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PROTECTIVE EFFECTS OF MEATS AND OFFALS ON DNA DAMAGE

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BACKGROUND

Many kinds of mutagens/carcinogens are produced in foods following cooking . In the case of cooked meats, more than 10 mutagens such as heterocyclic amines have been detected and most of all were shown to be carcinogenic[1]. On the other hand, many reports showed that there were various kinds of anti-mutagens in plant foods, but few in animal products[2].

The single cell gel electrophoresis (comet) assay detects DNA single and double strand breaks, alkali labile sites, incomplete excision repair sites and genomic structural discontinuities. This method has been used to quantify the effects of low doses of γ-Rays[3,4]. Recently this assay was used to evaluate DNA single- strand breakdown of culture cells by chemical mutagen.[5] We have shown that comet assay is effective method to screen protective effect of offals on DNA damage by chemical mutagen.[6] In this report we tried to find protective effect of meats and offals on DNA damage.

MATERIALS AND METHODS

Meats and offals. Meats and offals used in this experiment were beef, horse flesh, pork, bovine heart, bovine liver, bovine kidney, swine stomach, swine heart, swine liver, swine kidney, swine spleen. They were prepared as soon as possible after slaughter. After fat and connective tissues were removed, each of the materials was cut into small pieces. They were homogenized with an equal weight of water and centrifuged at 11,000 $\times g$ for 15 min. The supernatant was then freeze-dried. The dried product was dispersed with a culture medium and the sample solution was sterilized by passage through a 0.22-μM filter.

Cell treatment by mutagen with sample solution. HL60 (human promyelocytic leukemia) was used in this experiment. They were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FCS. The cells (1 x 10^6 cells/ml) were incubated with *N*-methyl *N*-nitro *N*-nitroso guanidine (MNNG, final conc. 0.3 μg/ml) and each of the sample solution (final conc. 0.1 ~ 10 mg dried product /ml) at 37℃ in a humidified incubator containing 5 % CO_2 and 95 % air. After 3 hour-incubation, the cell suspension was submit to comet assay.

Cell treatment by mutagen before sample addition. HL60 (1 x 10^6 cells/ml) was incubated with MNNG for 3 hours. After the medium was changed to DMEM, each of the sample solution was added to the medium and then incubated for 1 hour. The cell suspension was submit to comet asssay.

Analysis of DNA damage by comet assay. Electrophoresis of whole cell nuclei was carried out according to the procedure originally developed by Singh et al[3]. Mutagen-treated cells were suspended in prewarmed agarose (0.5% in PBS) and the suspension was put on a slide precoated with agarose (0.5%). After gelling at 0°C, the slides were treated with sodium sarcosinate (pH 10.0, 1hr). The slides were submersed in electrophoresis buffer (pH 13.0, 20min) in a flat bed apparatus. Electrophoresis was carried out at 20V for 30 min. The slides were washed in 0.4 M Tris-HCl (pH7.5) for 15 min, and then stained with 50 μg/ml propidium iodide in PBS for 10 min. After washed with water, the slides were examined with an Olympus microscope equipped with a fluorescent filter.

Expression of Comet-type cells. After examination by microscope, we divided cells into 3 types; (A) normal shape, (B) short-tailed shape, (C) long-tailed shape. We expressed comet-type cell ratio as follows.

$$\text{Comet-type cell ratio} = \frac{\text{Number of B+C with sample and MNNG - Number of B+C without sample}}{\text{Number of B+C with MNNG - Number of B+C without sample}}$$

Protein Assay. Protein content of sample solutions was analyzed using DC Protein Assay Kit (Bio-Rad).

RESULTS AND DISCUSSION

Sensitivity of HL60 cells against MNNG. First, we examined DNA damage of HL60 cells treated by MNNG (0.1-10 μg/ml). The number of comet-type cells was less than 10 % below 0.1 μg/ml MNNG addition, about 50 % by 0.3 μg/ml , and almost 100 % by

1.0 μg/ml addition. We selected 0.3 μg/ml MNNG as positive control.

DNA damage protection by meats and offals added with MNNG. Fig.1 shows the results of the protective effect of meats and offals on DNA damage when HL60 cells were treated with MNNG and samples for 3 hours. Addition of meats and offals decreased the comet-type cell ratio to 47 - 77 %. Among them, swine liver, horse flesh, beef and swine stomach had high activity. The protein content of freeze-dried samples were between 50.2 % (beef liver) to 70.2 % (swine spleen). There were no relationship between their protective effect and protein content. It is possible that they adsorbd MNNG and protected DNA from MNNG.

DNA damage protection by meats and offals when they were added after MNNG treatment. Fig.2 shows the effect of meats and offals on DNAdamage when samples were incubated with HL60 cells after 3h-treatment by MNNG. Among 11 samples, swine stomach and swine liver had higher effect and they decreased comet type cell ratio to 50 %. They had some effect on DNA repair process, but the mechanism has not known yet.

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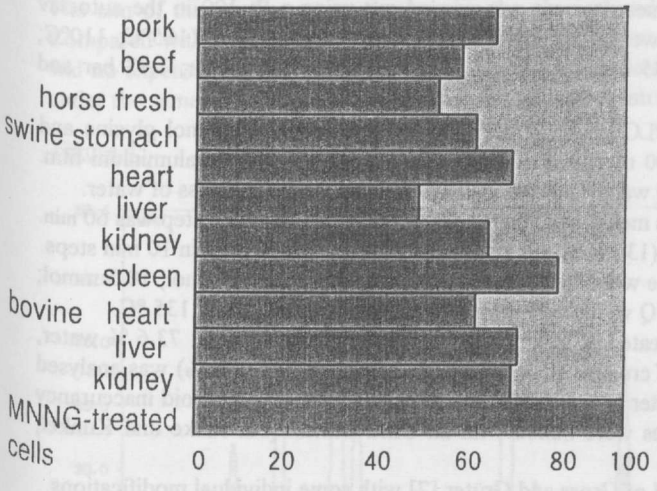

Fig.1 DNA damage protection by meats and offals (3h-treatment)

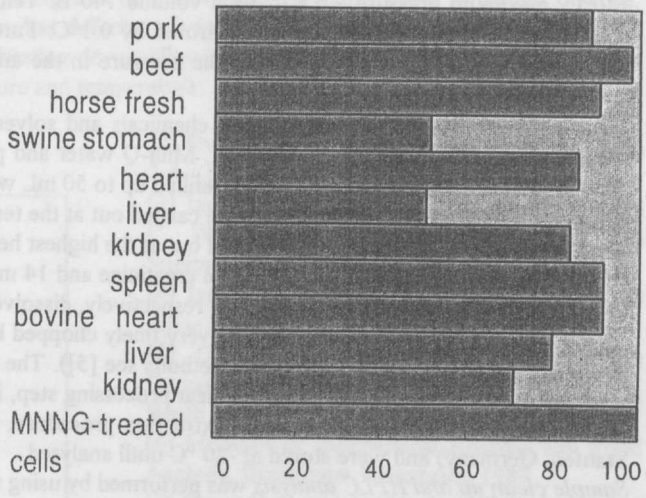

Fig.2 DNA damage protection by meats and offals (3h-treatment, 1h-incubation)