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Induction of nitric oxide synthase *in vivo* and cell injury in rat duodenal epithelium by a water soluble extract of *Helicobacter pylori*

[1]Dominique Lamarque, [1]József Kiss, [2]Jacques Tankovic, [3]Jean François Flejou, [1]Jean-Charles Delchier & [4,5]Brendan J.R. Whittle

[1]Institut National de la Santé et de la Recherche Médicale (INSERM U.99) et Service d'Hépatologie et de Gastroentérologie, Hôpital Henri Mondor, F-94010 Créteil; [2]Service de Microbiologie, Hôpital Henri Mondor, F-94010 Créteil, [3]Service d'Anatomo-Pathologie, Hôpital Beaujon, F-92 Clichy, France and [4]William Harvey Research Institute and St. Bartholomew's & Royal London School of Medicine & Dentistry, Charterhouse Square, London EC1M 6BQ

1 *Helicobacter pylori (Hp)* infection, which involves the gastric antrum and duodenal mucosa, may be involved in peptic ulceration by stimulating the local release of cytoxic or pro-inflammatory factors.

2 Nitric oxide (NO) is known to be cytotoxic at high concentration. The aim of the present study was therefore to investigate the ability of a water soluble extract of *Hp* to induce NO synthase in duodenal mucosa and epithelial cells following its administration *in vivo* in rats and determine its association with cell damage.

3 Administration of *Hp* water extract (4 ml kg^{-1}) led to the expression of the calcium-independent inducible nitric oxide synthase (iNOS) after 4 h in the duodenum, determined as [^{14}C]-arginine conversion to citrulline.

4 This iNOS activity was not reduced by pretreatment with anti-neutrophil serum (0.4 ml kg^{-1}, i.p., 3 h before challenge). However, dexamethasone pretreatment (1 mg kg^{-1}, i.v., 2 h before the extract), or administration of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, i.v., 2.5 h after the extract) reduced this activity.

5 Furthermore, iNOS was expressed in duodenal isolated epithelial cells 4 h after the i.v. challenge with the extract, at a time when the cellular viability was also reduced, as assessed by trypan blue exclusion.

6 Dexamethasone pretreatment, administration of L-NAME, or pretreatment with polymyxin B (1 mg kg^{-1}, i.v.) which binds endotoxin, reduced both the iNOS activity and epithelial cell damage.

7 The induction of NO synthase by the *Hp* extract thus results in duodenal epithelial cell injury and such actions could play a role in pathogenesis of peptic ulcer disease.

Keywords: Nitric oxide; inducible nitric oxide synthase (iNOS); duodenal epithelial cells; duodenum; *Helicobacter pylori*

Introduction

Helicobacter pylori (H. pylori) infection is considered a predominant pathogenic factor in peptice ulcer disease (Baldwin *et al*., 1991). Thus, this bacterium colonizes the gastric antrum and sites of gastric metaplasia in the duodenum, and induces local inflammation with activated neutrophils (Carrick *et al*., 1989; Blaser, 1990). The processes by which *H. pylori* infection may provoke damage in the stomach and duodenum are unclear, but the bacteria could release soluble cytotoxic factors that activate inflammatory cells such as neutrophils, that are implicated in tissue damage (Nielsen & Andersen, 1992a) and microvascular leakage (Kurose *et al*., 1994). Such activation of neutrophils could provoke the release of free radicals and other reactive species, including superoxide (Mooney *et al*., 1991) and nitric oxide (NO) (McCall *et al*., 1989). High concentrations of NO are known to be cytotoxic and in combination with the superoxide radical, lead to the subsequent formation of the moities, peroxynitrite and the hydroxyl radicals, which are highly injurious to cells (Beckman *et al*., 1990; Ischiropoulos *et al*., 1995).

