Effect of Nicotine, Alcohol and Caffeine Pretreatment on the Gastric Mucosal Damage Induced by Aspirin, Phenylbutazone and Reserpine in Rats

Narayan S. PARMAR, Mohammad TARIQ and Abdulrehman M. AGEEL
College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Saudi Arabia
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Abstract—The effects of nicotine (2.5 mg/100 ml), alcohol (25% v/v) and caffeine (30 mg/100 ml base) and their combination (nicotine, 2.5 mg/100 ml; alcohol, 25% v/v; and caffeine, 30 mg/100 ml base) fed in drinking water ad libitum for 21 days were studied on the gastric mucosal damage induced by aspirin, phenylbutazone and reserpine in rats. When given alone, none of them produced any visibly discernible gastric lesions. Their concurrent administration, however, produced some injury to the gastric mucosa which was far less severe than the lesions induced by any of the ulcerogenic drugs used in this study. Pretreatment with nicotine, alcohol and caffeine and their combination resulted in a significant augmentation of gastric lesions produced by aspirin, phenylbutazone and reserpine. These results establish an association between nicotine, alcohol and caffeine in the pathogenesis of gastric ulcers and also implicate them as modifying factors in the genesis of gastric lesions induced by aspirin, phenylbutazone and reserpine.

Nicotine has been considered to be responsible for many pharmacological effects produced by tobacco smoking (1) including its potential to produce and aggravate peptic ulcer disease (2, 3). Recently, we have shown that nicotine feeding ad libitum in drinking water for 10 days could exacerbate the gastric lesions produced by aspirin and reserpine (4). However, it is also possible that nicotine may exert its effect on the gastric mucosa in association with other agents. Alcohol and caffeine consumption have been associated with cigarette smoking (5) and also with the pathogenesis of peptic ulcer in man (6). Habituation to each of them is widespread, and many people have the dubious distinction of consuming all three substances simultaneously. It was therefore considered worthwhile to study the effect of nicotine, alcohol and caffeine given alone and in combination on the gastric lesions induced by aspirin, phenylbutazone and reserpine.

Materials and Methods
Wistar albino rats of either sex, approximately of the same age, weighing 200–220 g and fed on standard chow diet were used. They were divided in groups of 6–10 animals each. The distribution of the animals in groups, the sequence of the trials and the treatment allotted to each group were randomized. The following groups were constituted:

- Group 1. Control (Normal)
- Group 2. Nicotine control
- Group 3. Alcohol control
- Group 4. Caffeine control
- Group 5. Nicotine+alcohol+caffeine
- Group 6. Aspirin alone
- Group 7. Nicotine+aspirin
- Group 8. Alcohol+aspirin
- Group 9. Caffeine+aspirin
- Group 10. Nicotine+alcohol+caffeine+aspirin
- Group 11. Phenylbutazone alone
- Group 12. Nicotine+phenylbutazone
- Group 13. Alcohol+phenylbutazone
- Group 14. Caffeine+phenylbutazone
- Group 15. Nicotine+alcohol+caffeine+phenylbutazone
- Group 16. Reserpine alone
Group 17. Nicotine + reserpine
Group 18. Alcohol + reserpine
Group 19. Caffeine + reserpine
Group 20. Nicotine + alcohol + caffeine + reserpine.

The animals in groups 2, 7, 12 and 17 were treated with nicotine bitartrate (BDH) in their drinking water (25 µg/ml) available ad libitum through 100 ml Kimax drinking tubes.

Groups 3, 8, 13 and 18 were treated with 25% v/v ethanol in their drinking water given ad libitum according to the method described by Oei et al. (7).

Groups 4, 9, 14 and 19 were treated with 60 mg/100 ml caffeine sodium benzoate in their drinking water given ad libitum which was equivalent to 30 mg/100 ml of caffeine base.

Groups 5, 10, 15 and 20 were given the combination of 25 µg/ml of nicotine bitartrate, 25% v/v ethanol and 60 mg/100 ml of caffeine sodium benzoate in the drinking water available ad libitum.

