THE PROTECTIVE EFFECTS OF KEFIR IN ASPIRIN-INDUCED GASTRIC MUCOSAL DAMAGE: AN EXPERIMENTAL STUDY

GURSEL A. ACARTURK1, ALTUG SENOL1, METE AKIN2, RECEP SUTCU1, ONDER SAHIN1, MEHMET ISLER3
1Department of Gastroenterology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar - 2Department of Gastroenterology, Faculty of Medicine, Suleyman Demirel University, Isparta - 3Department of Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta - 4Department of Pathology, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

ABSTRACT

Aims: Aspirin has a side effect of mucosal damage even at low doses. The aim of the present study was to investigate the efficacy of kefir in the prevention of gastric damage produced by aspirin.

Materials and methods: In the present study, 32 male Wistar-Albino rats were divided into four equal groups. First group (Control) and 2nd group (Aspirin) were administered 2 ml serum physiologic for seven days. Third group (Kefir) and 4th group (Kefir + Aspirin) were administered 2 ml kefir solution instead of serum physiologic. On the eight day, aspirin and Kefir + Aspirin groups were administered 200 mg/kg aspirin, three hours before being sacrificed. All stomach mucosa was examined and mucosal damage scores were evaluated.

Results: In Kefir + Aspirin group, macroscopic damage score was higher than Control (P=0.002) and Kefir (P=0.028) groups, and lower than Aspirin group (P=0.005). Histological damage scores in Kefir + Aspirin group were similar to those in control and kefir groups and significantly lower than Aspirin group (P<0.001).

Conclusion: It was established that kefir prevents aspirin-induced gastric damage in experimental model.

Key words: Aspirin, kefir, mucosal damage, probiotic.

Received February 18, 2014; Accepted March 24, 2014

Introduction

Aspirin (ASA) exerts antiaggregant effect in cardiovascular diseases by inhibiting cyclooxygenase-1. However, ASA may decrease levels of gastroprotective prostaglandin (PG) at doses as low as 10 mg/day, leading to possible damage in gastric mucosa and consequential gastrointestinal bleeding. Therefore, the prevention of gastric mucosal damage associated with ASA is clinically important.

Probiotics are food products that contain living microorganisms and exert beneficial effects on health when consumed in adequate amounts. Kefir is a complex probiotic consisting of various lactic acid bacteria and yeast. It has favorable effects in gastrointestinal system diseases such as antibiotic associated diarrhea and functional constipation.

At present it is an industrial product that is increasingly used due to its positive impact on health.

The studies addressing the effect of probiotics on gastric mucosa mostly focus on suppression of Helicobacter pylori (Hp) by probiotics and decrease in gastritis activity associated with Hp. However, the main causes of the formation of gastric mucosal injury are Hp infection and consumption of nonsteroidal anti-inflammatory drugs (NSAIDs). There are few studies regarding the effect of probiotics in preventing gastric mucosal injury due to ASA. In addition, the effect of kefir on gastric mucosal damage associated with ASA, is not known.

The aim of the present study was to investigate the efficacy of kefir in the prevention of gastric damage produced by ASA.
Materials and methods

The study was carried out upon approval of animal experiments local ethics committee. In the present study, 8-12 week old male 32 wistar-Albino rats, weighing between 156-246 g, were used. Rats were obtained one week before experiment and their adaptation to environment was ensured. Care of the rats was made in accordance with “Guideline for the care and use of laboratory animals” prepared by Laboratory animals source institute and issued by National Institute of health and with “Care principles of experimental animals” developed by national association for medical investigations. They were cared in experimental animals laboratory in rooms which have clean air and at 22-24 degrees temperature under seasonal day light rhythm and with 50-60% humidity in special cages. Rats were fed with rat pellet food and tap water.

Preparation of kefir: Lactobasillus ssp., Leuconostoc ssp, Lactococcus lactis ssp. lactis, Streptococus thermophilus and kefir yeast were present in commercial kefir culture branded Danisco®, Poland. This culture was mixed with sterile milk and kefir was obtained by keeping it for 16 hours in autoclave at 26 ºC.

32 rats were randomized into four equal groups one day before the experiment.