The calcium-independent inducible isoform of NO synthase (iNOS) is capable of generating high sustained levels of NO, and can be expressed in inflammatory cells and in a number of other cell types, including gastro-intestinal epithelial cells (Tepperman *et al*., 1993; Brown *et al*., 1994). The expression of iNOS in the gastrointestinal mucosa following challenge with bacterial endotoxin is associated with microvascular injury (Boughton-Smith *et al*., 1993; Laszlo *et al*., 1995) and damage to epithelial cells (Tepperman *et al*., 1993; 1994). It is feasible, therefore, that since *H. pylori* can likewise elaborate an endotoxin (Moran *et al*., 1992), expression of iNOS in the duodenum or inflammatory cells could also play a role in the pathogenesis of mucosal lesions related to infection by this organism.

Recent *in vitro* studies have shown that extracts of *H. pylori* can lead to the expression of iNOS in murine and human macrophage cell lines in culture (Perez-Perez *et al*., 1995; Shapiro *et al*., 1996; Wilson *et al*., 1996). However, such *in vitro* studies show only a low potency of *H. pylori* to induce iNOS, and its actions on other cell types, particularly gastrointestinal cells, and its effects *in vivo* on iNOS have not been determined. The aim of the present study was therefore to investigate the ability of a water soluble extract of *H. pylori* to induce iNOS in duodenal mucosa and in duodenal epithelial cells following its administration *in vivo*, and determine its association with epithelial cell damage in the rat.

[5] Author for correspondence.

Methods

H. pylori *extract preparation*

H. pylori extracts were prepared from strains isolated from the gastric antrum from a single patient with antral gastritis and peptic ulcer or with the reference strain, CCUG-17874 obtained from the culture collection, University of Göteborg. The bacteria was grown on blood agar plates under sterile conditions, as previously described (Rauws *et al*., 1989). Briefly, the growth medium was Columbia agar (Oxoid, Basingstoke, U.K.) containing 5% (vol/vol) horse blood, Skirrow antibiotic selective supplement (Oxoid, Basingstoke, U.K.) and amphotericin B. After inoculation, all plates were incubated at 37°C under microaerophilic conditions for a total of 3 days. Purity of *H. pylori* cultures were assessed by positive urease and oxidase tests and morphological recognition (spiral shape gram negative bacillus). The bacteria were harvested with a sterile curved glass scraper into distilled water, 1.0 ml plate (10^7-10^8 colony forming unit; CFU). As previously described, the suspension was then kept at room temperature for 20 min before centrifugation at 17,000 *g* for 15 min (Kurose *et al*., 1994). The supernatant, referred to as the *H. pylori* water extract, was stored at −20°C. Before use, the extract was allowed to reach room temperature and then centrifuged at 17,000 × *g* for 15 min. The pellet was discarded and the supernatant passed through a 0.2 μm syringe adapted filter. Water extracts from *Helicobacter felis* (10^7-10^8 CFU), used as control, were prepared in a similar manner.

Animal preparation

Male Wistar rats, weighing 200–250 g, were fasted overnight but allowed free access to water. The animals were pretreated with 4 ml kg^{-1} *H. pylori* water extract or with isotonic saline (4 ml kg^{-1}), administered via the tail vein under transient anaesthesia induced by ether. In control experiments, rats were pretreated with *Helicobacter felis* water extract (4 ml kg^{-1}).

Duodenal epithelial cell isolation

Duodenal epithelial cells were isolated as described previously (Lentze *et al*., 1985). A 5 cm segment of duodenum was slowly flushed with 50 ml of a solution containing 0.15 M NaCl and 0.1 mM dithiothreitol (DTT). The segment was then filled with 5 ml of a solution containing (in mM): KCl 1.5, NaCl 96, sodium citrate 27, KH_2PO_4 8 and Na_2HPO_4 5.6 (pH 7.3), and the proximal and the distal ends were ligated. The segment was then immersed in phosphate-buffered saline (PBS) kept at 37°C, which was bubbled with 95% O_2–5% CO_2. After 15 min, the instilled solution was removed and another solution containing 1.5 mM EDTA and 0.5 mM DTT was instilled over 5 min, as described previously (Tepperman *et al*., 1993). The epithelial cells were collected in suspension in this solution. The cells were washed twice with PBS (pH 7.4) and centrifuged for 5 min at 800 *g*. The cells were suspended in a buffer containing N-2-hydroxyethylpiperazipine-N′-2-ethanesulphonic acid (HEPES) (10 mM), sucrose (320 mM), DTT (1 mM), soybean trypsin inhibitor (10 μg ml^{-1}), leupeptin (10 μg ml^{-1}) and aprotonin (2 μg ml^{-1}). To assess the purity of the epithelial cells in the aliquots isolated from duodenum, in some experiments the cells were fixed with formaldehyde, stained by haematoxylin-eosin-safran (HES) and counted under light microscopy and expressed as a percentage of epithelial cells.

