URSODEOXYCHOLIC ACID DOES NOT INTERFERE WITH *IN VIVO HELICOBACTER PYLORI* COLONIZATION

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A low frequency of *Helicobacter pylori* in the gastric mucosa of patients with alkaline gastritis has been reported. At the same time, it can be noted that the growth of bacteria can be inhibited by bile acids. We studied 40 patients with chronic gastritis related to *Helicobacter pylori* in order to determine the effect of ursodeoxycholic acid on this infection. Diagnoses of the infection and the inflammatory process were obtained by histologic study of gastric biopsies collected during endoscopy. Two groups were studied: group I received ursodeoxycholic acid - 300 mg/day, and group II received the placebo, twice a day, both for 28 days. The colonization by *Helicobacter pylori* and the intensity of the mononuclear and polymorphonuclear inflammatory infiltrate were determined before (time 1) and after (time 2) treatment. Ursodeoxycholic acid had no effect on the *Helicobacter pylori* infection. A significant reduction in the intensity of the mononuclear inflammatory infiltrate of the gastric antrum mucosa was observed in patients from group I, when we compared not only times 1 and 2 but also groups I and II. However, this was not the case with the body mucosa. We concluded that ursodeoxycholic acid had no action on the colonization by *Helicobacter pylori* or on the polymorphonuclear inflammatory infiltrate, but it caused a significant reduction in the intensity of the mononuclear inflammatory infiltrate of the gastric antrum.

DESCRIPTORS: ***Helicobacter pylori.*** **Gastritis. Bacteria. Ursodeoxycholic. Endoscopy.**

Helicobacter pylori (Hp) colonization has been reported to be infrequent in gastritis secondary to duodenogastric reflux (DGR)[1, 2].

Bile acids (BA) are an important part of DGR, and it has been considered that they may damage the gastric mucosal barrier and also reduce local bicarbonate secretion in patients with abundant reflux, as is the case with gastrectomized patients[2]. Consequently, it would be easier for the hydrogen ion to diffuse towards the mucosa, leading to a fall in pH level at the cell surface, which would make the environment unfavorable for Hp[2, 3]. It has been demonstrated *in vitro* that BA[4, 5] have an inhibitory effect on Hp growth, and studies have been reported[6, 7] concerning the use of BA *in vivo* in an attempt to eradicate these bacteria.

The purpose of this study was to determine the effect of ursodeoxycholic acid (UDCA) on Hp colonization of the gastric mucosa, as well as to evaluate the occurrence of changes in the mononuclear inflammatory infiltrate (MNII) and in the polymorphonuclear inflammatory infiltrate (PMNII).

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PATIENTS AND METHOD

The study was conducted on outpatients with upper digestive complaints who were referred for endoscopy of the esophagus, stomach, and duodenum and who had a histologic diagnosis of gastritis related to infection with Hp.

During endoscopy, 6 biopsies were obtained for anatomopathology and for the detection of Hp. The first 3 biopsies were taken from the gastric antrum at a 2.0 cm distance from the pylorus: 1 from the small curvature, 1 from the anterior wall, and 1 from the posterior wall. The other 3 biopsies were obtained from the gastric body about 4.0

cm above the incision: 1 from the large curvature, 1 from the anterior wall, and 1 from the posterior wall.

The fragments were fixed in 10% formalin and sent to the Department of Pathological Anatomy. The slides were stained by 2 methods: 1) the modified Giemsa method for the detection and quantification of Hp infection; and 2) by the hematoxylin-eosin method for histology with diagnosis and classification of gastritis according to the criteria of the Sydney System Classification[8]. Only the histological results were considered for the diagnosis of gastritis, presence of Hp, and for inclusion in the protocol. A semiquantitative criterion based on counts of bacterial numbers per microscopic field was used to determine the intensity of infection. The results were scored as follows: (+) up to 10 bacteria, (++) 11 to 30 bacteria, and (+++) countless bacteria in 1 or more foveolae or on the cell surface.

The same professionals performed, respectively, the endoscopic examination and the histopathological study for the diagnosis of gastritis and the detection of Hp. The pathologist was unaware of the endoscopy results.

The patients included in the protocol were divided at random into 2 groups on a double-blind basis, with the following order of entry:

GROUP I: Patients who received UDCA, 300 mg/day/28 days taken in 2 daily doses, one at 9:00 a.m. and the other at 4:00 p.m.

GROUP II: Patients who received a placebo administered as for Group I.

On the first visit (time 1), medication was prescribed and all patients were advised as to the possible occurrence of side effects. A second upper digestive endoscopy was performed at the end of their treatment (time 2) so as to collect material for Hp detection, followed by a new histological study.

