1 INTRODUCTION

The consumption of moderate amounts of alcohol is documented to be inversely correlated with the mortality following myocardial infarction episodes\(^1\)–\(^3\). Such mortality is at its lowest when moderate amounts (2–3 glasses or 40mL per day) of alcohol is consumed\(^4\),\(^5\). Higher alcohol intake elevates the risk of death due to sudden cardiac arrests. Moreover, overall the mortality rate increases due to an increased incidence of the liver cirrhosis and cancer\(^6\). The lower incidence of coronary artery disease in the French and other Mediterranean populations, despite a diet rich in saturated fat (the "French paradox"), has been attributed to the high rate of red wine consumption by these populations\(^7\),\(^8\). Much of the focus of research into the mechanism of this effect has been on the antioxidant and free radical scavenging properties\(^9\)–\(^11\) and vasorelaxation\(^12\)–\(^14\) of phenolic compounds present in wine.

In 1963, it was reported that wine and wine samples obtained during fermentation exhibited in vitro contractile effects\(^15\) on guinea pig ileum. The conclusion of that study implicated histamine as the major component causing the observed biological effect. Histamine, a biogenic amine\(^16\), is generated following malolactic fermentation by the action of yeast in primary fermentation and is mainly present in red wine\(^17\),\(^18\). In our preliminary experiments, both red and white wine evoked similar contractile effects on isolated rat duodenum. Furthermore, pyrilamine, a H1-receptor antagonist, failed to suppress the contractile responses evoked by

---

**Contractile and Extensile Effects of Red and White Wine on Rat and Mongolian gerbil Gastrointestinal Smooth Muscle**

Hideo Shimamura\(^1\), Mikako Hirota\(^2\), Mitsuo Miyazawa\(^4\), Noriko Kinjo\(^5\) and Satoru Mineshita\(^6\)

Abstract: The contractile and extensile effects of red and white wine on rat and Mongolian gerbil (gerbil) gastrointestinal smooth muscle were investigated. Both wines elicited contractile responses on rat and gerbil duodenum and ileum but had no such effects on the colon or rectum. Dichloromethane extracts derived from either wine showed extensile responses only on rat duodenum and ileum, and did not elicit extensile effects on the colon or rectum. In contrast, wine dichloromethane extracts did not elicit any extensile effects on either gerbil duodenum or ileum. Moreover, dichloromethane extracts had suppressive effects on acetylcholine-induced contractile responses. Red and white wine has been documented to contain a number of organic acids such as tartaric, malic, lactic, and citric acid. Individually, such compounds evoked contractile response on rat duodenum with an order of contractile potency: citric > tartaric > malic > lactic acid. The abundance of such compounds in either wine implicates them as the active component responsible for gastrointestinal smooth muscle responses.

**Key words**: wine, gastrointestinal smooth muscle, citric acid, lactic acid, malic acid, tartaric acid
red or white wine. These preliminary observations indicated that other components present in wine may be responsible for the observed contractile effects in rat duodenum.

The aim of the present study was to investigate the gastrointestinal effects of red and white wine on different gastrointestinal preparations such as rat duodenum, ileum, colon, and rectum; and gerbil duodenum and ileum. The same biological preparations were also examined with dichloromethane extracts derived from wine. In those cases where no contractile effects were seen, the extract ability to suppress acetylcholine responses was examined.

2 MATERIALS AND METHOD

2.1 Materials

Acetylcholine was purchased from Daiichi Pharmaceutical Co. Ltd., DL–lactic and citric acids and pyrilamine were from Sigma Chemical Co. (St. Louis, MO, USA), DL–tartaric and DL–malic acids were from Tokyo Kasei Kogyo Co. Ltd. (Tokyo), dimethyl sulfoxide were from Wako Pure Chemical Industries Ltd. (Osaka). Red wine used in this study was Kiyomi (ethanol concentration about 12% alcohol by volume) and white wine was Seiorosamu (ethanol concentration about 12% alcohol by volume), both from Hokkaido (Japan). In preliminary experiments, the final bath concentration of ethanol was maintained at less than 0.05% where it had no effect on the tonus of the preparations or agonist–induced contraction. Consequently, the quantity of either wine did not exceed 40 µL in this study. The neutralization of organic acid samples was accomplished with 0.5 M sodium hydroxide.

2.2 Dichloromethane extraction of red and white wine

Red wine (1440 mL) was partitioned with dichloromethane (300 mL × 5), and subsequently concentrated under reduced pressure to yield a dichloromethane extract (1770 mg). A dichloromethane extract (1550 mg) was obtained from 1440 mL of white wine using the same procedure.

2.3 5 times concentrated red and white wine

Five mL of red and white wine were evaporated to dryness under a stream of nitrogen at room temperature, and individually reconstituted in 1.0 mL of distilled water.

