EFFECT OF PRETREATMENT WITH ETHANOL OR AMMONIUM HYDROXIDE ON HELICOBACTER PYLORI COLONIZATION IN THE STOMACH OF RATS

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Abstract: ***Animal models for H. pylori infection have been developed to clarify the pathogenesis, testing new therapies and developing vaccines against human H. pylori infection. Although rats have been used extensively for gastric ulceration and acid secretion studies, the animal is not normally infected with H. pylori.***

Several chemicals such as ethanol and ammonium hydroxide can induce gastric erosion and interact with gastric mucosal defense mechanisms. The aim of the present study was to investigate the effects of pretreatment with the gastroinvasive agents on colonization of H. pylori in not germ-free rats in order to overcome the resistance against H. pylori in rats. After 24 h fasting, the rats were divided into three major groups. Animals in the first group were not pretreated with any chemicals .The two other groups were pretreated with ethanol (60 %) or ammonium hydroxide (1 %) before inoculation of 1 mL H. pylori suspension (3 × 108 cfu/mL). The results showed that H. pylori could not colonize in rats, even with ethanol or ammonium hydroxide pretreatment. An understanding of the mechanism of this resistance can help researchers to develop new therapeutic or preventive drugs against H. pylori and it is recommended to perform more investigation to clarify the reason of this resistance.
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Key words*: Helicobacter pylori, ethanol, ammonium hydroxide, gastric erosions*

INTRODUCTION

Helicobacter pylori (H. pylori) plays a causal role in the pathogenesis of gastritis, which may be the underlying condition for subsequent development of various gastrointestinal disorders, such as peptic ulcer disease, gastric adenocarcinoma and lymphoma (1-4). Eradication of *H. pylori* can cure peptic ulcer disease and lead to regression of low graded gastric lymphoma (5). Over the last decade animal models has been used extensively to investigate disease process and therapy for *H. pylori* infection. *H. pylori* has a limited host range and does not easily colonize rodents. However a number of these animals using germ-free as well as specific-pathogen-free mice have been successfully colonized with this organism and the histopathologic feature seems to be very similar to that found in the *H. pylori* infected humans (6-9). Furthermore gnotobiotic rats can be infected with *H. felis* (8,10). Since the high cost of germ-free animals, attempts to develop a not-germ-free model for *H. pylori* infection were made. Mature not-germ-free rats are not usually infected with *H. pylori* (11). An understanding of the mechanism of this resistance can help the development of new therapeutic or preventive drugs against *H. pylori*. Recently it has been shown that *H. pylori* can temporarily colonize non-germ-free immature rats but not mature animals (12). Although the mentioned study did not clarify the reason of resistance against *H. pylori* colonization in mature rats, but revealed that there was no absolute resistance against *H. pylori* colonization in rat species. Ross et al. studied the effect of daily intragastric administration of *H. pylori* suspension in mature rats with normal mucosa and with surgically produced experimental gastric ulcers (11). They showed that *H. pylori* alone causes no erosion on an intact gastric mucosa in rats but resulted in continuous presence of *H. pylori* in stomachs of rats with pre-existent ulcer (11).

Several chemicals such as ethanol, ammonium hydroxide and aspirin-like drugs can induce gastric

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erosion and interact with gastric mucosal defense mechanisms. Mucosal defense consists of a complex network of components that function in concert with one another. This network includes: 1) the extramucosal components such as acid, mucus, surface-active phospholipids, and bicarbonate; 2) the epithelium itself; 3) the microcirculation and sensory afferent neurons beneath the epithelium; 4) the mucosal immune system; and 5) the ability of the mucosa to undergo repair (13).The aim of the present study was to investigate the effect of pretreatment with gastroinvasive agents (such as ethanol and/or ammonium hydroxide) on colonization of *H. pylori* in not germ-free rats in order to overcome the resistance against *H. pylori* in rats.

MATERIALS AND METHODS

Animals

Male albino Wistar rats weighing 180-220 g were used. The animals were given a standard pellet chow and tap water *ad libitum*. They were fasted with free access to water 24 h before bacterial inoculation.

Ethanol-induced gastric damage

Ethanol was administered orally (1 mL/rat) with a feeding needle as a concentration-dependent manner of 50, 60, 70 and 96%. One hour after ethanol administration the animals were killed with cervical dislocation, the stomachs were removed for evaluations of gastric damage. The concentration of 60 % was considered for induction of gastric erosions before *H. pylori* inocu-lation.

Ammonium hydroxide-induced gastric damage

Ammonium hydroxide was administered orally (1 mL/rat) with a feeding needle as a concentration-dependent manner of 0.5, 1, 2 and 4 %. One hour after ammonium hydroxide administration the animals were killed with cervical dislocation and the stomachs were removed for evaluations of gastric damage. The concentration of 1% was considered for induction of gastric erosions before *H. pylori* inoculation.