We adopted a score system in order to define, in an increasing order of severity, the Hp colonization density (HpD) and the intensity of MNII and PMNII. We attributed scores 0, 1, 2, and 3 respectively to grades 0, +, ++, +++, or to absent, mild, moderate, and intense, depending on whether the variables were HpD or histology. Score points were compared in terms of the significance of their variation between times 1 and 2 for HpD, chronic gastritis, and activity of the inflammatory process between groups I and II for all variables.

In agreement with the Declaration of Helsinki [9], upon admission to the study, the patients were informed about the study project in detail and they gave written consent to participate.

Data were analyzed by nonparametric methods. The classifying variables are presented in contingency tables containing absolute and relative frequencies. The proportions of these variables in both groups were analyzed by the Fisher exact test. The continuous variables are presented in tables containing means and standard deviations. Both groups were compared by using the Wilcoxon rank sum test. Variation of mean score for the histological variables between times 1 and 2 was compared within each group. The level of significance was set at p=0.05.

RESULTS

We studied 53 patients, but only 44 completed treatment. We excluded 4 from these 44 because faulty data hindered analysis.

Table 1 shows the 40 patients distributed by group, age, and sex. Group I consisted of 23 patients: 9 males (39.13%) and 14 females (60.87%), mean age (yrs) 27.47. Group II consisted of 17 patients: 9 males (52.94%) and 8 females (47.06%), mean age (yrs) 28.82.

An individual study of each patient shows that the elimination of infection by Hp at time 2 after the use of UDCA happened only in 2 cases. It is interesting to note that these patients also had a reduced intensity of the inflammatory infiltrate.

Table 2 compares the mean scores for HpD, MNII, and PMNII in the antrum and gastric body at time 1 between groups I and II. An analysis of the 2 tables shows that both groups were homogeneous in terms of age, sex, and variables analyzed at time 1, except antrum MNII, whose mean score was significantly higher in group I.

Variables were evaluated by the score system in the antrum and body at times 1 and 2 in both groups. In reference to HpD, no difference was observed between times or between groups in either type of mucosa (Table 3).

With respect to MNII, table 4 shows that the chronic inflammatory process in the antral mucosa was significantly reduced in intensity in group I - patients who used UDCA - when times 1 and 2 were compared, with a score variation of -0.48 ($p < 0.001$). In contrast, group II presented a positive variation of 0.06 ($p = 1.000$). When both groups were

Table 1 - Patient distribution by sex and age per groups I (treated with ursodeoxycholic acid) and II (control).

	Group I				Group II				
Variable	Male		Female		Male		Female		p
	n	%	n	%	N	%	n	%	
Sex	9.00	39.13	14.00	60.87	9.00	52.94	8.00	47.06	0.385
Age	27.47 ± 6.16				28.82 ± 5.67				0.485

±: mean ± standard deviation
p: probability level

Table 2 - Comparison of the mean scores for the variables between groups I (treated with ursodeoxycholic acid) and II (control) in the basal condition.

Variable	Group I	Group II	p
HpD antrum	2.35 ± 0.78	2.35 ± 0.70	0.940
HpD body	2.26 ± 0.92	2.06 ± 1.09	0.615
MNII antrum	2.17 ± 0.65	1.59 ± 0.62	0.009
MNII body	1.22 ± 0.80	0.94 ± 0.75	0.302
PMNII antrum	1.30 ± 0.70	1.00 ± 0.61	0.216
PMNII body	0.87 ± 0.81	0.76 ± 0.75	0.714

±: mean ± standard deviation
p: probability level
HpD: *Helicobacter pylori* colonization density
MNII: mononuclear inflammatory infiltrate
PMNII: polymorphonuclear inflammatory infiltrate

Table 3 - Variation in mean score for colonization by *Helicobacter pylori* between times 1 and 2 in the antrum and body of groups I (treated with ursodeoxycholic acid) and II (control).

Variable		Group I	Group II	p**
HpD antrum	Mean ± SD	-0.22 ± 1.44	0.00 ± 0.79	0.691
	p*	0.562	1.000	
HpD body	Mean ± SD	-0.61 ± 1.50	-0.06 ± 0.97	0.276
	p*	0.081	0.906	

SD: standard deviation
p*: probability level - comparison at basal conditions
p**: probability level - comparison of variability between groups
HpD: *Helicobacter pylori* colonization density

Table 4 - Variation in mean score for mononuclear inflammatory infiltrate between times 1 and 2 in the antrum and corpus of groups I (treated with ursodeoxycholic acid) and II (control).

Variable		Group I	Group II	p**
MNII antrum	Mean ± SD	-0.48 ± 0.51	0.06 ± 0.90	0.049
	p*	<0.001	1.000	
MNII body	Mean ± SD	-0.22 ± 0.95	0.12 ± 0.70	0.314
	p*	0.255	0.726	

SD: standard deviation
p*: descriptive probability level - comparisons at basal conditions
p**: descriptive probability level - comparison of the variation between groups
MNII mononuclear inflammatory infiltrate

Table 5 - Variation in mean score for the polymorphonuclear inflammatory infiltrate between times 1 and 2 in the antrum and corpus of groups I (treated with ursodeoxycholic acid) and II (control).

