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EFFECT OF GABOB IN THE INFLAMMATORY RESPONSE OF RHEUMATOID ARTHRITIS PATIENTS WITH *H. PYLORI* AND NSAIDS TREATMENT

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ABSTRACT

Helicobacter pylori (Hp) and non-steroidal anti-inflammatory drugs(NSAID) are the major causes of gastric-disorders. Oral GABA-treatment down-regulates inflammatory-responses in rheumatoid-arthritis (RA) models. We investigate the anti-inflammatory effect of oral-ingestion of GABOB in dyspeptic-patients with RA and NSAID intake to establish if Hp-colonization contributes to the association between NSAID and peptic damage. 26 patients with RA who took NSAIDs and dyspeptic-symptoms were recruited. Patients were tested for Hp-infection. TNF-α, TGF-α, TGF-β, IL-10, IL-8, NO expression and secretion were determined in antrum-duodenum biopsy and plasma before-after GABOB-treatment. Immohistochemistry and Sydney evaluation were also conducted. Severity of dyspepsia assessment (SODA) test was applied. Ten patients

were Hp+ and 16 Hp-. IL-8 expression presented higher-values in antrum from patients Hp+, and it correlated with greater inflammatory-infiltration. Data didn´t show significant changes in cytokine-levels as a result of GABOB, except IL-8 secretion that increased in Hp+ duodenum biopsy. Moreover, it was observed an increase of Smad3 expression in Hp- duodenum and a decrease in TGF-β plasma-levels in the same patients. Apparently, Hp colonization doesn´t contribute to increased damage, since we didn´t find any correlation among dose, NSAID type, time consuming and gastropathy. These findings suggest that GABOB-treatment improves dyspepsia-tolerability at 60% and 69% of Hp+ and Hp- patients. However, 4 weeks of GABOB-treatment didn´t improved inflammatory-mediators in gastric-biopsies nor in plasma-levels.

KEYWORDS: cytokines, antrum, duodenum, Sydney evaluation, SODA test.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are highly effective agents for the control of musculoskeletal pain and inflammation, and are among the most widely used drugs worldwide. It is well recognized that marked improvements in quality of life occur among patients with rheumatoid arthritis (RA) receiving NSAIDs. Although careful patient selection and monitoring for potential adverse effects is essential, these drugs are well tolerated by most patients. NSAID-induced gastrointestinal events are reported more commonly than adverse effects from any other class of drugs.

Patients with RA are known to have an increased propensity for developing a variety of upper gastrointestinal disorders. Long-term use of NSAIDs is accepted as being the most important factor in the development of peptic ulceration, although surprisingly, few studies have concentrated on patients with RA. The prevalence of gastric ulcer and duodenal ulcer have been reported to be 13% and 11%, respectively, in NSAID-consuming patients with chronic arthritis but, only 0.3% and 1.4% in the population without regular NSAID use.[1] In a British study, peptic ulcer disease was detected in 36% RA patients, but, unexpectedly, no difference was observed in NSAID treatment between the ulcer and non-ulcer groups.[2]

Helicobacter pylori infection is the main cause of chronic gastritis, which is also the commonest histologic finding in patients consuming NSAIDs, and is closely associated with peptic ulcer.[3] It is present in more than 90% of patients with duodenal ulcer.[4] The role of Hp in patients receiving NSAIDs has been investigated with conflicting results, as the so-

called reactive gastritis has been suggested as a characteristic prevalent finding in patients consuming NSAIDs, but it may also be detected in association with bile reflux, high alcohol intake, or gastric surgery.[5-6] Few studies have examined the potential interaction between NSAIDs and Hp, with controversial findings.

It has been shown that gamma-aminobutyric acid (GABA), and various GABAergic agents are important regulators of gastrointestinal functions in the central nervous system.[7] There are some evidence that central administration of GABA agonists and antagonist caused significant alteration in gastric motility, gastric secretion, and intestinal electrolyte transport.[8-9] Others suggest gastric acid secretion modulation in rats and influence the formation of experimental gastric ulcers, apparently due to their inhibitory effect on mucosal defensive factors. GABA is also able to modulate gastric tone acting peripherally through neural $GABA_A$ and $GABA_B$ receptors.[9] Baclofen, a $GABA_B$ receptor agonist, attenuates functional dyspepsia and appears to act by central mechanisms.[10] However, there is a general disagreement in the literature concerning the characteristics of the GABAergic effects on gastric acid secretion and ulceration. GABA by itself has been reported to increase, decrease or not influence gastric acid secretion.[11-12] In recent years, it has become clear that there is an extensive cross-talk between the nervous and the immune system. Indeed, the immune cells themselves express components of the neuronal neurotransmitters systems. The role that neurotransmitters and, their ion channels, receptors and transporters have in immune function and regulation is an emerging field of study. Several recent studies have shown that the immune system is capable of synthesizing and releasing the classical neurotransmitter GABA, which has a number of effects on immune cells such as activation or suppression of cytokine secretion, modification of cell proliferation, even affect cellular migration. The GABA also appears to have a role in autoimmune diseases like multiple sclerosis, type 1 diabetes and RA and may modulate the immune response to infections.[13-15] Oral GABA treatment down regulates inflammatory responses in a mouse model of rheumatoid arthritis.[16] Kelley et al. propose that GABA may down regulate p38 MAPK activity to reduce peripheral production of proinflammatory cytokines in joints affected by RA. 4-Amino-3-hydroxybutanoic acid (GABOB) is an endogenous ligand found in the central nervous system in mammals[17] and it is considered a metabolic product of GABA. GABOB has anticonvulsant properties. More studies have been suggested that it may improve learning and memory function, probably through a cholinergic mechanism, as well as boosting growth hormone release.[9] Recently Liu et al. demonstrated that GABOB, presumably by activation

of $GABA_B$ receptors, significantly attenuates behavior and visceromotor responses to gastric mechanical distention.[10] They consider the GABAergic pathway as a potential target for the treatment of functional dyspepsia and related conditions.[9]

The present study aimed first to estimate the effect of oral ingestion of GABOB in dyspeptic patients with RA and to establish whether or *Helicobacter pylori* colonization contributes to the association between NSAID intake and peptic damage.

