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Nitazoxanide in Treatment of *Helicobacter pylori*: a Clinical and In Vitro Study

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Nitazoxanide (NTZ) is an antibiotic with microbiological characteristics similar to those of metronidazole but without an apparent problem of resistance. The aim of this study was the prospective evaluation of NTZ given as a single agent in the treatment of *Helicobacter pylori* infection. Twenty culture-positive patients with dyspepsia who had previously failed at least one course of *H. pylori* eradication therapy were enrolled. Subjects received 1 g of NTZ twice daily for 10 days. The safety and tolerability of the drug were assessed by physical examination, monitoring of adverse events, and clinical laboratory evaluation. Urea breath tests (UBTs) were performed 6 weeks posttreatment. *H. pylori* was isolated from UBT-positive patients by the string test or endoscopy with biopsy, and the MICs for these isolates were compared to those for isolates obtained pretherapy. The levels of tizoxanide, the active deacylated derivative of NTZ, were measured in blood, saliva, and tissue from two patients during treatment. The UBT results were positive for all 20 patients after completion of NTZ therapy. The MIC results demonstrated that the NTZ susceptibilities of none of the strains isolated from the patients posttherapy had changed significantly. No major adverse reactions were observed, but frequent minor side effects were observed. In conclusion, NTZ did not eradicate *H. pylori* when it was given as a single agent.

Nitazoxanide (NTZ), a nitrothiazolyl-salicylamide compound first described by Rossignol and Cavier (J. F. Rossignol and R. Cavier, Chem. Abstr. **83:**28216n, 1975), has a broad spectrum of activity against microaerobic and anaerobic bacteria, anaerobic protozoa, and helminths (1, 6). It is the first antiparasitic agent reported to be effective against both protozoa and helminths, particularly in the treatment of intestinal parasitic infections (8, 13). The pharmacokinetics and metabolism of NTZ have been studied after the administration of single oral doses to healthy subjects (18), as well as after administration of radiocarbon-labeled NTZ (4). The main circulating metabolites have been identified as deacetyl-NTZ or tizoxanide (TIZ) and the corresponding acyl-glucuronide.

Previous studies showed that the incidence of adverse events was dose related, while vital signs, electrocardiograms, and laboratory test results remained unchanged (19). Side effects included loose stools, diarrhea, abdominal pain, flatulence, nausea, vomiting, dyspepsia, xerostomia, discolored urine (yellow-green), and headache (19, 20).

NTZ was the first effective treatment for cryptosporidial diarrhea in patients with AIDS, eradicating *Cryptosporidium parvum* from the intestinal tract (5, 7, 14, 21), and has been successful in the treatment of microsporidiosis in a patient with AIDS (3). Diarrhea caused by *Giardia intestinalis* and *Entamoeba histolytica* or *Entamoeba dispar* has also been shown to respond to NTZ (12, 15). Furthermore, the drug was efficacious in a hamster model of antibiotic-induced diarrhea caused by *Clostridium difficile* compared to the standard vancomycin and metronidazole (MTZ) treatments (10).

NTZ has an spectrum similar to that of MTZ in vitro and so is regarded as a possible alternative to MTZ in *Helicobacter pylori* eradication regimens (11). In one study, NTZ has been shown to be effective in vitro against MTZ-sensitive and -resistant strains of *H. pylori* (23). Recent research has identified three enzymes in *H. pylori* that activate NTZ, and two of these also mediate susceptibility to MTZ (17). The third enzyme, pyruvate oxidoreductase, seems to be responsible for most of the bactericidal effects of NTZ against *H. pylori*. The investigators also showed that NTZ, unlike MTZ, did not cause DNA damage and was not mutagenic and that *H. pylori* was unable to mutate to clinically significant levels of resistance to NTZ.

In a clinical study, NTZ was found to be well tolerated by humans, with a high rate of eradication of *H. pylori* when it was administered with omeprazole (11). An eradication rate of 83% was reported among 91 patients using NTZ in combination with omeprazole (1 g of NTZ twice daily with 20 mg of omeprazole once daily) for seven consecutive days. Resistance could not be observed, despite in vivo exposure during the course of treatment and long-term in vitro exposure of *H. pylori* strains to NTZ.

These data prompted us to evaluate NTZ as a single-agent therapy for patients who had already failed at least one attempt at *H. pylori* eradication. NTZ was used as a monotherapy, as it was a new drug that was unlikely to be affected by the antibiotic resistance of strains in patients who had failed standard proton

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pump inhibitor (PPI)-based combination therapies. Even the partial success of NTZ as a single-agent therapy would be an excellent base for further studies of combination therapy. Also, we believed that the extra expense of adding a PPI to NTZ therapy could not be justified without knowing for certain that the drug would fail as a single agent.

