Cytotoxicity and Clastogenic Activity of Ribose–Lysine Browning Model System

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The cytotoxic and clastogenic activity on human lymphocytes of the Maillard reaction products in the heated ribose–lysine model system was investigated. A significant increase in the percentage of aberrant cells in treated samples was observed. However, the browning mixture showed a strong cytotoxic effect which limited the maximum amount testable.

INTRODUCTION

Nonenzymatic browning reactions of the Maillard system, between carbonyl and amino compounds, taking place during the processing of foods at the relatively mild heating temperature of 100 °C, are very important in the food industry since they affect sensory characteristics such as flavor, aroma, and color and they decrease the nutritional value and contribute to genotoxic properties of heat-treated foods (Adrian, 1974; Fors, 1983; Krone et al., 1986; Labuza and Saltmarch, 1981; Nagao et al., 1983; Shibamoto, 1983; Spingarn and Weisburger, 1979; Warren and Labuza, 1977).

Since the complexity in chemical composition of foods renders study of the many different chemical and biological properties of browning reactions extremely difficult, different simple browning model systems have been used (Ashoor and Zent, 1984; Shibamoto, 1982; Spingarn and Garvie, 1979). Model systems based on mixtures of sugars, amino acids, and creatine revealed that creatine is essential for the production of the imidazole moiety of the aminoimidazoarene type of heterocyclic amines, which are the most studied mutagens and carcinogens so far identified in heat-processed foods (de Meester, 1989; Jägerstad et al., 1984; Nagao et al., 1983; Skog and Jägerstad, 1990).

In some simple browning model systems composed of only an amino acid and a sugar, mutagens formed by amino carbonyl reactions showed a different strain specificity in the Ames test as compared to that of heterocyclic amines, suggesting a different chemical structure (Shinohara et al., 1980, 1983).

We have previously demonstrated that a browning solution of ribose with different amino acids had a strong mutagenic activity of direct acting type in the Ames test toward *Salmonella typhimurium* TA 100 strain; moreover, lysine was shown to be the most active amino acid in producing mutagenic derivatives in the browning reaction with ribose (Gazzani et al., 1987; Cuzzoni et al., 1988, 1989).

The products of the Maillard reaction in the ribose–lysine browning model system induced a significant increase in the frequency of his+ revertants in the Ames test and of mitotic crossing-over in *Saccharomyces cerevisiae* 6117 strain (Gazzani et al., 1987).

In the ribose–lysine browning model system the influence of water activity and reaction temperature on mutagenicity, absorbance, and content of furfurals was investigated (Cuzzoni et al., 1988). The same model system was also used to investigate stability with time of browning mixtures (Cuzzoni et al., 1989).

To add further support to the possible involvement of Maillard reaction products in human carcinogenesis (Ames et al., 1987; Hatch, 1986; Hatch et al., 1988; Weisburger et al., 1983), we extended the study to the cytotoxic and clastogenic effects of a ribose–lysine mixture on human lymphocytes.

MATERIALS AND METHODS

Chemicals. D-Ribose and L-lysine were analytical grade reagents purchased from Fluka (Buchs, Switzerland).

Browning Model System. The browning model system consisted of ribose–lysine mixtures, prepared in water at a 0.2 M concentration of each reactant. Flame-sealed glass ampules containing the sugar–amino acid mixture were heated in a forced convection oven at 160 °C for 1 h. Since the browning reaction of ribose–lysine is accompanied by a drop of the pH of the solution to acidic values, to avoid any cytotoxic or clastogenic effect due to low pH values, samples were neutralized with 1 M NaOH.

Cytotoxicity Tests. (*A*) *Assay on Monolayer.* FT cells (HeLa clone) (2×10^4) were plated in 10-mm wells of microtiter plates (Nunc) with Ham's F12 medium supplemented with 10% newborn calf serum (Gibco) and maintained overnight at 37 °C in a CO_2 incubator. The medium was then removed, and cells were maintained at 37 °C in Hank's solution (Gibco) containing different concentrations of ribose–lysine browning mixture. After 2 h of exposure, the test solution was removed and replaced with fresh Ham's F12 medium supplemented with 10% newborn calf serum. After 5 days, the number of cells in each well was counted.

(*B*) *Assay on Isolated Cells.* FT cells (500) were plated in 50-mm Petri dishes (Nunc) and maintained in Ham's F12 medium supplemented with 10% newborn calf serum at 37 °C. The treatments with ribose–lysine mixture at different concentration were performed in Hank's solution for 2 h. The test solutions were then removed and replaced with the conditioned medium. After 5 days of incubation at 37 °C, plating efficiency and colony size were evaluated. Since the ribose–lysine browning solution is an equimolar mixture of sugar and amino acid, the amount tested is expressed as microliters of mixture per milliliter of culture medium.