MATERIALS AND METHODS

Study Cohort

26 patients with early diagnosis of RA from the Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" Hospital (INCMNSZ), Mexico City, were considered in this study. All patients presented dyspeptic syntoms and abdominal pain or discomfort for at least one week of evolution and were under NSAIDs treatment for more than two weeks. Patient selection was based on clinical examination, endoscopy, and anti-Hp IgG test. Ten of them were Hp positive and sixteen negative. After diagnosis, patients were treated with 250 mg GABOB daily for a month. All patients had a standardization diet and, free of irritants, one week prior beginning to take the GABOB. They all signed an informed consent before entry to the protocol. The study was approved by the Ethical Committee of the INCMNSZ (approval number 1524).

SODA Scale

Severity of Dyspepsia Assessment (SODA) scales that measures the change in dyspepsia-related health, which takes into account the multidimensional nature of dyspepsia using three scales (pain, non-pain symptoms, and satisfaction with dyspepsia-related health) and demonstrates good psychometric properties with respect to validity, reliability and sensitivity to change in the measurement of dyspepsia-related health[18] were applied at visit 1 and when patients finished the study.

Biopsy Specimens

Two biopsies samples were collected during endoscopy study from each patient by a certified gastroenterologist, one biopsy from antral of the stomach and another one from duodenum previously to GABOB treatment initiation. Procedure was repeated after 4 weeks of treatment. Biopsies from each patient were divided into three sections, the first one was stored in RNA later at -70^{o}C, the second one for ELISA and Western blot analysis, and the

third was fixed with 10% formaldehyde for immunohistochemistry and Haematoxylin-Eosin staining. The histological variables were graded according to the Updated Sydney System visual analogue scale to generate a score (0 = absent, 1 = mild, 2 = moderate and 3 = marked).

Cytokine assays

Biopsy samples were washed, homogenized and centrifuged. Supernatants were obtained from centrifugation at 12000xg for 10 min at 4°C. Plasma was also separated by centrifugation (2000xg for 15 min at 4°C). Samples were frozen at -20°C until used for IL-8, IL-10, TGF-β, TGF-α, TNF-α and NO measurements by ELISA (Biolegend, San Diego, CA; BD Bioscences, New Jersey) according to manufacturer´s instructions. In each patient, every determination was run by duplicate. Total concentrations in samples were expressed as pg/mg of protein. Plasma values were expressed as pg/ml.

Quantitative RT-PCR assays for cytokines and others molecules

Biopsies from 21 patients with Hp+ or Hp-, were subjected to RNA isolation and qRT-PCR of IL-8, IL-10, CGRP, inducible NO synthase, TNF-α, TGF-α, TGF-β, COX-1, COX-2, using primers and probes listed in table 1 (table 1 as shown in ESM) and following protocol reported by Trejo.[19] mRNA levels were expressed as cytokine mRNA/GAPDH mRNA. Each assay was performed in duplicate and each cytokine assay was performed testing all RNA samples in the same experiment.

Western blot analysis

Total proteins were isolated from antrum and duodenum biopses with Tissue Extration Buffer (T-PER) (Pierce Rockford, IL, EU) containing complete protease inhibitor and PhosphoStop tablet (Roche Indianapolis, EU). 70 µg of total protein were subjected to Western blotting as previously reported Gomez-Quiroz et al.,[20] using anti-ciclooxygenase-1/PTGS1/COX-1 and anti-ciclooxygenase-2/PTGS2/COX-2 (Imgenex, San Diego, CA), anti-phospho-NF-κB p65 and phospho-Smad 3 (Cell Signaling Technology, Boston, EU) antibodies.

Apoptosis detection

Biopsy specimens from patients were fixed in 10% formalin, and apoptotic cells were detected with immunostaining of active caspase 3 according to the instructions of the manufacturer (Biocare Medicall, USA; CP 229 A,B,C.).

Immunohistochemistry

10 gastric biopsies (5 Hp+ and 5 Hp-) previous to GABOB treatment were fixed in formaline and embedded in paraffin. Sections of 1.5 microns (Hyrax M25, EPC-1200PC, Zeizz, Germany) were prepared and subjected to immunohistochemistry,[20] using primary antibody against COX-1 or COX-2 (Abcam, USA; Imgenex).

Evaluation of COX-1 and COX-2 was performed according to the following scoring system: staining intensity was graded as absente (0), weak (1), moderate (2), or strong (3), area of staining positivity as <10% (0) of all cells stained in the cytoplasm, 10-40% (1), 40-70% (2), or ≥70% (3), and gland of staining positivity as absente (0), surface (1) intermediate (2) and deep (3). Total scores for grade and area of three or more, were defined as positive expression and less than three as negative.[22]

Statistical Analysis

The quantitative variables will summarize in terms of median and intervals (minimum and maximum values). In the case of RNAm determination in figure 2, data are means + SD. The analysis of the change was made by the proof of the ranks of Wilcoxon and the change between groups was made by Mann-Withney test while analysis between different groups by Kruskal-Wallis test. A p value <0.05 was considered statistically significant.

RESULTS

Patients

26 patients with RA were included in the study. Of these subjects 10 patients (10 F) were *Helicobacter pylori* positive (Hp+) and had a median age of 37 years old (range 23-64 years old) and 16 patients (15F/1M) were *Helicobacter pylori* negative (Hp-) and had a median age of 52 years old (range 36-69 years old). All patients were treated with NSAID's, 31% indomethacin, 31% diclofenac and the remaining patients other NSAIDs (ibuprofen, sulindac, naproxen, piroxicam, acetaminophen). NSAID's were taken from different periods and dose from 2 months to 10 years. Gastropathy was evaluated by endoscopy: nodular and erosive gastropathy, mild chronic superficial gastritis antrum and body, erythematous and erosive gastropathy antral gastropathy chronic alkaline reflux changes in antrum and body or chronic follicular gastritis inactive. No relationship was found between treatment of NSAID's, gastropathy and the presence or absence of *Helicobacter pylori*. SODA, which is a multidimensional, reliable and clinically meaningful valid instrument used as a measure of

dyspepsia tolerability, showed that 60% of patients Hp+ and 69% of patients Hp- considered an improvement in dyspepsia tolerability as result of GABOB treatment.

