

## Mutagenicity of Cooked Hamburger is Reduced by Addition of Ascorbate and Erythorbate to Ground Beef

Tetsuta Kato, Kazuyuki Hiramoto, and Kiyomi Kikugawa\*

School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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The addition of phenolic antioxidants, cysteine, *N*-acetylcysteine, unsaturated fatty acids, ascorbate and erythorbate has been evaluated in the reduction of free radical Maillard intermediates, the pyrazine cation radicals, and also the mutagenicity of a heated model system composed of glucose/glycine/creatinine. The aim of the present study was to determine whether these components were useful to reduce the mutagenicity of cooked hamburger. The effect of these components, added at low concentrations to ground beef, on the generation of the mutagenicity of cooked hamburger was examined. Mutagenicity of hamburger was assayed by the Ames test using *Salmonella typhimurium* TA98 strain with metabolic activation after the mutagens were purified by the use of blue rayon. Mutagenicity of hamburger was reduced to a half by the addition of ascorbate or erythorbate at 0.33%, whereas the mutagenicity was not reduced by epigallocatechin gallate (EGCG), cysteine, *N*-acetylcysteine, soybean oil or lard at the low concentrations.

**Key words** — ascorbate, erythorbate, mutagenicity, hamburger

### INTRODUCTION

Hamburger cooked by heating ground beef contains heterocyclic amines mutagenic to *Salmonella typhimurium* TA98 strain with metabolic activation.<sup>1–3)</sup> Heterocyclic amines are considered to be probable human carcinogens.<sup>4)</sup> The heterocyclic amines are generated at a temperature above 125°C in the reaction of the model systems composed of

sugars/amino acids/creatinine.<sup>5–7)</sup> The radical pathway, including condensation of pyrazine cation radicals generated by the Maillard reaction of sugars/amino acids with creatinine, has been proposed for the formation of the heterocyclic amines.<sup>8–12)</sup>

One way to reduce the mutagenicity of cooked hamburger would be by scavenging the free radical Maillard intermediates, pyrazine cation radicals. Previous studies have shown that the addition of phenolic antioxidants to the model systems composed of glucose/glycine/creatinine decreased the pyrazine cation radical<sup>10–12)</sup> and mutagen formation.<sup>10,12–14)</sup> More recently, cysteine, *N*-acetylcysteine, unsaturated fatty acids,<sup>11)</sup> ascorbate and erythorbate<sup>15)</sup> are found to scavenge the pyrazine cation radical and decrease the mutagenicity of the model system.

In the present study, the effect of the addition of these food components to ground beef on the generation of the mutagenicity of cooked hamburger was investigated. It was found that the mutagenicity of cooked hamburger is reduced by the addition of ascorbate and erythorbate to ground beef at the low concentrations.

### MATERIALS AND METHODS

**Materials** — Blue rayon was purchased from Funakoshi Company (Tokyo, Japan). Epigallocatechin gallate (EGCG) (purity more than 80%) was obtained from Oriental Menthol Industry (Okayama, Japan). Ground beef was obtained at a local market in Tokyo. Other reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan). **Preparation of Hamburger** — Thirty grams of ground beef were used for each experiment. Cysteine, *N*-acetylcysteine, sodium ascorbate, sodium erythorbate at 0.03–0.1 g (0.1–0.33% equivalent to ground beef) or 1 g of soybean oil or lard (3.3% equivalent to ground beef) were added to the ground beef. Each mixture was well blended, plated and heated on a hot plate regulated at 200°C for 20 min to prepare the hamburger.

**Extraction of Mutagens from Hamburger** — Each hamburger from 30 g ground beef was added into 60–70 ml of boiling water, then ground in a mixer. The mixture was filtered through glass-wool, and the filtrate was made up to 200 ml with water. The mutagens in the solution were extracted by the blue rayon method.<sup>16)</sup> Thus, blue rayon (0.2 g) was added to the solution and the solution was shaken at room temperature for 1 h. The blue rayon

