PREVENTIVE EFFECTS OF ORALLY ADMINISTRATED PORCINE SKELETAL MUSCLE COMPONENTS ON CIGARETTE SMOKE-INDUCED DNA DAMAGE IN MOUSE ORGANS

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Background

Many epidemiological studies have proved that cigarette smoking causes human cancer, especially lung cancer. Smoke-induced carcinogenesis are related in part to the genotoxic activities of the chemicals (e.g. nitrosoamines). In addition, clastogenic action by cigarette smoking is thought to be intimately involved in tumor promotion. It seems likely that cigarette-induced DNA single-strand breaks are relating to the formation of cancer. Cigarette smoke induces DNA single-strand breaks in lung and liver of mice and in human cells in vitro. Those are ascribed to free radicals generated from cigarette smoke.

Recently, Tsuda et al. (2000) reported that oral administration of antioxidants (ascorbic acid and alfa-tocopherol acetate) prevent the cigarette smoke-induced DNA single-strand breaks by the alkaline single cell gel electrophoresis assay with mouse multiple organs. Their results suggest that free radicals would be a source of the damage of organ DNA. Thus, antioxidants could be utilized for protecting from DNA damage and carcinogenesis. Successful interdiction and neutralization of free radicals have been shown to occur, in addition to the human's own defence mechanism, through diet, especially fruits and vegetables (Maza, 1998). That beneficial action was attributed to the antioxidant potency of compounds including ascorbic acid, vitamin E, beta-carotene, and polyphenolic compounds, which are naturally occurring flavonoids of varying structure. The consumption of fruit and vegetables antioxidants, such as carotenoides, phenolics, may decrease the risk of cancer by providing enhanced antioxidant protection in the human body.

Several endogenous antioxidants (e.g., carnosine) also has been found in skeletal muscle (Decker, 2000; Gopalakrishnan et al., 1999). However, little effort has been directed to evaluate the anticarcinogenic activities of these components (Miwa and Hongo, 1998).

Objectives

In the present study, we examined the effect of oral administration of porcine skeletal muscle homogenate and skeletal muscle components on cigarette smoke-induced DNA single strand breaks in mouse lung and liver. Such activity of meat could be utilized for producing new healthy meat products, which might open up a new market in the meat industry.

Materials and Methods

Animals

Male ICR mice were purchased from Charles River Japan Inc. (Yokohama, Japan) at 6 weeks of age and used after 1 week of acclimatization. They were fed a standard laboratory diet (CE-2; Clea Japan, Inc., Tokyo, Japan), and tap water was freely available. The animal room was maintained at 24C and 50 to 60% humidity with a 12-h light-dark cycle.

Cigarette smoke exposure

Two mice (combined body weight, ca. 66g) were put into a 920 ml polypropylene whole-body inhalation chamber containing 4 inlet pores at the top and an exhaust pore at the side. Smoke was generated by one 2-s, 35-ml puff per cigarette holder from an unfiltered commercial cigarette (Seven Stars, Japan Tobacco, Tokyo; containing 15 mg nicotine and 1.3 mg tar, according to the manufacturer). Smoke from four cigarettes was introduced to the chamber from the inlet pores and the pores were closed immediately. After 1 min, the mice were removed from the chamber and returned to the original cage. They were sacrificed by cervical dislocation 5 min after the exposure. Lung and liver were removed and used for the alkaline single cell gel electrophoresis assay.

Alkaline single cell gel electrophoresis assay

The lung and liver were minced, suspended in chilled homogenizing buffer (pH 7.5) containing 0.075 M NaCl and 0.024 M EDTA, and then homogenized gently using a Potter type homogenizer. To obtain nuclei, the homogenates were centrifuged at 700 x g for 10 min, and the precipitates were resuspended in chilled homogenizing buffer. Agarose solution (1%) was quickly layered on a slide, covered with another slide, and permitted to solidify. The nuclear preparation was mixed 1:1 with low melting point agarose solution (2%), and the mixture was quickly layered over the agarose after removal of the covering slide. Finally, another layer of agarose (1%) was added on top. The slides were immersed immediately in a chilled lysing solution (pH 10) of 2.5 M NaCl, 100mM EDTA, 10 mM Trizma, 1% sarkosyl, 10% DMSO, and 1% Triton X-100 and kept at 0C in the dark for 60 min. The slides were placed on a horizontal gel electrophoresis platform and covered with chilled alkaline solution made up of 300 mM NaOH and 1mM EDTA (pH13) for 10 min in the dark at 0C to allow DNA unwinding and expression of alkali-labile sites. Electrophoresis was conducted at 0C in the dark for 15 min at 1 V/cm and 250 mA. Each slide rinsed with Trizma was stained with ethidium bromide and covered with a coverslip.