MATERIALS AND METHODS

Study subjects. Consecutive dyspeptic outpatients who had previously failed at least one course of *H. pylori* eradication therapy were enrolled in this study. The presence of *H. pylori* infection was based on a positive culture result at any time in the preceding 12 months. If the previous detection of *H. pylori* was made more than 3 months prior to entry, a ^{14}C urea breath test (UBT; PYtest; Tri-Med Distributors Pty. Ltd., Perth, Australia) was performed in the month before enrollment in the study to confirm the patient's *H. pylori* infection status. The study protocol was approved by the Institutional Ethics Committee of the Sir Charles Gairdner Hospital (Perth, Australia), and all patients signed informed consent forms.

Study design. Patients received 1 g (two 500-mg tablets) of NTZ twice daily for 10 days and were instructed to take the medication with food. The use of other medications for gastrointestinal conditions, especially PPIs and H2 receptor blockers, was suspended during the study. NTZ was supplied by Romark Laboratories, L.C. (Tampa, Fla.). The safety and tolerability of the drug were assessed by physical examination, including vital signs (supine systolic and diastolic blood pressures and pulse rate), and clinical laboratory evaluation (urea and electrolyte levels; full blood count; urinalysis; and serum iron, ferritin, and β-human chorionic gonadotropin levels). Laboratory data and physical examination findings were within normal limits for all subjects prior to treatment. Adverse events were recorded and documented by duration and relationship to the study drug. A ^{14}C UBT was scheduled for 6 weeks posttreatment to confirm eradication. If the follow-up UBT was still positive, a string test or endoscopy with biopsy was performed.

UBT. The ^{14}C UBT (PYtest) contained 37 kBq of [^{14}C]urea. The fasting subject swallowed a capsule and delivered a single breath sample 10 to 15 min later. The breath sample was analyzed at Tri-Med Laboratories. The test is considered positive if the result is >200 dpm and negative if it is <50 dpm; results between 50 and 200 dpm are considered indeterminate.

Strains. An *H. pylori* isolate was collected from all patients by endoscopy or the string test (Enterotest Hp; HDC Corporation, San Jose, Calif.) during the 12 months prior to inclusion in the trial. Gastric antral biopsy specimens were homogenized in 0.2 ml of normal saline with a sterile scalpel blade and plated onto three agar plates (22). The selective plates used were Skirrow's agar and Wilkins-Chalgren agar with Dent supplement (Oxoid, Basingstoke, England), and the nonselective plates used were Columbia agar base with 5% horse blood. *H. pylori* strains were isolated from the strings used in the string test as described previously (16). All inoculated plates were incubated at 37°C in an atmosphere of 10% CO_2 and a relative humidity of 95% for 3 to 10 days. Isolates were identified as *H. pylori* on the basis of colonial morphology; positive urease, catalase, and oxidase tests; as well as a Gram stain. The bacterial isolates from each patient were tested to determine their levels of susceptibility to MTZ, clarithromycin, amoxicillin, tetracycline, and ciprofloxacin by the disk diffusion method. The strains were frozen at −85°C until they were needed for the NTZ agar dilution MIC study. Whenever possible, a second bacterial isolate was obtained by one of the collection methods described above during the 2 months posttherapy.

Determination of MICs. The MICs of NTZ were determined by the agar dilution method, as described by Megraud et al. (11), for paired pre- and posttherapy *H. pylori* isolates. In brief, a stock solution of 256 mg of NTZ (Romark Laboratories) per ml was prepared by dissolving the antibiotic in dimethyl sulfoxide. This solution was further diluted with sterile highly pure distilled water to concentrations ranging from 0.6 μg/ml to 2.56 mg/ml. After incorporation of 1 ml of antibiotic solution into 19 ml of freshly poured Wilkins-Chalgren agar with 5% horse blood, the plates had concentrations ranging from 0.03 to 128 μg/ml. Frozen *H. pylori* isolates were cultured on the blood agar plates, and the growth was harvested after 48 h. A suspension with a turbidity equal to that of a McFarland 3.0 standard was prepared for each of the isolates. A 1-in-10 dilution of each suspension was also prepared, and both of these suspensions were used to inoculate the Wilkins-Chalgren plates with a Steers replicator. A suspension of 48-h-old *H. pylori* cells with a turbidity equal to that of a McFarland 3.0 standard was previously determined to have a concentration of approximately 3×10^8 CFU/ml. The plates were incubated at 37°C in 10% CO_2 with 95% relative humidity for at least 5 days before the results were recorded. The MIC was defined as the lowest concentration of NTZ that inhibited *H. pylori* growth.