2.4 Preparation of gastrointestinal smooth muscle and mounting

Male wister rats weighing 200–250 g and Mongolian gerbils (60–65 g) were used in this study. Care of animals was conducted in accordance with the Guide to the Care and Use of Experimental Animals (Tokyo medical and Dental University). The animals were housed in stainless steel cages in a room regulated for temperature and humidity on a 12–h light–dark cycle with free access to laboratory food and water. The animals were killed by decapitation under anaesthesia, and the abdomen was opened to obtain the duodenum, ileum (rat and gerbil), colon, and rectum (rat) 3.0 cm in length. The organs were quickly removed and cleaned of adhering fat and connective tissue. The organs were mounted on a 10 mL organ bath filled with physiological salt (modified Krebs–Henseleit solution) at 37°C and continuously aerated with air. Recording was performed using an isotonic transducer (KW–259, Natsume Seisakusho Co. Ltd., Tokyo USA) connected to an electronic recorder (R–642A, Rikadenki Kogyo Co. Ltd., Tokyo USA). The solution had the following composition (mM): NaCl 119, KCl 4.7, CaCl2·2H2O 2.5, KH2PO4 1.2, MgSO4·7H2O 1.2, NaHCO3 25, Glucose 11.1, EDTA 2Na 0.027. Measurements were typically obtained from two different preparations derived from the same animal. Isolated tissues were allowed to equilibrate for 45 min with the equilibration buffer that was changed every 15 min under a tension of 1 g. The tension was adjusted to 0.3g for gerbil ileum. Consequently, the isolated tissues were examined by obtaining acetylcholine dose response curves until constant and reproducible contractions were observed. Maximum contraction evoked by acetylcholine was at 7.6 × 10–6 M. The concentration of 7.6 × 10–7 M (60% of maximum contraction) was used as a 100% contraction in this study.

2.5 HPLC analysis

The HPLC analysis of organic acids in red and white wine was performed on a Hitachi Model L–6200 liquid chromatography equipped with a diode array detector. Isocratic separations were carried out on a Shodex Rspak KC–811 (8 × 30 mm) 6-mm column with 2.0 mM of HClO4 as the mobile phase. The flow–rate was adjusted to 0.75 mL/min and the column temperature was 60°C. Organic acids were derivatized with Bromothymol blue (0.2 mM) to
generate chromophores that can be detected at 440 nm.

2.6 GC–MS analysis

The dichloromethane extracts was analyzed by GC–MS on a Hewlett– Packard 5972A series mass spectrometer interfaced with a Hewlett– Packard 5890 gas chromatograph in EI mode (70 eV). A split ratio of 1:10 and HP–5MS capillary column (30m × 0.25mm i.d.; 0.25µm) was used. The temp. program was from 140°C to 280°C at the rate of 4°C/min. As carrier gas, He was used at a flow rate of 1.0 mL/min. The identification of the components was based on comparison of their mass spectra with those of the Wiley MS database and from the literature data, and confirmed by GC analysis of authentic samples from previous work.

2.6 Statistics

Results are expressed as means ± S.E.M. of three experiments. Statistical significance between mean values of different experiments was estimated with Student’s t-test and ANOVA according to Fisher and Scheffe. A value of P < 0.05 was considered significant.

3 RESULTS AND DISCUSSION

Either wine partially evoked dose-dependent contractile responses in rat duodenum and ileum. In contrast, no detectible responses were observed on colon and rectal tissues. Maximum tension developed within 5 to 10 s, but was not sustained in the rat duodenum and ileum. The initial increase in tone slowly decayed to baseline levels within 10 to 30 s. Red wine elicited partial contraction of the duodenum and ileum at the 0.4% concentration of acetylcholine (Fig. 1a). Similarly, white wine elicited partial contractile responses in rat duodenum and ileum, but failed to elicited any contractions in the colon or rectum (Fig. 1b). Five times concentrated wine (containing no alcohol) were further investigated for contractile response in the tissues. Five times concentrated red wine exhibited 55.8 ± 2.9% of contractile response (relative to acetylcholine) in the duodenum at a final concentration of 1.0%, and 32.4 ± 3.5% in the ileum. Weak contractile responses were observed in the colon and rectum following addition of concentrated red wine to a final concentration of 1.0%. Similarly, concentrated white wine showed 58.8 ± 3.4% contractile response in the duodenum and 37.8 ± 9.2% in the ileum, and weak con-

Fig. 1 Contractile Responses of Red and White Wine and 5 Times Concentrated Red and White Wine on Isolated Rat Duodenum, Ileum, Colon, and Rectum.
1a: contractile response of red wine, 1b: contractile response of white wine, 1c: contractile response of 5 times red wine, 1d: contractile response of 5 times concentrated white wine. (●) duodenum; (■) ileum; (○) colon; (△) rectum. Each point represents the mean of 3 experiments; vertical lines show S.E.M.
tractile responses in the colon and rectum (Fig. 1c and 1d).

