Influence of Ibuprofen-Arginine on Serum Levels of Nitric Oxide Metabolites in Patients with Chronic Low Back Pain – A Single-Blind, Placebo Controlled Pilot Trial (ISRCTN18723747)

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ABSTRACT. ***Objective.*** To determine whether ibuprofen-arginine has a cyclooxygenase-independent pain modulating property in addition to its known antiinflammatory effect.

Methods. Patients with chronic low back pain were randomly divided into 2 groups treated either with oral ibuprofen-arginine (400 mg) or with placebo. Blood was drawn from study subjects before, during, and after treatment and they were asked each time about the intensity of their pain. Concentrations of nitric oxide (NO) metabolites were determined.

Results. Twenty minutes after intake of ibuprofen-arginine, but not after placebo, there was a relevant and significant reduction of NO metabolites in serum. In both groups there was significant analgesic effect compared to baseline.

Conclusion. An early lowering of the serum NO metabolite levels after ibuprofen-arginine administration could be detected in patients with chronic low back pain. (First Release Oct 1 2006; J Rheumatol 2006;33:2515–8)

Key Indexing Terms:
NITRIC OXIDE IBUPROFEN-ARGININE CHRONIC PAIN

Ibuprofen [2-(4-isobutyl-phenyl) propionic acid], an effective and well-tolerated nonsteroidal antiinflammatory drug (NSAID), is widely used to treat acute and chronic pain[1]. Arginine, a nonessential amino acid, appears to improve the pharmacokinetic profile of ibuprofen by enhancing its rate of absorption. Ibuprofen-arginine achieves meaningful pain relief in acute pain twice as rapidly as ibuprofen[2]. The mean plasma concentration 5 minutes after treatment with ibuprofen-arginine proved to be similar to the mean plasma concentration observed 1 hour after treatment with ibuprofen alone[3]. Interestingly, results of another study showed that some patients with migraine experienced pain relief within 10 minutes after oral administration of ibuprofen-arginine[4]. This response seems to be more rapid than that obtained from conventional NSAID, which relieve pain by blocking cyclooxygenases. Therefore, the rapid analgesic effect of oral ibuprofen-arginine might be mediated through not yet specified cyclooxygenase-independent pathways that we investigated in this study.

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In looking for possible targets (pronociceptive molecules) exerting a change of their concentrations in the peripheral blood soon after ibuprofen-arginine administration, nitric oxide (NO) revealed such an effect. It is readily diffusible in tissues and freely permeates cell membranes[5]. A NO reduction might be able to primarily alleviate pain before secondary mechanisms, e.g., inhibition of proinflammatory cytokines, take place.

MATERIALS AND METHODS

Outcomes. The primary outcome of our study was NO concentration in serum of patients with low back pain at different times before and after oral ingestion of ibuprofen-arginine compared to placebo. The secondary end point was pain intensity measured on a visual analog scale (VAS, 0-100) at the same times.

Subjects. Thirty-two consecutive patients in our outpatient department who presented with chronic low back pain were asked to participate in the study. Twelve patients could not be included due to less pain (n = 8), current opioid or NSAID medication (n = 3), or hospitalization for other reasons (n = 1). One patient in the placebo group withdrew without explanation after randomization. Nineteen patients (12 women, 7 men) with nonspecific chronic low back pain (VAS = 60) participated in the study. They were randomly allocated to the verum group [n = 10, 6 women, 4 men, mean age: 59 ± 4.96 (SEM) yrs] or to the placebo group (n = 9, 6 women, 3 men, mean age: 60 ± 3.63 yrs). The study was approved by the local ethics committee. All subjects gave their informed consent prior to inclusion in this study.

Drugs. The 500 mg placebo tablets consisting of lactose and amylum solani were purchased from Hänseler AG, Herisau, Switzerland (Art. 13-3425-2). Ibuprofen-arginine (Spedifen® 400) was provided by Zambon, Cadempino, Switzerland.

The study was carried out according to a single blind design. The placebo tablet was a different shape than the Spedifen® 400 tablet.

Drug administration. Patients were asked to remain in a reclining position for 10 minutes until a first sample of blood was drawn (T-10). Ten minutes later (T0) placebo or ibuprofen-arginine 400 mg (verum) was administered orally with water as a tablet while a second sample of blood was drawn. Third and

fourth samples of blood were drawn 10 (T10) and 20 minutes (T20) after intake of the medication.

Blood sampling. For determination of serum NO concentration, 10 ml blood was drawn from the antecubital vein into a Vacutainer™ (Becton Dickinson, Plymouth, USA) at the above time points. Blood was centrifuged for 10 minutes at 1300 g at room temperature. Serum was placed in KryoTube™ vials (NUNC A/S, Roskilde, Denmark) and stored at -80˚C until NO determination.