NO synthase activity

NO synthase activity in the whole duodenal tissue or in duodenal epithelial cells was measured as the conversion of L-[^{14}C]-arginine monohydrochloride to [^{14}C]-citrulline, based on the method described previously (Knowles *et al*., 1990). Tissues were homogenized (30 s, Ultra-Turrax; 5 mm blade) in buffer (pH 7.4, 100 mg ml^{-1}) containing HEPES (10 mM), sucrose (32 mM), DTT (1 mM), leupeptin (10 μg ml^{-1}), soybean trypsin inhibitor (10 μg ml^{-1}) and aprotonin (2 μg ml^{-1}).

Following centrifugation (10,000 *g*, 4°C), an aliquot of the supernatant (40 μl) was used for the determination of the enzymatic activity and the remaining kept for protein content measurement by Bradford's method. The aliquot was placed in 100 μl of the pre-warmed incubation buffer containing (final concentration) potassium phosphate (50 mM; pH 7.4), L-valine (50 mM), $MgCl_2$ (1 mM), $CaCl_2$ (200 μM), (±-dithiothreitol (1 mM), L-citrulline (1 mM), NADPH (0.3 mM), FAD (3 μM) FMN (3 μM), BH_4 (3 μM) L-[^{14}C]-arginine monohydrochloride (15.5 nM; Amersham France, Les Ulis) and incubated for 10 min at 37°C. The incubation was terminated by binding arginine by the addition of 500 μl of 1:1 suspension of Dowex (AG 50W-8; Sigma) in water. The resin was allowed to settle (30 min) and 975 μl of supernatant taken for scintillation counting (3 ml Ready safe, Beckman).

Product formation that was inhibited by *in vitro* incubation with ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA; 1 mM) and the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA; 300 μM; Sigma Chemical Co.) was taken as an index of constitutive NO synthase activity. Activity of iNOS was taken as that which was inhibited by *in vitro* incubation with L-NMMA but not with EGTA (Salter *et al*., 1991). The constitutive NO synthase activity was calculated from the difference between the iNOS and the total activity. NO synthase activity was expressed as pmol min^{-1} mg^{-1} protein.

Myeloperoxidase activity

The myeloperoxidase (MPO) activity was determined by the method described by Bradley *et al*. (1982) with minor modifications. A segment of the duodenum was homogenized in ice-cold phosphate buffer (50 mM, pH 6) containing 0.5% hexadecyltrimethylammonium-bromide (HTAB), freeze-thawed three times and centrifuged (10 000 × *g*, 15 min, 4°C). Then, 30 μl of the supernatant was mixed with 710 μl phosphate buffer (50 mM, pH 6) containing 0.167 mg ml^{-1} O-adenosine dihydrochloride and 0.0005% hydrogen peroxide and assayed spectrophotometrically (500 nm). MPO activities were expressed as mu g^{-1} wet tissue.

Effects of H. pylori *and* Helicobacter felis *extract on NO synthase activity*

At 2, 4 or 6 h after administration of *H. pylori* water extract, the animals were killed by cervical dislocation and the duodenum removed. In two preliminary series of experiments, the effects of extracts prepared from the two different strains were compared. In all further experiments the use of these extracts was randomized. In control experiments, rats were pretreated with saline or, in further studies, *Helicobacter felis* water extract, and the duodenum removed 4 h later. The NO synthase activity was determined in these tissues following homogenization.