Cytokine content in antrum and duodenum biopsy

The antral and duodenal biopsies were taken at visit 1 before starting treatment with GABOB and at visit 4 at the end of treatment with the drug. TNF-α, TGF-α, IL-10, IL-8, TGF-β and NO were determined (Figure 1).

Results showed a significant difference between IL-8 protein expression values in the antrum versus duodenum (Figure 1D) being higher in the case of antral biopsies in patients Hp+ and Hp-, with a median value of 201.85 pg/mg (range 109-700) and 114.04 pg/mg (range 77-128), respectively. After GABOB treatment, IL-8 protein expression did not change in antrum biopsies. However, in duodenal biopsies, IL-8 protein expression was increased as a result of treatment in both Hp+ [median value 99.26 pg/mg (38-193)] and Hp- [median value 58.19 pg/mg (range 38-88)] patients. It is notably that IL-8 concentration was higher in Hp+ than Hp- in antrum independent of treatment of GABOB.

qRT-PCR assays of gastric damage mediators in biopsies of antrum and duodenum

IL-8, IL-10, CGRP, inducible NO synthase (iNOS), TNF-α, TGF-α, TGF-β, COX-1, COX-2 gene expression were determined. None of the determined variables presented a statistically significant change. There is no difference between antro and duodenum biopsies in Hp+ and Hp- samples, neither before and after GABOB treatment (Figure 2).

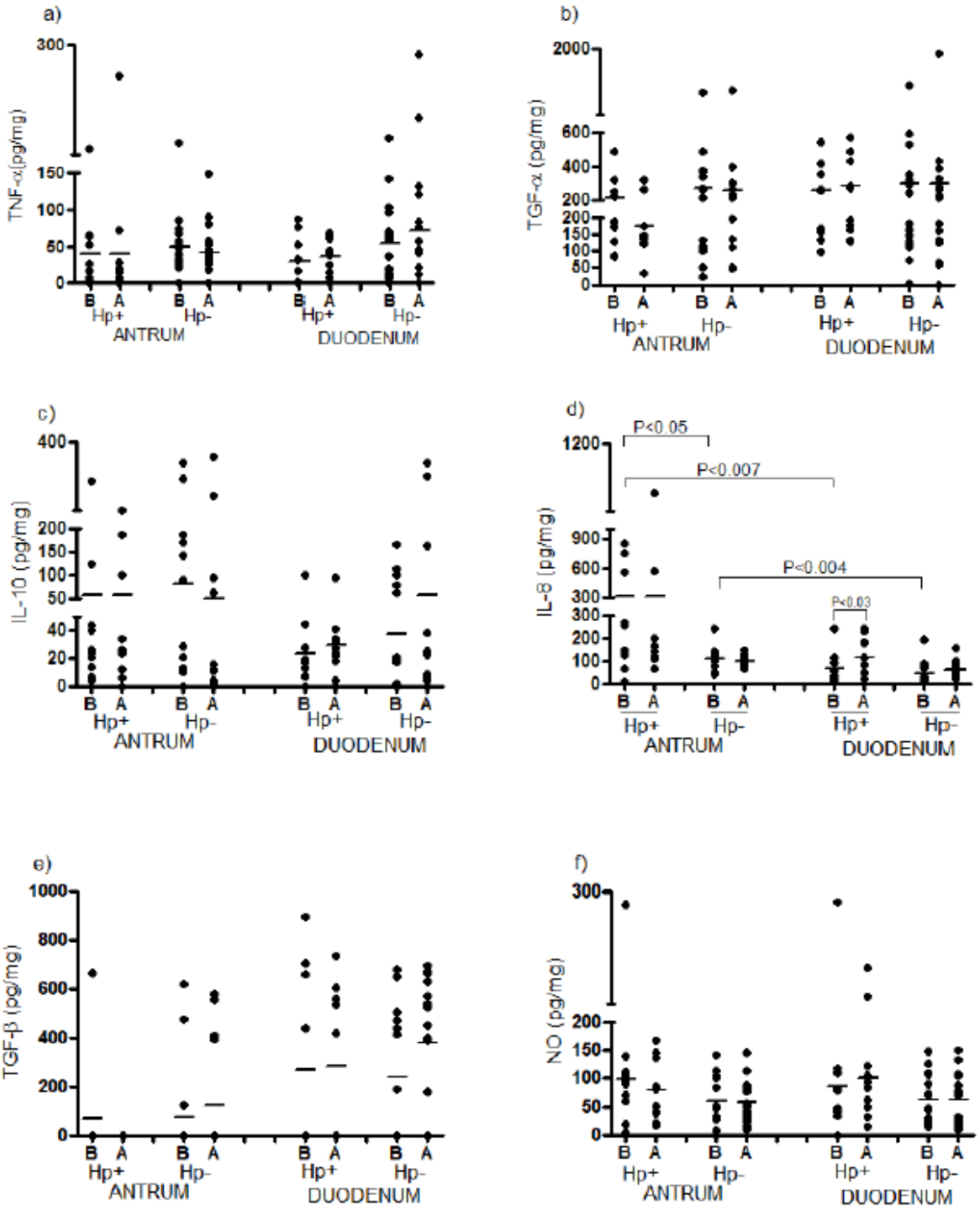

Figure1- Cytokines and NO concentration in antrum and duodenum biopsies of *Helicobacter pylori* positive (Hp+) and negative (Hp-) patients before (B) and after (A) GABOB treatment. a)TNF-α, b)TGF-α, c)IL-10, d)IL-8, e)TGF-β and f)NO concentration. Data from each patient were plotted and median is shown. Inflammatory status, measured by IL-8 presented significant difference between Hp+ and Hp- antrum biopsies before treatment and between antrum versus duodenum Hp+. GABOB treatment only changes IL-8 expression in duodenum Hp+ biopsies.