Chromosome Aberration Test. PHA-stimulated peripheral blood lymphocytes from an adult healthy donor were cultured in RPMI 1640 medium supplemented with 15% fetal calf serum. Forty-eight-hour cultures were treated with the ribose–lysine mixture, at the indicated concentrations, for 2 h. After 24 h of

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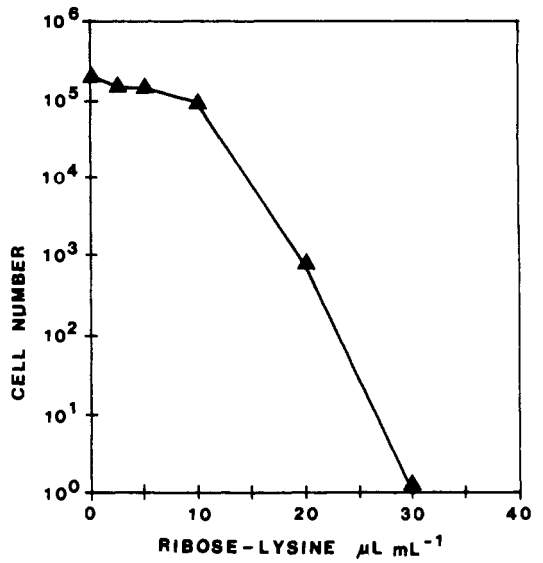

Figure 1. Cytotoxic effect of the ribose–lysine reaction products on FT cells evaluated on high-density cultures. The results are means of two separate determinations.

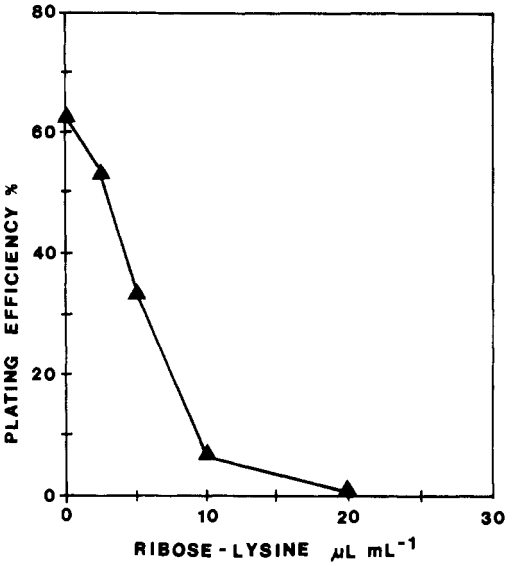

Figure 2. Plating efficiency of FT cells at different concentrations of the ribose–lysine mixture. The results are means of two separate determinations.

recovery in the conditioned medium, cultures were treated with 0.03 μg mL^{-1} Colcemid (Gibco) for 2 h. Chromosome preparations were performed according to the standard protocol. After Giemsa (Merck) 5% staining in Soerensen buffer, the mitoses were scored for rearrangements, which were classified as chromosome or chromatid type. Deletions were identified as a nonstaining region greater than the width of a chromatid or by dislocation of the deleted fragment. A minimum of 100 cells was analyzed for each concentration of the test solution. For statistical analysis Fischer's exact test was used. Data are represented as means ± standard deviation.

RESULTS

The ribose–lysine browning solution was processed at the temperature and heating time previously shown to produce an advanced stage Maillard reaction and a strong mutagenic effect in microorganisms (Cuzzoni et al., 1989). Cytotoxic effects of the ribose–lysine browning mixture were evaluated both on monolayer and on single-cell cultures. The cytotoxic effect was evident with both methods, although a higher sensitivity was shown by single-cell plating (Figures 1–3). The results obtained in these experiments allowed us to select a proper range of doses to be used in the chromosome aberration test, which included two concentrations giving 50% survival and one subtoxic concentration. Mitomycin C (0.5 μg mL^{-1}) was used as a positive control. To prevent any possible reaction of the Maillard mixture products with serum components, we incubated cells in Hank's solution for 2 h. The mitotic indices of the treated cultures are reported in Table I; concentrations of 20 and 30 μL mL^{-1} almost completely inhibited the mitotic activity, whereas 5 and 10 μL mL^{-1} decreased the mitotic index to 50% that of the control and were therefore used in the analysis of chromosome aberrations. Samples of at least 100 metaphases per dose were analyzed in two parallel experiments; the results are shown in Table II. Most of the chromosome aberrations were identified as breaks; data on metaphases, where the only detectable lesions were chromatidic or isochromatidic gaps, have been recorded separately. A dose-related effect was observed up to 10 μL mL^{-1}.

