The role of nitric oxide in gastric protection by honey

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Abstract Objectives: To investigate the role of nitric oxide in the protective mechanism of honey against ethanol-induced gastric lesions in rats.
Setting: Department of Medical Pharmacology, College of Medicine, King Saud University, Riyadh, Saudi Arabia.
Materials and methods: Male wistar rats were used in all experiments. Honey orally and drugs intraperitoneally were administered to 24-hour fasted rats 30, 45 or 60 minutes before oral administration of one ml ethanol. Gastric mucosal lesions were assessed one hour after ethanol by modification of the method of Schiantarelli et al, 1984. The severity of gastric lesions was analyzed statistically either using unpaired Student's t-test or by analysis of variance.
Results: Pre-treatment with NG-nitro-L-arginine methyl ester (12.5-5-50 mg/kg) which is reported to inhibit nitric oxide synthesis in various cells, dose dependently aggravated ethanol-induced gastric lesions. The enhancement of lesions was time dependent and maximum effect occurred if it was given 45 minutes before ethanol. Pre-treatment with honey (1.25 mg/kg) afforded protection against ethanol-induced lesions by 78%. Pre-treatment with NG-nitro-L-arginine methyl ester 12.5 and 25 mg/kg brought down the protective effect of honey to 63% and 43% respectively. The attenuating effect of NG-nitro-L-arginine methyl ester (25 mg/kg) on honey-induced gastric protection was reversed by L-arginine (200 mg/kg) but not by D-arginine (200 mg/kg).
Conclusion: The study suggests that NG-nitro-L-arginine methyl ester aggravated gastric lesions by inhibiting biosynthesis of nitric oxide and the gastric protection by honey may be due to modulation of nitric oxide system since pre-treatment with NG-nitro-L-arginine methyl ester reduced the protective effect of honey which was reversible by L-arginine but not by D-arginine.

Keywords: Ethanol; honey; nitric oxide; NG-nitro-L-arginine methyl ester.

Natural honey (honey) is widely available throughout the world and has formed an integral part of traditional medicine for centuries. Unfortunately, the therapeutic potential of honey is grossly underutilized in modern medicine due to lack of systematic scientific study. However, interest is gradually growing on the therapeutic effectiveness of honey. Thus scientific support on the effectiveness of honey in several experimental and clinical conditions is beginning to emerge. Although honey has been shown to prevent experimentally-induced gastric lesions in rats, the mechanism of its protective effect has not been fully elucidated.

Nitric oxide (NO) which is biosynthesised from the amino acid L-arginine has recently been attributed to the gastroprotective effect of antulcerogenic drug, sucralfate. Furthermore it has been found that honey possesses many of the features of sucralfate in the protection against antral ulcers in rats. In view of this, we investigated the possible role of endogenous NO in the protective mechanism of honey against ethanol-induced gastric lesions in rats. For this purpose, NG-nitro-L-arginine methyl ester (L-NAME), an established inhibitor of NO biosynthesis in various tissues including the stomach, L-arginine, the substrate for NO synthase and D-enantiomer, D-arginine were used. Some of the preliminary results have been communicated at the International Conference on Nitric Oxide held in Los Angeles.

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Materials and methods

Materials Natural honey was obtained from Abha region of the Kingdom of Saudi Arabia. The constituents of honey used were identical to that of published reports for pure honey.\(^n\) Absolute ethanol (BDH, UK) NG-nitro-L-arginine methyl ester (L-NAME), L-arginine and D-arginine were purchased from Sigma Chemical Co., UK. L-NAME, L-arginine and D-arginine were dissolved in distilled water. All agents were administered by intraperitoneal (i.p.) route. Ethanol and honey were given orally (p.o.).

Animals Male Wistar rats, of approximately same age, weighing between 220 and 250 g were obtained from the Animal House, College of Medicine, King Saud University, Riyadh. The rats were housed individually in metabolic cages with a shield to prevent coprophagy. The animals were fasted for 24-hours before the experiments but water was allowed ad libitum. Following 24-hour starvation, the rats were randomly coded in groups of 6-10 animals, and water was withdrawn. The groups were then subjected to various treatment schedules as detailed below.

Methods

Study 1: Control groups received water (1 ml/kg i.p.) and test groups were given different doses of L-NAME (12.5, 25 and 50 mg/kg i.p.) 45 minutes before p.o. administration of one ml ethanol.

Study 2: L-NAME (25 mg/kg i.p.), L and D-arginine (each 200 mg / kg i.p.) were administered, 15, 30, 45 and 60 minutes before p.o. administration of one ml ethanol.

Study 3: Honey (1.25 mg/kg p.o.) and/or L-NAME (12.5 or 25 mg/kg i.p.) and/or L and D-arginine (each 200 mg/kg i.p.) were administered 30, 45 and 60 minutes before p.o. administration of one ml ethanol, respectively.