Effects of L-NAME or dexamethasone on NO synthase activity

In further experiments, rats were pretreated with the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 5 mg kg^{-1}, i.v.) or saline, 2.5 h after challenge with *H. pylori* water extract. A separate group of rats was pretreated with dexamethasone (1 mg kg^{-1}, i.v.; Soludecadron, Merck Sharp and Dohme) administered 2 h before *H. pylori* water extract. The NO synthase activity was determined in duodenal tissue, taken 4 h after challenge with *H. pylori* water extract. The doses and timing of administration of these agents were taken from previous studies on rat gastrointestinal tissue (Boughton-Smith *et al*., 1993; Tepperman *et al*., 1991; 1993).

Effects of polymyxin B or rabbit anti-rat neutrophil serum on NO synthase activity

A group of rats was pretreated with polymyxin B (1 mg kg^{-1}, i.v.) 15 min before extract injection in order to bind and neutralize any lipopolysaccharide (LPS) (Baldwin *et al*., 1991) derived from *H. pylori* in the extract. In further studies, rabbit anti-rat neutrophil serum (0.4 ml kg^{-1}, i.p.) was injected 3 h before administration of the extract, in a dose which reduces the circulating neutrophil count by more than 90% (Tepperman *et al*., 1994; Lamarque & Whittle, 1995). The number of circulating neutrophils was determined on a blood smear 4 h after antibody injection (haematoxylin-eosin stain). The NO synthase activity was determined in duodenal tissue, 4 h after *H. pylori* water extract administration.

Effects of H pylori *extract on NO synthase activity and cell viability*

In further experiments, the cell viability was examined and the NO synthase activity was determined in duodenal epithelial cells collected from rats that had been challenged, 1 and 4 h previously, with *H. pylori* water extract or saline. In further experiments, a group of rats was treated with N^G-nitro-L-arginine methyl ester (L-NAME; 5 mg kg^{-1}, i.v.), 2.5 h after *H. pylori* water extract administration. In other experiments, a group of rats was pretreated with dexamethasone (1 mg kg^{-1}, i.v.) administered 2 h before *H. pylori* water extract. A separate group of rats was pretreated with polymyxin B (1 mg kg^{-1}, i.v.) 15 min before extract injection. The viability of cells was determined by trypan blue dye exclusion (0.5% trypan blue in PBS) as described previously (Tepperman *et al*., 1991). The number of non-viable cells was determined by light microscopy ($\times 40$ magnification) by counting those cells that failed to exclude the dye. Cells were counted in a random manner with a haemocytometer.

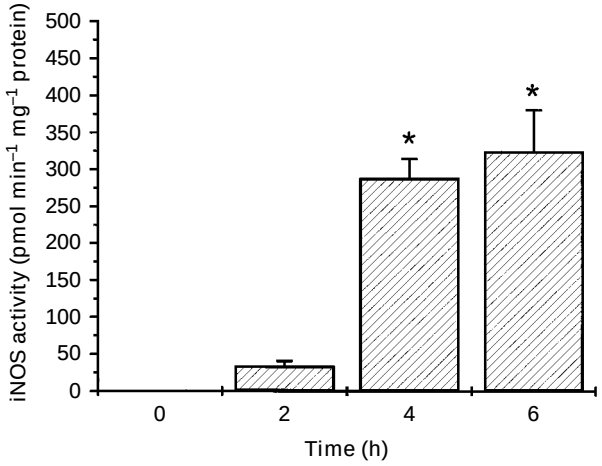

Figure 1 Inducible nitric oxide synthase (iNOS) activity in duodenal tissue following challenge with *H. pylori* water extract (4 ml kg^{-1}, i.v.) in rats. Data shown as the Ca^{2+}-independent activity (pmol min^{-1} mg^{-1} protein) are means $\pm$ s.e. of 9–14 experiments. *Denotes significant difference from the control at time 0 ($P<0.005$).

Statistical analysis

The data are expressed as the mean $\pm$ s.e.mean of n rats per group. Statistical comparison were made by Student's t test or by Mann-Whitney nonparametric data test for unpaired data; $P<0.05$ was taken as significant.

Results

Induction of NO synthase in duodenal tissue after H. pylori *extract administration*

Basal NO synthase activity, which was abolished by addition of L-NMMA (300 μM) or EGTA (1 mM), was detected in the supernatants of homogenates of duodenum (638 ± 102 pmol min^{-1} mg^{-1} protein, $n=9$).