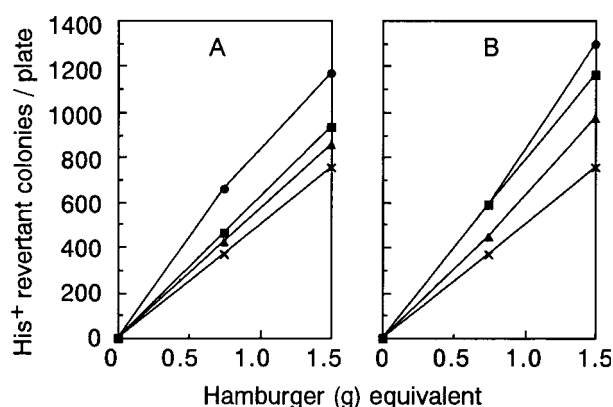
\*To whom correspondence should be addressed: School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan. Tel.: +81-426-76-4503; Fax: +81-426-76-4508; E-mail: kikugawa@ps.toyaku.ac.jp

was recovered. New blue rayon (0.15 g) was added to the solution, and the solution was worked up similarly. Two lots of recovered blue rayon were combined and shaken in 80 ml of methanol : concentrated ammonium hydroxide (1000 : 1, v/v) at room temperature for 30 min. Blue rayon was again shaken in 80 ml of a solution of the methanol–ammonium hydroxide solution. The extracts were combined and evaporated to dryness under reduced pressure. The residue was dissolved into 2.0 ml of dimethyl sulfoxide for the mutagenicity assay.

**Mutagenicity Assay** — Mutagenicity was assayed according to the preincubation method of Yahagi *et al.*<sup>17)</sup> using *Salmonella typhimurium* TA 98 strain<sup>18)</sup> with an S9 mix. The microsomal S9 system prepared from liver microsomes of a rat treated with phenobarbital and 5,6-benzoflavone and cofactor-1 was obtained from Oriental Yeast Company (Tokyo, Japan). A 0.1-ml aliquot of the dimethyl sulfoxide solution of the test sample or a 2-fold diluted solution of the test sample in dimethyl sulfoxide was introduced into a plate. Duplicate plates were used for each assay. The number of His<sup>+</sup> revertant colonies/plate was obtained after subtracting the background number of spontaneously formed His<sup>+</sup> revertant colonies (24–32) /plate. For determination of the mutagenicity of each test sample, the mutagenicity of the test sample linearly increased, depending on the dose of the sample. The number of His<sup>+</sup> revertant colonies of cooked hamburger obtained from ground beef without the addition of food components was 1200–1300/1.5 g ground beef.

## RESULTS AND DISCUSSION

Ground beef (30 g) was mixed with different amounts of EGCG, cysteine, *N*-acetylcysteine, sodium ascorbate or sodium erythorbate at up to 0.33%, or soybean oil or lard at up to 3.3%. Hamburger was cooked by heating ground beef at 200°C for 20 min. Mutagenicity of hamburger was tested on *Salmonella typhimurium* TA98 strain with metabolic activation after extraction of the mutagens by blue rayon. The number of His<sup>+</sup> revertant colonies of cooked hamburger obtained from ground beef without the addition of the food components was 1200–1300/1.5 g ground beef. Mutagenicity of the cooked hamburger was not affected by the addition of EGCG, cysteine or *N*-acetylcysteine at 0.1, 0.2 and 0.33%, or by soybean oil or lard at 3.3% to ground beef. By contrast, the mutagenicity of cooked ham-

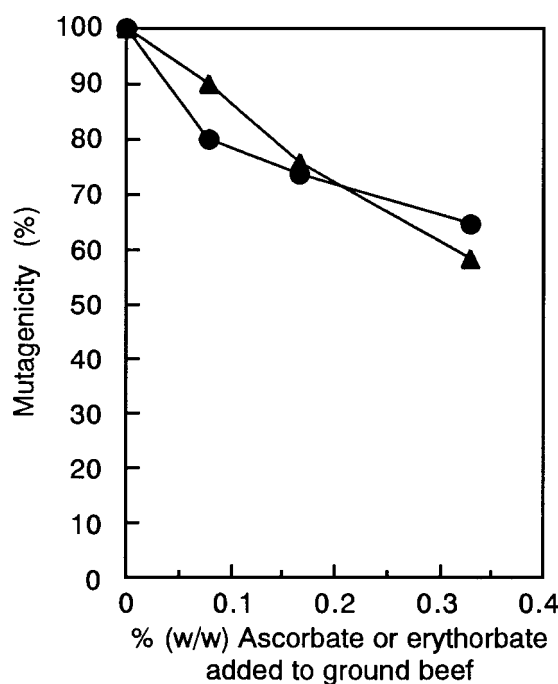


**Fig. 1.** Dose–Response Curves of the Mutagenicity of Cooked Hamburger Prepared by Addition of Sodium Ascorbate (A) and Sodium Erythorbate (B) to Ground Beef