TABLE 1. Patient data and posttherapy *H. pylori* status[a]

Patient no.	Age (yr)	Sex	Posttherapy result by:	
			UBT (dpm)	Culture
1	33	M	2,832	+
2	47	F	3,030	+
3	49	F	2,268	+
4	69	F	826	+
5	61	F	1,628	+
6	36	F	1,710	+
7	24	F	915	+
8	63	M	565	−[b]
9	69	M	467	+
10	52	M	599	NA
11	47	F	2,287	+
12	43	F	753	+
13	55	F	4,091	−[b]
14	30	F	1,720	+
15	45	F	1,562	NA
16	41	F	2,475	NA
17	66	F	2,160	NA
18	67	F	5,717	+
19	58	M	401	+
20	43	M	1,076	+

[a] Abbreviations: M, male; F, female; NA, not performed.
[b] PCR positive.

Plasma and tissue NTZ levels. On the 10th day of NTZ therapy, two patients underwent endoscopy 12 h after the receiving an NTZ dose on the previous evening. Samples of blood, saliva, and duodenal and gastric juices and gastric mucosal biopsy specimens were collected. These were assayed for TIZ by high-performance liquid chromatography by a modification of the method of Stockis et al. (18). A 0.1-ml aliquot of the fluid specimen was added to 1.0 ml of acetonitrile containing mefloquine as an internal standard. After centrifugation, 0.5 ml of the supernatant was evaporated to dryness in a water bath at 45°C under a stream of nitrogen. The residue was reconstituted in the mobile phase, and a 0.1-ml aliquot was injected onto the column. A calibration curve was prepared by using blank plasma spiked with TIZ over a concentration range of 0.15 to 2 μg/ml. Tissue samples were weighed and homogenized with 0.2 ml of 10% dimethyl sulfoxide in ethanol. A 0.1-ml aliquot was evaporated, reconstituted, and injected onto the column as described above. Separation was achieved with a Merck LiChrospher 60 RP Select B 5-μm column (25 by 0.46 cm) and a mobile phase of acetonitrile in phosphate buffer (pH 3.0; 40:60) at a flow rate of 1.5 ml/min. Detection was performed with a UV detector monitored at 320 nm. The intraday coefficients of variation for recovery from plasma with concentrations of 0.2 and 1 μg/ml were 10.5 and 6.7%, respectively, and the rate of recovery of TIZ added to plasma was 92%.

Further gastric biopsy specimens for histological examination were collected from these two patients at endoscopy to estimate the levels of *H. pylori* colonization.

RESULTS

Twenty dyspeptic patients (6 males, 14 females) with an average age of 49 years took part in this study. At the end of the trial, frequent minor adverse events were reported, but none of these had caused a participant to cease treatment prematurely. Fifteen patients reported diarrhea, 14 patients reported nausea, and 13 patients reported abdominal pain, with a few patients reporting headache, anorexia, dizziness, taste disturbance, yellow skin discoloration, skin rash, vomiting, myalgia, constipation, and insomnia.

The UBT results for all participating subjects were positive after treatment (Table 1). No indeterminate or negative results were obtained. Four patients withdrew from further follow-up

TABLE 2. MICs for pairs of *H. pylori* strains isolated before and after treatment with NTZ

Patient no.	MIC (μg/ml) for isolates obtained:	
	Pretherapy	Posttherapy
1	0.06	0.125
2	4	4
3	1	2
4	4	4
5	4	4
6	4	4
7	1	2
9	0.5	0.5
11	0.5	1
12	2	4
14	1	2

TABLE 3. TIZ concentrations in samples from two patients collected during NTZ therapy

Sample	Concn[a]	
	Patient 19	Patient 20
Plasma	0.855	0.135
Gastric juice	<0.1	0.2
Duodenal juice	<0.1	1.0
Saliva	<0.1	<0.1
Corpus tissue[b]	2.5	2.0
Antrum tissue[b]	3.2	5.8

[a] Concentrations are in micrograms per milliliter unless indicated otherwise.
[b] Concentrations are in micrograms per gram.

after realizing that the treatment had been unsuccessful and refused to undergo the string test or endoscopy. *H. pylori* was cultured from 10 patients by use of samples obtained by the string test and from 4 patients by use of samples obtained by endoscopy and biopsy. Two individuals were culture negative for *H. pylori* by use of samples obtained by the string test, but PCR of the washings from the string test were positive for *H. pylori* DNA (16).

In summary, 20 (100%) of the patients were UBT positive, and the presence of *H. pylori* was confirmed by a second method in 16 of these patients.