NO synthase activity which was inhibited by L-NMMA but not by *in vitro* incubation with EGTA (1 mM), was detectable 4 h after extract injection, while it was not detected under basal conditions or 2 h after challenge (Figure 1). This Ca^{2+}-independent activity remained at a similar level for the remainder of the 6 h period.

The increase in Ca^{2+}-independent NO synthase activity measured 4 h after challenge, was comparable with the two strains used to prepare the *H. pylori* extract, being 361 ± 21 pmol min^{-1} mg^{-1} protein ($n=6$) with the strain isolated from a single patient and 390 ± 95 pmol min^{-1} mg^{-1} protein ($n=5$) with the reference strain, CCUG-17874. In further experiments, the two strains were administered randomly to the rats and the results pooled.

In contrast, Ca^{2+}-independent activity was not detectable 4 h following challenge with *Helicobacter felis* extract as compared to control (13 ± 4 pmol min^{-1} mg^{-1} protein, $n=11$ and 0.3 ± 0.1 pmol min^{-1} mg^{-1} protein, $n=9$, respectively).

Effect of H. pylori *extract administration on myeloperoxidase activity in duodenal tissue*

After *H. pylori* extract administration, duodenal myeloperoxidase activity, measured 6 h later, did not increase significantly (199 ± 94 mu g^{-1} tissue; $n=5$) as compared to control (102 ± 32 mu g^{-1} tissue; $n=5$).

Effect of L-NAME or dexamethasone on NO synthase activity in duodenum

The increase in Ca^{2+}-independent NO synthase activity determined in duodenum 4 h after challenge with *H. pylori* extract was suppressed by administration of L-NAME (5 mg kg^{-1}, i.v.), administered 2.5 h after the extract injection (Figure 2).

Pretreatment with dexamethasone (1 mg kg^{-1}, i.v.), 2 h before *H. pylori* extract challenge also prevented the increase in Ca^{2+}-independent NO synthase activity in duodenum (Figure 2).

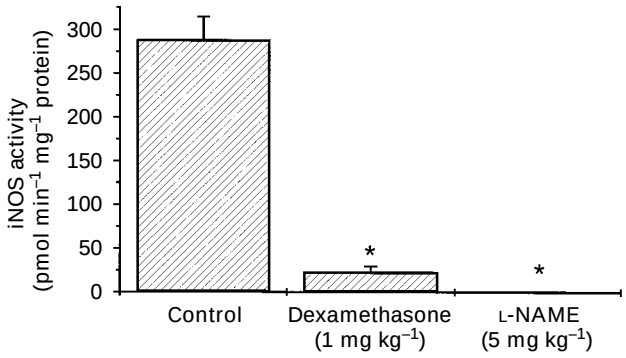

Figure 2 Inducible nitric oxide synthase (iNOS) activity in duodenum following challenge with *H. pylori* water extract (4 ml kg^{-1}, i.v.) in rats treated with saline (control) or with dexamethasone (1 mg kg^{-1}, 2 h before the extract) or with N^G-nitro-L-arginine methyl ester (L-NAME; 5 mg kg^{-1}, i.v., given 2.5 h following the extract). Data shown as the Ca^{2+}-independent activity (pmol min^{-1} mg^{-1} protein) are means ± s.e. of 5–9 experiments. *Denotes significant difference from control ($P<0.01$).

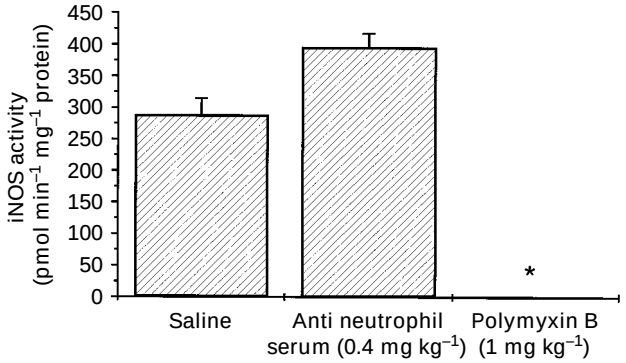

Figure 3 Inducible nitric oxide synthase (iNOS) activity in duodenum following challenge with *H. pylori* water extract (4 ml kg^{-1}, i.v.) in rats treated with saline (control), rabbit anti-rat neutrophil serum (0.4 ml kg^{-1}, i.p. administered 3 h before challenge) or polymyxin B (1 mg kg^{-1}, i.v. administered 15 min before challenge). Data shown as the Ca^{2+}-independent activity (pmol min^{-1} mg^{-1} protein) are means ± s.e. of 9–18 experiments. *Denotes significant difference from control ($P<0.01$).

