administration of the μ -opioid agonist fentanyl leads, as with other opiates, to acute tolerance. Behavioral experiments carried out in our laboratory showed that, when fentanyl was administered continuously by means a perfusion pump to rats, acute tolerance developed in 3 days. However, the tolerance was not observed when fentanyl was administered in combination with NCX-701 (Gaitan et al. 2005). Both drugs were administered at the subanalgesic doses and, although further experiments are needed to elucidate the mechanisms underlying the interaction of the two drugs, this finding suggests a useful therapeutic approach to the prevention of acute opiate tolerance.

Mechanism of Action of NCX-701

Most of the experiments described here demonstrate the antinociceptive effect of NCX-701 in different types of pain and under various experimental conditions. They suggest that NCX-701 possesses a mechanism of action different from that of its parent compound and that this difference is likely to be due to a combined action of paracetamol and a low but sustained level of NO. Experiments carried out in our laboratory have shown that NCX-701 is an effective antinociceptive agent in noninflamed animals, a condition at which paracetamol, like COX inhibitors, is not effective, possibly because COX enzyme levels were not high enough for the drug to generate an action (Mazario et al. 2001). NCX-701 was also effective in reducing the centrally mediated wind-up phenomenon. In addition, NCX-701 kept its analgesic effectiveness in spinalized animals. Paracetamol itself penetrates easily into the central nervous system (Courad et al. 2001); it does not, however, modulate the wind-up phenomenon, supporting the idea that NCX-701 has a different mechanism of action. All these observations indicate that NCX-701 most likely acts in the central nervous system, mainly at the spinal cord level. Because wind-up depends on spinal NMDA and NK1 receptors (Davies and Lodge 1987; Dickenson and Sullivan 1987; De Felipe et al. 1998; Herrero et al. 2000), we hypothesized that one of the mechanisms of action of NCX-701 may involve the modulation of these two systems. Even though descending influences might modulate spinal cord wind-up (Herrero and Cervero 1996), it is unlikely that the main action of NCX-701 is at the supraspinal levels, because, as mentioned above, NCX-701 is very effective in spinalized monoarthritic animals (Romero-Sandoval et al. 2003).

The simplest explanation for the different actions of NCX-701 when compared to those produced by paracetamol would be that NO is responsible for such effects. The involvement of NO in the processing of nociceptive information is controversial. Certainly, disparate effects of NO have been reported. NO seems to induce either antinociception or pronociception in a concentration-dependent manner (Kurihara and Yoshioka 1996; Sousa and Prado 2001), although the clearest effect in spinal cord-mediated withdrawal reflexes seems to be antinociceptive (Sousa and Prado 2001). However, when we studied the possible antinociceptive effect of NOC-18 (DETA NONOate; ((Z)-1-[2-(2-aminoethyl)-N-(2-ammonio-ethyl)amino]diazene-1-ium-1,2-diolate)), a NO donor with similar kinetics as NCX-701, we did not observe any depression of nociceptive responses, suggesting that in the nociceptive system the amount of NO released was not efficacious on its own. In these experiments (Romero-Sandoval et al. 2002), NOC-18 and paracetamol were studied in comparison to NCX-701 and, whereas the administration of NCX-701 reduced the responses to noxious mechanical and electrical stimulation, paracetamol and NOC-18 were not effective. These data lead us to the hypothesis that the analgesic effect of NCX-701 is due to the combined effect of both compounds rather than their individual actions.

Obviously, it is expected that the mechanism of action of NCX-701 is related to that of its parent compound. However, it is not yet fully clear what the mechanism of action of paracetamol is. It has been suggested that the inhibition of prostaglandin production by the blockade of COX-2 and/or of acetaminophen-sensitive COX-2 variant is the mechanism by which paracetamol exerts its analgesic effects (Simmons et al. 1999; Graham and Scott 2005; Kis et al. 2005a, 2005b). In keeping with this, we have observed that NCX-701 is a more potent and effective analgesic in normal animals previously treated with all-trans retinoic acid (ATRA; ED₅₀: 46 μ mol/kg in the presence of ATRA treatment versus 147 μ mol/kg in the absence of ATRA treatment; Romero-Sandoval et al. 2006b). ATRA

