
Direct Action of Helicobacter pylori on the Freshly Isolated Rat Gastric Mucosa Cells (GMCs)

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Additional information is available at the end of the chapter

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9.1. General introduction

The etiological role of *H. pylori* has been suggested in the development of gastric and duodenal ulcer and it later advances to chronic atrophic gastritis and gastric cancer. Consequently, everyone tried to find suitable treatment for *H. pylori*-induced disorders. Finally, “evidence-based” therapeutic recommendations were established consisting of antibiotic combination, metronidazole and proton pump inhibitors (PPI). The efficacy of various therapeutic schemes differing by small modification has been widely studied by randomized, multiclinical, multinational studies and their meta-analysis (www.cancer.gov/cancertopics/factsheet/Risk/p-pylori-cancer; Wong et al., 1998; Molloy et al., 1998; Molloy and Sonneberg, 1997; Mabe et al., 2009).

The researchers all over the world can be divided into two groups based on their view of *H. pylori*'s importance. A larger group of researchers totally accepts the role of *H. pylori* in all abovementioned pathological conditions, including the etiological role and eradication treatment in these *H. pylori*-induced diseases. A smaller group of researchers shows a little skepticism toward the *H. pylori* as being a factor in gastric and duodenal ulcer, gastric atrophic gastritis and gastric cancer development (Mózsik et al., 2014). We did a critical analysis of gastric secretory responses (BAO, MAO) on patients with duodenal ulcer with respect to their age and duration of complaints ($n = 120$) (Mózsik et al., 1981f). Surprisingly, their MAO values remained the same in old age as those in the younger age, meanwhile the BAO values increased with respect to their age and duration of complaints. We had no information about the presence of *Helicobacter pylori* infection at that time (for details, see Mózsik et al., 2014 a, b, c, d).

The aim of this chapter is to give a summary of the observations that are not in agreement with the main line of *H. pylori* infection and the necessity of its eradication treatment (Mózsik et al., 2014 a, b, c, d).

9.2. Testing of *Helicobacter pylori* (*H. pylori*) cultures on the isolated gastric mucosal cell

As the letters of Warren and Marshall in *Lancet* (1983) were published, a very active research chain was started throughout the world. Researchers tried to find the explanation on how *H. pylori* can lead to inflammation in the stomach and peptic ulcer.

The existence of *H. pylori* as the cause for direct damaging effect in the stomach was one of the possibilities. To answer the question, we analyzed the direct cellular effect of sonicated *H. pylori* cultured (at the level of membrane, mitochondrion and DNA) alone and with different combinations on the freshly isolated gastric mucosal cells of rat. The aggravation effect of this sonicated *H. pylori* was also examined in ethanol-induced cell injury model to evaluate the changes in cell resistance.

9.2.1. *H. pyloric culture*

Bacteria were obtained from human gastric and duodenal biopsy samples. Urease test and histological positivity were both required to be definite in the tissue samples. Bacterial suspensions (10^6 bacteria/mL in 20 Tris/HCl buffer, pH 7.0) were sonic on ice (30 W) in six consecutive treatments lasting 30 seconds.

9.2.2. Preparation of mixed Gastric Mucosal Cells (GMCs)

Gastric mucosal cells from 1 or 2 unfasted Sprague-Dawley strain rats were isolated by the method of Nagy et al. (1994). Briefly, the segments of glandular stomach without blood vessels surrounding the connective tissue were sequentially incubated in a physiological solution containing 0.5 mg/mL Pronase E (Type XXV Sigma Chemical Co.) and 10^{-3} EGTA. After washing for several times, the cells were resuspended and kept in shaking water bath at a temperature of 37°C in a solution (0.157 M, pH 7.4) freshly produced with the following ingredients: 98.0 mM NaCl, 5.8 mM KCl, 2.5 mM NaH_2PO_4 , 5.1 mM Na pyruvate, 6.9 mM Na fumarate, 2 mM glutamine, 24.5 mM HEPES Na, 1.0 mM Trizma base, 11.1 mM D-glucose, 1.0 mM CaCl_2 , 1.0 mM MgCl_2 and 2 mg/mL (w/v) bovine serum albumin. Mixed population of isolated rat GMCs contained at least three types of cells: parietal (20–25%), chief (40%) and epithelial cells (40–45%). An initial viability of 85–95% of the isolated cells was maintained for 6–7 hours. The examinations were carried out through the same media of solution.