The daily intake of water, nicotine, alcohol, caffeine and their combination in solutions were recorded. The treatments continued for a period of 21 days.

Experimental gastric lesions: Following the treatments as outlined above, the animals were starved for 24 hr with access to water ad libitum before subjecting them to one of the procedures mentioned below:

Aspirin: Aspirin (Bayer) was suspended in 1% carboxymethyl-cellulose in water 20 mg/ml) and administered orally in the dose of 200 mg/kg (1 ml/100 g). Four hours after the aspirin administration, the animals were sacrificed (8).

Phenylbutazone: Phenylbutazone (Geigy) was administered s.c. as an aqueous solution (20 mg/ml) of the sodium salt. Two doses of 100 mg/kg were given at an interval of 15 hr. Six hours after the second dose, the animals were sacrificed (9).

Reserpine: Reserpine (CIBA) was administered in a dose of 5 mg/kg, i.m., and the animals were sacrificed 24 hr later. Twenty-five mg of reserpine with 25 mg of citric acid was dissolved in 0.2 ml of benzyl alcohol by slight warming. To this solution 1 ml of polysorbate 80 was added, and the volume was made up to 10 ml with distilled water (10).

The solutions of the ulcerogenic drugs were freshly prepared before injections. The animals were sacrificed using anaesthetic ether, the stomach was removed, opened along the greater curvature, washed with saline and examined with a 6.4× binocular magnifier. Lesions were assessed by two observers unaware of the experimental protocols. Gastric lesions induced by all three drugs used in this study were multiple in each stomach. They were evaluated singly according to their dimensions and severity, and they were scored between 0 (no visible ulcers) and 10 (deep lesions with diameter greater than 8 mm) in each stomach. The scores for each single lesion were then summed up so that the total score per stomach far exceeded the value of ten (11).

The results refer to the average lesion score±S.E.M. Statistical analysis of the severity of gastric ulcers was done by Student’s t-test.

Results

The average daily intakes of different fluids including water, nicotine, alcohol, caffeine and the combination of nicotine, alcohol and caffeine at 5 day intervals during the study have been summarized in Table 1. The average fluid intake was almost the same in the control, nicotine and caffeine fed animals, but it markedly declined in the alcohol and the combination of nicotine, alcohol and caffeine fed animals. The intake of alcohol markedly declined during the first few days of the pretreatment with the combination of nicotine, alcohol and caffeine. Thus the consumption of nicotine and caffeine also remained low in the combined pretreatment group as the volume of fluid intake decreased due to the presence of alcohol.

There was no visibly discernible evidence of any inflammation or cellular abnormality denoting gastric mucosal damage in any of the specimens from the normal, nicotine, alcohol or caffeine pretreated groups. The group treated with the combination of nicotine, alcohol and caffeine showed the presence of few lesions in the glandular region (Table 2).

The administration of aspirin, phenyl-
butazone and reserpine resulted in the production of gastric mucosal damage mainly in the glandular segment of the stomach in 100% of the animals. The mean scores for the intensity of lesions for aspirin, phenylbutazone and reserpine were 23.20±2.16, 28.70±2.94 and 24.50±2.38, respectively. There were no statistically significant differences between these groups. They showed small to medium sized lesions that were 2-4 mm in diameter, the haemorrhagic lesions being more common in the aspirin treated rats.