In all studies, animals were killed one hour after ethanol by cervical dislocation and stomachs were dissected out, opened along the greater curvature and randomized so that the examiner had no knowledge of the treatment given. The severity of the gastric mucosal lesions was scored by a modification of the method of Schiantarelli et al., according to the following arbitrary scale: 0 = normal mucosa; 1 = hyperemic mucosa; or up to 3 small patches; 2 = from 4 to 10 small patches; 4 = more than 10 small patches; 6 = up to 6 medium patches; 8 = more than 6 medium of up to 3 large patches; 10 = from 4 to 6 large patches; 12 = from 7 to 10 large patches; 14 = more than 10 large patches; 16-20 = extensive covering of the whole mucosa with hemorrhages.

“Small” was defined as up to 2 mm; “medium” as between 2 and 4 mm and “large” as more than 4 mm across. The sum of the scores in each group of rats was divided by the number of animals and expressed as mean lesion score. The percentage decrease or increase of lesions was calculated by comparing with the appropriate control values.

Statistical analysis The results are expressed as mean ± SEM. The significance of difference between means was calculated by Student’s t-test for unpaired data when comparing two groups and analysis of variance (ANOVA) followed by Kruskal-Wallis test for multiple comparisons. A probability of p<0.05 was considered significant.

Results The effect of different doses of L-NAME given at a fixed time before administration of ethanol is presented in Table 1. Administration of ethanol alone produced multiple hemorrhagic lesions. These lesions were characterized by red patches of different sizes along the long axis of glandular stomach. Pre-treatment of rats with L-NAME dose dependently aggravated these lesions and that at a dose of 50 mg/kg, caused a rise of 48% above that achieved with ethanol alone.

<table>
<thead>
<tr>
<th>Drug and dose</th>
<th>Lesion score ± s.e.m.</th>
<th>% increase (+) or decrease (−) of lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>12.3 ± 0.8</td>
<td>−</td>
</tr>
<tr>
<td>L-NAME 12.5 mg/kg</td>
<td>14.1 ± 0.7</td>
<td>+ 15a</td>
</tr>
<tr>
<td>L-NAME 25.0 mg/kg</td>
<td>17.5 ± 0.8a*</td>
<td>+ 42a</td>
</tr>
<tr>
<td>L-NAME 50.0 mg/kg</td>
<td>18.2 ± 0.4a</td>
<td>+48a</td>
</tr>
</tbody>
</table>

* compared to control; * p<0.05. Student’s t-test

Table 2 shows the effect of L-NAME (25 mg/kg i.p.), L or D-arginine (each 200 mg/kg i.p.) given at different times before ethanol. The aggravation of lesions was maximum if L-NAME was given 45 minutes before ethanol. However, there was also significantly higher values when L-NAME was given 30 minutes before ethanol. L-arginine or
D-arginine did not cause any significant changes of ethanol-induced gastric lesions if given 30 or 60 minutes before ethanol.

**Table 2 - Effect of L-NAME (25 mg/kg i.p.) or L or D-arginine (each 200 mg/kg i.p.) given at different times before oral administration on ethanol-induced gastric lesions (rats were killed 1 hr after ethanol).**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Time in minutes</th>
<th>Lesion score ± s.e.m.</th>
<th>% increase (+) or decrease (-) of lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td></td>
<td>11.8 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>L-NAME</td>
<td>15</td>
<td>12.2 ± 0.4</td>
<td>+03a</td>
</tr>
<tr>
<td>L-NAME</td>
<td>30</td>
<td>14.6 ± 0.8a</td>
<td>+24a</td>
</tr>
<tr>
<td>L-NAME</td>
<td>45</td>
<td>16.7 ± 0.7a</td>
<td>+42a</td>
</tr>
<tr>
<td>L-NAME</td>
<td>60</td>
<td>13.1 ± 0.7</td>
<td>+11a</td>
</tr>
<tr>
<td>L-arginine</td>
<td>30</td>
<td>11.3 ± 0.8</td>
<td>-04a</td>
</tr>
<tr>
<td>L-arginine</td>
<td>60</td>
<td>10.7 ± 1.1</td>
<td>-09a</td>
</tr>
<tr>
<td>D-arginine</td>
<td>60</td>
<td>11.6 ± 0.9</td>
<td>-02a</td>
</tr>
</tbody>
</table>

*compared to control: *p<0.05. Student’s t-test

**Discussion** The results of this study demonstrate that NO biosynthesis inhibitor, L-NAME, causes enhancement of ethanol-induced gastric lesions and reduces the gastroprotective effects of honey.

The pathogenesis of ethanol-induced gastric lesions is complex. However, depletion of non-protein sulfhydryls (NP-SHs) concentration, modulation of NO system, reduction of microvascular circulation and mast cell degranulations besides other factors have been suggested to be involved in the pathogenic mechanisms. Therefore, it might be expected that interference with any of these mechanisms may reduce the severity of lesions.