9.2.3. Toxicological studies

9.2.3.1. *H. pylori culture*

Cells were incubated with sonicated *H. pylori* (10^6 – 10^8 bacteria /mL) for 30 minutes in the shaking water bath at 37°C. Equivalent volume of both diluted toxic agent and cell suspension were added. At the end of 30 minutes of incubation, the cells were separated from the

supernatant by centrifugation. Cell pellet was carefully resuspended and incubated for 10 minutes in the shaking water bath, before it was centrifuged again (500 g, 10 minutes) to obtain the toxic-agent-free supernatant. Cells were resuspended for further biochemical examinations.

9.2.3.2. Ethanol

Pretreated and washed cells of sonicated *H. pylori* were incubated with 15% ethanol (EtOH) for 5 minutes in shaking water bath at 37°C after the cells were treated as described above.

9.2.3.3. Indomethacin

Pretreated and washed cells were incubated with 10^{-8} – 10^{-4} M indomethacin (IND) (Chinoin, Hungary) for 5 minutes in shaking water bath at 37°C. Then the cells were treated as described above.

9.2.3.4. Determination of cell viability by Trypan Blue exclusion test

Trypan Blue (TB) is excluded by viable cells, while being taken up by damaged cells, staining the cytoplasm blue (Bauer et al., 1972). A solution of 0.4% TB (Sigma Chemical Co., St Louis, USA) was mixed with the same volume of cell suspension, and 5 minutes later the rate of stained (dead) and unstained (viable) cells were calculated as percentage of counting 100 cells in a hemocytometer.

9.2.3.5. Biochemical assays

9.2.3.5.1. Lactate dehydrogenase assay

The enzyme, lactate dehydrogenase (LDH) can be found in the cytoplasm of the cells. If it is present in the supernatant, it indicates membrane damage of cells. Its activity was determined in samples of both toxic-agent-free supernatant and the cell pellet destroyed by freezing. The calorimetric assay was based on the reduction of NAD^+ to NADH, catalyzed by LDH in the presence of lactate as a substrate (Bergmayer and Bern, 1972). The color produced by the reduction of phenazine methosulfate (Sigma Chemical Co., St. Louis, USA) and tetrazonium salt isonicotinic acid hydrazide (Sigma Chemical Co., St. Louis, USA) was measured at 520 nm by a Hitachi 124 spectrophotometer. The results were expressed as $\text{mU}/\text{min}/10^6$ cells.

9.2.3.5.2. Succinic dehydrogenase assay

The enzyme succinate dehydrogenase (SDH) can be found in the mitochondria. The mitochondrial integrity was tested in 2×10^6 previously treated and redispersed cells (Mosmann, 1983). The callus formazon product was quantified using a Hitachi 124 spectrophotometer at 500 nm and calculated as $\text{nmol}/\text{min}/2 \times 10^6$ cells.

9.2.3.5.3. Ethidium bromide-DNA fluorescence assay

The nuclear damage of cells due to ethidium bromide–DNA-binding was assessed by nuclear fluorescence (Bányai and Majumder, 1988). To the ethidium bromide (EB) solution, 10^7 cells were mixed with a Chemical Co., St. Louis, USA), and the fluorescence intensity was measured by a Hitachi F-3000 spectrophotometer at 325–385 nm (excitation–emission). The results were expressed as arbitrary fluorescence units / 10^7 cells.

9.2.3.6. Direct cellular effect of *H. pylori*, EtOH and IND

9.2.3.6.1. *H. pylori* culture alone

Sonicated 10^6 – 10^8 bacteria/mL had no direct cellular toxicity on freshly isolated rat GMCs by Trypan blue exclusion test (Figure 279), LDH activity (Figure 280) or ethidium bromide–DNA fluorescence (Figure 281) (Bódis et al., 1995 a, b; 1996, 2000; Mózsik et al., 1996c).

