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Short communication

Effects of nimesulide on nitric oxide-induced hyperalgesia in humans—a neurophysiological study

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to induce analgesia mainly via the inhibition of cyclo-oxygenase. Several reports suggest that chronic pain is mediated by central sensitization, an *N*-methyl-D-aspartate (NMDA)-mediated phenomenon influenced by cyclo-oxygenase activity and nitric oxide (NO). In this double-blind study, we evaluated the effects of a preferential inhibitor of the inducible isoform of cyclo-oxygenase-2, nimesulide, on the spinal nociceptive flexion reflex (RIII reflex) before and after administration of an NO donor in healthy volunteers. Nimesulide caused a reduction of the RIII reflex area, which persisted after NO donor administration. Conversely, in the placebo group the RIII reflex area significantly increased following the administration of the NO donor. These data suggest a central effect for nimesulide, possibly related to a reduction of nociceptive activity at spinal level.

Keywords: Nimesulide; Cyclo-oxygenase; Nitric oxide (NO); Pain; RIII threshold

1. Introduction

The analgesic action of nonsteroidal anti-inflammatory drugs (NSAIDs) has been attributed to the peripheral inhibition of prostaglandin synthesis via the blockade of the enzyme cyclo-oxygenase and prevention of bradykinin- and cytokine-induced hyperalgesia via inhibition of tumor necrosis factor-alpha (TNF-α) release (Ferreira, 1993). However, it is becoming increasingly evident that NSAIDs exert their analgesic effect through a variety of other peripheral and central mechanisms (Cashman, 1996; Sandrini et al., 1992). The recent localization in the brain of the inducible cyclo-oxygenase isoform (cyclo-oxygenase-2) (Yamagata et al., 1993) provides an additional central mechanism of action.

Cyclo-oxygenase-2, together with nitric oxide (NO), is likely to play an important role in chronic pain states, which are mediated by a constellation of changes, collectively termed as central sensitization (Woolf, 1983). Physiological changes similar to those associated with central sensitization are evoked in spinal nociceptive neurons by repetitive, low frequency, noxious electrical stimulation of C-fibers (Wall and Woolf, 1986). This type of stimulation also produces wind-up, a centrally mediated increase in both the frequency and duration of spinal nociceptive responses (Mendell, 1966). Wind-up depends on the activation of *N*-methyl-D-aspartate (NMDA) receptors located on dorsal horn neurons and is also mediated by cyclo-oxygenase-2 and NO activity (Herrero et al., 2000).

The nociceptive flexion reflex (RIII reflex) is a useful model in neurophysiological investigation of pain (Willer, 1977; Sandrini et al., 1993). This model allows the study of changes in the activity of central pain system in different physiological and pathological conditions and it has proved very useful for investigating the effects on pain transmission of several drugs, including NSAIDs, able to modulate

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nociceptive activity (Willer et al., 1989; Sandrini et al., 1990, 1992, 1993).

In the present study, we investigated the effects of nimesulide—a preferential cyclo-oxygenase-2 inhibitor—on RIII reflex as a means to further elucidate the possible central mechanisms of this drug, with particular focus on NO mediation.

2. Materials and methods

Ten healthy subjects (four female and six male) with a mean age of 29.8 ± 7.6 years participated in the study. At the first session, all procedures were explained and informed consent obtained. All procedures had been previously reviewed and approved by the Institutional Ethical Review Board.

The study consisted in a double-blind, placebo-controlled, crossover trial. Each subject randomly underwent treatment with nimesulide (100 mg p.o.) and placebo during two different sessions. The interval between placebo and nimesulide sessions was of at least 4 days. In each session, a nitric oxide donor (nitroglycerin, 0.9 mg s.l.) was administered 140 min after nimesulide or placebo.

The RIII reflex was elicited by a 20-ms train of shocks at 300 Hz (1 ms rectangular pulse), randomly delivered every 5–20 s on the sural nerve at the retromalleolar site and the responses derived from the ipsilateral biceps femoris muscle (*capitis brevis*) with electromyographic technique (Willer, 1977). Once the RIII reflex threshold was established, three consecutive responses were recorded at 1.5-fold the threshold (Willer, 1977). The RIII reflex was performed before nimesulide/placebo administration and 15, 30, 60, 90 and 120 min after nimesulide/placebo administration, and before nitroglycerin administration and 15, 30, 60, 90 and 120 min after. Each reflex response was amplified, digitized, full-wave-rectified and integrated. The resulting integrals were used to calculate the area of the reflex and its percent changes from baseline. The mean of three to five responses recorded at 1.5-fold intensity of the threshold was considered for each time-point. Temporal window restrictions were applied to avoid integration of tactile reflex component and artifacts by involuntary movements.