Effects of polymyxin B or rabbit anti-rat neutrophil serum on NO synthase activity

Administration of polymyxin B (1 mg kg^{-1}, i.v.), prevented the appearance of Ca^{2+}-independent activity in duodenal extracts induced by *H. pylori* extract determined 4 h after challenge (Figure 3).

In contrast, in a further group, this activity was not inhibited by pretreatment with the rabbit anti-rat neutrophil serum (0.4 ml kg^{-1}, i.p.), administered 3 h before challenge, in a dose that reduced the neutrophil count by 95 ± 2%, 4 h after its administration (Figure 3).

Effects of H pylori *extract on NO synthase activity in duodenal epithelial cells*

Ca^{2+}-independent NO synthase activity was not detected in duodenal epithelial cells 1 h after *H. pylori* water extract ($n=6$), whereas this activity was present 4 h after challenge. This NO synthase activity was significantly reduced by pretreatment of the rats with dexamethasone (1 mg kg^{-1}) 2 h before the removal of the duodenum, or by L-NAME administration, given 2.5 h after the extract (Figure 4).

The administration of polymyxin B (1 mg kg^{-1}, i.v.), likewise substantially diminished the Ca^{2+}-independent activity induced 4 h after challenge with *H. pylori* extract (Figure 4).

Effects of H. pylori *extract on viability in duodenal epithelial cells*

The percentage of epithelial cells in the cell suspension isolated from the duodenum by dispersion was 98 ± 2% ($n=4$), as determined by microscopy, the other cells identified by morphological analysis being mastocytes.

The percentage of non-viable intestinal cells isolated from rats treated 1 h previously with *H. pylori* water extract, assessed by trypan blue staining, was not different from basal values compared with cells taken from control rats (12.5 ± 5%; $n=5$ compared with 12 ± 1%, $n=4$). Four hours after the extract injection, the percentage of damaged cells was significantly increased (28 ± 5%, $n=4$ as compared to 10 ± 2%, $n=4$ in the corresponding control group; $P<0.05$). However, this increase in non-viable cells was abolished by pretreatment of the rats with dexamethasone (1 mg kg^{-1}, i.v.) 2 h before challenge or by L-NAME (5 mg kg^{-1} , i.v.) given 2.5 h after *H. pylori* water extract (Figure 5). Likewise, pretreatment of the rats with polymyxin B (1 mg kg^{-1}, i.v.), reduced ($P<0.01$) the percentage of damaged cells (Figure 5).

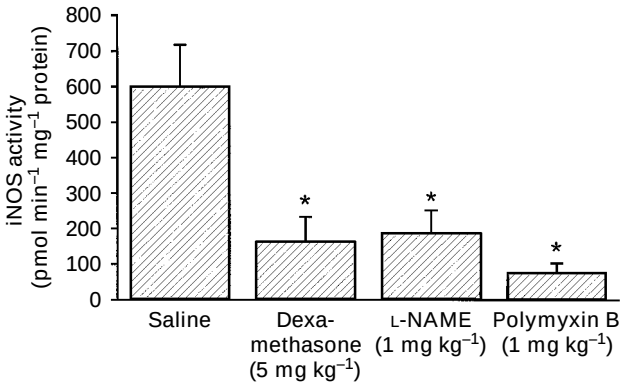

Figure 4 Inducible nitric oxide synthase (iNOS) activity in duodenal isolated epithelial cells following challenge with *H. pylori* water extract (4 ml kg^{-1}, i.v.) in rats treated with saline (control), dexamethasone (1 mg kg^{-1}, s.c., 2 h before the extract), N^G-nitro-L-arginine methyl ester (L-NAME; 5 mg kg^{-1}, i.v., given 2.5 h following the extract) or polymyxin B (1 mg kg^{-1}, i.v., administered 15 min before challenge). Data shown as the Ca^{2+}-independent activity (pmol min^{-1} mg^{-1} protein) are means ± s.e. of 9–22 experiments. *Denotes significant difference from control ($P<0.01$).