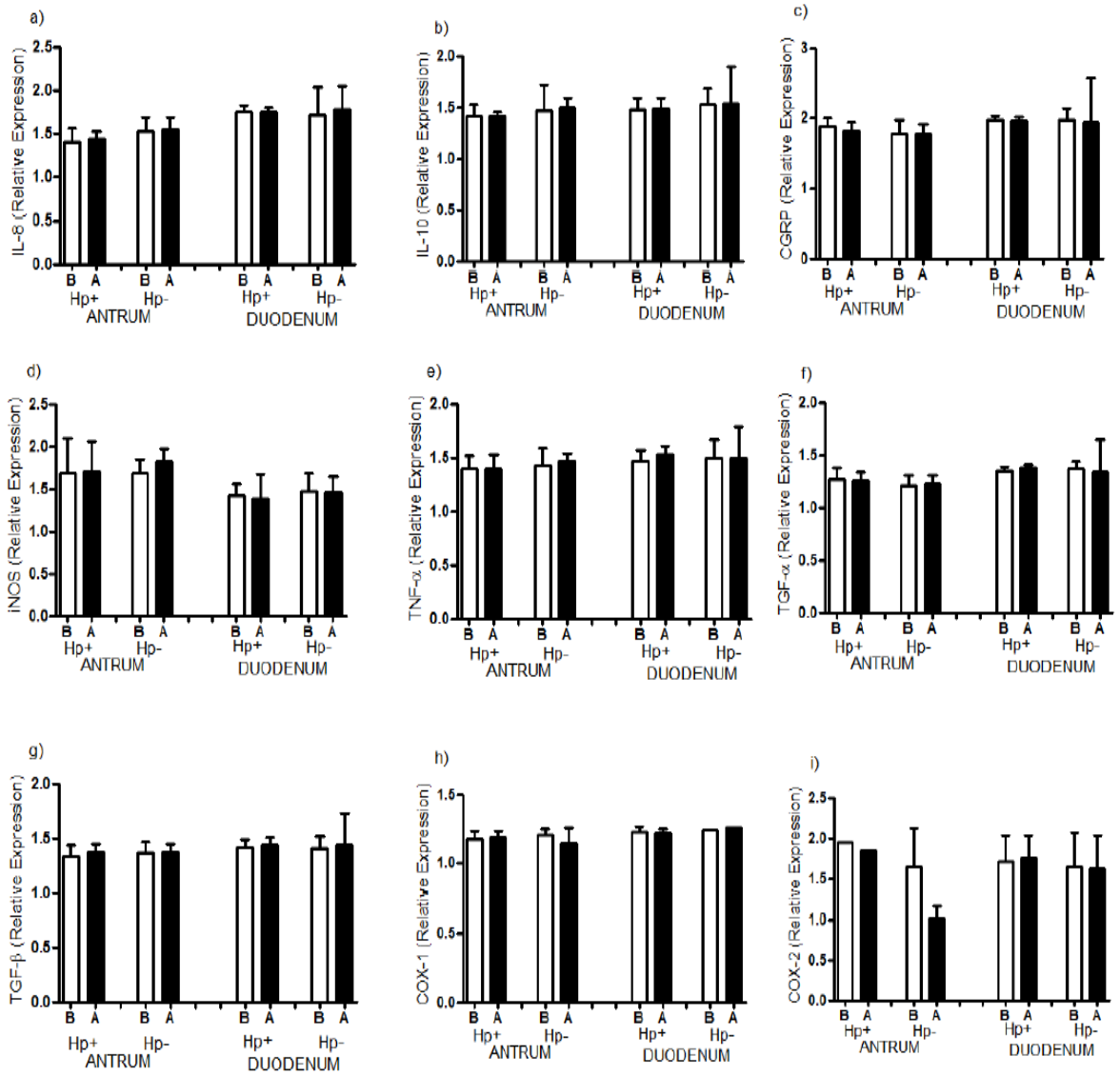

Figure 2- a)IL-8, b)IL-10, c)CGRP, d)inducible NO synthase (iNOS), e)TNF-α, f)TGF-α, g)TGF-β, h)COX-1, i)COX-2 gene expression in antrum and duodenum biopsies *Helicobacter pylori* positive (Hp+) and negative (Hp-) of patients before (B) and after (A) GABOB treatment. Data are expressed as mean + SD.

COX-1, COX-2, NF-κB and Smad3 Western blot analysis

Western blot analysis for COX-1, COX-2, NF-κB and Smad3 expression were carried out in antrum and duodenum biopsies Hp+ and Hp- (Figure 3). COX-2 was not detectable by this technique in antrum Hp+ nor Hp- in basal condition or after GABOB treatment. The results showed no change in COX-1, COX-2, NF-κB and Smad3 expression between Hp+ versus Hp- in antrum and duodenum, except a statistically significant change observed in Smad 3 expression in duodenum Hp-, due to GABOB treatment.