Table 1. Average daily intake of fluids: water, nicotine, alcohol, caffeine and combination of nicotine, alcohol and caffeine in ml recorded at 5 day intervals during the study

| Period (day of the study) | Drinking water | Nicotine (25 μg/ml in drinking water) | Alcohol (25% v/v in drinking water) | Caffeine sodium benzoate (60 mg % in drinking water) | Nicotine+Alcohol+Caffeine (in drinking water) |
|--------------------------|----------------|---------------------------------------|------------------------------------|-----------------------------------------------------|---------------------------------------------|
| 1                        | 48.80          | 44.50                                 | 26.60                              | 48.00                                               | 20.40                                       |
| 5                        | 48.20          | 48.60                                 | 28.00                              | 49.50                                               | 24.60                                       |
| 10                       | 52.00          | 46.50                                 | 34.20                              | 52.00                                               | 32.50                                       |
| 15                       | 49.40          | 50.20                                 | 38.40                              | 48.60                                               | 38.60                                       |
| 20                       | 52.60          | 52.50                                 | 30.60                              | 54.00                                               | 34.70                                       |

The values denote ml of fluids taken during 24 hr by the groups of 6 rats in each case. Each value is the average of 4 such groups.

Table 2. Effect of pretreatment with nicotine, alcohol, caffeine and their combination on the gastric mucosal damage induced by aspirin, phenylbutazone and reserpine

| Treatment                              | Gastric mucosal damage | Student’s t-test |
|----------------------------------------|------------------------|------------------|
|                                        | Intensity mean score ±S.E.M. | Incidence percentage |                       |
| Control (Normal)                       | 0.00 ±0.00 n=8          | 62.5%             | —                   |
| Nicotine control                       | 0.00 ±0.00 n=8          | —                 | —                   |
| Alcohol control                        | 0.00 ±0.00 n=8          | —                 | —                   |
| Caffeine control                       | 0.00 ±0.00 n=8          | —                 | —                   |
| Nicotine + Alcohol + Caffeine          | 8.46±2.44 n=8           | 62.5%             | <0.01** d.f.15      |
| Aspirin alone                          | 23.20±2.16 n=10         | 100%              | <0.01* d.f.17       |
| Nicotine + Aspirin                     | 48.50±3.44 n=6          | 100%              | <0.01*** d.f.15     |
| Alcohol + Aspirin                      | 64.40±7.80 n=6          | 100%              | <0.01** d.f.15      |
| Caffeine + Aspirin                     | 36.60±5.20 n=8          | 100%              | <0.05** d.f.15      |
| Nicotine + Alcohol + Caffeine + Aspirin| 98.60±8.20 n=6          | 100%              | <0.01** d.f.15      |
| Phenylbutazone alone (PBZ)             | 28.70±2.94 n=10         | 100%              | <0.01* d.f.17       |
| Nicotine + PBZ                         | 52.80±4.90 n=6          | 100%              | <0.01** d.f.15      |
| Alcohol + PBZ                          | 58.66±4.96 n=6          | 100%              | <0.01** d.f.15      |
| Caffeine + PBZ                         | 42.80±5.20 n=6          | 100%              | <0.05** d.f.15      |
| Nicotine + Alcohol + Caffeine + PBZ    | 94.20±8.60 n=8          | 100%              | <0.01** d.f.15      |
| Reserpine alone                        | 24.50±2.38 n=10         | 100%              | <0.01* d.f.17       |
| Nicotine + Reserpine                   | 54.20±8.55 n=6          | 100%              | <0.01** d.f.15      |
| Alcohol + Reserpine                    | 58.64±4.98 n=6          | 100%              | <0.01** d.f.15      |
| Caffeine + Reserpine                   | 38.90±4.96 n=6          | 100%              | <0.05** d.f.15      |
| Nicotine + Alcohol + Caffeine + Reserpine| 98.40±9.40 n=8          | 100%              | <0.01** d.f.15      |

n: Number of animals used in each group. *: As compared to the control. **: As compared to the respective ulcerogenic drug control.
the three agents individually produced a significant augmentation of gastric lesions induced by aspirin, phenylbutazone and reserpine. Among them, alcohol produced a comparatively more severe augmentation of aspirin induced lesions, and the augmentation of gastric lesions in general was less severe in the caffeine pretreated rats.

The animals pretreated with the combination of nicotine, alcohol and caffeine showed a highly significant augmentation of gastric lesions produced by all the three ulcerogenic drugs. The ulcer indices in all the three groups were significantly higher than the respective controls and those seen in the nicotine, alcohol or caffeine pretreated groups.

