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SPECIAL REPORT

Protective effect of NO on gastric lesions and inhibition of expression of gastric inducible NOS by flurbiprofen and its nitro-derivative, nitroflurbiprofen

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Nitroflurbiprofen (NFP) causes significantly less gastric lesions than flurbiprofen (FP), probably because of its capacity to release nitric oxide (NO) in the stomach. Lipopolysaccharide (LPS), which induces the expression of an inducible type of NO synthase (iNOS) in rat stomach, also reduces gastric mucosal damage elicited by FP. Furthermore, both FP and NFP decrease significantly the amount of mRNA encoding iNOS induced by LPS in the stomach. The inhibitory effect of NFP seems to be due at least in part to its ability to release NO.

Keywords: Nitric oxide; nitric oxide synthase; flurbiprofen; nitroflurbiprofen; gastric lesions

Introduction Flurbiprofen (FP), a well-established non-steroidal anti-inflammatory drug (NSAID), causes severe gastrointestinal lesions that limit its clinical use. NFP, a new anti-inflammatory drug obtained by the incorporation into FP of a nitroxybutyl moiety through an ester linkage to its carboxylic group, has shown good gastrointestinal tolerability associated with an excellent anti-inflammatory action (Wallace *et al.*,1994). Recently, we have reported data indicating the *in vivo* release of NO from NFP, and the possible counteracting action of exogenous NO to the LPS-elicited induction of iNOS expression in neutrophils (Mariotto *et al.*, 1995a,b). In this work, we try to elucidate the protective effect of both exogenous and endogenous NO against FP-elicited gastric ulcer formation. Moreover, since LPS administration to rats has been shown to induce iNOS expression in the stomach, experiments are presented, which may suggest a putative NO-mediated inhibitory action of NFP on the LPS-elicited induction of gastric iNOS expression.

Methods Female Sprague-Dawley rats were treated as previously described (Mariotto *et al.*, 1995a,b). The degree of lesions was graded according to an arbitrary scale: (0), no lesion; (1), mild lesions; (2), small ulcers; (3), severe and numerous ulcers. Total RNA was extracted from the stomach by the guanidine thiocyanate method (Chirgwin *et al.*, 1979) and Northern blot hybridization was performed using rat liver iNOS cDNA (Adachi *et al.*, 1993) as a probe. The statistical analysis of the data was performed by one-way analysis of variance followed by Dunnett's multicomparison test.

Results and Discussion As shown in Table 1, while FP induced severe stomach lesions, NFP caused in comparison significantly less damage, although NFP was reported to be as potent as FP in inhibiting cyclo-oxygenase (Wallace, 1993). LPS-administration to rats 1 h after FP-treatment also reduced significantly the stomach lesions elicited by FP. The administration of sodium nitroprusside (SNP) or LPS alone, and LPS plus NFP or LPS plus SNP produced no significant stomach damage. Treatment of rats with LPS or NFP alone caused an increase in nitrite/nitrate plasma levels (Mariotto *et al.*, 1995b). For LPS this is due to induction of the iNOS mRNA expression in a variety of tissues including stomach (Figure 1) followed by a high production of NO; for NFP to the release of NO from the drug (Wallace *et al.*, 1994). Wallace *et al.* (1994) have recently suggested the possible importance of exogenous NO in protecting the stomach from ulcer formation induced by NSAID. Thus, on the basis of the present data, we believe that either exogenous NO released from NFP or endogenous NO produced after LPS-treatment protects the gastric mucosa against FP-elicited damage.

SNP failed to counteract gastric ulcer formation induced by FP (Table 1) probably because this drug is unable to release NO in the stomach (Schumacher, 1966), being more stable in acidic solution. Moreover, SNP was unable to decrease the LPS-induced level of iNOS mRNA in the stomach (Figure 1).

In rats co-treated with LPS plus FP or LPS plus NFP, the amount of iNOS mRNA was reduced to less than 50% of that elicited by LPS-treatment alone (Figure 1). Although the mechanism of the inhibitory action of FP on the gastric iNOS expression awaits further elucidation, a reason could be the cyclo-oxygenase inhibition induced by FP, as well as the inflammation triggered by gastric lesions.

Regarding the inhibition by NFP of LPS-elicited gastric iNOS expression, a role should be played not only by FP but

Table 1 Evaluation of stomach lesions in control rats and in rats receiving lipopolysaccharide (LPS) and/or the drugs

Groups	*Damage score*
Controls ($n=17$)	0.24 ± 0.43
Flurbiprofen ($n=16$)	2.62 ± 0.62**
Nitroflurbiprofen ($n=15$)	0.73 ± 0.88*
Sodium nitroprusside ($n=13$)	0.15 ± 0.27
LPS ($n=25$)	0.48 ± 0.50
LPS + flurbiprofen ($n=18$)	1.11 ± 0.67●●◇◇
LPS + nitroflurbiprofen ($n=15$)	0.53 ± 0.64
LPS + sodium nitroprusside ($n=9$)	0.11 ± 0.33
Flurbiprofen + sodium nitroprusside ($n=8$)	2.00 ± 0.93

The values are expressed as mean ± s.d. (*) NFP vs controls, $P<0.01$; (**) FP vs controls, $P<0.001$; (●●) LPS plus FP vs FP, $P<0.001$; (◇◇) LPS plus FP vs LPS, $P<0.001$.

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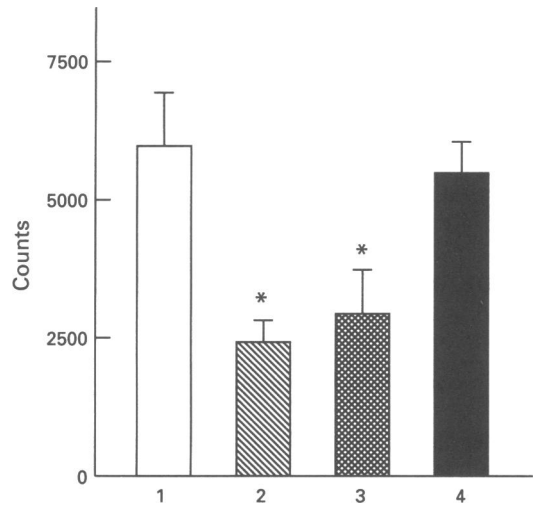

Figure 1 Amount of mRNA encoding iNOS in the stomach of rats treated with lipopolysaccharide (LPS) alone or LPS plus examined drugs. Treatment: (1) LPS ($n=19$); (2) LPS plus FP ($n=11$); (3) LPS plus NFP ($n=11$); (4) LPS plus SNP ($n=11$). The values are expressed as mean ± s.e. One way ANOVA: $P<0.01$. (*) FP or NFP vs LPS, $P<0.05$.

also by the NO, both gradually released from the drug. The inhibitory action of NFP on the LPS-elicited induction of iNOS expression has already been described in the intestine (A. Carcereri de Prati, L. Cuzzolin, M. Menegazzi, A. Adami, A. Chiamenti, S. Mariotto, H. Suzuki, G. Benoni, unpublished observations).

In conclusion, the results obtained in this work suggest that not only exogenous NO released from NFP but also endogenous NO synthesized by LPS-induced gastric iNOS may protect against stomach lesions elicited by FP. Furthermore, both FP and NFP have been shown to counteract significantly the expression of iNOS induced in the stomach by LPS-treatment. While the observed inhibitory effect of NFP may be partly mediated by exogenously released NO from NFP, further work is needed on the inhibitory action of FP on gastric iNOS expression.

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