ANTIMUTA GENICITY OF HOM NIL RICE AND BLACK GLUTINOUS RICE USING SOMATIC MUTATION AND RECOMBINATION TEST

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ABSTRACT: Raw, cooked and fermented Hom Nil rice (*Oryza sativa*) and black glutinous rice (*Oryza sativa* var. *glutinosa*) were assessed for their mutagenicity and antimutagenicity against *in vivo* formed nitrosomethylurea in *Drosophila melanogaster*. In antimutagenic assay, 3-day old, trans-heterozygous (*mwh flr*$^{+}$*/mwh TM3*) larvae derived from mating virgin females (Oregon wing flare strain) and males (multiple wing hair stain) were fed on experimental media that contained each rice sample substituted for corn flour and two mutagen precursors, namely methylurea and sodium nitrite and then the mutant wing spots of surviving flies were analyzed. This study found that all samples did not significantly induce the frequencies of mutant spots at any testing amounts to be higher than that of the negative control. This indicated that none of them was mutagenic. All of rice samples exhibited the antimutagenicity against *in vivo* formed nitrosomethylurea and the fermented black glutinous rice possessed the highest effect. The antimutagenic activity of these pigmented rices might possibly be due to their antioxidative substances including phenolic compounds which might scavenge the free radicals derived from the mutagen during mutagenesis. Therefore, this study suggested that Hom Nil rice and black glutinous rice may be a good choice for health concerning consumers. Further study should be conducted to investigate the active compound(s) and mechanism of antimutagenic activity of these types of pigmented rice.

Keywords: Hom Nil rice, black glutinous rice, fermentation, antimutagenicity, somatic mutation and recombination test

INTRODUCTION

Rice is the main staple food in many parts of the world [1]. Some varieties of pigmented rice can promote human health due to the presence of antioxidants that can counteract oxidative damage or reduce the concentrations of reactive cell-damaging free radicals [2, 3]. Anthocyanin, one of the most important antioxidants in the pigmented rice [4], promotes beneficial effect in reducing the risk of chronic diseases such as cardiovascular diseases, diabetes and cancers with its antioxidant and anti-inflammatory effects [5]. Numerous studies using cell culture and also animal models showed that anthocyanin possessed anticancer activities [6-8].

Direct acting mutagens could be synthesized endogenously in the stomach from the reaction of nitrite and the precursors of mutagens such as primary, secondary, tertiary amines and amides, these products were possible candidates as causes of gastric cancer [9, 10]. *N*-nitroso compounds present in several kinds of food such as bacon, cured meats, cheese, sausage [11] and nitrite also found in human gastric juice [12]. Therefore, the product of nitrite and methylurea will be used as positive mutagen in this study.

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Since some varieties of pigmented rice, namely Hom Nil rice and black glutinous rice are currently consumed in Thailand and Sadabpod et al. [13] have recently reported that raw, cooked and fermented Hom Nil rice and black glutinous rice extracts were high in antioxidant activity and had antimutagenicity in the Ames test. It was, therefore, interested to evaluate whether such two varieties of pigmented rice could modify the mutagenicity induced by a mutagen namely, nitrosomethylurea which was the product formed *in vivo* in the reaction between sodium nitrite and methylurea, in the somatic mutation and recombination test (SMART) using *Drosophila melanogaster*.

MATERIALS AND METHODS

Chemicals

Urethane was furnished by Sigma Chemical (St. Louis, MO, and U.S.A.). Sodium nitrite was supplied by BDH Chemical Ltd. (Poole, England). *N*-Methylurea was purchased from Fluka AG (Buch, Switzerland). Other chemicals were of laboratory grade.

Sample preparations

Hom Nil rice and black glutinous rice were purchased from a supermarket in Bangkok. Each sample (900 g) was equally divided into three portions. The first portion was wash with tap water,

left dry at room temperature and served as raw. The second portion was mixed with distilled water (2:1 w/v) and cooked in an electric rice cooker. The home-made fermented rice was prepared by mixing cooked rice (the third portion) with 0.3% (w/w) a traditional yeast cake (containing both fungi and yeast) and left for 48 h at room temperature. Each sample was lyophilized, protected from light and stored in a desiccator. They were grounded in an electrical blender to fine powder before use.

Drosophila Medium

The medium which prepared accordingly to Robert [14] was used for maintaining the stock of *Drosophila* tester strains, mating and collecting larvae for mutagenesis study. The standard medium containing corn flour 0.25 g, sugar 0.2 g, baker's yeast 0.3 g, agar 0.1 g and water 2 ml was also used as a negative control. A positive control for mutagenic evaluation, the water in standard medium was replaced with urethane (20 mM 2 ml). The medium containing each ground Hom Nil rice or black glutinous rice (20%, 40%, 60%, 80% or 100%) substituted for corn flour in standard medium was used as a sample medium for mutagenic evaluation and was also used as experimental medium when the combination of sodium nitrite (36 mM, 1 ml) and methylurea (10 mM, 1ml) were added instead of water. The combination of sodium nitrite (36 mM, 1 ml) and methylurea (10 mM, 1ml) was substituted for water in standard medium was used as the positive control medium in antimutagenicity evaluation [15].

