APPLICATION OF THE SINGLE CELL GEL ELECTROPHORESIS (COMET) ASSAY TO SCREEN ANTIMUTAGENS IN MEATS AND OFFALS

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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 it was used to evaluate DNA single- strand breakdown of culture cells by chemical mutagens[5]. Here, we tried to find antimutagens in meats or offals using comet assay.

MATERIALS AND METHODS

Meats and offals. Meats and offals used in this experiment were prepared immediately 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 x *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.

Cells. HL60 (human promyelocytic leukemia), U937 (human histiocytic lymphoma), RAW264.7 (mouse monocyte-macrophage) were used in this experiment.

Cell treatments by mutagen. Cells were irradiated at 1, 10 and 100G in a Gammacell 220 (Nordion International Inc., Canada). MeIQx was dissolved in DMSO and then sterilized by passage through 0.22μm-filter. Each of the cells (1 x 10^6cells/ml) was incubated with MeIQx (500ng/ml), S9mix and with or without sample solution (0.1, 1, 10 mg/ml) for 6 hours at 37°C in CO_2 incubator. Then medium was changed to mutagen-free one and the cells were incubated for another 18 hours. Cyclophosphamide (25 μg/ml) was also used as a mutagen in a same way as MeIQx.

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 120 mA for 30 min. The slides were washed in 0.4M Tris buffer for 15 min, and then stained with 50 mg/ml propidium iodide in PBS for 10 min. After washed with water, the slides were examined with a Olympus microscope equipped with a fluorescent filter.

RESULTS

Sensitivity of cells against mutagens

First, we tested the sensitivity of cells (HL60, U937, RAW264.7) against several mutagens. Gamma-ray irradiation (> 1G) could induce DNA single-strand breaks of HL60, U937 and RAW264.7 cells. Photomicrographs of DNA migration pattern of those three cells were clearly different from that of non-treated cells. Fig.1 shows typical DNA migration pattern of cultured cells treated with and without mutagens.

MeIQx is one of the strong mutagens found in meats following the cooking at high temperature. It needs to be activated by S9 mix to express mutagenicity. MeIQx (+ S9 mix) treatment induced clear difference of DNA migration pattern of RAW264.7, slight difference in U937, and no difference in HL60 under the conditions used here (Table I). Cyclophosphamide had no effect against these three ce4lls. We used RAW264.7 for screening of antimutagens in meats and offals.

Antimutagenicity in meats and offals

The DNA migration pattern of RAW264.7 cells treated with MeIQx + S9mix was spindle-shape (typical comet shape). Table II shows the antimutagenic effect of each of the samples (cattle : liver, heart, kidney and pork : liver, heart, kidney, spleen, stomach). The migration pattern of the cells treated with water extracts of pork heart (10 mg/ml) and pork stomach (0.2, 2 mg/ml) were clealy defferent from that of treated MeIQx. The shape were round and there were no tails.

These results suggeted that there could be antimutagenic activity in pork heart and pork stomach. Hayatu et al.[5] reported that porphyrins could adsorb heterocyclic amines and diminish mutagenicity of them. We have not clarified the compounds which contributed to antimutagenicity in pork heart or pork stomach. Porphyrins is one of the candidates.

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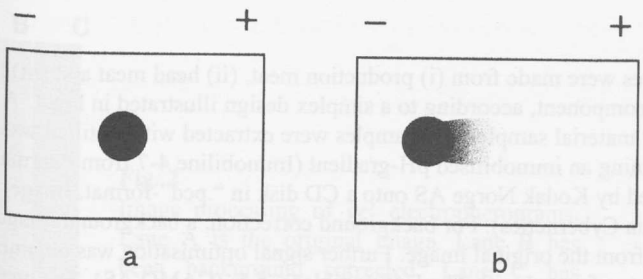

Fig.1 Typical DNA migration pattern of cultured cells in comet assay

a : non-treated cells , b : mutagen- treated cells

Table I Sensitivity of cells againsrt several mutagens

mutagens	HL60	U937	RAW264.7
γ-irradiation (1G)	+	+	+
MeIQx (500ng)	−	±	+
Cyclophosphamide (25 μg/ml)	−		−
no treatment	−	±	−

Table II Antimutagenic Activity of Organs

		concentration (mg/ml)		
		0.1	1	10
Cattle	heart	+	+	+
	liver	+	+	+
	kidney	+	+	+
Pork	heart	+	±	-
	liver	+	+	+
	kidney	+	+	+
	spleen	+	+	+
		0.02	0.2	2
	stomach	+	-	-