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Antimicrobial effect of local anesthetics on Helicobacter pylori

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Abstract

Background/Aim: Helicobacter pylori (HP) is a gram-negative bacillus, with a prevalence of 50% throughout the world. HP infection is considered the strongest etiological factor for gastric cancer (GC). Therefore, early diagnosis and treatment of HP can be considered as the primary prevention strategy for the development of GC. This study aimed to reveal the antimicrobial effect of commonly used local anesthetics (LAs) on HP. The literature review in English has revealed no study on this subject.

Methods: This *in vitro* laboratory study was conducted in a University laboratory between 25 October 2019 and 1 November 2019. In the study, antimicrobial effect of 1 mL sterile saline, 20 mg/mL lidocaine, 1 mg/mL adrenaline, 10 mg/mL prilocaine, 50 mg/mL bupivacaine, and 20 mg+0.0125 mg/mL lidocaine plus adrenaline against *H. pylori* strain NCTC 11637 obtained from National Collection of Type Cultures (NCTC) were tested *in vitro*. The groups were designated as follows: Group C (Control), Group B (Bupivacaine), Group L (Lidocaine), Group A (Adrenaline), Group P (Prilocaine), and Group LA (Lidocaine plus adrenaline). HP strain NCTC 11637 was cultured in Mueller Hinton agar (Oxoid, UK) supplemented with 5% sheep blood plates at 37°C under microaerophilic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen gas combination) for 72 hours.

Results: Antimicrobial activity was observed in Group L and Group LA. No antimicrobial action against HP was observed in other groups. The comparison of groups in terms of inhibition zone diameters showed that there was a statistically significant inhibiting effect in Group L and Group LA compared to Group C (P=0.002 and P<0.001, respectively).

Conclusion: Among the LAs used in the present study, lidocaine and lidocaine + adrenaline had antimicrobial action against HP whereas bupivacaine and prilocaine had no statistically significant effect. Therefore, we believe that lidocaine can be used in the eradication therapy of HP. If the findings obtained from the present study are supported by clinical trials, lidocaine can help break the increasing resistance mechanisms in the treatment of HP or can be used in its treatment, which may help to improve treatment success and reduce treatment costs.

Keywords: Helicobacter pylori, Antimicrobial, Lidocaine, Adrenaline, Bupivacaine, Prilocaine

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Introduction

Helicobacter pylori (HP) is a gram-negative bacillus, with a prevalence of 50% throughout the world [1]. HP infection is considered the strongest etiological factor for gastric cancer (GC) [2]. In various studies, gastritis from a chronic active form to chronic atrophic form, intestinal metaplasia (IM), dysplasia, and gastric cancer (GC) due to HP have been reported to develop within 16–24 years [3]. Therefore, early diagnosis and treatment of HP can be considered as the primary prevention strategy for the development of GC.

The traditional treatment of HP is based on the combination of proton pump inhibitors (PPI) with dual antibiotics [1]. However, the development of drug resistance is reported after repeated treatments [4]. Eradication therapy of HP becomes more difficult due to increased antimicrobial resistance, leading to an increase in treatment costs [5]. Therefore, there is a need for alternative or supportive therapies that reduce antimicrobial resistance, facilitate treatment, and reduce costs.

The primary aim of this study was to reveal the antimicrobial effect of commonly used local anesthetics (LAs) on HP. The literature review in English has shown no study on this subject.

Materials and methods

Determination of *in vitro* antimicrobial effect

In the study, antimicrobial effect of 1 mL sterile saline, 20 mg/mL lidocaine, 1 mg/mL adrenaline, 10 mg/mL prilocaine, 50 mg/mL bupivacaine, and 20 mg+0.0125 mg/mL lidocaine plus adrenaline against *H. pylori* strain NCTC 11637 obtained from National Collection of Type Cultures (NCTC) were tested in an *in vitro* environment. The groups were designated as follows: Group C (Control), Group B (Bupivacaine), Group L (Lidocaine), Group A (Adrenaline), Group P (Prilocaine), and Group LA (Lidocaine+adrenaline). *H. pylori* strain NCTC 11637 was cultured onto Mueller Hinton agar (Oxoid, UK) supplemented with 5% sheep blood plates at 37°C under microaerophilic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen gas combination) for 72 hours. Colonies from these plates were suspended in sterile saline and a 2 McFarland turbidity standards suspension of each isolate was prepared. Each labeled Mueller-Hinton agar (Oxoid, UK) containing 5% sheep blood plate was uniformly seeded with a test organism by means of a sterile swab rolled in the suspension and streaked on the plate surface. *In vitro* antimicrobial activity of 1 mL sterile saline, 20 mg/mL Lidocaine, 1 mg/mL adrenaline, 10 mg/mL prilocaine, 50 mg/mL bupivacaine, and 20 mg/mL Lidocaine plus Adrenaline were evaluated by modified disc diffusion method with determination of inhibition zones. Each sterile disc (Merck, Germany) was impregnated with the anesthetics and dried. After dried, they were placed and incubated on Mueller-Hinton Agar containing 5% sheep blood for 72 hours at 37°C under microaerophilic conditions. The zone of inhibition was measured at the 72nd hour. Each experiment was repeated ten times **[6].**