Discussion

The results of this study reveal that nicotine, alcohol and caffeine administered separately and in combination for three weeks significantly potentiate the gastric mucosal damage induced by aspirin, phenylbutazone and reserpine in rats. These findings are in agreement with the earlier reports on the exacerbation of restraint+cold, aspirin and reserpine induced gastric lesions in rats following pretreatment with nicotine (5 μg/ml) in drinking water for 10 days (3, 4). The combination of all three agents produced significantly greater gastric mucosal damage than any one of them alone or a combination of any two of them could produce in these experiments (11 and N.S. Parmar, unpublished observations), suggesting an additive effect when used together.

The observations on the alcohol induced augmentation of experimental gastric lesions confirm the association between alcohol consumption and aspirin ingestion in inducing upper gastrointestinal bleeding (12). Similarly the caffeine induced exacerbation of aspirin, phenylbutazone and reserpine lesions confirms the contention that cigarette smoking and APC (aspirin, phenacetin and caffeine) ingestion could be strongly associated with the development of peptic ulcers, particularly gastric ulcers (13).

Chronic administration of nicotine in the dose used by us can lead to a significant decrease in total mucus cell population, neck cell mucus volume fraction and mucosal depth; and to an increase in mucosal surface area (2). These alterations can render the gastric mucosa more susceptible to the damaging effects of ulcerogenic drugs which depress the synthesis of gastric mucus (14). Alcohol, unlike nicotine, is a gastric mucosal barrier breaker (15), a property which is shared with aspirin (16). Caffeine, on the other hand, does not have any effect on the gastric mucosal barrier (17). It is considered a moderate stimulant of gastric acid secretion in dog and man (18).

The enhancement of aspirin lesions in the alcohol pretreated groups was apparently more severe and this could be attributed to the additive effect of both aspirin and alcohol, resulting in the impairment of the gastric mucosal barrier allowing the back diffusion of intraluminal hydrogen ions into the mucosa (16). Although acute administration of alcohol in concentrations of 20% has been shown to produce a significant cytoprotective effect by many workers (19), it should be pointed out here that this study differs from them in the administration of 25% alcohol for a chronic period of 21 days. Moreover, acute administration of 25% alcohol has been shown to have a deleterious effect on the mouse gastric epithelium (20) and 5–49 g/100 ml of alcohol acutely or chronically administered to rats also produces slight intestinal damage in rats (21).

The inhibition of mucopolysaccharide synthesis by gastric mucosal cells or a reduction in their mitotic activity or both and increase in mucosal histamine level have been implicated in the genesis of phenylbutazone induced gastric ulcers (22, 23). The deleterious effect of nicotine on the gastric mucosa (2), the disruption of the gastric mucosal barrier by alcohol (15) and the increased gastric acid secretion by caffeine (18) could be responsible for enhancement of phenylbutazone induced gastric ulcers. The mechanism of reserpine in the production of acute gastric lesions has not been clearly delineated. It has not been shown to have any deleterious effect on the gastric mucosal barrier. Histamine and 5-HT, both released by gastric mast cell degranulation, appear to be the main cause of stomach
glandular ulceration evoked by reserpine (24). Reserpine as well as nicotine both produced the gastrointestinal effects mainly due to parasympathetic stimulation, and single doses of reserpine consistently stimulate gastric secretion (25). The gastric secretory effect of caffeine (18), the reduction of pancreatic bicarbonate secretion induced by nicotine which has a gastric acid neutralizing effect (26, 27) and the disruptive effect of alcohol on the gastric mucosal barrier (6) may be involved in the exacerbation of reserpine induced gastric lesions. Further studies on the functional changes like mucosal blood flow, gastric acid secretion and pancreatic secretion under similar conditions appear necessary to reveal the exact mechanisms of the augmentation of gastric lesions.

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