The duodenum of Mongolian gerbil was tested with the same tension (1.0 g) of rat experiments, while tension of the ileum was changed 1.0 g to 0.3 g to sustain the motility and the acetylcholine contraction. Red wine evoked 27.6 ± 1.5% of the contractile effects in the ileum at a final concentration of 0.4%. The contractile response elicited by white wine was 23.7 ± 2.2% at same final concentration (Fig. 2a and b). Either red or white wine evoked similar partial contractile responses in gerbil duodenum. As shown in Fig. 2c and 2d, 5 times concentrated red wine showed 71.9 ± 5.0% of contractile responses in the ileum at the concentration of 1.0%, and 35.8 ± 5.2% in the duodenum. The concentrated white wine showed 74.3 ± 2.6% of contractile response in the ileum and 47.9 ± 4.7% in the duodenum at the same concentration.

Both red and white wine extracts evoked slow, but sustained relaxation response in the duodenum and ileum (Fig. 3a and b). The threshold concentration to this relaxation response was approximately 0.03%. As shown in Fig. 4a and 4b, red and white wine extracts also showed the suppressive effects on the acetylcholine contraction (7.6 × 10⁻⁷ M) in the four tissues. Red and white wine extracts did not show significantly different suppressive effects to acetylcholine contraction in the rat duodenum, ileum, colon, and rectum. Dichloromethane extracts of red wine suppressed 58.1 ± 8.2% of the acetylcholine contraction in the duodenum, 67.6 ± 10.4% in the ileum, 47.1 ± 5.9% in the colon, and 37.5 ± 15.5% in the rectum at the concentration of 0.03% (Fig. 4a). Similarly, white wine extract suppressed 65.8 ± 5.5% of the acetylcholine contraction in the duodenum, 53.1 ± 7.5% in the ileum, 45.4 ± 9.3% in the colon, and 38.3 ± 6.8% in the rectum at the concentration of 0.03% (Fig. 4b). Interestingly, the dichloromethane extracts did not elicit any extension on gerbil duodenum or ileum. As shown in Fig. 5a and 5b, both extracts partially suppressed the acetylcholine contraction in gerbil duodenum and ileum.

The organic acid content of red and white wine is shown in Table 1. Lactic and tartaric acids had the highest content (> 1000 mg/mL) in red wine. The total organic acid content in red wine was 7522.7 mg/L. Alternatively, white wine had a higher concentration of tartaric and malic acids. Similarly, the total organic content of white wine was 6471.2/L.

Tartaric, malic, lactic, and citric acids elicited contractile effects in isolated rat duodenum (Fig. 6). Maximum ten-

Fig. 2  Contractile Responses of Red and White Wine and 5 Times Concentrated Red and White Wine on Isolated Gerbil Duodenum and Ileum. 2a: contractile response of red wine, 2b: contractile response of white wine, 2c: contractile response of 5 times red wine, 2d: contractile response of 5 times concentrated white wine. ( ● ) duodenum; ( ■ ) ileum. Each point represents the mean of 3 experiments; vertical lines show S.E.M.
Contractile and Extensile Effects of Red and White Wine on Smooth Muscle

Sion developed within 5 to 10 sec following the addition of the various organic acids to the media. However the initial contractile response was not sustained and slowly decayed to baseline levels within 10 to 30 s. The contractile responses due to the organic acids were similar to those observed with red and white wine. Moreover, organic acid did not suppress the contractile responses elicited by pyrilamine. There was a significant difference on the contractile responses observed between citric acid and lactic (P < 0.05) only at the concentration of $5.5 \times 10^{-4}$ M. There was no significant contractile responses difference between these organic acids at a concentration of $1.1 \times 10^{-3}$ M.

Regional differences in the responses due to red and white wine were observed between “duodenum and ileum” and “colon and rectum” in rat. Specifically, either wines evoked contractile responses in the duodenum and ileum, but failed to do so to the colon and rectum. Failure of pyrilamine (a H1-receptor antagonist) to inhibit the contractile responses, indicated that the observed effects were not due to the presence of histamine in either alcoholic beverage. Moreover, the observed contractile responses (initial contraction followed by relaxation) due to red and white wine are not similar to those observed with histamine. Tugrul et al.\textsuperscript{19} reported that (−)-tartaric acid at $4.0 \times 10^{-8}$ M (highest concentration tested) evoked contractions in isolated rat gastric fundus, that was partially abolished by

Fig. 3 Extensile Response of the Dichloromethane Extracts on Isolated Rat Duodenum, Ileum, Colon, and Rectum.  
3a: extensile response of the red wine extract, 3b: extensile response of the white wine extract. (●) duodenum; (■) ileum; (○) colon; (△) rectum. Each point represents the mean of 3 experiments; vertical lines show S.E.M.

