Effect of glutamine on the intestinal permeability changes induced by indomethacin in humans

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SUMMARY

Background: Long-term non-steroidal anti-inflammatory drug (NSAID) intake may induce increased intestinal permeability, eventually resulting in enteropathy. Because increased permeability might be related to cell damage resulting from energy depletion, it was hypothesized that glutamine—the major energy source of the intestinal mucosal cell—might prevent permeability changes.

Methods: The 6-h urinary excretion of ^{51}Cr-EDTA after an oral load of ^{51}Cr-EDTA was used in this study as a measure for intestinal permeability. Healthy volunteers underwent a series of permeability tests: (i) basal test; (ii) test following NSAID (indomethacin); (iii) test following NSAID in combination with glutamine and/or misoprostol.

Results: The NSAID induced increased permeability in all volunteers. Pre-treatment with glutamine (3×7 g daily, 1 week before NSAID-dosing) did not prevent the NSAID-induced increase in permeability. Multiple doses of glutamine close in time to NSAID-dosing resulted in significantly lower permeability compared to the NSAID without glutamine. Co-administration of misoprostol with the multiple-dose scheme of glutamine resulted in a further reduction in the NSAID-induced increase in permeability.

Conclusions: Glutamine decreases the permeability changes caused by NSAID-dosing when it is administered close in time, and misoprostol has a synergistic effect.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have proved their efficacy as analgesic, antipyretic and anti-inflammatory agents. Yet, there is growing concern about their side-effects. Sixty to 70% of patients on long-term therapy may suffer from enteropathy, which can be characterized by intestinal bleeding, protein loss, bile acid malabsorption and occasionally small intestinal strictures.[1]

The interest in the relationship between NSAIDs and small intestinal damage was actuated by the finding that patients with rheumatoid arthritis or osteoarthritis who were treated with these drugs developed increased intestinal permeability.[2–5] Experiments in healthy volunteers confirmed that permeability changes are induced by NSAIDs.[6, 7]

The pathogenesis of NSAID-associated enteropathy is still uncertain. It is thought that increased intestinal permeability is an important triggering factor in the development of inflammation, thus facilitating invasion of bacteria or action of other aggressive agents such as bile acids or certain food components. A first possible mechanism for increased permeability might be a reduced mucosal prostaglandin synthesis by the inhibition of cyclooxygenase, which leads to cell damage.[6, 7] Secondly, it was postulated that NSAIDs inhibit the oxidative phosphorylation in mitochondria[8, 9] and that shortage of ATP causes damage to the cell and the intercellular junctions.

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Based on these mechanisms, two possible agents to restore permeability changes can be suggested. Misoprostol (a synthetic prostaglandin) may be able to substitute for the shortage of endogenous prostaglandins. Glutamine (the energy source accounting for approximately 35% of the total CO_2 production by the epithelial cells of the intestine)[10–12] may serve as an alternative source of energy for the mucosal cell. The aim of the present project was to study whether glutamine and/or misoprostol is able to prevent the NSAID-induced increase in intestinal permeability in healthy volunteers.

MATERIALS AND METHODS

Subjects

All volunteers were healthy subjects without intestinal complaints. They did not take any medication for the week prior to testing and alcohol was strictly forbidden for 24 h preceding each test. Repetitive testing in the same subject was separated by a wash-out period of at least 4 days. All tests were performed after at least an 8-h fast. Tests were not performed in random order.

Permeability test

Permeation of ^{51}Cr-EDTA was measured as follows: at 08.30 hours, after an overnight fast, the subject drank 160 mL of Nutridrink® (Nutricia, Bornem, Belgium) with 50 μCi of ^{51}Cr-EDTA (Amersham International, Amersham, UK) and the glass was rinsed with 90 mL of water.[13] Eating or drinking was not allowed for the next 2 h and urine was collected for 6 h. Volumes of urine were recorded and 1-mL aliquots were counted for radioactivity in a β liquid scintillation counter (Packard, model 4430, Downers Grove, Illinois, USA) within 48 h after sampling. Results were expressed as the percentage urinary excretion of the ^{51}Cr-EDTA that was orally administered.