Determination of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The broth microdilution method was used to determine the MIC values on brain heart infusion broth (BHIB) (Oxoid, UK) supplemented with 10% horse serum and 0.25% yeast extract using 96 well microplates (Hachem et al., 1996). The anesthetic agents were prepared by dilution in BHIB containing 10% horse serum and 0.25% yeast extract and 100, 80, 60, 50, 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 mg/mL concentrations of lidocaine, lidocaine plus adrenaline, prilocaine, bupivacaine, and adrenaline were tested. Anesthetics were added to the wells of a 96-well microplate containing HP strain NCTC 11637. Each test was repeated two times for each microplate. Microplates were incubated at 37°C under microaerophilic conditions for 72 hours (3 days). The OD600 (wavelength of 600 nm) was measured after 72-hour incubation by using Epoch spectrophotometer (BioTek Inst. Inc. Vermont, USA). Wells without anesthetic agents were used as growth control and wells with BHIB containing 10% horse serum and 0.25% yeast extract alone served as negative control. Amoxicillin and Clarithromycin were also tested for control (twofold serial dilution 16-0.002 mg/L).

To determine the MBC, each well exhibiting no visible growth (viability) after 72 hours was tested for viable organisms by subculturing 10 µL samples of each well onto Mueller-Hinton Agar containing 5% sheep blood. The plates were incubated at 37°C under microaerophilic conditions to observe the growth of any colony after 72 hours.

Statistical analysis

Descriptive statistics were used to define continuous variables (mean, standard deviation, minimum, median, and maximum). Two continuous independent variables not following normal distribution were compared using the Mann–Whitney U test whereas Kruskal–Wallis test was used for the comparison of more than two continuous independent variables not following normal distribution. A p value of <0.05 was considered statistically significant. Statistical analysis was performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013.

Results

Mean inhibition zone diameters of all groups are shown in Table 1.

Table 1: Mean inhibition zone diameters of groups

	Mean (SD)	Mean (min-max)
Control	0(0)	0(0-0)
Lidocaine	1.2(0.8)	1(0-2)
Lidocaine+Adrenaline	1.9(0.7)	2(1-3)
Bupivacaine	0(0)	0(0-0)
Prilocaine	0(0)	0(0-0)
Adrenalin	0(0)	0(0-0)

Kruskal Wallis test, P<0.001

Antimicrobial activity was observed in Group L and Group LA whereas no antimicrobial activity was observed against *H. pylori* in other groups. The comparison of groups in terms of inhibition zone diameters showed that there was a statistically significant inhibiting effect in Group L and Group LA compared to Group C (P=0.002 and P<0.001, respectively). No statistically significant difference was observed between Group L and Group LA (P=0.074).

Table 3: Distribution of MIC values and the effect of different concentrations of anesthetic agents against H.pylori NCTC 11637 strains

Lidocaine (mg/mL)	0.078	0.156	0.313	0.625	1.25	2.50	5	10	20	40	50	60	80	100
H. pylori NCTC 11637	+	+	+	+	+	+	+	+	+	+	+	+	-*	-
Lidocaine+adrenaline (mg/mL)	0.078	0.156	0.313	0.625	1.25	2.50	5	10	20	40	50	60	80	100
H. pylori NCTC 11637	+	+	+	+	+	+	+	+	+	+	+	-*	-	-
Bupivacaine (mg/mL)	0.078	0.156	0.313	0.625	1.25	2.50	5	10	20	40	50	60	80	100
H. pylori NCTC 11637	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prilocaine (mg/mL)	0.078	0.156	0.313	0.625	1.25	2.50	5	10	20	40	50	60	80	100
H. pylori NCTC 11637	+	+	+	+	+	+	+	+	+	+	+	+	+	-*
Adrenaline (mg/mL)	0.078	0.156	0.313	0.625	1.25	2.50	5	10	20	40	50	60	80	100
H. pylori NCTC 11637	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amoxicillin (mg/L)	0.002	0.004	0.008	0.016	0.032	0.064	0.128	0.256	1	1	2	4	8	16
H. pylori NCTC 11637	+	+	+	-*	-	-	-	-	-	-	-	-	-	-
Clarithromycin (mg/L)	0.002	0.004	0.008	0.016	0.032	0.064	0.128	0.256	1	1	2	4	8	16
H. pylori NCTC 11637	+	+	-*	-	-	-	-	-	-	-	-	-	-	-

*MIC values

A significant difference was observed between Group C and Group B, Group P, and Group A (P=1.00). Table 2 shows the comparison of inhibition zone diameters of the groups. The MIC values in all groups are shown in Table 3 in comparison with Amoxicillin and Clarithromycin. The MIC and MBC values in all groups are shown in Table 4 in comparison with amoxicillin and Clarithromycin.

