Lung cancer in women is the leading cause of cancer death in Taiwan. Most Chinese women are non-smokers and 60% of female lung cancer patients have adenocarcinomas. Epidemiological data indicate that the incidence of lung cancer among Chinese women may be correlated with cooking fumes. However, the carcinogenic compound(s) in cooking fume aerosols is not defined. In the present study, the cooking aerosols from Chinese stir-frying of fish were prepared under domestic conditions. To determine the mutagenic compounds in the cooking aerosol, mutagens were purified by two steps of high-performance liquid chromatography (HPLC), and their mutagenicity was monitored with *Salmonella typhimurium* TA98. The chemical structure of the major mutagenic fraction of cooking aerosol extract was determined to be 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). An amount of 0.25 ng MeIQx/g of meat per min was estimated based on the mutagenic response. These data indicated that significant amounts of MeIQx (268.1 ng/Chinese dish of frying fish) were present in cooking aerosol in a short time. Chinese women spend ~1 h preparing meals everyday, thus, they may be exposed to significant amounts of MeIQx from cooking aerosols in the kitchen.

Introduction

Lung cancer in women is the leading cause of cancer death in Taiwan. Most Chinese women are non-smokers and 60% of female lung cancer patients suffer from adenocarcinomas (1,2); adenocarcinoma is the most common form of lung cancer in women who are non-smokers. This percentage closely agrees with the 64.5% of non-smokers reported in a summary of 16 studies from six countries (1). Epidemiological studies of lung cancer in Chinese women indicate that factors other than cigarette smoking are related to lung cancer risk (1,2,4). Much interest has been focused on identifying other potential risk factors of lung cancer, including passive smoking, incense burning, mosquito coil smoke, domestic coal fires and cooking oil emissions (5–9). Multiple conditional logistic regression analyses of risk factors of lung cancer in Taiwan reveal that cooking is a significant risk factor associated with adenocarcinoma of the lung (4). A possible explanation is the high exposure to volatile mutagenic/carcinogenic components formed during cooking (10). The mutagenic compounds in cooking aerosols have still not been characterized, although the mutagenicity of smoke formed during pan-baking and frying of lean pork has been demonstrated (11,12). In the present study, the mutagenicity of cooking fumes from stir-frying fish was examined by a *Salmonella* microsomal test.

To determine the major mutagenic compounds in this cooking aerosol, mutagens were purified by two steps of high-performance liquid chromatography (HPLC*), and their mutagenicity was monitored with *Salmonella typhimurium* TA98. The chemical structure of the major mutagenic fraction of cooking aerosol from frying of fish was characterized by UV spectra and liquid chromatography-mass spectrometry (LC-MS).

**Materials and methods**

**Collection and preparation of cooking fumes from stir-frying of fish**

The cooking aerosol from Chinese frying of fish was prepared under domestic conditions. Soybean oil (30 ml) was first heated in a wok until the oil temperature reached to 180°C. Shredded fish (pomfret, 150 g) was quickly placed in the wok and stir-fried for 5 min. The cooking aerosol was collected for 5 min when the fish was cooked in the wok. The aerosol from heating soybean oil (30 ml) alone was also prepared according to the conditions of frying fish. The temperature changes during the frying of fish and heating soybean oil alone were detected by Portable Digital Thermometer (JENCO Electronics Ltd, Model 7000CH, Taiwan). The temperature changes are shown in Figure 1. The aerosol of cooking fumes was collected with a high volume air sampler through the hood at a flow rate of 1 m³/min. The cooking aerosol was filtered through glass filters (Whatman, EPM1000) and extracted with acetone in a shaker as described previously (13). The acetone extracts (1 mg) was dissolved in 4 ml of redistilled water and were reacted with 10 mg of blue cotton for 30 min. The aromatic compounds in acetone extracts adsorbed by the blue cotton were then eluted with methanol/ammonium (50:1, v/v) three times (14). The extract was dissolved in methanol and stored at ~80°C for mutagenicity test and HPLC analysis.