Mutagenic and Antimutagenic Assay

Each rice sample was firstly evaluated for its toxicity and mutagenicity using Somatic mutation and recombination test as described by Graf et al. [16]. The toxicity of each sample was determined from the survival rate of adult files from 100 of 3-day old larvae (obtained from mating virgin females of *ORR/ORR; flr3/In(3LR)TM3, ri pp sep l(3)89Aa bx34e e Bds* and *mwh/mwh* males on the standard medium) that fed on each sample medium. The Institute of Toxicology (Swiss Federal Institute of Technology, and the University of Zurich) kindly provided both stains. The highest content of each sample medium that provided more than 50% survival of flies and did not express their genotoxicity was evaluated in antimutagenic assay.

Three-day old larvae were fed on each experimental medium containing the combination of sodium nitrite and methylurea and the treated flies were incubated at 25 ± 1^{o}C until pupation. The round wings (both dorsal and ventral surface) of trans-heterozygous flies were analyzed on microscope at 400x magnification for the presence of clones of cells showing malformed wing hairs. The procedure according to Frei and Würgler [17] was used to decide whether a result was positive, weakly positive, inconclusive or negative with significance level of $\alpha = \beta = 0.05$. In antimutagenic assay, the reduction of frequency of mutant spots per wing induced by *in vivo* formed nitrosomethylurea was calculated as following:

$$\text{Percentage of inhibition} = 100(a - b)/a$$

Where a is the frequency of spots per wing obtained from flies brought up on positive control medium that contained the combination of sodium nitrite and methylurea; b is the frequency of spots per wing obtained from flies brought up on each sample medium containing the combination of sodium nitrite and methylurea as modified from Abraham et al [18]. It is proposed that percentages of inhibition between 0-20 represent a negligible effect while expression of percentages of inhibition between 20-40, 40-60 and more than 60 are the evidences of weak, moderate and strong antimutagenicity, respectively.

RESULTS

Raw Hom Nil rice (RH), cooked Hom Nil rice (CH), fermented Hom Nil rice (FH), raw black glutinous rice (RB), cooked black glutinous rice (CB) and fermented black glutinous rice (FB) were neither mutagenic nor too toxic on the 3-day old larvae. They gave numbers of surviving flies higher than 50% (data not shown) and they did not significantly induce the frequencies of mutant spots at any testing amounts to be higher than that of the negative control medium (Table 1). The counteracting effect of each rice sample on the mutagenicity of *in vivo* formed nitrosomethylurea is shown in Figures 1(A) and 1(B). The result indicated that raw, cooked and fermented Hom Nil rice had negligible inhibitory effect while black glutinous rice had weak to moderate inhibitory effect. In addition, fermented black glutinous rice possessed the highest antimutagenicity against *in vivo* formed nitrosomethylurea.

DISCUSSION

The results indicated that the pigmented rice samples showed the inhibiting effect against the mutagenicity of *N*-nitrosomethylurea that might be available from the reaction of nitrite and methylurea. Since Sadabpod et al. [13] has recently determined that all the extracts from raw, cooked and fermented Hom Nil rice and black glutinous rice contained antioxidants and phenolic compounds. Antioxidants might be helpful in diminishing the formation of the mutagen occurred from the reaction between methylurea and sodium nitrite [19]. Some antioxidants such as vitamins C and E could inhibit the formation of N-nitroso compound in the study done by Pourazranga et al. [20]. In this study, each sample might act as a NO• scavenger and diminished this radical during the formation of *N*-nitrosomethylurea in the gut of *Drosophila* larva. Therefore, it is postulated that all

Table 1 Mutagenicity of each rice sample reported as wing spot induction on *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross using the somatic mutation and recombination test.

Hom Nil rice	Amount of Sample* (%)	Total spots per wing[a]		Black glutinous rice	Amount of Sample* (%)	Total spots per wing[a]	
		Trial 1	Trial 2			Trial 1	Trial 2
Raw				Raw			
	20	0.225 (9)-	0.225 (9)-		20	0.250 (10)-	0.250 (10)-
	40	0.225 (9)-	0.200 (8)-		40	0.100 (4)-	0.250 (10)-
	60	0.275 (11)-	0.225 (9)-		60	0.225 (9)-	0.250 (10)-
	80	0.325 (13)-	0.375 (15)-		80	0.375 (15)-	0.375 (15)-
	100	0.375 (15)-	0.575 (23)i		100	0.550 (22)i	0.475 (19)-
Cooked				Cooked			
	20	0.375 (15)i	0.450 (18)+		20	0.375 (15)i	0.100 (4)-
	40	0.200 (8)-	0.075 (3)-		40	0.100 (4)-	0.025 (1)-
	60	0.200 (8)-	0.075 (3)-		60	0.250 (10)i	0.250 (10)i
	80	0.125 (5)-	0.225 (9)i		80	0.300 (12)i	0.475 (19)+
	100	0.150 (6)-	0.25 (10)i		100	0.150 (6)-	0.225 (9)i
Fermented				Fermented			
	20	0.100 (4)-	0.325 (13)i		20	0.175 (7)i	0.200 (8)i
	40	0.150 (5)i	0.050 (2)-		40	0.050 (2)-	0.050 (2)-
	60	0.075 (3)-	0.075 (3)-		60	0.250 (10)i	0.250 (10)i
	80	0.050 (2)-	0.100 (4)-		80	0.075 (3)-	0.150 (6)i
	100	0.050 (2)-	0.075 (3)-		100	0.050 (2)-	0.075 (3)-