Control group (A) (n = 8): They were fed with standard rat pellet food and tap water for seven days and were administered 2 ml serum physiologic once a day between 9-10 a.m. via gastric gavage. On the eighth day, they were administered 2 ml serum physiologic and were sacrificed with the administration of intramuscular 100 ml/kg ketamine hydrochloride (Ketalar®, Parke-Davis, Eczacibasi, Istanbul, Turkey) and 25 mg/kg xylazine hydrochloride (Rompun®, Bayer, Germany) and their stomachs were removed.

ASA group (B) (n = 8): In addition, on the eight day, they were administrered ASA at the dose of 200 mg/kg and then sacrificed at the third hour.

Kefir group (C) (n = 8): They were administered 2 ml kefir once a day between 9-10 a.m. via gastric gavage instead of serum physiologic.

Kefir + ASA group (D) (n = 8): They were administered 2 ml kefir once a day between 9-10 a.m. via gastric gavage instead of serum physiologic. On the eighth day, they were administered ASA at the dose of 200 mg/kg and then sacrificed at the third hour.

Macroscopic examination: After the rats were sacrificed, their stomachs were marked in a way that their esophageal and duodenal ends would not be confused and removed. With a section towards greater curvature, stomach was opened from cardia to pylorus. Inner aspect of the stomach was washed with SF saline and its sides were fixed with toothpick on milimetric paper. Mucosa was examined macroscopically for hemorrhage, erosion and ulcer, and digital picture of each stomach was taken. Damage score of mucosa was performed according to the definition of Coleman et al (11).

Histopathological examination: After macroscopic examination, tissue sample at the size of approximately 1 cm2 was obtained from corpus region. Stomach tissue underwent fixation with 10% buffered formalin following dehydration steps, tissue samples were blocked with paraffin. Four μm thick section were obtained with microtome. And sections were stained with hematoxylin-eosin and preparations were covered with entallan. Sections were examined with light microscope (Nicon Eclipse E600W, Tokyo, Japan). In sections, the severity of mucosal damage and inflammation, changes in vessels, intramucosal hemorrhage and glandular cell necrosis and sloughing severity scores were evaluated (12).

Statistical analysis

Variables were expressed as mean ± standard standart deviation or as median (interquartile range; 25-75%). Paired t test, One Way ANOVA, Mann Whitney U test and Kruskal Wallis test were used to compare variables. P value of < 0.05 was considered as statistically significant.

Results

No significant difference was found between baseline and final weights of rats, which were respectively 196.3 ± 23.0 g vs 194.9 ± 21.6 g. Weight changes during experiment was not significant among study groups.

Macroscopic evaluation

There were significant difference among groups in terms of macroscopic mucosal damage score (P < 0.001) (Table 1).

Inner aspect of the stomach was completely normal in control group. In ASA group, normal stomach mucosa was not observed (as shown in Fig. 1) and mucosa damage score was 4.00 (3.00-
4.75) [median (25-75%)]. In Kefir group, mucosa was generally normal with mild findings such as edema, congestion and bleeding in two specimens. In Kefir + ASA group mucosa was damaged less than ASA group (as shown in Fig. 2), and mucosal damage score was 1.00 (1.00-2.75).

Table 1: Comparison of macroscopic and microscopic damage between groups.

<table>
<thead>
<tr>
<th>Data</th>
<th>Control (n=8)</th>
<th>ASA (n=8)</th>
<th>Kefir (n=8)</th>
<th>Kefir+ASA (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic Mucosal Damage</td>
<td>0.00 (0.00-0.00)</td>
<td>4.00 (1.00-4.75)</td>
<td>0.00 (0.00-0.75)</td>
<td>1.00 (1.00-2.75)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: The distribution of histological damage scores in study groups.

In ASA group, marked mucosal damage and glandular epithelium necrosis, marked vascular changes and intramucosal hemorrhage and pronounced inflammation were present. In Kefir ± ASA group, histological damage was found to be lower than that in ASA group.

Discussion

In the present study, it was demonstrated that the use of kefir prevented the formation of gastric damage associated with ASA in rat according the evaluation of macroscopic lesions of the stomach and histological damage scoring.