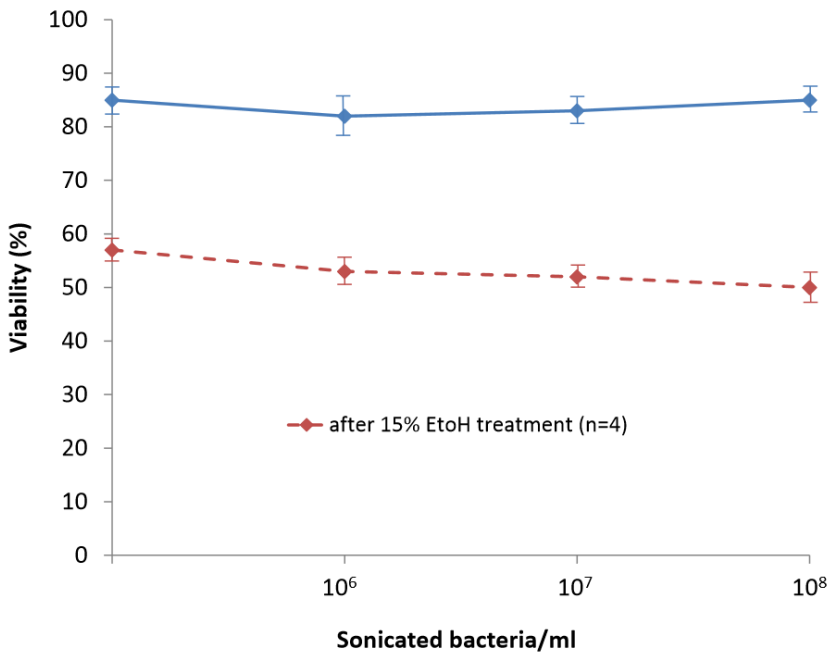


Figure 279. Effect of 30 minutes incubation with sonicated 10^6 – 10^8 bacteria/mL *H. pylori* on the viability of freshly isolated gastric mucosal cells of rat detected by Trypan blue exclusion test with or without 5 minutes of 15% ethanol treatment. [Bódis, Karadi, Voros, Mózsik (1995b): Has sonicated *Helicobacter pylori* any direct cellular effect? In: Bajtai A., Papp J., Rác I., Simon L. (Eds.). *Helicobacter pylori*. Hungarian Society of Gastroenterology. Medicom, Budapest. pp. 137–142 (with kind permission).]

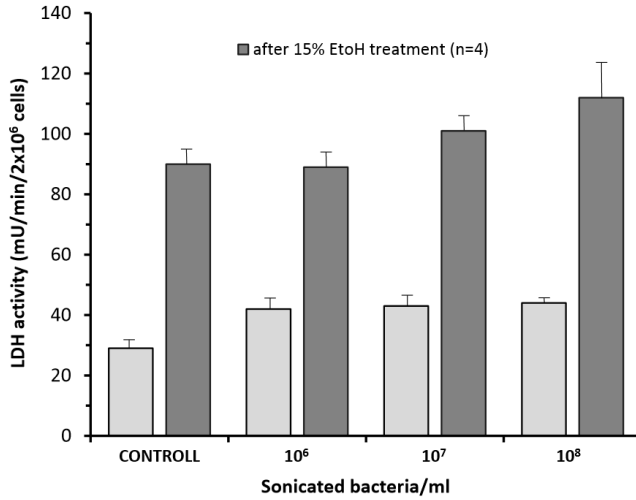


Figure 280. Changes in lactate dehydrogenase activity of freshly isolated gastric mucosal cells of rat after 30 minutes incubation with sonicated 10⁶–10⁸ bacteria/mL *H. pylori* with or without 5 minutes of 15% ethanol treatment. [(ódi-sBoKaradi, Voros, Mozsik (1995b): Has sonicated *Helicobacter pylori* any direct cellular effect? In: Bajtai A., Papp J., Rác I., Simon L. (Eds.). *Helicobacter pylori*. Hungarian Society of Gastroenterology. Medicom, Budapest. pp. 137–142) with kind permission.]