Fig. 4 Suppressive Effects of the Dichloromethane Extracts on the Acetylcholine Induced Contraction in Rat Tissues.  
4a: suppressive effect of the red wine extract, 4b: suppressive effect of the white wine extract. (●) duodenum; (■) ileum; (○) colon; (△) rectum. Each point represents the mean of 3 experiments; vertical lines show S.E.M.
Moreover the observed contractile response was completely abolished by indomethacin, although the tissue remained sensitive to prostaglandin E2. In 1996, Kristev et al. showed that sodium butyrate (a short fatty acid) influenced the spontaneous bioelectric and contractile activity of gastrointestinal smooth muscle preparation derived from rat and guinea pig. The butyrate–induced contractions were significantly reduced by indomethacin (a PG synthesis inhibitor) and by SC19220, an inhibitor of the excitatory action of PGF₂α, PGE₂ and PGI₂. Koley et al. reported that lactic acid evoked biphasic intrarectal pressure changes (i.e. initial relaxation followed by contraction which decays to baseline levels), and the epicardial lactic acid–induced rectal contractile phase was absent in atropinised cats, whereas the relaxation phase was abolished in NG–nitro–L–arginine (nitric oxide blocker) pretreated animals. Lactic acid was applied to the epicardial surface of the left ventricle and not direct to the cat rectum. Such observations may support the results obtained in our studies where either wine and the various organic acids elicited contractile responses followed by a decrease in tension to baseline levels.

Organic acids reported here (especially tartaric, citric and malic acids) are present in the pulp of grape. Lactic acid is mainly formed by bioconversion of malic to lactic acid. These four organic acids are present in other varieties of wine. The specific content ratio is characterized by the type of grape or by the way it was fermented.

Dichloromethane extracts of red and white wine suppressed the contractile effect elicited by acetylcholine. These results suggest that dichloromethane extracts of either red or white wine contain an acetylcholine receptor antagonist. To identify the major chemical constituents, both extracts were submitted to gas chromatography–mass spectrometry (GC–MS) analysis (Table 2). The major components included butanediolic acid monoethyl ester (48.1% in red wine; 34.5% in white wine) and phenylethyl alchohol (27.8% in red wine; 19.6% in white wine). Positive identification of such effective compounds on gastrointestinal extensible responses and acetylcholine induced contractile responses are the subject of further investigations.
4 Conclusion

In this study we compared: 1) the effects of red and white wines in rat isolated duodenum, ileum, colon, and rectum, 2) the effects of the dichloromethane extracts of red and white wine in rat isolated duodenum, ileum, colon, and rectum, 3) the effects of red and white wine in Mongolian gerbil isolated duodenum and ileum, and 4) the effects of the dichloromethane extracts of red and white wine in Mongolian gerbil isolated duodenum and ileum. Especially, the central finding of the present study is that organic acids in red and white wines are the major effective components to the contractile response in isolated rat duodenum.

References

**Table 1** Determination of Tertaric, Malic, Lactic, and Citric Acid in Red and White Wine.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kiyomi (red wine)</th>
<th>Seiorosamu (white wine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maleic acid</td>
<td>59.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>Citric acid</td>
<td>n.d.</td>
<td>244.2</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>1572.5</td>
<td>1666.3</td>
</tr>
<tr>
<td>Malic acid</td>
<td>234.5</td>
<td>3475.6</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>761.3</td>
<td>403.0</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>4452.7</td>
<td>478.3</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>442.5</td>
<td>203.8</td>
</tr>
<tr>
<td>Total acids (mg/mL)</td>
<td>7522.7</td>
<td>6471.2</td>
</tr>
</tbody>
</table>

n.d.: not detected

**Table 2** Major Chemical Constituents of the Dichloromethane Extracts by C-MS.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Compounds</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wine</td>
<td>4-hydroxy-4-methyl-2-pentanone</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>1-hexanol</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1-butanol, 3-methyl, acetate</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>4-hydroxy-butanoic acid</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Phenylethyl alcohol</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>diethyl-butanodinoate</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>butanedioic acid, monoethyl ester</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>butanedioic acid, hydroxy-, diethyl ester</td>
<td>1.6</td>
</tr>
<tr>
<td>White wine</td>
<td>1-butanol, 3-methyl-, acetate</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>4-hydroxy-butanoic acid</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>phenylethyl alcohol</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>butanedioic acid, diethyl ester</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Octanoic acid</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Octanoic acid, ethyl ester</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>butanedioic acid, monoethyl ester</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>fumaric acid, monoethyl ester</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>butanedioic acid, hydroxy-, diethyl ester</td>
<td>21.4</td>
</tr>
</tbody>
</table>

a) Retention time