The most important effect of kefir and other probiotics on GIS gastrointestinal tract is the regulation of mucosa by suppression of pathogens bacteria[9]. They attract attention due to their positive effects on Lactose intolerance, antibiotic associated diarrhea, diarrhea related to Clostridium difficile, rotavirus enteritis, functional constipation, pouchitis treatment, and maintenance of the remission of...
ulcerative colitis, and their effect on various GIS disorders is being investigated\(^\text{[16,35]}\).

Some of studies investigating the effect of probiotics on Hp eradication and Hp associated gastritis. In this context, it was reported that; L. Acidophilus and L. casei subsp. Rhamnosus inhibit Hp via lactic acid and lactic acid products\(^{[16]}\), and that L. Johnsonii decreases IL-8, proinflammatory cytokine, and lymphocytic infiltration and neutrophilic infiltration in gastric lamina propria\(^{[17]}\). In clinical studies, it was reported that in Hp positive subjects, fermented milk with L. Johnsonii added to clarithromycin monotherapy decreases the activity of Hp associated gastritis\(^{[18]}\), and its long term use increases gastric mucus thickness and decreases severity of gastric inflammation and Hp density\(^{[19]}\).

However, in the development of gastric mucosal damage, while one main cause is Hp infection, the other cause is the consumption of NSAIDs\(^{[20]}\). Gastric mucosal damage caused by aspirin is essentially the consequence of gastroprotective prostaglandins due to inhibition of cyclooxygenase-1. In addition, due to unionized weak acidic characteristics, they freely penetrate gastric barrier and damage tight connections in basolaterals of mucosal cells\(^{[21]}\). According to several studies, probiotic use decreases gastric mucosal damage associated with NSAIDs, and gastroprotective effects of probiotics are explained with various mechanisms such as decrease in mucosal permeability, regulation of systemic or local immune response, local and systemic effects of exopolysaccharide produced by them, and increase in mucosal PGE2, mucus and HCO3 synthesis\(^{[22-25]}\).

In the present study, kefir was started to be administered seven days before the development of gastric model and it was discontinued one day before they were sacrificed and gastroprotective characteristics of kefir was demonstrated by macroscopic and histological investigations.

Kefir culture used in the present study had, Lactobacillus ssp., Leuconostoc ssp, Lactococcus lactis ssp. lactis, Streptococcus thermophilus and also kefir yeast was present. Gastroprotective properties of exopolysaccharides synthesized by Streptococcus thermophilus were previously reported in two studies\(^{[24,25]}\). In one of these, similar to present study, fermented milk containing Streptococcus thermophilus was given for long term before ASA and gastroprotective effect was explained by the reduction of IFN interferon and increase in IL-10 by fermented milk, i.e. by its immunmodulatory effect\(^{[24]}\). Although the methodology of the present study was not based upon the investigation of potential immunomodulatory effect of kefir, gastroprotective effect found with use of kefir may be associated with its regulation of immune response, increase in gastric mucus thickness or reduction of gastrointestinal permeability. We believe that whether kefir influences, proinflammatory or anti-inflammatory cytokines should be investigated with properly performed further studies.

In conclusion, decreased gastric mucosal damage related to ASA with kefir was demonstrated in this experimental study. In the literature, there are few studies investigating the gastroprotective effects of probiotics against NSAIDs. However, to our knowledge, this is the first study in the literature investigating the gastroprotective effect of kefir, which is one of the oldest traditional probiotics. When trying to produce probiotics including strains effective on gastric damage associated with H. pylori gastritis and NSAIDs, it should be borne in mind that kefir includes many strains depending on its production conditions and is available in daily life. Therefore, gastroprotective effect of kefir were demonstrated experimentally in the present study, should be investigated through clinical studies in order to be translated into medical practice.

References

The protective effects of Kefir in aspirin-induced gastric mucosal damage...


21) Gill HS, Ratherfur DJ, Prasad J, Gopal PK. Enhancement of natural and acquired immunity by Lactobacillus rhamnosus (HN001), Lactobacillus acidophilus (HN017) and Bifidobacterium lactis (HN019). Br J Nutr 2000; 83: 167-76.