**HPLC purification**

The purification procedures for the major mutagen(s) from blue cotton extracts were as described previously. Briefly, the blue cotton extracts of cooking aerosol from fish-frying were first injected into a semi-preparative Nucleosil column (10 μm particle, 10×250 mm) and eluted at a flow rate of 2.5 ml/min with a gradient of acetonitrile in 10 mM phosphate/sodium hydroxide (pH 7.2) using the following concentrations of acetonitrile: for 0–5 min a linear gradient of 10–25%; for 5–10 min a linear gradient of 25–35%; for 10–20 min a linear gradient of 35–55%; for 20–25 min a linear gradient of 55–60%. Fractions were collected at 1-min intervals for mutagenicity testing. The mutagenic fractions were pooled and loaded onto a Nucleosil CN column (5 μm particle, 4.6×250 mm). The mobile phase was acetonitrile/water/diethylyamine (12:88:0.1) at a flow rate of 1 ml/min. Finally, the fractions showing mutagenicity from the second HPLC purification were injected into a Nucleosil C18 column (5 μm particle, 4.6×250 mm). The mobile phase was the same as the second HPLC analysis. The peak of the sample with retention time corresponding to standard mutagenic compound was monitored with a photodiode array detector to compare them with the UV spectrum using standard MeIQx. The authentic MeIQx was purchased from Toronto Research Chemicals Inc. (Ontario, Canada).

**Mutagenicity assay**

The samples were tested for mutagenicity by the plate-incorporation assay as described by Maron and Ames (15). An aliquot of 2 ml of molten top agar was melted in a water bath at 80°C. After solidification, 0.1 ml of the test sample was added to the molten agar and thoroughly mixed. The melted medium was then poured into Petri dishes (9 cm diameter) and left to solidify. Then, 0.1 ml of molten S9 mix was added to each plate, and the plates were incubated at 37°C for 48 h. The plates were then scored to determine the presence or absence of *Salmonella* colonies.

*Abbreviations:* HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; DMSO, dimethylsulfoxide; ES, electrospray; dbaha, dibenzo[a]anthracene; bap, benzo[a]pyrene.
The amounts of cooking aerosol and acetone extracts collected from cooking and processing of fried fish are shown in Table I. An amount of 39.8 mg of cooking aerosol was collected from frying 150 g of fish for 5 min. The amounts of cooking aerosol, acetone extracts and blue cotton extracts obtained from heating 30 ml of soybean oil alone for 5 min were 3.8 mg, 2.3 mg and 0.97 mg respectively.

Table I. The amounts of cooking aerosol and acetone extracts collected from cooking and processing of fried fish

<table>
<thead>
<tr>
<th>Frying fish</th>
<th>Cooking aerosol</th>
<th>Acetone extracts</th>
<th>Blue cotton extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amounts (mg)</td>
<td>39.8</td>
<td>34.5</td>
<td>29.0</td>
</tr>
<tr>
<td>Percent of aerosol (%)</td>
<td>–</td>
<td>86.8</td>
<td>72.8</td>
</tr>
</tbody>
</table>

Fish (150 g) was fried with 30 ml soybean oil for 5 min. The meat was added into the wok when the oil temperature reached 180°C. The cooking aerosol, acetone extracts and blue cotton extracts obtained from heating 30 ml of soybean oil alone for 5 min were 3.8 mg, 2.3 mg and 0.97 mg respectively.

agar was added to various concentrations of samples in 50 µl of dimethylsulfoxide (DMSO) and 100 µl of each overnight culture of TA98. The mixture was gently mixed and poured onto minimal glucose agar plates. The plates were incubated at 37°C for 48 h and revertant colonies were counted. All experiments were performed at least twice with triplet plates for each dose. The linear regression equation from the dose–response curve of mutagenicity was used to calculate the mutagenic activity of 1 mg of cooking aerosol extract from fish-frying.