Test conditions

Each volunteer performed a series of permeability tests: (i) at baseline ($n = 54$); (ii) after NSAID-dosing ($n = 54$): 75 mg indomethacin (Indocid, Merck Sharp & Dohme, Haarlem, the Netherlands) at 22.00 hours the day prior to the permeability test and 50 mg at 08.00 hours the day of the permeability test, which was

performed at 08.30 hours; (iii) after NSAID-dosing in combination with a possibly protective agent. In order to study the protective effect of glutamine and/or misoprostol, several schemes of administration of glutamine (pre-treatment vs. co-administration; multiple-dose vs. single-dose) were tested and the addition of misoprostol was also studied. In the following paragraphs, the different schemes of glutamine and/or misoprostol administration are described.

High-dose pre-treatment with glutamine. Six healthy volunteers (4 females, 2 males; mean [± S.E.M.] age $= 22.1 \pm 0.5$ years) ingested 3×7 g of glutamine per day on the 7 days preceding NSAID-dosing as indicated in Figure 1(A). Glutamine was obtained from ICN Biomedicals (Ohio, USA) and was given as powder dissolved in water. The solution had a neutral taste and gastro-intestinal tolerance was good. The dose was in accordance with a clinical trial that we performed to study the effect of oral glutamine supplements (3×7 g per day) on small intestinal permeability in patients with Crohn's disease.[14] The choice of dose in the latter was based on clinical trials with total parenteral nutrition (TPN), in which between 0.2 and 0.6 g glutamine/kg body weight was administered.[15, 16]

Single-dose co-administration of glutamine. The effect of 7 g of glutamine half an hour prior to NSAID-dosing was studied. Twelve volunteers (7 females, 5 males; age $= 21.2 \pm 0.6$ years) performed the test as indicated in Figure 1(B) and thus ingested 7 g of glutamine at 21.30 hours on the day before (NSAID intake at 22.00 hours) and 7 g of glutamine at 07.30 hours on the day of the test (NSAID intake at 08.00 hours).

Multiple-dose co-administration of glutamine. Twelve healthy volunteers (5 females, 7 males; age $= 25.5 \pm 1.9$ years) followed the scheme presented in Figure 1(C). They started with 7 g of glutamine half an hour prior to NSAID-dosing and took several doses of 1 g of glutamine together with and after NSAID-dosing.

Multiple-dose co-administration of glutamine in combination with misoprostol. The combined effect of glutamine and misoprostol (Cytotec, Searle, Brussels, Belgium) was also studied. Twelve healthy volunteers (7 females, 5 males; age $= 25.6 \pm 1.1$ years) combined the 'multiple-dose
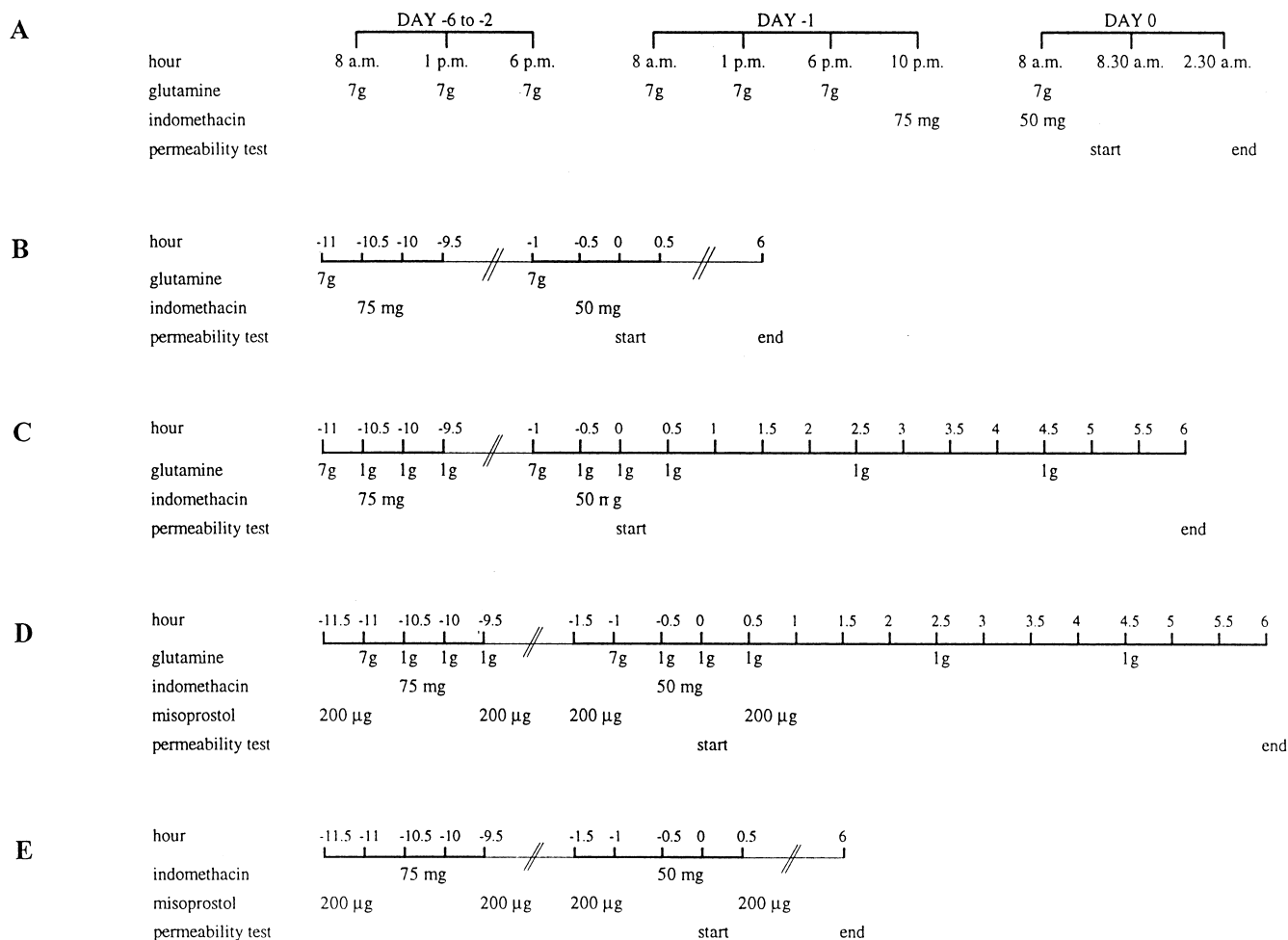
Figure 1. Time schedule for glutamine, indomethacin and misoprostol administration in (A) high-dose pre-treatment of glutamine; (B) single-dose co-administration of glutamine; (C) multiple-dose co-administration of glutamine; (D) multiple-dose co-administration of glutamine in combination with misoprostol; (E) misoprostol administration.

