Antimutagenicity of Herbs and Spices Used as “Yakumi” against Different Mutagens

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The antimutagenic activities of 10 kinds of herbs and spices used as “yakumi” (black and white peppers, Japanese pepper, red pepper, welsh onion, onion, ginger, garlic, and white and black sesame) were investigated. The antimutagenicity was examined by the suppression of mutagenicity induced by Trp-P1, 1-nitropyrene (1-NP), and activated metabolites of Trp-P1 (act. Trp-P1) and Trp-P2 (act. Trp-P2) toward Salmonella typhimurium TA98 according to the Ames method. The mechanism for the antimutagenic activity was also examined by comparing the antimutagenic intensity against different types of mutagens. Six samples (black pepper, Japanese pepper, red pepper, welsh onion, onion and ginger) of 10 kinds of methanol extract from “yakumi” showed inhibitory effects on the mutagenicity induced by 1-NP, while 6 extracts (white pepper, red pepper, welsh onion, onion, ginger and garlic) were also active against Trp-P1.

Furthermore, the antimutagenic activities of the hexane, benzene, ethyl acetate and methanol fractions from the methanol extracts of several samples were also observed against Trp-P1, 1-NP, act. Trp-P1 and act. Trp-P2. In most cases, the extracts inhibited not only the microsomal activation-dependent mutagen, but also the direct-acting mutagens such as 1-NP and act. Trp-P1. These results indicate that several types of “yakumi” inhibited the mutagenicity induced by various mutagens.

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Keywords: antimutagenicity, Trp-P1, 1-nitropyrene (1-NP), herbs and spices.

INTRODUCTION

The food eaten is one of the main causes of human chemical cancer,1,2, because carcinogens and/or mutagens like heterocyclic amines, aflatoxin B1, pyrrolidine alkaloid and nitrosoamine are contained in the food which we eat every day. These substances can be divided into two groups; one group which directly induces mutagenicity and the other group which indirectly induces it by disrupting drug-metabolizing enzymes. It is difficult to remove many of the dietary carcinogens even by taking extreme care in our dietary habits.

On the other hand, it has also been proven that edible plants contain many kinds of biologically active substances, some of which can inhibit the action of carcinogens and/or mutagens.3,4 Dan-no et al. have reported that flavonoid compounds were very strong antimutagens against Trp-P2 which induced mutagenicity by the action of drug-metabolizing enzymes and were contained in such herbs as sage, peppermint, thyme and oregano.5,6 They presumed that flavonoid compounds inhibited the action of those enzymes. The authors have previously reported that the extracts of such edible plants as tomato, onion and kiwi fruit showed antimutagenicity against some mutagens.

However, most of the studies hitherto reported have dealt with the antimutagenicity against a single mutagen. It is very important to investigate the mechanism for antimutagenicity with different types of mutagen. This study deals with the antimutagens in herbs and spices used as “yakumi” against two types of direct and indirect mutagens (1-nitropyrene and Trp-P1).

MATERIALS AND METHODS

Materials
The herbs and spices used as “yakumi” (black and white peppers (ground), Japanese pepper (ground), red pepper (ground), onion (flaked), welsh onion (flaked), ginger (flaked) and garlic (flaked)) were
kindly provided by K. Kobayashi & Co. Ltd. (Kobe, Japan), and black and white sesame were provided by Kuki Sangyou & Co. Ltd. Trp-P1 and Trp-P2 were purchased from Wako Pure Chemical Ind. Ltd., 1-nitropyrene (1-NP) from Aldrich Chemical Co. Ltd., and agar and nutrient broth (extracts from beef and yeast) for the culture of bacteria were from Difco Laboratories. All other chemicals were of high grade.

Extracts from herbs and spices used as “yakumi”

Each sample was homogenized by a Waring Blender in 3 volumes of methanol for 5 min. The methanol extract was evaporated to dryness and submitted to the Ames test. To fractionate the active substances, the residue from the methanol extract was extracted with 10 volumes of several different solvents for 20 min, and then each resulting extract was evaporated to dryness. The solvents used were hexane, benzene, ethyl acetate and methanol in order of polarity.

After extracting with the first solvent three times, the residue was successively extracted with the next solvent. Each resulting extract was dissolved at a suitable concentration in dimethyl sulfoxide (DMSO) and submitted to the bioassay for antimutagenicity.