Determination of NO_x levels. NO, which is a free, highly unstable radical, is transformed into various NO products [NO_2^- (nitrite) and NO_3^- (nitrate)] by reacting spontaneously with molecular oxygen. NO_x products in sera were measured with the Nitric Oxide Colorimetric Assay (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's protocol. In brief, serum was diluted 1:1 with potassium phosphate buffer provided in the kit and then ultra-filtrated by centrifugation at 1250 g at 20°C for 45 minutes with the Centrisart® filtration system (cut-off 10,000, Sartorius AG, Göttingen, Germany). NO_3^- in the filtrate was converted to NO_2^- by nitrate reductase. Subsequently, sulfanylamide and N-(1-naphthyl)-ethylene diamine dihydrochloride (Roche Diagnostics) were added to the resulting nitrite solution. These substances underwent a nitrite-dependent color reaction (Griess assay) measured by spectrophotometry at 560 nm[6].

Pain measurement. Pain intensity (lower back) was measured by VAS (0 = no pain; 100 = worst imaginable pain) at different times before and after drug administration.

Statistical methods. Arithmetic means and standard error of the mean (SEM) were calculated for all data. A detailed comparison of data was carried out using the Wilcoxon test for related samples (time course) and the Mann-Whitney U test for independent samples (comparison between the groups) using SPSS 11.5 (Chicago, Illinois, USA). A p value < 0.05 was considered significant.

RESULTS

Level of NO metabolites in serum mediated by ibuprofen-arginine. Ten minutes before drug application serum NO metabolite levels were lower in the placebo group than in the verum group. However, according to the Mann-Whitney U test this difference was not significant ($p = 0.34$) and was no longer present at the time of placebo or ibuprofen-arginine administration (Figure 1A). The verum group showed a significant reduction of the serum NO concentration 10 minutes ($p = 0.008$) and 20 minutes ($p = 0.008$) after ibuprofen-arginine administration. The group receiving placebo had increased levels of NO 20 minutes after treatment ($p = 0.021$) compared to T0. Consequently there were significant differences regarding the levels of serum NO metabolites between the placebo and the verum group at 10 minutes ($p = 0.002$) and 20 minutes ($p < 0.001$) after treatment.

Pain relief mediated by ibuprofen-arginine compared to placebo. Ten minutes before and during administration of placebo or ibuprofen-arginine, all study subjects indicated that they suffered from chronic low back pain (VAS about 60). A statistically significant deviation in the VAS was not observed in either the control or the verum group during this period. Ten minutes after drug application both groups showed a weak but not significant reduction in VAS pain scores. However, the analgesic effect became statistically significant in both groups 20 minutes after administration of placebo or ibuprofen-arginine ($p < 0.05$, respectively) compared to T-10 as well as to T0 (Figure 1B). However, differences in VAS scores between the placebo group and the verum group were not significant at any timepoint.