**Results**

The amounts of cooking aerosol, acetone extracts and blue cotton extracts from 150 g of frying fish for 5 min are shown in Table I. An amount of 39.8 mg of cooking aerosol was collected from frying 150 g of fish for 5 min. The amount of aerosol collected from 30 ml of cooking oil alone was 3.8 mg in 5 min. The amount of aerosol from fish-frying was 10.5-fold greater than that of oil-cooking alone. The cooking aerosol from fish-frying was recovered as acetone extracts with an 86.8% yield and as blue cotton extract with a 72.8% yield. The mutagenicity of blue cotton extracts of cooking aerosol from fish-frying and oil cooking alone, were examined with *S.typhimurium* TA98 in the presence and absence of S9 mix. The metabolic-acting mutagenicity of cooking aerosols extracted from frying was higher than that of direct-acting mutagenicity (Figure 2). No mutagenic response was detected in the extracts of soybean oil aerosol in the same dose ranges (data not shown).

To characterize the mutagenic compounds in cooking aerosol extracts, the mutagenic fractions purified from semi-preparative HPLC and an analytical Nucleosil CN column were further loaded onto a Nucleosil C18 column. The most potent mutagenic fraction was found at a retention time of 16 min, which corresponded to the retention time of authentic MeIQx (Figure 3). Comparison of the authentic MeIQx and the active mutagenic peaks collected from a C18 column using a photodiode array detector and ES mass spectrometry, confirmed the presence of MeIQx. The UV spectra (Figure 4) and mass spectra (Figure 5) of the mutagenic fraction with a retention time of 16 min corresponded to the authentic MeIQx. The amount of MeIQx in the cooking aerosol extracts from fish-frying was calculated from the total revertants of mutagenic fractions (Figure 3) based on the dose–response mutagenicity of authentic MeIQx (Figure 6). One milligram of blue cotton extract of cooking aerosol was estimated to contain 9.26 ng MeIQx (Table II). Therefore, 0.25 ng MeIQx/g of meat per min was calculated, based on the data presented in Table I.

**Discussion**

MeIQx has been shown to be carcinogenic in rodents by oral ingestion causing leukemia, and tumors of the liver, clitoral gland, zymbal gland and lung (16,17). In the light of previous reports (18,19), it is conceivable that dietary exposure to MeIQx may have significant human health implications. Layton *et al.* (20) indicated that MeIQx...
Berg et al. (12) have suggested that the nature of the mutagens present in the smoke, such as IQ-type mutagens, are common in the crust of pan residue and the aerosol fraction of smoke. Our data indicate that significant amounts of MeIQx (268.1 ng/Chinese dish of fish-frying) were present in cooking aerosols in a short time. Chinese women spend ~1 h preparing meals every day.

Our results indicate that much of the mutagenic compounds, which are acetone extractable and blue cotton adsorbable, were generated in cooking aerosols during preparation of a Chinese dish (Table I). In addition, greater amounts of cooking aerosols were generated from stir-frying pork using a small amount of cooking oil in a wok at 100–180°C than that from deep frying with large amounts of cooking oil at 170–200°C (unpublished data). In Taiwan, to prepare delicious and tender meats, women generally use the stir-frying method to cook julienne of meats with soybean oil at high temperature. Thus, they may be exposed to significant amounts of MeIQx from stir-frying cooking aerosols in the kitchen.