Table 2: Comparison of inhibition zone diameters between groups

	P-value
Control vs Lidocaine	0.002
Control vs Lidocaine+Adrenaline	<0.001
Lidocaine vs Lidocaine+Adrenaline	0.074
Control vs Bupivacaine	1.00
Control vs Prilocaine	1.00
Control vs Adrenalin	1.00

*Mann Whitney u test

Table 4: MIC and MBC values anesthetic agents, amoxicillin and clarithromycin against *H.pylori NCTC 11637*

H. pylori NCTC 11637	MIC	MBC
Lidocaine (mg/mL)	80.00	-
Lidocaine+adrenaline (mg/mL)	60.00	-
Bupivacaine (mg/mL)	-	-
Prilocaine (mg/mL)	100.00	-
Adrenaline (mg/mL)	-	-
Amoxicillin (mg/L)	0.016	2
Clarithromycin (mg/L)	0.008	4

MIC: Minimum inhibitor concentration, MBC: Minimum bactericidal concentration

Discussion

HP is a major public health problem affecting about 52.1–58% of the worldwide population [5] as gastric and extragastric diseases are reported in the HP-associated broad spectrum. It has been reported to particularly cause peptic ulcer disease, gastritis, gastric atrophy, GC, gastric mucosa-associated lymphoid tissue lymphoma, idiopathic thrombocytopenic purpura, iron deficiency anemia, colorectal cancer, esophageal adenocarcinoma, metabolic syndrome, Alzheimer's disease, or glaucoma [2, 5, 7, 8]. Histopathological studies have reported that gastritis from a chronic active form to chronic atrophic form, IM, dysplasia, and GC due to HP develop within 16 to 24 years [3]. Therefore, early identification and eradication therapy of HP have become more important. However, antibiotic resistance in HP has been increasing in many parts of the world [2, 7]. Increased antimicrobial resistance leads to the search for new treatment strategies [5]. Considering the existence of major *H. pylori*-associated diseases, the importance of eradication therapy is understood. The present study has shown the antimicrobial effect of commonly used LAs on HP.

Among the LAs used in the present study, lidocaine and lidocaine plus adrenaline were found to have an antimicrobial action against HP whereas bupivacaine and prilocaine did not. There has been no significant difference between lidocaine and lidocaine plus adrenaline in terms of antimicrobial effect, however, MIC levels have been lower in the lidocaine plus adrenaline group, suggesting that lidocaine plus adrenaline combination may have a clinically significant contribution to reducing the risk of dose-related adverse effects. The uncertainty regarding its clinical use increases since this is the first study in English literature and an *in vitro* study. However, considering the increase in HP antimicrobial resistance, it is understood that there is a need for new treatment options due to the difficulty in treatment and increased costs. Therefore, we believe that demonstrating the contribution of lidocaine to HP treatment through clinical trials is of great importance.

Various antibiotic-resistance mechanisms have been described for HP. One of them is the reduction of bacterial membrane permeability [9]. In the literature, LAs have been reported to inhibit the growth of live bacteria, to reduce the membrane-dependent enzymatic activity of living cells, to cause lysis in protoplasts, to change the membrane permeability, and to cause ultrastructural alterations [10, 11]. Considering these effects of LAs, their antimicrobial effects on HP can be utilized and the mechanism of action on membrane permeability can contribute to the elimination of mechanisms of antibiotic resistance in HP.

Limitations

Its in vitro design is the limitation of the present study. Findings of this study should be supported by clinical trials.

Conclusion

HP infection is considered the strongest etiological factor for GC. Therefore, eradication therapy of HP can be preferred as the primary treatment strategy in the prevention of GC. Considering the existence of other major HP-related diseases, eradication therapy is of great importance. However, HP antibiotic resistance has been increasing in many parts of the world. One of the various antibiotic-resistance mechanisms in *H. pylori* is the reduction of bacterial membrane permeability. Due to the mechanism of action of LAs on membrane permeability, they can contribute to the elimination of mechanisms of antibiotic resistance in HP. Among the LAs used in the present study, lidocaine and lidocaine plus adrenaline have an antimicrobial effect on HP whereas bupivacaine and prilocaine have no statistically significant effect. Therefore, lidocaine is thought to be used in the eradication therapy of HP.

The findings obtained from the present study should be supported by clinical trials. Thus, lidocaine can be used to break the increasing resistance mechanisms in HP or be used in the

treatment of HP, which may help to improve treatment success and reduce treatment costs.

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