Determination of antimutagenicity

The assay was carried out according to the modified Ames test with Salmonella typhimurium TA98. One hundred microliters of DMSO containing 10 μg or 100 μg of each sample was preincubated with 150 ng of Trp-P1 in 100 μl of DMSO at 37°C for 20 min. TA98 and 500 μl of an S9 mixture were then added to the preincubated mixture which was further incubated at 37°C for 20 min. One hundred microliters of DMSO alone was added to the control mixture. The incubation mixture was mixed with 2 ml of molten-top agar and then poured on to a minimal-glucose agar medium. The revertant colony number was measured after 2 days of incubation at 37°C.

The antimutagenicity of each test substance is expressed as the ratio of the colony number on the plate containing Trp-P1 to that without a herb or spice extract.

The determination of antimutagenicity against 1-NP, another type of mutagen, was done in a 0.1 M Na-phosphate buffer solution (pH 7.4) instead of the S9 mixture.

The incubation was also carried out by changing the procedure in order to confirm whether the antimutagenic activity was of desmutagenic type or not. To do this, act. Trp-P1 and act. Trp-P2, which are direct mutagens, were prepared by reacting Trp-P1 or Trp-P2 with the S9 mixture. The antimutagenicity of each sample solution was then examined against these direct mutagens.

RESULTS AND DISCUSSION

The results of the antimutagenic assay with Trp-P1 and 1-NP as the mutagenic agents are shown in Table 1. The methanol extract from the herbs and spices used as “yakumi” showed antimutagenic activity,
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Table 3. Antimutagenic activities of the fractions from the methanol extract of “yakumi” against various mutagens

<table>
<thead>
<tr>
<th></th>
<th>Trp-P1</th>
<th>1-NP</th>
<th>act. Trp-P1</th>
<th>act. Trp-P2</th>
</tr>
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<tr>
<td>White pepper</td>
<td></td>
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<tr>
<td>Hexane</td>
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<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benzene</td>
<td>−</td>
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<td>+ +</td>
<td>−</td>
</tr>
<tr>
<td>Ethyl acetate</td>
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<td>+</td>
<td>+ +</td>
<td>+ +</td>
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<tr>
<td>Methanol</td>
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<td>−</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Red pepper</td>
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<td>−</td>
</tr>
<tr>
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<td>+</td>
<td>−</td>
<td>+ +</td>
<td>−</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
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<td>−</td>
<td>+</td>
<td>+ +</td>
<td>−</td>
</tr>
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<td>+</td>
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<td>−</td>
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<td>+</td>
<td>+ + +</td>
<td>−</td>
</tr>
<tr>
<td>Methanol</td>
<td>−</td>
<td>+ +</td>
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</tbody>
</table>

+ + + : very strong antimutagenic activity (75-100%), + + : strong antimutagenic activity (40-74%), + : antimutagenic activity (10-39%), − : no or weak antimutagenic activity (0-9%). The antimutagenic measurement was carried out for a solution containing 100 μg of the sample. The numbers of revertants of Trp-P1 and 1-NP were almost equal to the values shown in Table 1. The revertants induced by act. Trp-P1 and act. Trp-P2 were 575±68 (n=9) and 575±68 (n=9), respectively.

agreeing with many data from past studies. The methanol extracts from white pepper, red pepper, onion, welsh onion and ginger inhibited the Trp-P1-induced mutagenicity (+S9 mixture) toward S. typhimurium TA98. The antimutagenic effect against 1-NP, which induced mutagenicity by a different mechanism (−S9 mixture), was also observed for the “yakumi” extracts, with exception of white pepper, garlic, and white and black sesame. The welsh onion extract showed strong antimutagenic activity against Trp-P1 and 1-NP (a 40% and 50% decrease in mutagenic activity, respectively), while the other extracts resulted in a 10−15% decrease against Trp-P1 and a 15−25% decrease against 1-NP in mutagenic activity, respectively. Thus, many extracts of “yakumi” showed greater antimutagenicity against 1-NP than against Trp-P1. These results indicate that more methanol extracts of “yakumi” suppressed the mutagenicity produced by direct-acting mutagens (−S9 mixture) than by microsomal activation-dependent mutagens (+S9 mixture). This result agrees with the findings of Saudamini et al. We have previously reported that aqueous extracts of radish (leaves), garland chrysanthemum, sweet pepper, cucumber and garden asparagus showed high antimutagenicity. The mutagenic content of normal Japanese daily meals has been estimated to be equivalent to 150 μg of Trp-P1, and this would be suppressed by 200−300 g of these vegetables. Comparing the present results for “yakumi” with those for methanolic extracts of vegetables and fruits, the effects of “yakumi” are obviously greater than...
those of the aqueous vegetable extracts and are equivalent to the activity of other herbs.15)