co-administration of glutamine' with 2×200 μg misoprostol on the day before the permeability study and 2×200 μg misoprostol on the day of testing as indicated in Figure 1(D).

Administration of misoprostol. The effect of misoprostol alone (4×200 μg) was studied in 12 volunteers (9 females, 3 males; age = 23.0 ± 0.7 years) (see Figure 1E).

Statistical analysis

Data are presented as mean $\pm$ S.E.M. Normal distribution was checked by a Shapiro–Wilks test and a logarithmic transformation was performed if data were not normally distributed. Permeability in the different test conditions was compared by repeated measures analysis of variance (ANOVA). Statistical analysis was performed with the SAS program on PC[17] and the 5% level was considered significant.

RESULTS

In all experiments, intake of NSAID resulted in a significant increase of permeability of the small bowel compared to baseline. The overall baseline permeability ($n = 54$) was $0.66 \pm 0.04\%$; permeability after NSAID-dosing was $1.69 \pm 0.13\%$.

An overview of the ^{51}Cr-EDTA excretion data in the different test conditions is given in Table 1.

Table 1. Mean (± S.E.M.) intestinal permeability (six-hour urinary excretion of ^{51}Cr-EDTA, expressed as percentage of the dose) in different experimental designs

Baseline	NSAID	NSAID + gln	NSAID + gln + mp	NSAID + mp	ANOVA
High-dose pre-treatment of glutamine (n = 6)					
0.42 ± 0.07%	1.02 ± 0.23%*	1.12 ± 0.22%*	—	—	$P = 0.004$
Single-dose co-administration of glutamine (n = 12)					
0.78 ± 0.07%	2.11 ± 0.34%*	1.90 ± 0.27%*	—	—	$P = 0.003$
Multiple-dose co-administration of glutamine (n = 12)					
0.56 ± 0.10%	1.61 ± 0.21%*	1.06 ± 0.13%*#	—	—	$P = 0.001$†
Multiple-dose co-adminstration of glutamine in combination with misoprostol (n = 11)					
0.63 ± 0.10%	1.65 ± 0.19%*	1.26 ± 0.19%*#	0.87 ± 0.17#‡	—	$P = 0.001$
Administration of misoprostol (n = 12)					
0.73 ± 0.08%	1.71 ± 0.23%*	—	—	1.28 ± 0.21%	$P = 0.002$

* Significantly different from baseline.
# Significantly different from NSAID.
‡ Significantly different from NSAID + glutamine.
† Statistical analysis after logarithmic transformation.