Variable		Group I	Group II	p**
PMNII antrum	Mean ± SD	-0.09 ± 0.79	0.06 ± 0.66	0.617
	p*	0.794	1.000	
PMNII body	Mean ± SD	-0.22 ± 0.95	0.00 ± 0.50	0.472
	p*	0.297	1.000	

SD: standard deviation
p*: descriptive probability level - comparison at basal conditions
p**: descriptive probability level - comparison of the variation between groups
PMNII: polymorphonuclear inflammatory infiltrate .

compared at the two different times, the reduction in inflammatory infiltrate that was observed in group I was significant (p = 0.049). In contrast, no significant differences were detected in the mucosa of the body.

Concerning PMNII, table 5 shows no significant variation between times 1 and 2 within the same group or between groups.

DISCUSSION

A low frequency of Hp colonization has been reported in patients with abundant DGR[10] undergoing partial gastrectomy as a treatment for peptic ulcers. The absence of bacteria may be related to high indices of alkaline gastritis and to elevated concentrations of BA in gastric content[1, 2, 3, 11]. In 1989, O'connor et al.[3] also observed that surgeries for the correction of DGR led to recolonization of gastric mucosa by Hp. The disappearance of the bacterium may be attributed to modifications in the characteristics of the gastric epithelium, but is primarily related to a lesion of the gastric mucosal barrier by constituents of refluxed bile, which may expose Hp to the action of bile and acid [2, 3, 11]. Some investigators have also reported an inhibitory action of bile[4], especially of BA [5], on Hp growth *in vitro*. Therefore, the absence of Hp in stomachs with persistent DGR may be caused by its intolerance to bile. Based on these observations, the use of BA was suggested in an attempt to eradicate Hp [5].

In another *in vitro* study, Nilius et al. (1993) [12] observed that UDCA and chenodeoxycholic acid can induce some ultrastructural changes on the cell wall of Hp, which confirmed the toxic and bactericidal power of BA on these bacteria. After these observations, *in vivo* studies using BA were undertaken [6] in an attempt to eradicate Hp, some of them indicating a possible therapeu-

tic action of BA, a result that stimulated further investigations [7].

In the present *in vivo* study, the inhibitory effect of UDCA on Hp growth that was observed by some investigators *in vitro* [4, 5] was not confirmed. The use of this specific bile acid neither led to the eradication of Hp, nor did it alter the HpD when compared to the placebo. A histological study of 2 patients from group I showed that Hp had disappeared at time 2 both in the antrum and in the body. This fact was accompanied by a decrease in MNII and PMNII intensity. In a study, Graham et al.[6] did not detect an effect of UDCA on gastric colonization by Hp. Silva et al. in a pilot study[7], found no infection by Hp in 1 out of 5 patients who used UDCA 10 mg/kg/day/30 days. Graham et al.[6] used this bile acid for just 2 days in only 2 patients, and they concluded that BA appears to be ineffective in the treatment of this infection, possibly because of the low solubility of glycine-conjugated BA, as is the case with UDCA at low pH values, such as the gastric pH values, which would inactivate them.

UDCA may act on Hp and on gastric mucosa by either a systemic or a local route, the latter being more probable. The local effect may occur at two different times. The first one would be when the acid passes through the stomach soon after administration in its non-conjugated form. Experimental data reported by Hoshita et al.[13] showed that ingested BA may remain in the stomach for about 1 hour, with levels rapidly decreasing thereafter. The second time would be when conjugated UDCA reflows into the stomach [14] as the major constituent of bile[15]. The systemic route is probably of little importance, since the serum levels of UDCA may be low and transitory, and the acid circulating is mostly bound to albumin and lipoproteins [16].

The present observations about an improvement in the inflammatory infiltrate at the antrum level along with the disappearance of Hp in 2 patients after the use of UDCA, may indicate that this non-conjugated acid, when passing through the stomach soon after administration, and then acting in its conjugated form with eventual reflux into the stomach, may have some effect on the infection.

Some other factors should be mentioned for their possible influence on the present results: the dose that was employed, which may not have reached adequate levels for bacterial inhibition, as well as the use of the medication for a sufficient extent of time. Mathai et al.[5] determined a minimum inhibitory concentration of more than 0.2% for UDCA. In the present series we did not detect significant changes in the gastric epithelium that are characteristic of alkaline gastritis. Since UDCA is a BA that leads to a low level of inhibition of Hp[5], and has a low level of damaging power to the gastric mucus layer and epithelium [17], the intragastric concentration that was reached in the present study, and the time of treatment may not have been sufficient to lead to changes in the mucosal barrier, or in the gastric epithelium typical of alkaline gastritis—changes such as those observed in patients with abundant DGR and incompatible with the presence of Hp [2, 3, 11].