is the major active metabolite of vitamin A, which induces inflammation-like changes in spinal cord neuronal nociceptive responses, enhances withdrawal reflexes in normal and inflamed rats, and upregulates the expression of COX-2 in the spinal cord (Romero-Sandoval et al. 2004, 2006a). These data suggest that NCX-701 might act centrally by inhibiting COX-2 activity. In similar experiments, we observed that paracetamol does not induce antinociception in noninflamed animals but, similarly to NCX-701, is effective in the absence of inflammation when the animals are pretreated with ATRA (Romero-Sandoval et al. 2006b), that is, when COX-expression is enhanced, as it occurs in inflammation. This is a strong indication that paracetamol and NCX-701 share a mechanism of action that involves the blockade of COX enzymes, probably COX-2. However, the fact that NCX-701 is an effective analgesic compound in animals without inflammation, and modulates the “wind-up” phenomenon, indicates that there are also important differences in the mechanisms of antinociceptive actions of NCX-701 and paracetamol (Romero-Sandoval et al. 2002, 2006b).

Other mechanisms proposed to explain the analgesic actions of paracetamol include a modulation of opioid receptors (Pini et al. 1997), an action mediated by serotonergic systems (Tjolsen et al. 1991; Bonnefont et al. 2007) and an interaction with cannabinoid CB1 receptors (Bertolini et al. 2006, for review). As far as we are aware, the analgesic effect of NCX-701 has been tested only in relation to a possible opioid component of its effect. In this case, a high dose of the nonselective opioid-receptor antagonist naloxone did not modify the analgesic effect induced by the systemic administration of NCX-701 in rats (Romero-Sandoval et al. 2002). It is, therefore, possible that NCX-701 shares a common mechanism of action with paracetamol that is related to COX-inhibition. However the differences in the pharmacological actions of the two compounds seem to be more important than the similarities in their mechanism of action.

Prostaglandin E2, one of the main products resulting from COX activity, enhances the release of glutamate and aspartate in the spinal cord (Nishihara et al. 1995). This means that COX inhibition reduces the synthesis of prostaglandin E2, which, in turn, would reduce glutamate release. In addition, it has been suggested that the NO release induced by NMDA receptor activation modulates negatively the release of glutamate by enhancing the amount of monoamines in the synaptic space (Kiss and Vizi 2001). Thus, we hypothesized (Romero-Sandoval et al. 2002) that the potent effect of NCX-701 observed in the reduction of wind-up (NMDA-dependent phenomenon; Herrero et al. 2000), and the more effective antinociception induced by this drug compared to its parent compound, might be due to a negative modulation of the activity of spinal cord NMDA receptors. This would be the result of a combined action of the maintained release of NO and the paracetamol molecule. Further experiments are needed to test this hypothesis.

NCX-701 appears to act not only in central areas of the nervous system but also in peripheral tissues, as suggested by its antiinflammatory effect in a model of soft tissue carrageenan-induced paw inflammation (al-Swayeh et al. 2000). The resolution of inflammation was observed to parallel the antinociceptive effect, and so the action of NCX-701 was related to a reduction in the sensitization of peripheral nociceptors. The antiinflammatory properties of NCX-701 have been related to a modulation of cytokines. In fact, NCX-701 reduces the proinflammatory cytokines IL-1 β and TNF- α in peripheral blood (Marshall and Moore 2004). Also, other NO-NSAIDs inhibit caspase-1 and thereby IL-1 β (Fiorucci 2001), providing a possible explanation of the molecular pathways targeted by NCX-701 to reduce IL-1 β and TNF- α (Fiorucci 2001). These cytokines are responsible for some of the

mechanisms involved in the generation of peripheral nociceptors. For example, their concentration is increased in the carrageenan-induced inflamed paw (Loram et al. 2007), they attract immune cells and have been shown to be pronociceptive in other peripheral inflammatory pain models (Twining et al. 2004; Romero-Sandoval et al. 2005). In addition, their administration in peripheral tissues reduces the threshold for mechanical stimulation and induces behavioral hypersensitivity (Fukuoka et al. 1994; Sorkin and Doom 2000; Cunha and Ferreira 2003). Therefore, the reduction in IL-1 β and TNF- α expression by NCX-701 would thereby prevent leukocytosis, inflammation, and pain, a peripheral mechanism of action of NCX-701 that might explain, at least in part, its analgesic effectiveness.