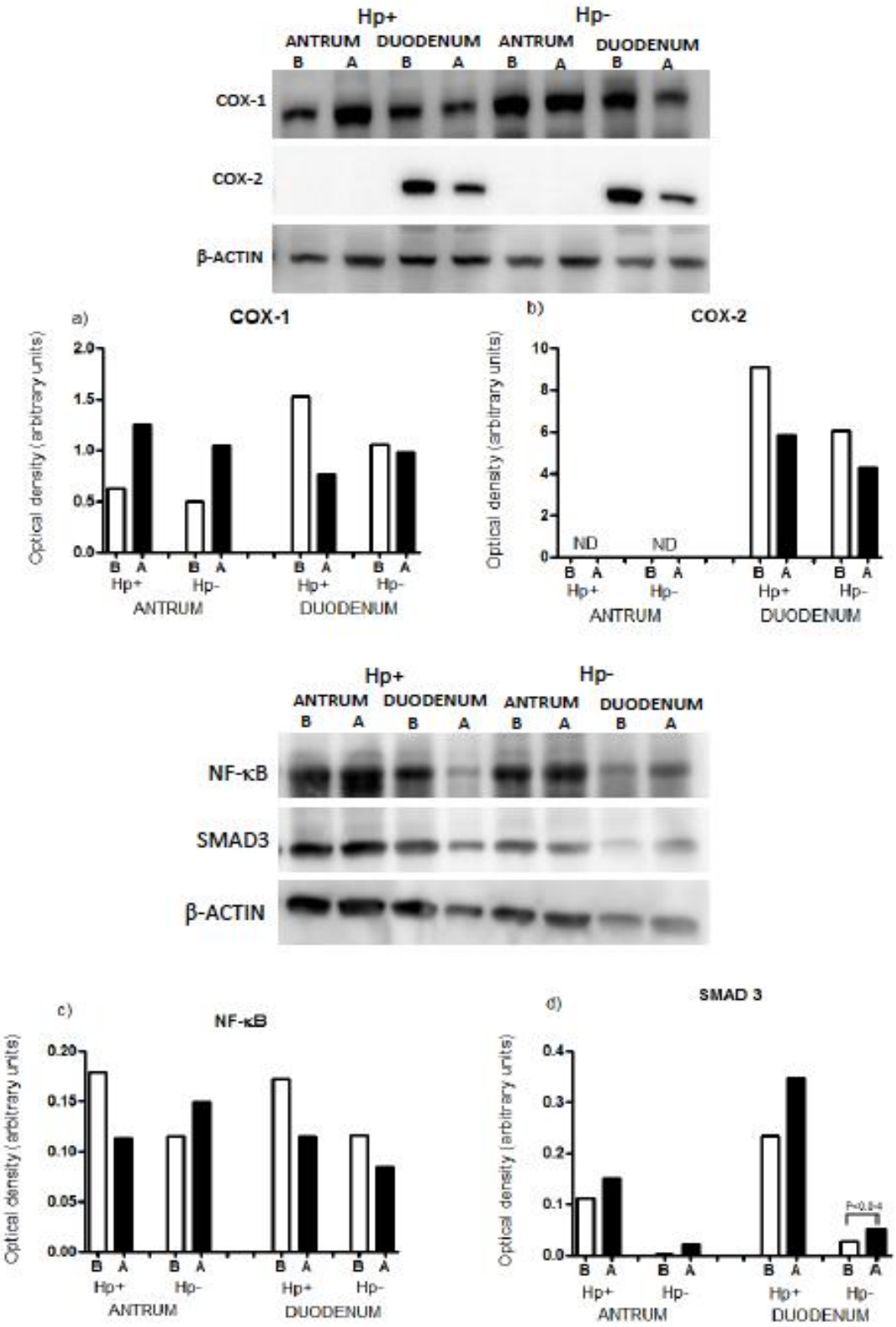

Figure 3- Protein content determined by Western blot a)COX-1, b)COX-2, c)NF-κB and SMAD3 from gastric antrum and duodenum biopsies *Helicobacter pylori* positive (Hp+) and negative (Hp-) of patients before (B) and after (A) GABOB treatment. Horizontal bars represent median expression.

Plasma cytokine concentration

IL-8, IL-10, TNF-α, TGF-α, TGF-β and NO concentration were determined in plasma. TGF-α and NO did not change after GABOB treatment, meanwhile the remaining cytokines were not detectable. Only TGF-β decreased in Hp- as a result of GABOB treatment (Figure 4).

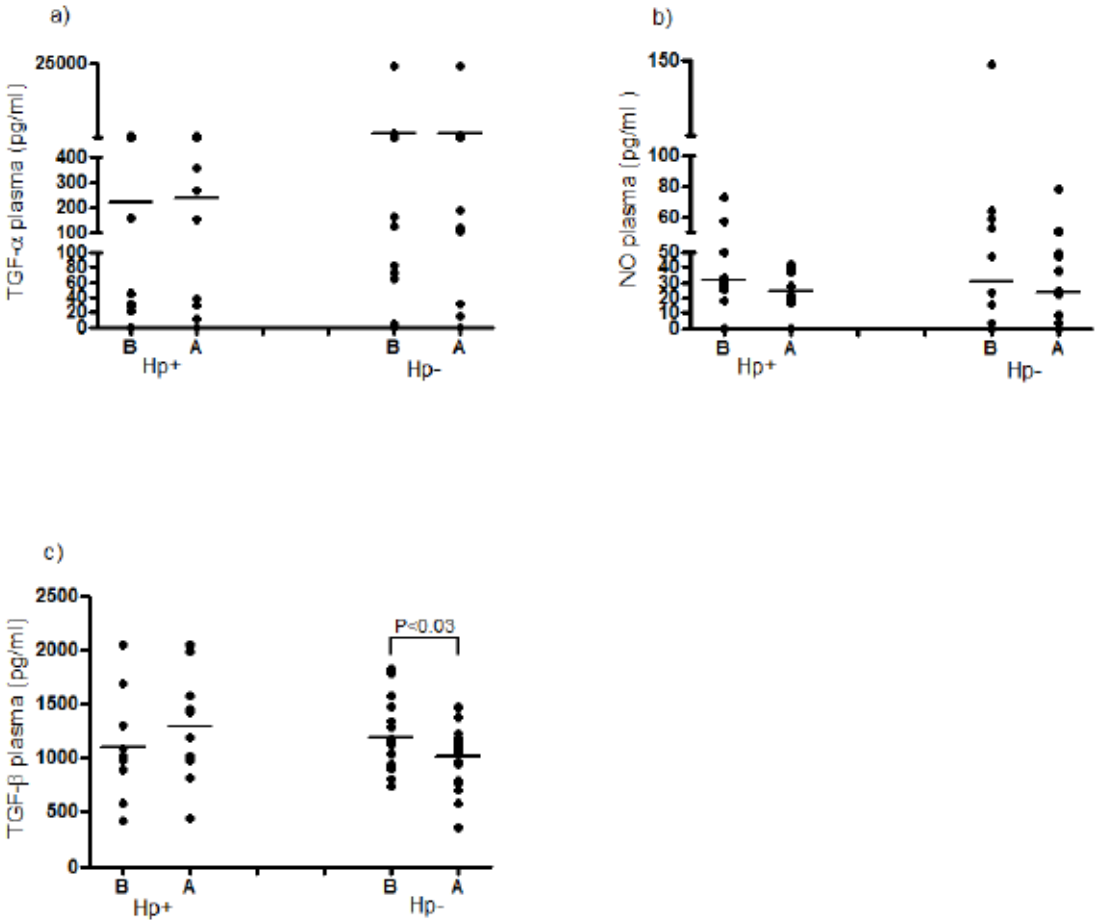

Figure 4- a)TGF-α, b)NO and c)TGF-β plasma concentration from *Helicobacter pylori* positive (Hp+) and negative (Hp-) of patients before (B) and after (A) GABOB treatment. Only TGF-β concentration in Hp- patients presented significant reduction as a result of GABOB treatment.