Before commencement of the study, all 20 patients were colonized with *H. pylori* isolates that were resistant to MTZ, and 14 of 20 (70%) were colonized with strains that were resistant to clarithromycin; but all the strains were sensitive to amoxicillin, tetracycline, and ciprofloxacin.

The MICs for 11 paired pre- and posttherapy *H. pylori* isolates are summarized in Table 2. The MICs ranged from 0.06 to 8 μg/ml. The MICs at which 50% of isolates were inhibited (MIC_{50}s) for isolates obtained pre- and posttreatment were 1 and 2 μg/ml, respectively, and the MIC_{90} was 4 μg/ml for isolates recovered both before and after therapy. The MICs for the strains isolated from the patients posttherapy were all within 1 dilution of the values obtained for the strains isolated pretreatment, showing that susceptibility to this antibiotic did not change significantly for any of the strains.

Pharmacokinetic assays were carried out, but with recognition of the limitations of such samples from clinical patients, i.e., the small sample size, contamination of gastric juice with saliva, and the inability to discriminate between the epithelium and submucosa in biopsy specimens. The levels of TIZ in both tissue and plasma from two patients studied suggested that the drug did not achieve adequate levels in gastric mucosal tissue (Table 3).

DISCUSSION

The treatment for *H. pylori* infections remains unsatisfactory. While in vitro the organism is sensitive to many antibiotics, the same does not apply in vivo. No single antibiotic has been shown to cure even 50% of infections (2). A meta-analysis has shown that success rates vary not only according to which combination is used and whether the assessments are intention to treat or per protocol but also according to the community studied. Factors that influence treatment success may also include the gender of the patient, the virulence of the organism, the antibiotic sensitivity of the particular strain, and patient compliance (9). Since MTZ is such a useful agent for therapy for susceptible strains of *H. pylori*, we investigated NTZ, a compound with a spectrum of activity similar to that of MTZ but without the problem of resistance.

Our study was designed to evaluate NTZ as a single agent since cure rates with NTZ as monotherapy had not been published. In addition, if the treatment had been successful, it would have been an excellent salvage therapy for our patients who had failed first-line combinations and were known to be infected with antibiotic-resistant strains.

During this study we did notice that the patients reported numerous side effects from the NTZ treatment, probably more than those reported from PPI-based triple therapies. We expected a high incidence of side effects in this study because the patients were dyspeptic and were unable to take their usual symptomatic therapy (e.g., an H2 blocker or PPI) during the study. Additionally there was no placebo control. The major side effects of diarrhea, nausea, abdominal pain, and headache were seen in most patients, convincing us that at the dose used, treatment with NTZ is more difficult than with most other antibiotics. On a relative scale we estimate that NTZ (1 g twice daily) is about twice as uncomfortable as high-dose MTZ or bismuth-based therapy for *H. pylori* infections. Nevertheless, all patients completed the study, and no abnormalities of clinical chemistry parameters were related to its use. The adverse effects were very similar to those reported by other studies with NTZ in vivo (19, 20), especially those noted by the patients taking a 1-g dose twice daily.

During the study it soon became evident that NTZ alone could not eradicate *H. pylori*. Breath tests were positive 6 weeks after therapy. To investigate this further we passed a protocol amendment that allowed us to study two patients in more detail. In these two individuals, who were biopsied immediately before administration of their final dose of NTZ, large and moderate numbers of *H. pylori*, respectively, were seen on histology. Plasma and tissue TIZ levels were all less than or close to the MIC_{50} of NTZ (2 μg/ml). These studies were problematic because patients were fasted for endoscopy and so saliva, gastric juice, and duodenal juice levels were low. The data for patient 20 appear to show that TIZ is present in bile, as described by Broekhuysen et al. (4), and this may have refluxed into the stomach.

Our investigation of the NTZ susceptibilities showed that the ranges of MICs obtained for 22 isolates of *H. pylori* from 11 Australian patients were similar to those obtained by other investigators testing isolates from France, Egypt, Japan, and Canada (11, 23). Although *H. pylori* was not eradicated, the NTZ susceptibilities of none of the strains isolated from the patients posttherapy had changed significantly, and the MICs for strains isolated posttherapy were all within 1 dilution of the values obtained for the pretreatment isolates.

Although a previous study (11) showed a promising high *H. pylori* infection eradication rate of 83% when 1 g of NTZ was given for 7 days in combination with omeprazole, in our study NTZ had no such effect when it was given as a single agent. Despite our negative outcome, there may still be a role for NTZ in the treatment of *H. pylori* infection in combination therapies.

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