2.1. Statistical analysis

Given the linear correlation between intensity of stimulus (i) and area (A) of RIII reflex (Sandrini et al., 1992), we used the ratio between the area and the square of the stimulus intensity (A/i^2), as an index for monitoring the neurophysiological effects of active drugs and placebo. The reliability of A/i^2 ratio had been tested in a separate group of healthy volunteers. The baseline mean A/i^2 ratios calculated before nimesulide/placebo and before nitroglycerin administration were equalled to 100; for the subsequent time-points (15 min through 120 min post-nimesulide/placebo and 15 min trough 120 min post nitroglycerin), A/i^2 ratios were calculated and expressed as percent changes from baseline values.

Influence of treatment order and period were tested with analysis of variance (ANOVA). Differences between treatments in A/i^2 at different time points were evaluated using the ANOVA for repeated measures with post hoc Duncan test.

3. Results

The sequence (nimesulide/placebo or placebo/nimesulide) and the period (first or second session) did not influence the results.

The A/i^2 ratio decreased in both groups during the first 60-min period (Fig. 1, upper panel). In the nimesulide group, this reduction was statistically different from baseline values at 15 min and persisted up to 2 h (ANOVA for

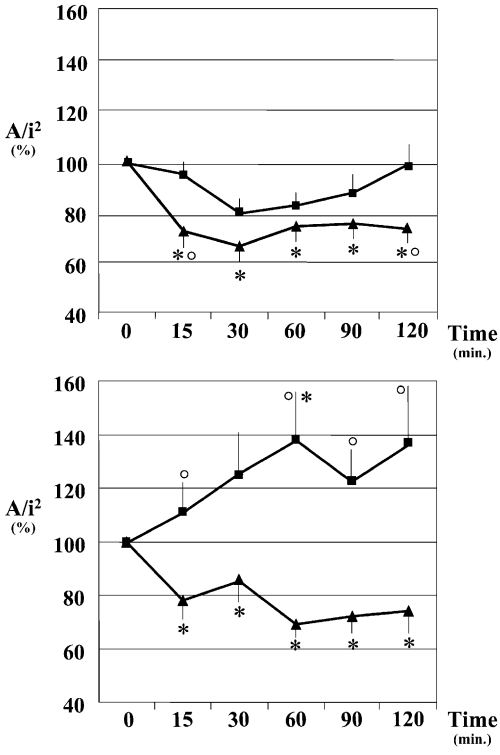

Fig. 1. Upper panel: Changes in RIII reflex (expressed as percent changes from baseline in A/i^2 ratio) after nimesulide/placebo. The RIII reflex was performed before nimesulide (▲) or placebo (■) administration (Timepoint 0) and 15, 30, 60, 90 and 120 min after. Data are represented as mean ± standard error. ANOVA for repeated measures: nimesulide, $F=3.89$, $P=0.005$; placebo, $F=1.91$, $P=0.11$. Post hoc Duncan test $^*P<0.05$ vs. baseline values, $^\circ P<0.05$ nimesulide vs. placebo. Lower panel: Effect of nitroglycerin administration on RIII reflex (expressed as percent changes from baseline in A/i^2 ratio) following (▲) or placebo (■) treatment. RIII reflex was performed at baseline (Timepoint 0), which corresponded to 2 h after nimesulide/placebo administration and 15, 30, 60, 90 and 120 min after nitroglycerin administration. Data are represented as mean ± standard error. ANOVA for repeated measures: nimesulide, $F=2.95$, $P=0.03$; placebo, $F=4.16$, $P=0.003$; Post hoc Duncan test $^*P<0.05$ vs. baseline values, $^\circ P<0.05$ nimesulide vs. placebo.

repeated measures $F=3.89$, $P=0.0052$). Conversely, in the placebo, the reduction in A/i^2 ratio never reached a significant level and disappeared by 120 min (ANOVA for repeated measures $F=1.91$, $P=0.11$). Comparison between groups showed statistically significant differences at 15 and 120 min.