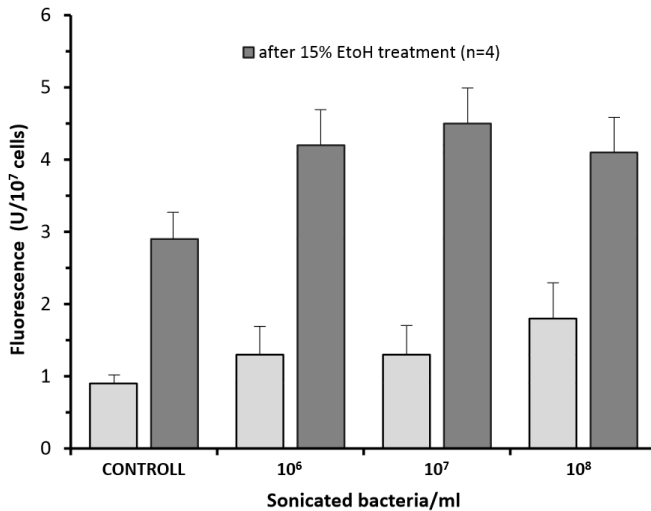


Figure 281. Changes in ethidium bromide–DNA fluorescence of freshly isolated gastric mucosal cells of rat after 30 minutes of incubation by sonicated 10⁶–10⁸ bacteria/mL *H. pylori* with or without 5 minutes of 15% ethanol treatment. [Bódis, Karadi, Voros, Mozsik (1995b): Has sonicated *Helicobacter pylori* any direct cellular effect? In: Bajtai A., Papp J., Rác I., Simon L. (Eds.). *Helicobacter pylori*. Hungarian Society of Gastroenterology. Medicom, Budapest. pp. 137–142 (with kind permission).]

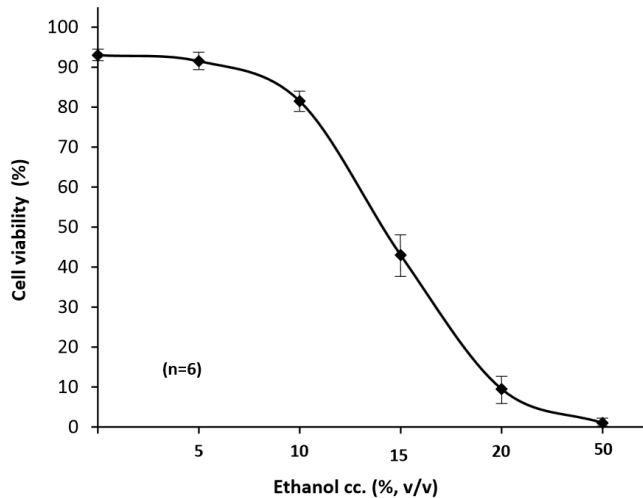


Figure 282. Changes in the viability of acutely (freshly) isolated rat gastric mucosal cells after 5 minutes incubation of 5–50% (v/v) ethanol detected by Trypan blue test. The $EC_{50}=13.5\%$. [Bódis et.al., (1996): *Dig.Dis.Sci.*41:430; Bódis, Németh, Mózsik (1998). Akadémiai Kiadó, Budapest (with kind permission).]

9.2.3.6.2. Combined effect of *H. pylori* and EtOH

Pre-incubation of cells with sonicated *H. pylori* (10^6 – 10^8 bacteria/mL) did not aggravate the 15% EtOH-induced cell injury detected by TB (Figure 282) and biochemical assays (Bódis et al., 1995b, 1996, 1997 a, b, 1998).

9.2.3.6.3. Combined effects of *H. pylori* and Indomethacin (IND)

After 5 minutes incubation of 10^{-8} – 10^{-3} M indomethacin, only high doses (10^{-4} – 10^{-3} M) of IND significantly decreased ($P < 0.01$) the number of viable cells (Figure 283).

When the IND and *H. pylori* (10^7 sonicated bacteria/mL) were applied in combination, the number of viable cells was not changed compared with IND treatments alone (Figure 283), and no changes were obtained by biochemical assays (Figures 284, 285).

9.2.3.6.4. Combined effect of EtOH and IND

Using these toxic agents, IND and all doses significantly aggravated the 15% EtOH-induced cell injury determined by Trypan blue exclusion (Bódis et al., 1995 a, b, 1996, 1998).

In these studies, the direct effects of sonicated *H. pylori* were examined under different experimental conditions (when it was given alone or in combination with EtOH and IND).

This model was suitable to eliminate the additional effect of immune systems, which is usually involved in a bacterial infection.

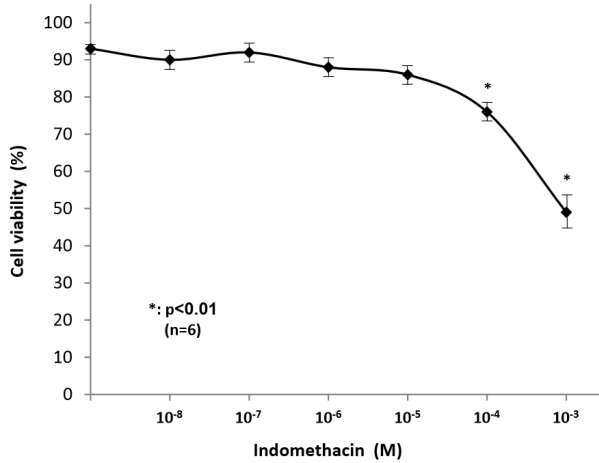


Figure 283. Changes in the viability of acutely (freshly) isolated rat gastric mucosal cells after 5 minutes incubation of 10⁻⁸–10⁻³ M indomethacin (IND) detected by Trypan Blue exclusion test. **P*<0.01 compared with untreated cells. [Bódis, Németh, Mózsik (1998). Akadémiai Kiadó, Budapest (with kind permission).]