With respect to the inflammatory process in the gastric antrum, a significant reduction of MNII intensity was observed at this dosage level in patients from group I compared to those from group II when time 1 was compared with time 2. This alteration did not recur in the body. The fact that these studied groups were not homogeneous in terms of the intensity of this MNII in the antrum does not invalidate this observation, since the statistical method that was used compared both groups in terms of score variation within each separate group. The negative variation in group I and the positive variation in group II were meaningfully different, reflecting the significant reduction in MNII intensity in the antrum of the group treated with UDCA when compared to the placebo group. This decrease cannot be explained by inhibition of Hp infection, since no inhibition occurred. Also, no significant change in PMNII intensity was observed. According to Genta et al. [18] and other investigators[19], the reduction of these cells may be the first indicator of successful combat against Hp, with a gradual reduction of MNII.

The following questions may then be formulated: why did a reduction of the mononuclear inflammatory process occur without a corresponding action against Hp? Is it likely that present results reflect some inhibitory power of UDCA against Hp? It is possible that UDCA acts on some of the pathogenic mechanisms of Hp, inhibiting the bacterium, or even that Hp is not the only factor that is responsible for the inflammatory process observed in these patients, and that the bile acid acted on this other factor. It is known that UDCA has some inhibitory power on urease release by the bacteria, with a consequent reduction of ammonia production[12]. Urease and ammonia are known to be pathogenic for the gastric epithelium[20]. Hp can release chemotactic proteins[20], which may attract mononuclear and polymorphonuclear cells, which in turn release substances such as interleukin-2, tumor necrosis factor (TNF), and free radicals. It has been demonstrated *in vitro* that BA[21] and UDCA[22] inhibit TNF, and interleukin-2 release by mononuclear cells. It is possible that UDCA has a similar action on Hp and on the mononuclear and polymorphonuclear cells located in the gastric mucosa, a fact that, allied to some decrease in urease activity, may explain the reduction in MNII observed here.

UDCA has little damaging power on the gastric mucosal barrier[17]. It is

not cytotoxic to most cells at any concentration because it binds minimally to the cell membrane, and its micelle has a low ability to solubilize membranes[24]. These observations, however, may be another explanation for the reduction in intensity of MNII. Assuming that the present patients had some degree of DGR, the predominance of UDCA in bile after its oral administration[15] would represent one less aggressive factor against the mucosa, leading to a regression in the inflammatory infiltrate.

In the present series, UDCA treatment of patients with Hp-induced chronic gastritis was followed by a reduced intensity of these MNII that was not related to infection of the gastric mucosa by the bacteria, which was not affected by this drug.

RESUMO

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SILVA JGN da e col. - O ácido ursodeoxicólico não interfere na colonização pelo *Helicobacter pylori, in vivo*. **Rev. Hosp. Clín. Fac. Med. S. Paulo 55**(6):201-206, 2000.

Tem sido relatada uma baixa freqüência da infecção pelo *Helicobacter pylori* na mucosa gástrica de pacientes portadores de gastrite alcalina. Ao mesmo tempo, foi observada inibição do crescimento da bactéria, *in vitro*, pelos ácidos biliares. Com o objetivo de avaliar o efeito do ácido ursodeoxicólico sobre a colonização da mucosa gástrica por este microorganismo foram estudados 40 pacientes com gastrite crônica relacionada ao *Helicobacter pylori*. O diagnóstico da infecção e do processo inflamatório foi realizado por estudo histológico de biópsias gástricas coletadas durante a endoscopia. Foram estudados dois grupos: o grupo I recebeu ácido ursodeoxicólico – 300mg/dia, e o grupo II placebo, duas vezes por dia, por 28 dias. A colonização pela bactéria e a intensidade do infiltrado inflamatório mono e polimorfonuclear foram quantificados antes (tempo 1) e depois (tempo 2) do tratamento. O ácido ursodeoxicólico não teve efeito sobre a infecção pelo *Helicobacter pylori*. Observou-se redução significante na intensidade do infiltrado inflamatório mononuclear na mucosa gástrica do antro nos pacientes do grupo I, tanto na comparação entre os tempos 1 e 2, quanto na comparação entre os dois grupos. Entretanto, isto não ocorreu na mucosa do corpo gástrico. Podemos concluir que o ácido ursodeoxicólico não tem ação na colonização pelo *Helicobacter pylori* ou na intensidade do infiltrado inflamatório polimorfonuclear, mas causou uma redução significante na intensidade do infiltrado inflamatório mononuclear na mucosa do antro gástrico.

DESCRITORES: ***Helicobacter pylori.* Gastrite. Bactéria. Ursodeoxicólico. Endoscopia.**

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