Sydney evaluation

Antrum biopsies were analyzed after standardization diet and before GABOB treatment (visit 0). Biopsies from each patient were staining with hematoxylin-eosin and histological evaluation was performed according to the Updated Sydney System. Mononuclear infiltrates in antrum biopsies were present in all Hp+ (30% mild, 70% moderate) biopsies, while Hp- only in 50% (43.75% mild and 6.25% moderate) at lamina propria of gastric mucosa and in

the cytoplasm as lymphoplasmocitic infiltrate. On the other hand, polymorphonuclear cell infiltrate was observed in 80% of Hp+ biopsies (70% mild, 10% moderate), meanwhile neutrophil infiltrate was presented in 12% of Hp- biopsies (6.25% mild and moderate) and were found in the lamina propria, particularly in the epithelium of glandular neck and foveolar lumen.

Cleaved caspase 3 immunohistochemistry

Apoptotic cells were quantified from antral biopsies in visit 0 by cleaved caspase-3 immunohistochemistry and determined in 10 high-power yields in each biopsy. There was no difference between Hp+ and Hp- patients finding an average of 23 apoptotic cells/10 high power yield (Figure 5).

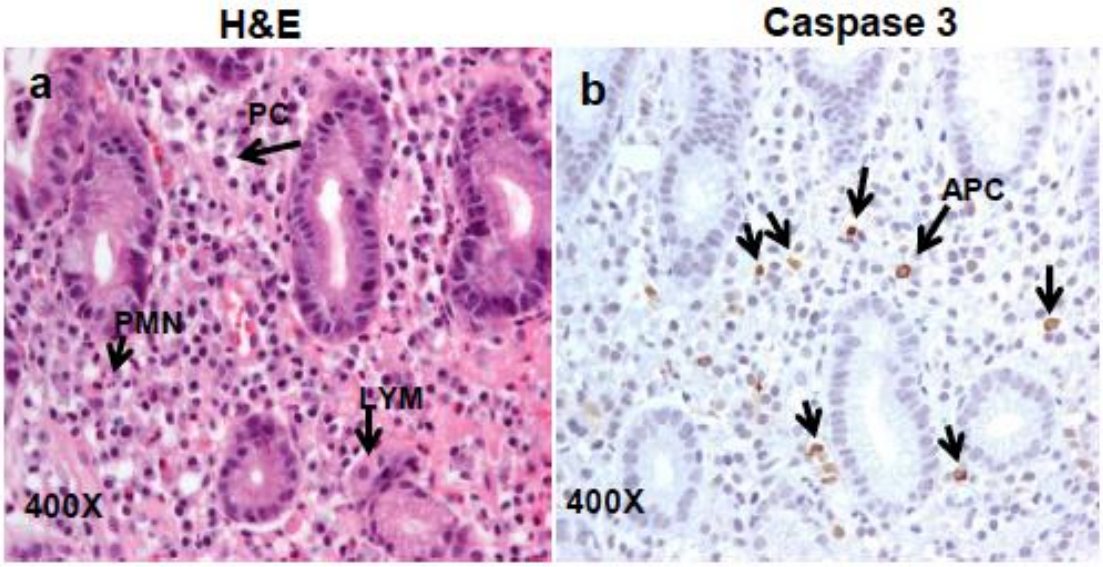

Figure 5- Photomicrographs of the a) haematoxylin and eosin (H&E)-stained tissue sections of antrum gastric tissues and active caspase 3 Hp+ before GABOB treatment. Biopsies from each patient were staining with hematoxilin-eosin and histological evaluation was performed according to the Updated Sydney System. Representative image of Hp+ patients that presented 100% of mononuclear infiltrate. Only 50% in Hp- presented mononuclear infiltrate (image not shown). b) Apoptotic cells were quantified from activated caspase 3 immunohistochemistry in Hp+ before GABOB treatment. There was no difference between Hp+ and Hp- patients (image not shown).

Immunohistochemistry assay for COX-1 and COX-2 and staining evaluation

Immunohistochemistry showed the presence of COX-2 in Hp+ and Hp- antrum biopsies before GABOB treatment. However, no difference in COX-1 or 2 could be observed,

although COX-2 was not detectable by Western blot. The overall presence of COX-1 and COX-2 in antrum biopsies was not significantly affected by presence of Hp (Figure 6).