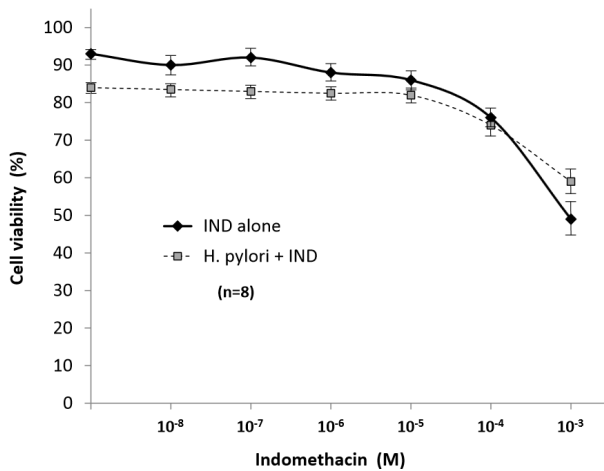


Figure 284. Effects of indomethacin (given in doses of 10⁻⁸–to 10⁻³ M concentrations) with and without co-administration of sonicated *H. pylori* (given in a dose of 10⁷ bacteria/mL together with all doses of indomethacin) on the viability of acutely (freshly) isolated gastric rat mucosal cells by Trypan Blue test. The incubation time was 30 minutes in all cases. [Bódis, Németh, Mózsik 1998. Akadémiai Kiadó, Budapest (with kind permission).]

We found that sonicated *H. pylori* had no direct cellular toxicity.

These types of observations with freshly isolated gastric mucosal cells from rat stomach were widely used to study the direct actions of various aggressive and protective compounds under experimental conditions.

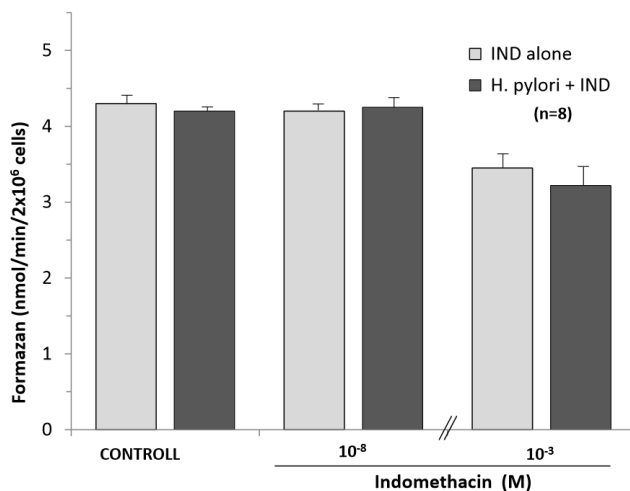


Figure 285. Effects of indomethacin (given in doses of 10^{-8} – 10^{-3} M concentrations) alone and in co-administration with sonicated *H. pylori* (given in 10^7 bacteria /mL together with all doses of indomethacin) on the succinate dehydrogenase activity of acutely (freshly) isolated rat gastric mucosal cells. The incubation time was 30 minutes in all cases. [Bódis, Németh, Mózsik (1998). Akadémiai Kiadó, Budapest (with kind permission).]

For the correct understanding, the results obtained with *H. pylori* (given alone or in combinations with EtOH or IND) have to be emphasized:

1. Rat (as animal strain) might not be the best model to study the possible gastric mucosal damaging mechanisms involved in *H. pylori* infection. Mongolian gerbil might be a better animal model to evaluate the mechanisms of *H. pylori*-induced pathology (Takahashi et al., 1998);
2. This *in vitro* model is an excellent model to study the direct cellular reactions of different chemical and hormonal, natural compounds (EtOH, IND, pentagastrin, histamine, body protective compound, BPC, etc.);
3. This experimental model is widely used in the research for preclinical selection of various drug candidates (Nagy et al., 1994);
4. No absolute correlation exists between the direct cellular damaging and defending effects of different biological agents and chemical compounds obtained in freshly isolated gastric mucosal cells and *in vivo* observations (Bódis et al., 1998).

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