* % sample substituted for corn flour in each sample medium. Total numbers of spots per wing of the negative control were between 0.175-0.550 while those of the positive control (containing 20 mM urethane) are between 11.025-13.000.

[a] Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler [17] for comparisons with distilled water: + = positive; - = negative; i = inconclusive. Probability levels: $\alpha = \beta = 0.05$ and one-sided statistical test.

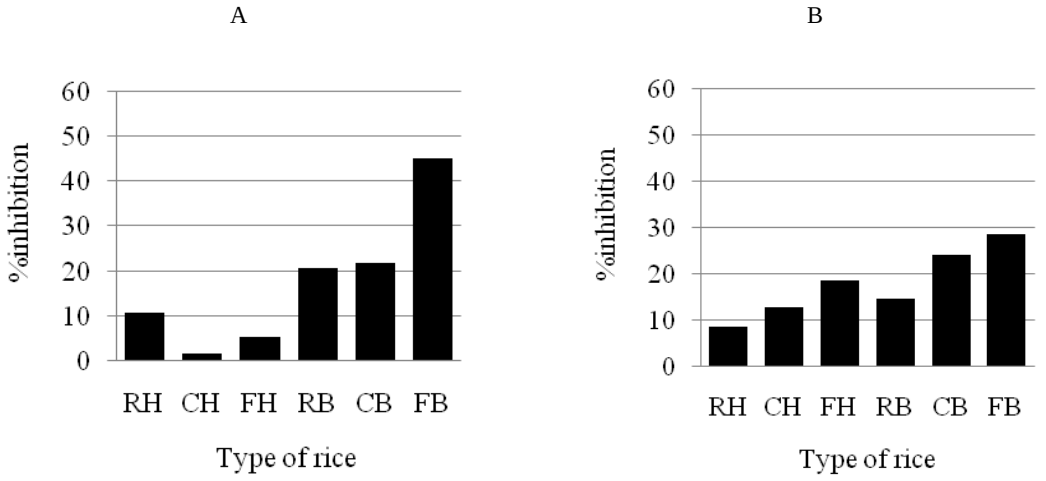

Figure 1 Antimutagenicity against the *in vivo* formed of nitrosomethylurea (expressed as %inhibition) of Hom Nil rice (0.25 g) and black glutinous rice (0.25 g) using Somatic Mutation and Recombination Test in trial 1(A) and trial 2(B); raw Hom Nil rice (RH), cooked Hom Nil rice (CH), fermented Hom Nil rice (FH), raw black glutinous rice (RB), cooked black glutinous rice (CB) and fermented black glutinous rice (FB)

of rice samples might be beneficial to consumers in terms of inhibition the formation of mutagen occurred during gastric digestion. Ferguson et al. [21] suggested that dietary antimutagen such as phenolic compounds, including anthocyanins, could protect against mutation by inhibiting nitrosation and blocking or competing with mutagens.

In addition, the result indicated that the antimutagenicity of Hom Nil rice was lower than that of black glutinous rice and the fermented black glutinous rice was surprisingly higher than that of its corresponding cooked rice. This might be due to higher content of total phenolics and aglycones in fermented rice. Sadabpod et al. [13] also indicated

that fermented rice had higher both antioxidant activity and phenolic contents than raw or cooked rice when their results were calculated based on their raw material dry weight. Phenolic compounds are commonly found in conjugated forms through hydroxyl groups with sugar and glycosides [22]. Some studies suggested that the increased action of β-glycosidase during fermentation by fungi contributed to the cleavage of the β-glycosyl bond in the black soybean glucoside and released aglycones [23, 24]. Yeast cake used as the starter organisms in the present study is the mixed cultures containing yeast, mold and bacteria. Lee and Chou [25] also reported that fermentation increased the content of aglycone (e.g. the bioactive isoflavone) in the same way as the activity of β-glucosidase. Furthermore, Juan et al. [26] found that the anthocyanins content of black soybean increased during fermentation, therefore, the fermentation might enhanced the antimutagenicity of black glutinous rice, which has been related to health benefits and well-being.

CONCLUSION

The results of this investigation indicated that Hom Nil rice and black glutinous were safe and had some health benefits to consumers because they could counteract the mutagenicity of the *in vivo* formed nitrosomethylurea. However the active compound(s) responsible for this antimutagenic activity will be required to verify. The present results might encourage food industry to substitute pigmented rice for white rice in their food products.

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