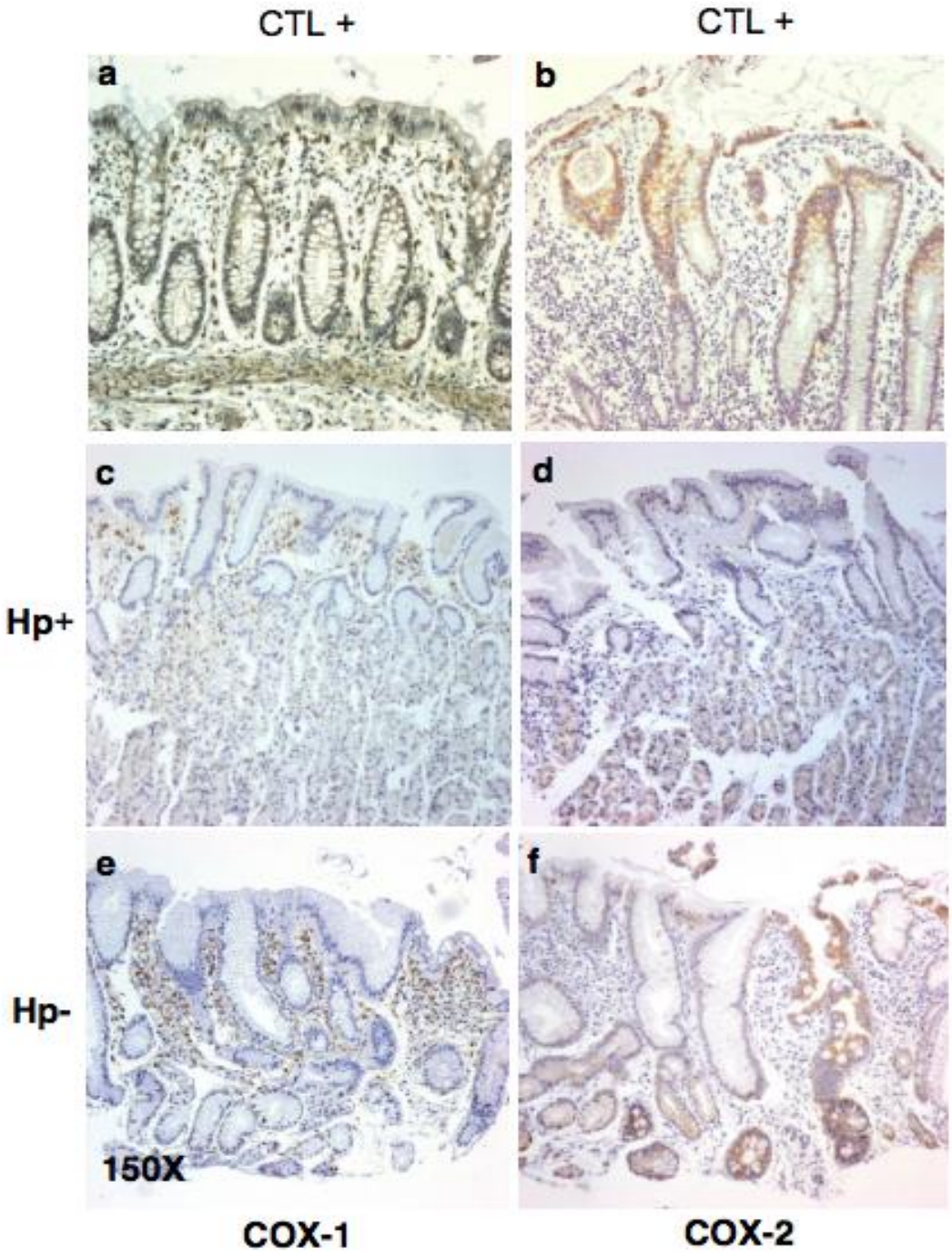

Figure 6- Photomicrographs of COX-1 and COX-2 in sections of antrum gastric tissues Hp+ and Hp- before GABOB treatment. a) and b) Positive controls for COX-1 and Cox-2 respectively, c) COX-1 in Hp+, d) COX-2 in Hp+, e) COX-1 in Hp- and d) COX-2 in Hp-. No difference in COX-1 and COX-2 were observed.

DISCUSSION

Our findings suggest that GABOB treatment improves tolerability in dyspeptic symptoms in 60% of Hp+ and 69% of Hp- patients. IL-8 expression presented higher values in antrum in Hp+, patients, that correlates with greater inflammatory infiltration. IL-8 secretion was increased in Hp+ duodenum biopsies as a result of GABOB treatment. Smad3 expression increased in Hp- duodenum; while a decreased of TGF-β plasmatic levels were observed in the same patients. Apparently, Hp colonization does not promotes damage, since we did not find any correlation among dose, NSAID type, time consuming and gastropathy. We suggest that 4 weeks of GABOB treatment is not enough to improve inflammation mediators in gastric biopsies, nor in plasma levels of patients with rheumatoid arthritis.

There are conflicting results regarding the role that *H. pylori* plays in the pathogenesis of ulcers induced by NSAIDs. Cross-sectional studies of chronic NSAID users from the general population of rheumatology patients have not shown a difference prevalence of ulcers among those with or without *H. pylori* infection.[23] Similar results have been reported in a follow-up endoscopic ulcer study of relatively highly selected chronic NSAID users. Tanaka et al observed a lower incidence of *H. pylori* infection in RA patients with chronic use of NSAIDs, and suggested that some NSAIDs have antibacterial effect against *H. pylori*. However, they concluded that *H. pylori* infection was a significant risk factor for upper gastrointestinal ulcer in Japanese patients with RA.[24] Kim and Graham reported similar results as in the present study; authors suggested that *H. pylori* does not confer increased risk of ulceration in arthritics receiving NSAIDs chronically.[25] However, Goggin et al. have shown that *H. pylori* infection is associated with increased dyspeptic symptoms in RA patients taking NSAIDs, but that infection does not predispose to NSAID gastropathy.[26] Vonkeman et al. in a recent controlled clinical trial, found that eradication of *H. pylori* infection did not reduce the incidence of endoscopic gastroduodenal ulcers in *H. pylori* seropositive patients currently taking NSAIDs for rheumatic diseases.[27]

There are some conflicting results about GABOB effect. Yano et al. reported that GABOB produced an increase in gastric acid secretion.[28] However, there are some reports that consider that GABA and GABA receptor agonists are involved in the regulation of mouse gastric tone, through modulation of intrinsic neurons[9,29] and that this stimulation impact in antrum which involves GABAergic transmission.,[30] which is not surprisingly since GABOB is considered an endogenous ligand found in the central nervous system in mammals which is

a metabolic product of GABA, and exerts similar effects. In this way, GABOB also can have not only an effect on gastric cells, but also an effect on cells of the immune system. In the present study, we found that GABOB treatment increased chemokine (IL-8) secretion at duodenum level concomitantly with *Helicobacter pylori* colonization. On the other hand, without *Helicobacter pylori* colonization we observed an increased of Smad3 expression at duodenum level, which decreases TGF-β plasma levels.