Discussion

A significant elevation of NOS activity was observed in rat duodenal tissue 4 and 6 h after challenge *in vivo* with a water extract of *H. pylori*. This NOS activity was Ca^{2+} independent and thus is considered to reflect the expression of iNOS (Salter *et al*., 1991). Furthermore, as found previously with the expression of iNOS (Salter *et al*., 1991; Boughton-Smith *et al*., 1993) the appearance of this Ca^{2+}-independent activity was prevented by dexamethasone pretreatment. In contrast to these effects of the *H. pylori* extract, a comparable water extract from *Helicobacter felis* failed to cause the expression of iNOS in duodenal tissue, indicating that this effect was not a non-specific property of all bacterial extracts prepared in this manner.

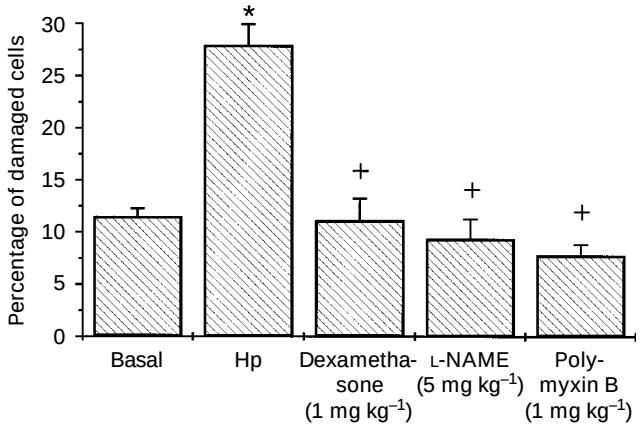

Figure 5 Percentage of duodenal isolated epithelial cells damaged following challenge with *H. pylori* (Hp) water extract (4 ml kg^{-1}, i.v.) in rats treated with dexamethasone (1 mg kg^{-1}, 2 h before the extract), N^G-nitro-L-arginine methyl ester (L-NAME; 5 mg kg^{-1}, i.v., given 2.5 h following the extract) or polymyxin B (1 mg kg^{-1}, i.v., administered 15 min before challenge). Data, are means ± s.e. of 5–13 experiments. *Significantly different from control ($P<0.01$) and $^{+}$significantly different from Hp group alone.

Sonicates of *H. pylori* have been shown to possess a chemoattractant activity towards human polymorphonuclear and monocytes (Nielsen & Andersen, 1992b). Such sonicates also induce an oxidative burst in these inflammatory cells, while an increased luminol chemiluminescence, which reflects the generation of reactive oxygen species, has been found in the antrum of patients infected by *H. pylori* (Nielsen & Andersen, 1992a; Davies *et al*., 1994). The local release of these cytotoxic moities has been suggested to play a role in the duodenal mucosal lesions observed in peptic ulcer disease associated with *H. pylori*. By interacting with NO, such oxygen species may form further damaging products such as peroxynitrite (Beckman *et al*., 1990).

Neutrophils and macrophages are known to express iNOS *in vitro* after prolonged exposure to bacterial endotoxin or cytokine administration (McCall *et al*., 1991), while induction of NO synthase after endotoxin challenge has been observed in gastrointestinal epithelial cells (Tepperman *et al*., 1993; 1994). In this present study, the involvement of neutrophils and epithelial cells in the elevated iNOS activity in the duodenum has been evaluated. Our findings suggest that circulating neutrophils are not the source of the iNOS activity detected in duodenal tissue after *H. pylori* extract administration, since anti-neutrophil serum, in doses which reduced these circulating leukocytes by 95%, did not modify the level of the iNOS activity in duodenum. Moreover, no elevation of myeloperoxidase activity was found in duodenal mucosa after challenge, suggesting there was no infiltration of neutrophils or macrophages. Whether resident neutrophils or other inflammatory cells contribute to the production of iNOS in the duodenum following challenge with the *H. pylori* extract is not yet known.