Examination of the nuclei and statistical analysis

Cells on slides from lung and liver were examined through a fluorescence microscope equipped with an excitation filter of 520-550 nm and a barrier filter of 580 nm. The frequency of cells with tail (tailed cells) was scored (Figure 1). The significance of differences between treatment and corresponding control was assayed by the Student's t-test. A p-value less than 0.05 was considered statistically significant.

Results and Discussion

Porcine skeletal muscle homogenates (muscle:water=1:4) were orally administrated (2 g muscle/kg body weight/day) to mice on each day for consecutive 7days before being exposed to smoke on day 8. Pretreatment with muscle homogenates prevented DNA single-strand breaks in mouse lung and liver significantly (Figure 2). Also, administration (100 mg/kg/day for 7 days) of actomyosin, enzymatic hydrolysate of actomyosin, carnosine and glutathione were examined. Carnosine and glutathione, which are endogenous skeletal muscle antioxidants (peptides), demonstrated the significant activities (Figure 3). Carnosine prevented single-strand breaks in both lung and liver. On the other hand, glutathione prevented single-strand breaks in liver.

The results of this study suggest that meat and meat components could be utilized for preventing DNA damage caused by free

radicals and the subsequent formation of cancer. In the dairy industry, many physiologically functional foods have been developed. However, there have been few such studies on meat products. We recently reported that the concept of probiotics has great potential in the meat industry (Arihara et al., 1998). Also, efforts were directed to find bioactive components (e.g., antihypertensive peptides) from meat (Arihara et al., 2001; Nakashima et al., 2002). By using such bioactive properties, meat products having potential health benefits can be developed. Such meat products could open up a new market in the meat industry.

Pertinent Literature

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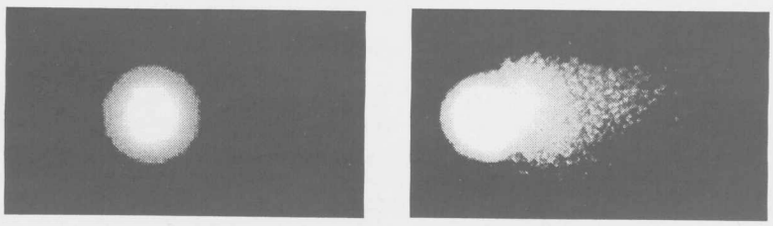

Figure 1. Photographs of control and tailed cells (alkaline single cell gel electrophoresis assay).

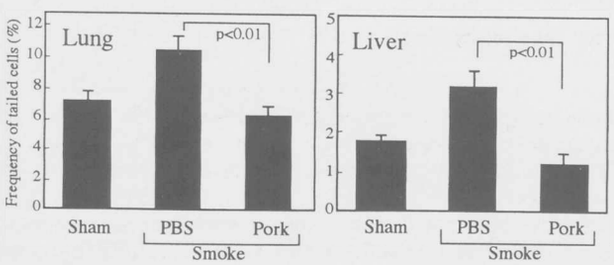

Figure 2. Effect of orally administrated porcine skeletal muscle homogenate on cigarette smoke-induced DNA single-strand breaks of mouse organs

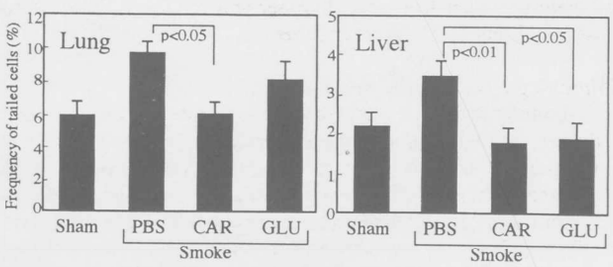

Figure 3. Effect of orally administrated carnosine (CAR) and glutathione (GLU) on cigarette smoke-induced DNA single-strand breaks of mouse organs.