Sodium ascorbate (A) and sodium erythorbate (B) were added at 0 (○), 0.08 (□), 0.17 (△) and 0.33% (×) to 30 g of ground beef, and the ground beef was heated at 200°C for 20 min. The mutagens in cooked hamburger were extracted by use of blue rayon, and the mutagenicity was tested using *Salmonella typhimurium* TA98 strain with metabolic activation.

burger was reduced effectively by the addition of sodium ascorbate and sodium erythorbate at 0.1–0.33%. Figure 1 shows the dose–response curves of the number of His<sup>+</sup> revertant colonies of cooked hamburger with sodium ascorbate (A) and sodium erythorbate (B). The mutagenicity of each hamburger linearly increased in a dose-dependent fashion of the hamburger up to 1.5 g equivalent. %Mutagenicity of the hamburger at 1.5 g equivalent was plotted *versus* the sodium ascorbate and sodium erythorbate content (Fig. 2). The mutagenicity of hamburger was reduced in a dose-dependent fashion: to 64.8% by the addition of 0.33% sodium ascorbate and 58.6% by addition of 0.33% sodium erythorbate. These experiments on the effect of sodium ascorbate and sodium erythorbate were repeated more than three times using different lots of ground beef, and similar results were obtained.

It has been shown that phenolic antioxidants inhibit the generation of the pyrazine cation radical in the heated model system composed of glucose/glycine,<sup>10)</sup> and that they inhibit the generation of mutagenicity in the heated model system composed of glucose/glycine/creatinine,<sup>10,12)</sup> whereas their concentrations required for an effective decrease of the pyrazine cation radicals and the mutagenicity were relatively higher.<sup>10,11)</sup> Practical application of green tea extract containing a larger amount of EGCG in a factory processing of smoked-and-dried bonito (katsuo-bushi) has no merit for the effective reduction of the mutagenicity.<sup>19)</sup> In the present experi-



**Fig. 2.** Mutagenicity of 1.5 g Equivalent Amount of Cooked Hamburger Prepared by Addition of Sodium Ascorbate (●) and Sodium Erythorbate (▲)

%Mutagenicity of 1.5 g equivalent amount of cooked hamburger obtained in Fig. 1 was plotted versus % sodium ascorbate or sodium erythorbate added to ground beef.

ment, EGCG at a practical concentration did not reduce the mutagenicity of cooked hamburger. While cysteine, *N*-acetylcysteine and unsaturated fatty acids reduce the pyrazine cation radical in the heated model system,<sup>15)</sup> cysteine, *N*-acetylcysteine and soybean oil with unsaturated fatty acids at practical concentrations did not reduce the mutagenicity of cooked hamburger. By contrast, ascorbate and erythorbate which effectively reduced the pyrazine cation radical in the heated model system<sup>15)</sup> were also found to be effective in reducing the mutagenicity of cooked hamburger at practically low concentrations.

A high content of reducing sugar can reduce the generation of pyrazine cation radicals and the mutagenicity in the heated model system,<sup>20)</sup> and the addition of 0.7% reducing sugars or onion with high reducing sugar content at 1.7% to ground beef reduced the mutagenicity of cooked hamburger to a half.<sup>21)</sup> Therefore, the addition of glucose at the concentration would be a practical way to reduce the mutagenicity of cooked hamburger. The addition of ascorbate and erythorbate at the above concentration would be another practical way to reduce the mutagenicity of cooked hamburger.

In conclusion, the addition of ascorbate or erythorbate to ground beef at 0.33% would be a practical way to reduce the mutagenicity of cooked hamburger to a half. The effect of the addition of these components was comparable to the effect of the addition of more than 0.7% glucose or a large amount of onion with 1.7% reducing sugars.

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