Cell separation studies indicated that iNOS activity could be detected in epithelial cells, determined 4 h after challenge with *H. pylori* extract. This iNOS activity was measured in cells isolated by a dispersion technique (Tepperman *et al*., 1993) that yields an essentially pure preparation of duodenal epithelial cells, as confirmed in the present study by microscopic examination. However, the technique employed did not allow separation of other cell types. Hence the contribution of cells other than epithelium to the total pool of iNOS in duodenal tissue cannot be evaluated from this present study. It is relevant that expression of iNOS immunoactivity in human gastric epithelial cells associated with *H. pylori* infection has been demonstrated (Crabtree *et al*., 1996).

Induction of iNOS in colonic and small intestinal epithelial cells, that follows *E. coli* endotoxin challenge, is associated with a reduction in the viability of epithelial cells (Tepperman *et al*., 1993; 1994). Likewise, in the present study, a reduction in duodenal epithelial cell viability, determined *ex vivo*, was observed four hours after *H. pylori* extract administration. This reduction in cell viability accompanied iNOS expression as it was not observed 1 h after extract administration and, hence, was not an early cytotoxic response to the extract. Moreover, dexamethasone pretreatment, which prevented the expression of iNOS by these cells, reduced the epithelial cell damage. In addition, administration of L-NAME at the time of the appearance of iNOS, likewise inhibited the subsequent cellular injury. These findings thus support the suggestion that the release of NO generated by iNOS is responsible for this duodenal epithelial damage.

The *H. pylori* water soluble factor which induces iNOS, could be an endotoxin, and a lipopolysaccharide (LPS) has been isolated from *H. pylori* (Mai *et al*., 1991). However, the potency of this LPS on neutrophil activation is not as great as that from *E. coli in vitro* (Rauws *et al*., 1989). Moreover, although *H. pylori* LPS can induce the production of tumour necrosis factor-α (TNFα) and NO from macrophages in culture, it was 2×10^4 fold less potent than that from *E. coli* (Perez-Perez *et al*., 1995; Shapiro & Hotchkiss, 1996). However, the difference in potency between LPS from *H. pylori* or *E. coli* in evoking responses in other cell types *in vitro* is not known. Polymyxin B, a known inactivator of LPS, has been shown to reduce the ability of LPS from *H. pylori* to induce TNFα release from human promyelomonocytic cell culture (Perez-Perez *et al*., 1995). In the present study, pretreatment of rats with polymyxin B reduced both the damage in epithelial cells and the iNOS activation following challenge, suggesting that an LPS-like product from *H. pylori* may be involved. This indicates that at least *in vivo*, the endotoxin produced by *H. pylori* can evoke potent responses, leading to cellular injury. The activation of inflammatory cells by a LPS-independent soluble factor released from *H. pylori* has also been suggested (Mai *et al*., 1991; Mooney *et al*., 1991; Nielsen *et al*., 1994) and may synergistically interact with LPS to provoke cell injury.

The present study thus identifies the ability of an *H. pylori* extract to provoke the expression of iNOS *in vivo*. Further studies in appropriate animal models with *H. pylori* infection should explore whether iNOS expression is evoked under such conditions. The expression of iNOS in duodenal epithelial cells could reflect a host-defence mechanism against the colonization of *H. pylori*, since NO can exert bacteriocidal actions (Granger *et al*., 1988; Evans *et al*., 1996). However, the release of *H. pylori* endotoxin and its passage into the circulation through damaged epithelium, could also play a role in a number of disease states. Indeed, expression of iNOS in cardiac tissue, with associated cytotoxic actions on the microcirculation (Laszlo *et al*., 1995), may contribute to the suggested increased rate of heart disease observed in patients infected by *H. pylori* (Mendall *et al*., 1994). The present findings thus give support to the concept that the release of LPS-like factors stimulating the local production of high concentrations of NO from epithelium and other cells, through the expression of iNOS, may play a role in the pathogenesis of peptic ulceration associated with *H. pylori* infection.

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