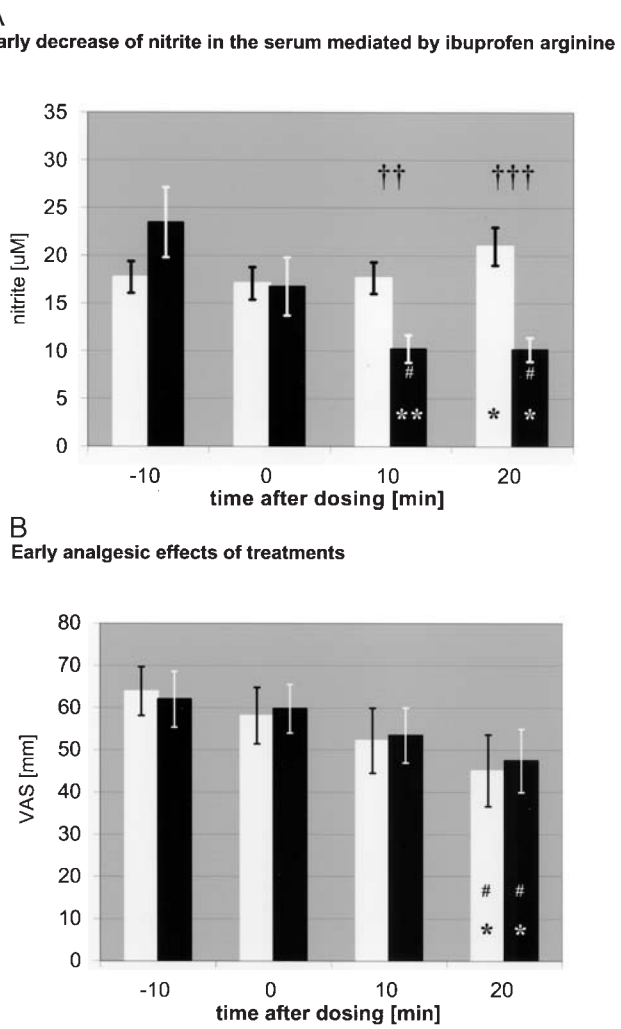

Figure 1. Patients with chronic low back pain received placebo (white boxes) or 400 mg ibuprofen-arginine (black boxes). Results are presented as arithmetic means ± SEM. Wilcoxon test: $^{\#}p < 0.05$ compared to T-10; $^{*}p < 0.05$; $^{**}p < 0.01$ compared to T0. Mann-Whitney U test: $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$ between verum and placebo.

DISCUSSION

Our results show an effect of ibuprofen-arginine on serum NO metabolite concentrations within 10 minutes after application. An arginine-mediated improved intestinal reabsorption due to better solubility in the acid milieu of the stomach is known. Even before this drug exerts its analgesic effect, a reduction of NO metabolite levels in serum can be shown[7]. A down-regulation of NO by ibuprofen has been reported by Vandivier and colleagues[7]. This effect might be provoked by blunting endothelial nitric oxide synthase (eNOS) responses, by decreasing low basal levels of inducible nitric oxide synthase (iNOS) expression, or both.

Therefore the question arises whether or not the observed reduction of NO metabolite concentration in serum might be responsible for pain relief experienced after ibuprofen-arginine, which appears to act faster than other NSAID after oral administration[8]. A number of observations suggest that NO

acts as a pronociceptive mediator in the periphery[9-12]. Intracutaneous injections of NO evoke pain in humans[13,14]. Endothelial and neuronal isoforms (eNOS and nNOS) are expressed constitutively: eNOS by endothelial cells and nNOS by neurons in the central and enteric nervous systems[15,16]. iNOS is expressed only in response to certain inflammatory stimuli such as bacterial lipopolysaccharides, cytokines, and lipid mediators, leading to a slow but sustained and high level production of NO[15,17-20]. In humans, NO evoked pain after intracutaneous or paravascular injection as well as by vascular perfusion of an isolated hand-vein segment[13,14].

Bradykinin, another well-known liberator of NO and a naturally occurring algetic[21], elicits pseudoaffective pain behavior after arterial injections in animals[22] and also evokes pain in humans after both intravenous and paravascular application[23].

Down-regulation of NO production has been reported after oral administration of 800 mg of conventional ibuprofen[7]. However, these authors observed a significant reduction ($p < 0.05$) only in the late period, i.e., after more than 3 h. Therefore, the question arises as to how the rapid down-regulation of NO in our study came about, i.e., a reduction within 10 minutes after intake of 400 mg of ibuprofen-arginine. In its formulation as the salt of the nonessential amino acid L-arginine, ibuprofen is absorbed much more rapidly because its solubility is enhanced. This supports the idea that ibuprofen is the agent mediating the reduction of the serum NO concentration. However accelerated bioavailability of ibuprofen does not sufficiently explain the much earlier downregulation of NO metabolites after ibuprofen-arginate: 180 minutes after intake of 800 mg ibuprofen vs 10–20 minutes after intake of 400 mg ibuprofen-arginate.

An autonomous effect of the arginine component appears to be unlikely. Although there is evidence that derivatives of L-arginine inhibit NOS and consequently lead to a decrease in NO production[24], this effect is probably due to competitive inhibition. L-arginine itself, the only natural substrate for NOS, should promote NO production rather than impeding it. eNOS and nNOS are the most probable targets explaining the observed reduction in serum NO concentration. The only alternative explanation would be the release of endogenous NO scavengers.

Some limitations of our study need to be addressed. There is a discrepancy between the rapid and marked effect of ibuprofen-arginine on serum levels of NO metabolites and the absence of an equally rapid effect in terms of clinical pain reduction. Ibuprofen-arginine has been shown to cause meaningful pain relief in acute pain within 30 minutes after oral intake[2]. Our patients, however, had chronic pain, which is more complex and frequently requires treatment with higher doses and longer latency time until pain reduction becomes manifest.

Moreover, the main objective of our pilot investigation was to assess the effects on NO metabolites. Analgesia in clinical terms was a secondary end point, and our experimental design was underpowered for detection of an early clinical effect (type 2 error). Adequately powered trials in acute pain are needed to ascertain whether early reduction of serum NO is associated with rapid clinical pain relief.

Further investigations, in particular with an ibuprofen-only arm, are needed before general conclusions can be formulated. The role of NO synthase isoforms, their concentrations, and their action should be investigated in this context. Future projects will also need to analyze a potential release of endogenous NO scavengers.

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