Recent data suggest a role for endogenous NO in the gastroprotection provided by sucralfate, aluminium containing antacids and morphine. In the present study, pre-treatment with L-NAME, a widely used NO biosynthesis inhibitor dose dependently aggravated ethanol-induced lesions which is consistent with previous reports suggesting a possible regulatory role of NO in the induction of ethanol-induced gastric lesions. Although the duration of action of L-NAME following its administration by different routes is not known, the severity of enhancement of ethanol-induced gastric lesion is found to be time dependent and the maximum effect occurs if it is given 45 minutes before ethanol. This may signify its possible short duration of action after i.p. route. Thus careful consideration regarding the route and time of administration should be taken into account in investigating the effect of L-NAME on this test system. On the other hand, administration of L-NAME before honey dose dependently produces reduction of gastric protection afforded by honey. Moreover, the attenuating effect of L-NAME on gastric protection elicited by honey is completely reversible by prior administration of substrate of NO synthase, L-arginine but not by D-arginine. Furthermore, the reduction of the effect of honey by L-NAME is not due to the difference of severity of lesions since a dose of L-NAME which does not enhance ethanol-induced gastric lesions also reduces the protective effects of honey indicating the specificity of blocking effect.

Failure of L-arginine alone to cause significant reduction in ethanol-induced gastric lesions agrees well with the previous finding of Konturek et al. However, it does not preclude the involvement of NO in the protective mechanism of honey since combined treatment with honey plus L-arginine reverses the blocking effect of L-NAME. Furthermore, modulation of nitric oxide system is the only one of the components in ethanol-induced gastric lesions. Thus the effect of L-arginine may...
not be evident as a protective agent as the other components remain unopposed. Moreover, the effect of L-arginine may only be prominent when there is sufficient inhibition of NO biosynthesis following the use of an inhibitor.

Taken all together, these findings suggest that L-NAME aggravates gastric lesions by inhibiting mucosal biosynthesis of NO and honey provides protection by modulation NO system which has recently been implicated in the maintenance of mucosal blood flow and gastric integrity.4,31,33

The mechanism by which honey regulates mucosal NO level and affords protection is not known. However, L-NAME attenuated but did not abolish completely the protective effects of honey which may indicate the involvement of other factors. Indeed, it has been reported previously34 that honey prevents ethanol-induced depletion of NP-SHs levels and the gastric protection effect of honey has been suggested to be mediated through SH-sensitive processes. Similarly, endogenous sulphydryls have also been implicated in the gastroprotective effects of sulcrate.4 Thus, maintenance of a critical level of gastric endogenous SHs compound appeared to be important for the gastric mucosa to resist challenge with ethanol.

It appears that the gastric protection afforded by honey against ethanol-induced lesions may be due to the combined beneficial effects exerted by restoration of both NO and NP-SHS levels. Both NO and NP-SHS may cause stabilization of mast cell membranes35,36 and increase in mucosal blood flow4,33,31,33,37 which would maintain mucosal integrity.

In summary, the protective mechanisms of honey against ethanol-induced gastric lesions seems to be mediated through the modulation of endogenous NO system since L-NAME-induced reduction of the protective effects of honey is reversible by the substrate of NO synthase, L-arginine but not by D-arginine. Furthermore, a dose of L-NAME which does not cause significant aggravation of ethanol-induced lesions also reduces honey-induced gastric protection indicating the specificity of blocking effect of L-NAME. However, further biochemical and histopathological studies are warranted to define the role of nitric oxide in the protective mechanism of honey.

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References


ملخص البحث:

الهدف: دراسة أهمية أكسيد النتريك في دور العسل في الحماية من حدوث القرحة المعدية المستحدثة بواسطة الكحول معمليا في الجرذان.

المكان: قسم علم الأدوية الطبي، كلية الطب، جامعة الملك سعود، الرياض، المملكة العربية السعودية.

التصميم: تم استخدام فرحة معدية بواسطة حاف (1 ملليتر) من الكحول عن طريق الفم إلى الجرذان التي منع منها الطعام لمدة 24 ساعة سابقة، وتم إعطاء هذه الجرذان جرعات من العسل من خلال الفم لمدة 30 أو 45 أو 60 دقيقة قبل استحداث القرحة بواسطة الكحول، وكذلك حفنت داخل الصفاق مشتبثات إفراز مادة أكسيد النتريك.

النتائج: تبين أن حقن مادة النتريوكجينين (12.5 – 2.5 ملجم/كجم) والمعروف بأنها إحدى مشتبثات إفراز أكسيد النتريك في الخلايا أدى إلى زيادة ملحوظة في حدة القرحة، خاصة بعد مرور 45 دقيقة من الوقت وقد لوحظ أن إعطاء العسل (1.25 ملجم/كجم) للجرذان قبل الكحول أدى إلى حمايتها من حدوث قرة المعدة بمقدار 78.78%، ولكن عندما تم حقن جرعتين من النتريوكجينين (25 و 25 كجم/كجم) ادى ذلك إلى نقص من مقدار حماية العسل من القرحة بنسبة 53% و 43% على التوالي.

هذا النقص في الحماية بواسطة النتريوكجينين تم عكسه بواسطة مادة الـ نتريوكجينين (200 ملجم/كجم) وليس د أرجينين.

الخلاصة: أن إفراز أكسيد النتريك في المعدة قد يكون له دوراً مهماً في مقدرة العسل على حماية معدة الجرذان من القرحة المستحدثة بواسطة الكحول.