In addition, it has been reported that NCX-701 reduces the expression of iNOS and COX-2 in lung and liver, in a model of LPS endotoxemia. These actions should be taken into consideration to further explain the central and/or peripheral mechanisms by which NCX-701 exerts its analgesic and antiinflammatory effects, because both iNOS and COX-2 are actively involved in the generation and maintenance of inflammation and nociception (Marshall et al. 2006).

Yet, the precise interaction of paracetamol and NO remains unclear and, although the benefits resulting from the combination of these two molecules are clear, further studies are needed to elucidate the mechanism of action of NCX-701.

Side Effects of NCX-701

As quoted previously, paracetamol is devoid of most of the gastrointestinal toxicity associated with aspirin-like drugs or COX inhibitors; however, it is not devoid of adverse effects, the main one being liver damage (Plevris et al. 1998; Poulin 2000). Therefore, it was important to determine whether or not the combination of paracetamol and a NO donor will modify the risk of liver toxicity. In a series of experiments in rats, paracetamol (5 mmol/kg, i.p.) caused an increase of plasma aspartate aminotransferase (AST), and alanine aminotransferase and glutamate dehydrogenase activities, indicating liver toxicity (Futter et al. 2001). However, at equimolar doses, NCX-701 did not modify plasma levels of any of these markers of liver damage. In another study, paracetamol (100, 300, or 500 mg/kg i.p.) caused a dose-dependent liver injury in mice, increased AST about 40-fold (at the highest dose) and produced extensive centrilobular necrosis (Fiorucci et al. 2002). NCX-701, however, was completely devoid of hepatotoxicity. Furthermore, when coadministered with paracetamol, NCX-701 protected liver from paracetamol-induced damage by acting in the Fas pathway (Fiorucci et al. 2002).

NCX-701 was also shown to be safe in mice with preexisting chronic liver disease (transgenic mice expressing the hepatitis B virus). These animals were, however, susceptible to paracetamol-induced liver toxicity (Fiorucci et al. 2002). Because liver injury caused by paracetamol has been attributed to the generation of toxic metabolites, one possible explanation for the lack of toxicity of NCX-701 is that it produces less of toxic metabolites. However, this is unlikely because plasma and liver levels of free paracetamol are similar in animals treated with NCX-701 or paracetamol (Futter et al. 2001). In addition, both drugs share the same metabolic pathway as shown by the time course of paracetamol-glucuronide levels in the plasma and liver. Therefore, the difference in the hepatotoxicity of the two compounds cannot be explained by their metabolism (Futter et al. 2001). Reduction of experimentally induced liver damage has also been associated with other

NO-releasing COX inhibitors, including nitroflurbiprofen (McLoughlin et al. 1999) and nitroaspirin (Fiorucci et al. 2000). The conclusion, so far, is that the hepatoprotective effects observed in rodents is due to the release of NO, possibly acting in combination with paracetamol. If this is also true in humans, NCX-701 may represent a safer alternative to paracetamol.

NO-NSAIDs have vasorelaxant effects of variable intensity in different *in vitro* preparations, such as rat aortic rings or mesenteric and kidney artery preparations. However, NO-NSAIDs do not seem to modify the mean arterial blood pressure (MAP) in acute *in vivo* experiments in normal rats (Keeble et al. 2001). In our laboratory we observed that NCX-701 did not induce any change in MAP in normal and monoarthritic anesthetized rats when administered i.v. (15–480 $\mu\text{mol/kg}$) at cumulative doses (Romero-Sandoval et al. 2002, 2003), except for a slight drop observed after the injection of the highest cumulative dose of 960 $\mu\text{mol/kg}$ in monoarthritic rats. In another study carried out in rats, NCX-701 did not modify the MAP during 7 days of daily i.p. injections of 15 mg/kg/day (Zhu et al. 2006). In addition, MAP was unaffected following 2 more days of treatment with NCX-701 in rats with experimental myocardial infarction (Zhu et al. 2006). All these data suggest that the acute vasorelaxant effects of NCX-701 are very limited. However, there is not much information about the effect of long-term treatment with NCX-701 on vascular reactivity. In fact, by chronic administration other NO-donors show vasodepressor or even vasopressor responses (Keeble and Moore 2002).