Accordingly to IL-8 levels, we observed higher concentration at antrum level in Hp+ patients compared to Hp-. This result could be correlated with greater inflammatory infiltrate found in Hp+ antrum biopsies quantified by Sidney System. Also, duodenum concentration for IL-8 were lower than the corresponding for antrum. GABOB treatment produced no change in this cytokine in antrum samples; however, only Hp+ patients significantly increased the value of IL-8 duodenal biopsies. Stromberg et al. have shown that duodenal ulcer (DU) patients have a significantly lower IL-8 response in the duodenum than asymptomatic carriers, which may be of importance for the pathogenesis of *H. pylori*-induced duodenal ulcers and most likely can be explained by host factors.[31]

TNF-α, TGF-α, IL-10, TGF-β and NO concentration were similar in infected and uninfected patients, also they have the same values in antrum as well as duodenum biopsies (Figure 1), but any of these parameters change after GABOB treatment. *H. pylori* infection is associated with increased expression of TGF-β1, TGF-β1RI, Smad-7, and CTGF in the gastric antrum.[32] Bontems et al. reported that IL-2, IL-4, IL-10 and TNF-α concentrations were similar in Hp+ and in uninfected adults in mucosal and duodenum biopsies.[33] IL-8, IL-10 TNF-α, TGF-α, TGF-β, iNOS, CGRP, COX-1 and COX-2 expression did not change in any case (Figure 2), which indicate that there is not a transcriptional change. These parameters showed similar values, that points out the lack of difference between the antrum and duodenal biopsies, nor due to the presence of *Helicobacter pylori* infection. Even mRNA IL-8 expression did not increase, IL-8 secretion in antrum Hp+ patients did increase, mainly based on the fact that the expression as well as secretion, were held in the same sample and possibly the induction of mRNA was at previous times. GABOB treatment did not produce any change. Subjects Hp- treated with ibuprofen during 3 days with gastric lesions or adverse reactions had lower PGE2 levels. COX-1, COX-2, eNOS, and iNOS were detectable in all subjects. The constitutive enzymes (COX-1 and eNOS) did not change after treatment. COX-2 was higher in corpus than antrum and it increased after ibuprofen treatment. iNOS tended to

increase mildly in the corpus in subjects with adverse reactions or endoscopic lesions.[34] Other studies have shown that *H. pylori* infection was associated with increased COX-2 expression in gastric antral mucosa for both NSAID users and nonusers, but not in gastric ulcer, where the effect of NSAID inhibition plays a major role. The authors concluded that *H. pylori* eradication does not interfere with gastric ulcer healing in NSAID users.[35] Conversely, in *H. pylori* infected mice, NSAID treatment significantly decreased the degree of gastric inflammation.[36] Therefore, it is possible that HP+ patients with concurrent NSAID treatment, may affect levels of gastric inflammation and consequently the serological response. There are some reports that indicate that *H. pylori* infection can activate NF-κB in gastric epithelium cells and subsequently up-regulate IL-8 gene transcription.[37] However, our data indicate that although we have an increase of pro-inflammatory IL-8 cytokine in *H. pylori* infected antral biopsy and an increased inflammatory infiltrate observed by microscopy, we did not find an increase in the activation of NF-κB. The results suggest that mononuclear cells in lamina propia were the major source of this pro-inflammatory cytokine. As seen in the different studies, there is a large discrepancy in the results obtained. Recent data indicated that *H. pylori* induced inflammation, up-regulate Hath1 expression via interleukin-8/STAT3 (IL-8) phosphorylation while suppressing Hes1, which provides a novel molecular connection between a *H. pylori* infection and intestinal metaplasia.[38] No results were found in the literature regarding the effect of GABOB in antrum and duodenal biopsies, but our data have suggested that GABOB does not contribute to ameliorate inflammatory response.

We determined plasma concentration of IL-8, IL-10, TNF-α, TGF-α, TGF-β and NO. IL-8, IL-10 and TNF-α were not detectable. TGF-α and NO did not change after GABOB treatment, whereas only TGF-β decreased in Hp- as a result of GABOB treatment (Figure 4). Notwithstanding, a few data are available on the circulating levels of cytokines in *H. pylori* infection. Russo et al. reported in asymptomatic people carrying *H. pylori* infection, a significantly higher TNF-α level than uninfected subjects.[39] On the contrary, IL-8 levels were significantly higher in the uninfected subjects than in *H. pylori* positive ones. Also they found that IFN-gamma and IL-10 circulating levels were not affected by *H. pylori* presence, being not significantly different in the two groups. Bayraktaroglu et al. could not be able to demonstrate any increase in circulatory cytokines in *H. pylori*- positive patients.[40] Although mucosal cytokine production was found to correlate well with the intensity of gastric mucosal inflammation, serum cytokine levels did not show any correlation with Sydney parameters,

including the gastric epithelial inflammatory scores, *H. pylori* density and activation. In the literature, some authors reported increased circulatory TNF-α levels while serum IL-8 levels remained normal in *H. pylori*-infected persons. Some others reported elevated serum TNF-α levels in patients with CagA positive *H. pylori* infection. IL-1β, IL-6 and IL-8 were elevated in Cag A-positive cases. We did not determine the Cag A status of our patients. Thus, we do not know whether most or all of our patients were infected with a Cag A-positive strain. However, we know that our patients had endoscopic sign of gastroduodenal disease. As a result of contradictory reports, including ours, we do not know whether the continuous spill-over of cytokines into the blood stream could be possible as a consequence of gastric mucosal cytokine activation and increased synthesis. We think that further studies are needed, but it could be difficult that *H. pylori* induced changes in the circulatory cytokine levels. Probably, only under specific conditions of acute events, gastric mucosal pro-inflammatory cytokines could be increased and be reflected in cytokine serum levels.

Our results showed, that COX-1 and COX-2 were detected by immunohistochemistry using the avidin-biotin complex. However, no difference was found in antrum biopsies Hp+ and Hp- in visit 0 (Figure 5). Wu et al. reported that *H. pylori* increased IL-8 and COX-2 in the antral mucosa, but did not influence COX-2 and local cytokines in gastric ulcer. NSAIDs inhibited COX-2 in gastric ulcer and delayed gastric ulcer healing.[41]

Our findings demonstrate that GABOB treatment improves dyspepsia tolerability in 60% and 69% of Hp+ and Hp- patients, respectively. Although, chronic NSAID users from rheumatology patients have not shown a difference in gastric disorders among those with or without *H. pylori* infection. We believe that 4 weeks of GABOB treatment did not improved inflammatory mediators in gastric biopsies nor in plasma levels.

Conflict of interest disclosure statement

The authors declare that they have no conflict of interest.

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