Bacterial inoculum

Freshly isolated strain of *H. pylori* obtained from gastric biopsy specimen of a patient with duodenal ulcer was used in this study. Bacterial isolate was confirmed as *H. pylori* on the basis of characteristics of the organism: Gram-negative spiral rod with oxidase, catalase and urease positive results, negative reaction for nitrate reduction, sensitivity to cephalothin and resistance to nalidixic acid (14-15). *H. pylori* was grown in Trypticase Soy Broth (Difco,UK) with 10% fetal bovine serum in a flask with a porous stopper put inside a GasPak jar at 37°C under a microaerobic condition (Anaerocult C, Merck) while stirring continuously by using a magnetic stirrer (Cenco Instrumenten B.V. breda, The Netherlands). 72-hour broth culture of *H. pylori* was administered as inoculum immediately after preparation. We also insured that bacteria had a typical morphology in Gram staining and corkscrew like motility in wet mount preparation**.**

Experimental design

After 24 h fasting, the rats were divided into three major groups.The first group was not pretreated with any chemicals but other groups were pretreated with ethanol (60%) or ammonium hydroxide (1%) before inoculation of 1 mL *H. pylori* suspension (3×108 cfu/mL). The major groups of the study are shown in table 1. After killing animals with cervical dislocation, the stomachs were removed under aseptic condition, and small specimens (2 mm square) of antrum and body were taken using sterile instruments and examined for *H. pylori* colonization by using rapid urease test (15) as well as culture.

Microbiology

The gastric samples were washed with 1 mL PBS, homogenized with 0.2 mL PBS and inoculated on Columbia agar plates containing 10% defibrinated sheep blood, supplemented with 10 mg/L vancomycin, 2500 IU/L polymyxine B, 2 mg/L amphotericin B and 5 mg/L trimethoprim. Plates were incubated at 37°C in a GasPak jar for 4-7 days in a microaerobic condition and bacterial isolates with typical characteristics of *H.pylori*, using biochemical tests as well as susceptibility to cephalothin and nalidixic acid were identified (14-15).

Statistical analysis

All data express the percent of animals with positive test. The Fisher's exact test was used for

analysis of data and a P-value less than 0.05 was considered to be statistically significant.

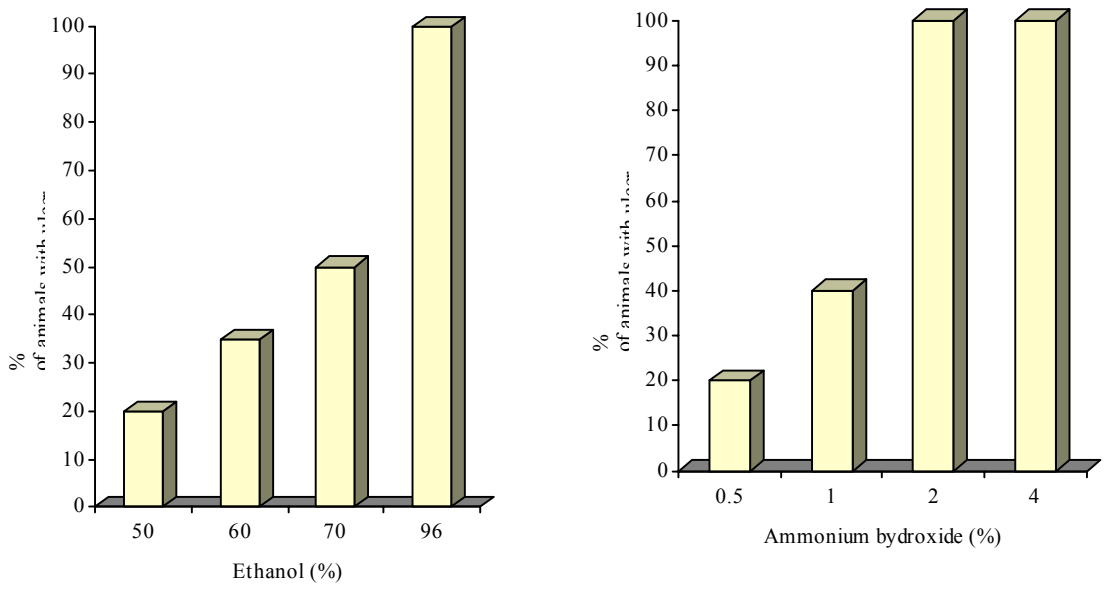
RESULTS

Figure 1 shows the effect of ethanol or ammonium hydroxide administration on induction of gastric erosions in animals. As shown in this figure both chemicals induced gastric erosions in a concentration-dependent manner.