The mortality rate of lung cancer in Chinese women is among the highest in the world (21). It seems possible that risk factors other than active cigarette smoking are involved in the development of adenocarcinoma (1,2). In a case-control study (4), both active and passive cigarette smoking are significantly associated with the development of three pathological types of lung cancer, i.e. epidermoid carcinoma, small-cell carcinoma and adenocarcinoma. While two types of indoor air pollutants, burning incense and cooking fuels, were not associated with the development of lung cancer. The association of exposure to volatile emissions of rapeseed cooking-oil with lung cancer risk in Chinese women has been reported (2). Shields et al. (22) demonstrated that condensates of the volatile emissions from heating Chinese rapeseed oil to 275–280°C contain different amounts of 1,3-butadiene (504 ng/l) and benzene (2391 ng/l), formaldehyde (71.2 µg/l), acetaldehyde (306.9 µg/l) and acrolein (391.8 µg/l). Among the five kinds of volatile organic compounds, the Chinese rapeseed oil yielded a higher emission rate of 1,3-butadiene than the peanut, soybean, lard, sesame and Canola oils. Pellizzari et al. (23) also reported that heating cooking oil to 265°C emitted higher amounts of 1,3-butadiene, benzene and other organic acids. In Shields’ experiment, soybean oil was heated at a designated temperature (260–265°C). However in our study, only 30 ml of soybean oil was heated in a wok and the temperature was controlled at 225–240°C (Figure 1). The cooking temperature is one of the critical factors for the mutagenicity and emission of cooking oils (12,22,23). This may cause the conflicting result on the mutagenicity of extracts of cooking soybean oil. Nevertheless, it is possible that the cooking aerosol from fish-frying may also contain detectable amounts of these volatile mutagens/carcinogens. On the other hand, Li et al. (24) indicated that soybean cooking oil fumes prepared at 265°C contained polycyclic aromatic hydrocarbons, especially dibenzo[a,j]anthracene (DBahA) (3725 ng/g) and benzo[a]pyrene (BaP) (341 ng/g). We also determined the amounts of DBahA and BaP in oil cooking using high-performance liquid chromatography.
Fig. 5. Mass spectra of authentic MeIQx (A) and the HPLC purified MeIQx (B) that were present in the blue cotton extracts of cooking aerosols from frying fish.

Fig. 6. The linear dose–response mutagenicity of authentic MeIQx in S. typhimurium TA98 with a S9 mix. The linear regression equation is shown at the top of figure.

Table II. The mutagenicity and the amounts of MeIQx in the blue cotton extracts of cooking aerosol from frying fish

<table>
<thead>
<tr>
<th>Cooking aerosol from frying fish</th>
<th>Mutagenicity (revertants)</th>
<th>Amount of MeIQx (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking fumes aerosols/mg</td>
<td>ND</td>
<td>6.54</td>
</tr>
<tr>
<td>Blue cotton extracts/mg</td>
<td>635</td>
<td>9.26</td>
</tr>
<tr>
<td>Total 150 g fish/min</td>
<td>4290</td>
<td>268.10</td>
</tr>
</tbody>
</table>

The number of revertants of blue cotton extracts/mg was calculated from the dose–response curve of the blue cotton extracts from cooking aerosols as shown in Figure 2. The total net number of revertants of four mutagenic fractions: 1605 revertants/2.1 mg of the blue cotton extracts in Figure 3 that corresponded to authentic MeIQx, were used to estimate the amounts of MeIQx in blue cotton extracts from the dose–response curve of authentic MeIQx as shown in Figure 6. The amounts of MeIQx in the blue cotton extracts were further corrected and based on the recovery of authentic MeIQx from HPLC processes. ND: No mutagenic response was detected.

The amount of DBahA and BaP in aerosol from heating soybean oil alone at 180–252°C (<1 ng/g for DBahA, 68 ng/g for BaP) and frying fish at 98–162°C (<1 ng/g
for DBahA and BaP) were much lower than those of Li et al.’s data (24).

In this study, we demonstrated that cooking aerosol contains significant amounts of MeIQx. The association between these mutagens/carcinogens from cooking emissions and human lung cancer is not clear at present. Further studies are needed to clarify the relationship between cooking aerosol, oil vapors and other environmental exposures, with genetic susceptibilities to lung cancer in Chinese populations.

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