High-dose pre-treatment with glutamine

Pre-treatment with glutamine, i.e. large doses of glutamine 1 week prior to NSAID-dosing, could not prevent permeability changes induced by the NSAID (Table 1).

Single-dose co-administration of glutamine

A single dose of 7 g of glutamine half an hour prior to NSAID intake was also not efficacious in the prevention permeability changes induced by the NSAID (Table 1).

Multiple-dose co-administration of glutamine

Small but frequent doses of glutamine administered close in time with NSAID-dosing significantly decreased permeability changes (1.06 ± 0.13% vs. 1.61 ± 0.21%) but the baseline value (0.56 ± 0.10%) was not reached (Table 1). Individual results are presented in Figure 2.

Multiple-dose co-administration of glutamine in combination with misoprostol

Permeability did not reach the baseline level after administration of glutamine supplements, therefore the question of whether the addition of misoprostol could result in any supplementary beneficial effect was studied. One volunteer was excluded because of
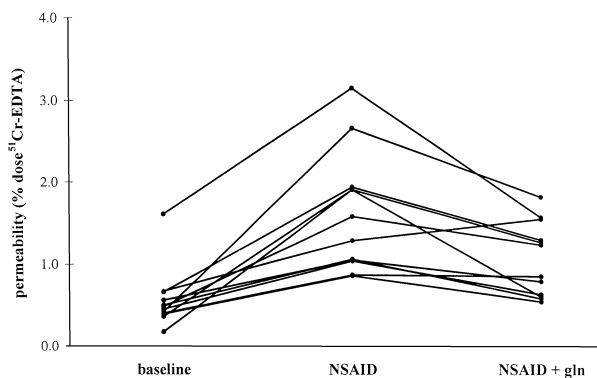

Figure 2. Baseline permeability, permeability after NSAID-dosing and permeability after NSAID-dosing + multiple-dose co-administration of glutamine in 12 healthy volunteers.

extremely high baseline values (4.93%). In the remaining 11 volunteers, the significant effect of repeated small doses of glutamine was confirmed: 1.26 ± 0.19% after NSAID + glutamine compared to 1.65 ± 0.19% after NSAID alone. The addition of misoprostol resulted in a further significant decrease in permeability change: 0.87 ± 0.17% (Table 1 and Figure 3).

Administration of misoprostol

The effect of misoprostol alone was not significant (Table 1). The effect of misoprostol was heterogeneous: misoprostol decreased permeability in most volunteers but increased permeability in some subjects, whereas glutamine decreased permeability in all subjects.
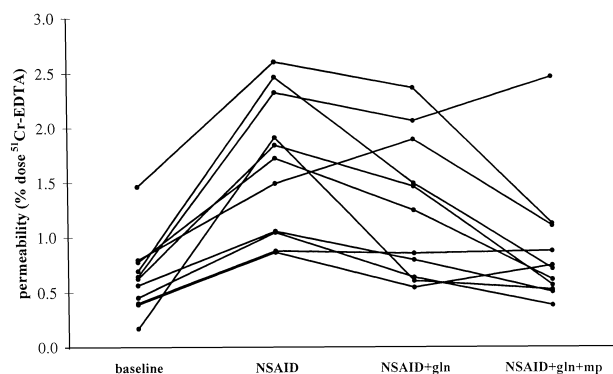
Figure 3. Baseline permeability, permeability after NSAID-dosing, permeability after NSAID-dosing + multiple-dose co-administration of glutamine, and permeability after NSAID-dosing + multiple-dose co-administration of glutamine + misoprostol in 11 healthy volunteers.