Like paracetamol, NCX-701 seems to lack renal toxicity (Futter et al. 2001); it is as effective as paracetamol in controlling fever (Futter et al. 2001) and seems to have the same cardioprotective effects as paracetamol (Zhu et al. 2006).

CLINICAL PERSPECTIVES

Recent clinical trials with NCX-701 provided data consistent with preclinical observations (Joshi 2004). NCX-701 was first tested in humans in 2001. This randomized, double-blind, placebo-controlled, crossover phase I study was designed to evaluate the general safety, tolerability, and pharmacokinetics of NCX-701. Five doses of an oral formulation of NCX-701 (100, 200, 400, 800, and 1,200 mg) were given at single escalating doses to young healthy male volunteers. Initially eight subjects (six active and two placebo) received 100 mg of NCX-701; subsequently eight subjects (six active and two placebo per dose level) received the drug in the crossover design at four dose levels (200, 400, 800, and 1,200 mg). All volunteers received the successive four dose levels of NCX-701 and the placebo only once. A washout period of minimum 7 days separated each dose level in the second part of the study. The absorption and tolerability of the drug were good at all doses during the whole study period. There were no serious or severe adverse events reported and no subject was withdrawn because of the adverse effects. NCX-701 bioavailability fit well with the pharmacokinetic data. Clinical parameters including hematology, clinical chemistry, and vital signs did not change significantly during the study (Kelly 2001; NicOx SA Press Release 2001).

In 2003 another randomized, double-blind, placebo-controlled, single-dose Phase II study was carried out. Four parallel groups were set up by randomizing 101 patients. The objective of the study was to evaluate the analgesic efficacy and safety of NCX-701 in moderate to severe postoperative dental pain after extraction of an impacted third molar tooth. Patients

received a single dose of NCX-701 of 1 or 2 g, 1 g of paracetamol (acetaminophen), or placebo (NCX-701, 2 g corresponds approximately to 1 g of paracetamol on a molar basis). Pain intensity difference, pain relief, time to perceptible pain relief, and time to rescue medication were the parameters evaluated before treatment and 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h posttreatment. In this study, NCX-701, 1 and 2 g, were equivalent to paracetamol 1 g and superior to placebo in terms of pain intensity difference, pain relief, and time to onset of perceptible pain relief. The results suggest that NCX-701 is a more effective analgesic agent than paracetamol on a mole-per-mole basis. As observed in the phase I study, no serious or severe adverse reactions were observed (Ongini 2003; NicOx SA Press Release 2003).

CONCLUSIONS AND FURTHER DIRECTIONS

NCX-701 has been shown to be a more effective analgesic and antiinflammatory agent than paracetamol in acute soft-tissue inflammation, monoarthritis, and neuropathic pain models. Moreover, NCX-701 has been shown to be safer than its parent compound, with liver protection properties and devoid of liver toxicity. In addition, NCX-701 remains effective in controlling fever, seems to induce cardioprotection, and does not alter MAP. The mechanism of action of NCX-701 is not fully understood. It appears to have central as well as peripheral sites of action. The mechanisms of action may be related to the inhibition of COX enzymes, but a reduction of iNOS, an action on spinal cord glutamate release, and the modulation of proinflammatory cytokines are also likely. The enhancement of the intensity and duration of the antinociceptive effect of fentanyl by NCX-701 in rodents should be studied further. Of special interest is the ability of NCX-701 to abolish fentanyl-induced acute tolerance. Because this is one of the major clinical limitations for the opioids, the confirmation of this effect in other pain conditions, and with different opioids, would have a tremendous impact on new strategies for the treatment of pain. In addition, further studies are needed to elucidate how NCX-701 prevents the development of acute tolerance to fentanyl. It has been shown that supraspinal descending pathways play an important role in morphine tolerance (King et al. 2005), whereas our experiments suggested that NCX-701 is acting mainly at the spinal cord level, rather than at supraspinal sites. Therefore, spinal cord mechanisms, which are actively involved in morphine tolerance/hyperalgesia, such as neuroimmune activation through glial cells (Raghavendra et al. 2002, 2004), should be explored.

In conclusion, NCX-701 looks like a promising compound for the treatment of different types of pain. Its side effect profile is superior to that of its parent compound, paracetamol. Although initial clinical trials with NCX-701 provided data consistent with the preclinical observations, further studies are needed to support its clinical use.

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