Table 2 shows the estimated amounts of “yakumi” herbs and spices necessary to suppress the mutagenicity of Trp-P1 (150 µg). The addition of 0.008 g of welsh onion (flaked) completely suppressed the mutagenicity, while white pepper showed the weakest antimutagenic activity with the addition of 0.8 g. This amount corresponds to one spoonful, while 0.03 g of red pepper corresponds to a pinch.

In order to make clear whether the effects of the extracts against the mutagenicity of Trp-P1 were due to their inhibitory effect on the S9 mixture (microsomal activation), desmutagenicity16) or bio-antimutagenicity,17) the antimutagenic effect against another type of mutagen was examined. The hexane, benzene, ethyl acetate and methanol fractions obtained from the methanol extract showed antimutagenic activity against Trp-P1, 1-NP, and the activated metabolites of Trp-P1 (act. Trp-P1) and Trp-P2 (act. Trp-P2) as shown in Table 3. The antimutagenicity of each fraction against act. Trp-P1 (−S9 mixture) was stronger than that against Trp-P1 (+S9 mixture). The hexane, benzene, ethyl acetate and methanol fractions from the methanol extract of ginger inhibited the act. Trp-P1-induced mutagenicity, with 76.1%, 63.4%, 84.5% and 84.5% inhibition, respectively. Although the data are not shown, every fraction from white and black sesame showed antimutagenic activity against 1-NP (−S9 mixture) but not against Trp-P1 (+S9 mixture). Similar results were obtained in the cases of white pepper, red pepper, onion and garlic. However, the fractions of the welsh onion extract showed weak or no activity. It is likely that the highest antimutagenic activity of the methanol extract was dependent upon a multiple effect of the active substances in welsh onion which is now under investigation. These data indicate that “yakumi” extracts suppressed the mutagenicity of 1-NP and act. Trp-P1, which are direct-acting mutagens. It is therefore suggested that the extracts of these “yakumi” herbs and spices did not act as bio-antimutagens but rather as direct-acting desmutagens. Further investigation will be needed to identify the active substances in “yakumi.”

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各種変異原物質に対する薬味として用いられる香辛料の抗変異原性

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10種類の薬味として用いられる香辛料（白コショウ，黒コショウ，サンショウ，トウガラシ，ネギ，タマネギ，ショウガ，ニンニク，黒ゴマ，白ゴマ）の抗変異原性を調べた。抗変異原性の測定は変異原物質としてTrp-P 1, 1-ニトロビレン（1-NP），薬物代謝酵素群により活性化されたact. Trp-P 1及びact. Trp-P 2に対する変異原性的抑制力をSalmonella typhimurium TA 98菌株を用いるAmes testで行った。また，これら試料の各種変異原物質に対する抗変異原性の強弱から活性発現メカニズムについても検討した。薬味10種類のメタノール抽出物の内，黒コショウ，サンショウ，トウガラシ，ネギ，タマネギ，ショウガの6種類の試料に1-NPに対する抗変異原性が認められた。一方，Trp-P 1に対しても白コショウ，トウガラシ，タマネギ，ネギ，ショウガ，ニンニクの6種類が活性を示した。

さらに，それぞれの薬味のメタノール抽出物をヘキサン画分，ベンゼン画分，酢酸エチル画分及びメタノール画分に分画し，Trp-P 1, 1-NP，act. Trp-P 1及びact. Trp-P 2に対する抗変異原性を検討した。その結果，多くの試料は薬物代謝酵素系ではなく変異原性を示す物質に直接に作用していた。この結果よりこれらの薬味は各種変異原物質に作用して抗変異原性を示す事が分かった。

キーワード：抗変異原性，Trp-P 1, 1-nitropyrene (1-NP)，香辛料。