Following nitroglycerin administration, significant changes were observed in both experimental groups, but with an opposite direction (Fig. 1, lower panel). In the nimesulide group, the A/i^2 ratio showed a significant reduction as compared to baseline at many time points after nitroglycerin administration (ANOVA for repeated measures $F=4.16$, $P=0.003$). At variance, the placebo group showed a progressive increase in the ratio (ANOVA for repeated measures $F=2.95$, $P=0.033$), which reached a statistically significant value at 60 min after nitroglycerin administration.

Comparison between groups showed statistically significant differences at 15, 60, 90 and 120 min after nitroglycerin administration.

In the placebo group, nitroglycerin administration induced headache in five subjects (50%), which spontaneously resolved after a mean duration of 48 ± 44 min. In the nimesulide group, the number of subjects who developed a headache was 3 (30%). The mean duration of their headache was of 10 ± 5 min. Also in this case, headache resolution was spontaneous.

4. Discussion

Nimesulide is a sulphonanilide drug showing anti-inflammatory, antipyretic and analgesic properties. This compound is considered a preferential cyclo-oxygenase-2 inhibitor (Hawkey, 1999), as it has found to be 5–26 times more selective for cyclo-oxygenase-2 than for cyclo-oxygenase-1 (Warner et al., 1999).

The present findings support a significant inhibitory effect of nimesulide on RIII reflex, expressed in terms of reduced RIII area and/or increased RIII threshold. A significant reduction in A/i^2 ratio is detected as early as 15 min after nimesulide administration, which confirms a fast-onset of its analgesic effect.

We can speculate that nimesulide exerts its analgesic activity via a central (spinal/supraspinal) mechanism, in analogy to what has been demonstrated for other NSAIDs in both human and animal studies (Willer et al., 1989; Sandrini et al., 1992; Jurna and Brune, 1990). This speculation seems further supported by the demonstration that the antipyretic action of nimesulide is primarily mediated through the selective inhibition of brain cyclo-oxygenase-2 (Taniguchi et al., 1997). Even in the absence of inflammation, significant cyclo-oxygenase-2 expression is found in the central nervous system (Gong et al., 2001), where cyclo-oxygenase-2 seems to play an important role in spinal nociceptive transmission (Vanegas and Schaible, 2001). It ensues that, at least partly, the analgesic effect of nimesulide in humans depends upon the central inibition of cyclo-oxygenase-2, probably at the spinal cord level, as suggested by previous animal data (Hay et al., 1997).

In the present model, exogenous nitroglycerin significantly increased RIII reflex response in subjects treated with placebo, while it did not interfere with the long-lasting (extending over 4 h) analgesic effect observed in the nimesulide-treated subjects. Nitroglycerin is a strongly lipophylic substance, that easily crosses blood brain barrier and accumulates in the cerebral tissue (Torfgard et al., 1989), where its biological effects last for several hours (Tassorelli and Joseph, 1995; Pardutz et al., 2000; Lambert et al., 2000). Nitroglycerin-derived NO is likely to facilitate neural transmission of afferent signals in the caudal trigeminal nucleus (Jones et al., 2001; Lambert et al., 2000), although other mechanisms are possibly involved (i.e. activation of the trigemino-vascular system) (Tassorelli et al., 1999; Reuter et al., 2001). Our present findings confirm and expand on previous studies showing a hyperalgesic action for nitroglycerin in both humans and animals (Thomsen et al., 1996; Lambert et al., 2000). The progressive increase in A/i^2 ratio, observed from 15 to 60 min after nitroglycerin administration, in the placebo group is intriguing and, though further studies are necessary for a thorough intepretation, it is suggestive for a sustained sensitization phenomenon, induced by NO and is in agreement with the observation that NO is involved in several potential pronociceptive mechanisms (Meller and Gebhart, 1993; Dolan et al., 2000).

It is noteworthy that, according to our findings, the analgesic activity of nimesulide proved effective in counteracting NO-mediate hyperalgesia. This seems to suggest that cyclo-oxygenase-2 inhibition is a limiting step for NO-mediated hyperalgesic mechanisms. The interactions between cyclo-oxygenase-2 activity and NO in the central nervous system are not fully known. Both substances are influenced by NMDA receptor activity in the mediation of inflammation-induced mechanical hyperalgesia (Laird et al., 1996; Ren and Dubner, 1993), and mechanical allodynia (Yaksh, 1989; Kim et al., 1997). NMDA receptor activation stimulates a number of intracellular second messengers such as NO and prostaglandins, and increases cyclo-oxygenase-2 expression by a calcium-dependent mechanism. Further studies will hopefully help unveiling the direct or indirect (NMDA-mediated) interactions between cyclo-oxygenase and NO.

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