DISCUSSION

The sugar–amino acid Maillard reaction system was known to produce mutagenic components, and it was

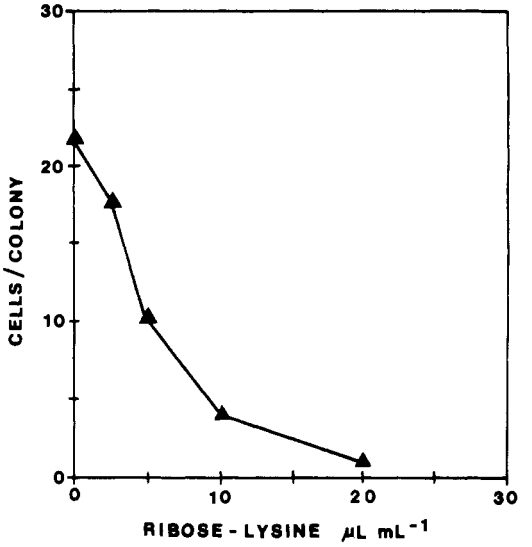

Figure 3. Cytotoxic effect of the ribose–lysine mixture evaluated as growth inhibition of plated FT cells. The results are means of two separate determinations.

Table I. Mitotic Index of Lymphocyte Culture Treated with Ribose–Lysine Mixture

concn	mitotic index,[a] % ± SD
control	5.1 ± 0.69
ribose–lysine, 5 μL mL^{-1}	2.9 ± 0.52
ribose–lysine, 10 μL mL^{-1}	3.8 ± 0.59
ribose–lysine, 20 μL mL^{-1}	1.2 ± 0.33
ribose–lysine, 30 μL mL^{-1}	0.7 ± 0.24
mitomycin C, 0.5 μg mL^{-1}	2.4 ± 0.68

[a] Mitotic index of 72-h lymphocyte cultures treated for 2 h with heated ribose–lysine mixture at different concentrations. The percentages were obtained from the analysis of 1000 cells in two parallel experiments.

observed that the greater the intensity of the brown color, the greater the mutagenic and clastogenic activities of the reaction products. In fact, Shinohara (1980) had shown that mutagenic activity as assayed by the Ames test on a heated (100 °C) glucose–lysine mixture increased with the intensity of the brown color. Since in the amino carbonyl reaction ribose is more reactive than glucose, it gives rise not only to a higher browning but also to a greater mutagenic activity (Gazzani et al., 1987). Thus, the ribose–

Table II. Chromosome Aberration of Ribose–Lysine Mixture on Human Lymphocytes

concn	aberrant mitosis,[a] % ± SD	
	−gaps	+gaps
control	1 ± 0.99	4 ± 1.95
ribose–lysine, 5 μL mL^{-1}	3.75 ± 1.89	6.25 ± 2.42
ribose–lysine, 10 μL mL^{-1}	10 ± 3	12 ± 3.25
mitomycin C, 0.5 μg mL^{-1}	20 ± 5.66	28 ± 6.35

[a] Aberrant mitosis was evaluated in 72-h lymphocytes cultures.

amino acid Maillard reaction system appears to be a useful model to study the production of mutagenic compounds during the cooking or processing of red, white, and fish meats where this sugar is present in relatively high amounts (Tarr, 1953; Cuzzoni and Gazzani, 1983).

To evaluate a possible mutagenic and carcinogenic risk of the dietary use of these compounds in man, we studied the induction of chromosome aberrations in human lymphocyte cultures treated with the heated sugar–amino acid mixtures. These showed a strong cytotoxic effect in cultured cells at concentrations higher than 10 μL mL^{-1}, which determines a drop of the mitotic index and a failure to detect scorable mitosis. The increase in the percentage of aberrant cells is evident at lower doses. A toxic effect of the browning mixture has also been reported in the *Salmonella* system. Gazzani et al. (1987), Cuzzoni et al. (1988), and Powrie et al. (1981) have already demonstrated a clastogenic effect of heated glucose– or fructose–amino acid solution on Chinese hamster ovary cells. The activity reported was higher than that observed in our assays on human lymphocytes. This is in contrast with the observation that ribose is one of the most reactive sugars and that the ribose–lysine mixture shows a higher mutagenic activity than glucose–lysine in the Ames test. A possible explanation would be that the toxic compounds in the browning model system used can mask a strong clastogenic activity. The positive results obtained in the chromosome aberration test with the ribose–lysine browning mixture suggest further investigation of the genotoxicity on different mammalian systems is needed. Since these compounds show a strong cytotoxicity in mammalian cells in culture, the analysis of interphase nuclei with chromosome-specified DNA probes could be a useful tool for the investigation of the chromosomal effects of the Maillard reaction products. Moreover, on the basis of the correlation between chromosome aberrations and carcinogenesis, we suggest that these Maillard reaction mixtures can be tested by an in vitro transformation assay.

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