Figure 2 shows effect of bacterial inoculation on later colonization of H. pylori in the stomach of group A animals using rapid urease test. There were no positive urease test after bacterial inoculation except at time 0. The results of culture were similar to the rapid urease test (data are not shown). Furthermore pretreatment with ethanol or ammonium hydroxide did not induce any positive urease test as well as culture in group B or C of the experiment.

Table 1. Three major groups of 24 h fasted rats were used in the study (A, B, C). Furtheremore, a number of rats without *H. pylori* inoculation were served as controls.

Group	n	Pretreatment (duration)	*H. pylori* inoculation	Killing animals
A	32	Without pretreatment	Single dose	0, 2, 3, 6, 24 and 48 h after bacterial inoculation
B	20	Ethanol 60% (2weeks)	At days 14, 16 and 18	At days 14, 17, 25 and 33
C	26	Ammonium hydroxide 1% (2weeks)	At days 7, 9 and 14	At days 20, 22, 28 and 33



(A) **(B)**

Fig. 1. Frequency of gastric erosions induced by ethanol (A) or ammoinum hydroxide (B). 8-12 rats were used in each group. For all groups P<0.01 in comparison with groups which were not received ethanol or ammonium hydroxide administration.

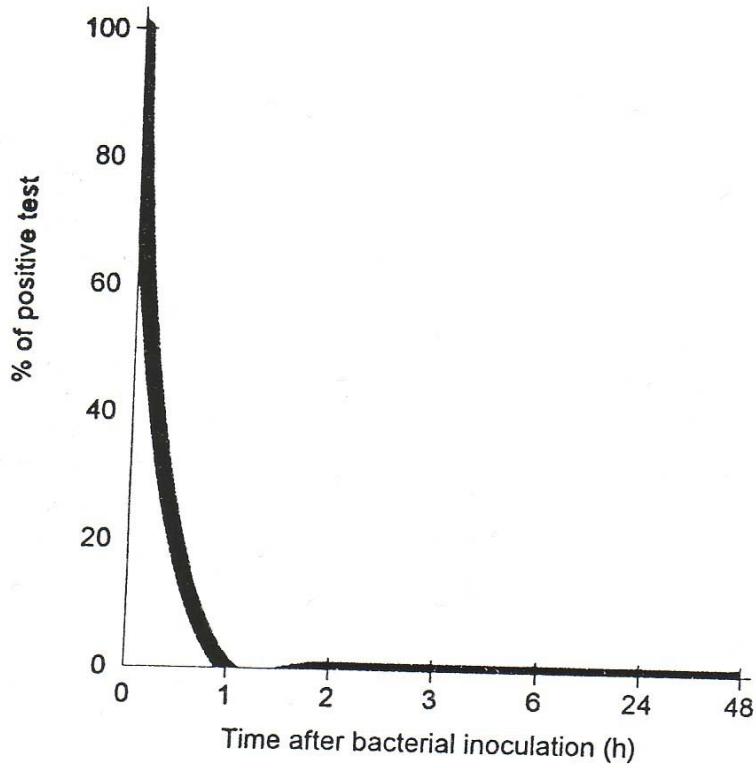


Fig. 2. Percent of H. pylori colonization after bacterial inoculation on group A.

DISCUSSION

Animal models for *H. pylori* infection have been developed to clarify the pathogenesis, testing new therapies and developing vaccines against human *H. pylori* infection (6-9). Although rats have been used extensively for gastric acid secretion studies,rats are not normally infected with *H.pylory*(11).Our study has demonstrated that *H.pylory* could not colonize not-germ-free rats even with ethanol or ammonum hydroxide pretreatment.The suppression of *H.pylory* growth in the rat's stomachs may be due to the immune system of the animals or other factors like inhibitory effect of gastric normal flora as reported in mice by some investigators(6,7).The present study showed that induction of gastric erosions before before bacterial inoculation could not overcome the resistance of animals against *H. pylori* colonization. Ross et al. studied the effect of oral administration of *H. pylori* suspension in rats with normal mucosa and with surgically produced experimental gastric ulcers, but they showed continuous presence of *H. pylori* in stomachs of rats with pre-existent ulcer (11). In our study, the pre-existent gastric erosions induced by the gastroinvasive chemicals could not provide a suitable environment for *H. pylori* colonization . Colonization of *H. pylori* in immature rats rather than mature animals (12) can be explained by less developed gastric mucosal defense mechanism in the immature animals. Understanding the mechanism of such development can possibly help to develop new drugs or vaccines against *H. pylori* infections .

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