DISCUSSION

In this study an important and reproducible increase in intestinal permeability after oral intake of indomethacin, as reported by other authors, was confirmed. The ratio 'permeability after NSAID-dosing' over 'baseline permeability', was 2.6, a value closely resembling the increase reported by Bjarnason.[6, 7, 18]

Indications to the use of glutamine as a protective agent to prevent an increase in small bowel permeability are twofold. First, glutamine was reported to influence gut permeability in lesions other than NSAID-induced ones. In animals,[19] as well as in humans,[20] standard TPN leads to deterioration of gut integrity, but the addition of glutamine to TPN is able to preserve jejunal villus height and to prevent an increase in permeability. Second, glutamine has been shown to reduce the occurrence of gastric mucosal lesions induced by aspirin in rats.[21]

The present study shows that glutamine decreases the NSAID-induced permeability changes in the gut. The effect of glutamine, however, was only found following multiple-dose co-administration, close in time to the intake of NSAID-dosing. Pre-treatment during 1 week or a single dose of glutamine did not show a significant effect. This indicates that glutamine has to be provided close in time with indomethacin and repetitive doses are needed, suggesting that its efficacy is limited in time.

One can only speculate about the mechanism for the protective role of glutamine. Bjarnason et al.[1] proposed the energy depletion hypothesis for NSAID damage to the intestinal cell. NSAIDs lead to uncoupling of the mitochondrial oxidative phosphorylation; the shortage of ATP results in loss of integrity and also in an efflux of calcium and hydrogen ions, which in turn leads to ATP depletion and promotes oxygen radical damage. Repair of mucosal integrity is hampered because the conversion of arachidonic acid to prostaglandins is decreased due to inhibition of cyclo-oxygenase. Loss of intestinal integrity in its turn facilitates the invasion of bacteria and increases the sensitivity for aggressive agents such as bile acids or certain food components, and eventually leads to inflammation.

Several observations support Bjarnason's hypothesis. First, the presence of bacteria in the gut is an important factor since NSAID cause less damage in germfree animals.[22, 23] Second, studies examining the effect of synthetic prostaglandins on intestinal permeability show a protective effect, although the results were inconsistent and large doses had to be administrated close in time to NSAID administration for a good protective effect.[6, 7, 24, 25] Third, energy depletion was prevented by supplementation with a glucose–citrate formulation. In normal conditions, glucose is degraded via the glycolysis and the tricarboxylic acid cycle and yields NADH, which generates ATP through the oxidative phosphorylation. Because NSAIDs inhibit the oxidative phosphorylation, no ATP is produced from NADH. This problem can be overcome by giving glucose and citrate simultaneously. Citrate is the inhibitory factor to phosphofructokinase—the key enzyme in glycolysis—and thus it creates a situation where glucose will be funnelled into the glucose monophosphate pathway where two molecules NADPH are produced from each molecule of glucose. In contrast to NADH, NADPH is not converted to ATP in oxidative phosphorylation, but is used directly in many endergonic reactions. Experiments in the rat[26] and in humans[18, 27] have indeed confirmed that the addition of glucose and citrate to an NSAID may prevent permeability changes.

Protection by glutamine may result from a similar mechanism. Although the major part of glutamine's energy is generated through the tricarboxylic cycle and thus depends on oxidative phosphorylation, the first two steps in the metabolism of glutamine, i.e. the conversion of glutamine to glutamate and further de-amination to α-ketoglutarate, yields one molecule NADPH. In addition to its role as fuel, the beneficial effect of glutamine in NSAID-induced damage might result from the fact that it provides amide nitrogen for nucleotide biosynthesis.[11]

Because permeability increase due to NSAID was not abolished completely with glutamine supplementation and because it is hypothesized that misoprostol works through a different mechanism than glutamine, it was postulated that the combination of glutamine and misoprostol might have a more beneficial effect than glutamine alone. The addition of misoprostol to glutamine indeed had a beneficial effect, however, a control test with misoprostol alone did not prevent the increase in permeability.

The role of prostaglandins in the prevention of increased intestinal permeability remains unclear. The first experiments with synthetic prostaglandins (prostaglandin E_2 analogues[24] as well as prostaglandin E_1 analogues[25]) failed to prevent the increase in permeability induced by NSAIDs. Later studies[6, 7] did show a significant effect and this was assigned to the stability of the medication and the fact that doses were given more frequently and closer in time with NSAID administration. In spite of the fact that medication was stable and that doses were given close in time with indomethacin, misoprostol failed to show a beneficial effect in this study. Possibly higher doses of misoprostol are needed to show any effect.

In conclusion, glutamine is able to partly prevent the increase in permeability after NSAID administration if it is administered close in time with the NSAID. A possible mechanism is the restoration of energy depletion and the supply of amide nitrogen for nucleotide biosynthesis. Misoprostol had a synergetic effect with glutamine.

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