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Reduction of Bacterial Mutagenesis of 2-Amino-3-Methylimidazo[4,5-f]quinoline by S-9 Fraction from Mice Treated with Conjugated Linoleic Acid (CLA)

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Abstract

Conjugated linoleic acid (CLA), when incorporated into mouse liver microsomal membranes, selectively inhibits the mutagenesis of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ). Nine-week old female ICR mice were given (p.o.) 0.1 mL olive oil alone (control), 0.1 mL olive oil plus 0.1 mL linoleic acid, or 0.1 mL olive oil plus 0.1 mL CLA, twice weekly for four weeks. The animals were then sacrificed and liver S-9 fractions were prepared. Activation of IQ for mutagenesis by the liver S-9 from CLA-treated mice was significantly reduced in comparison with liver S-9 from control or linoleic acid-treated mice. By contrast, the activation of 7,12-dimethylbenz[a]anthracene (DMBA) and benzo[a]pyrene (BP) was unaffected. Hence, CLA incorporated into phospholipids may selectively affect cytochrome P450 isozymes responsible for activating IQ, but not those which activate BP or DMBA. The addition of free CLA or the methyl esters of CLA, linoleic acid, or oleic acid, to control S-9 inhibited the activation of all three mutagens (IQ, BP, and DMBA).

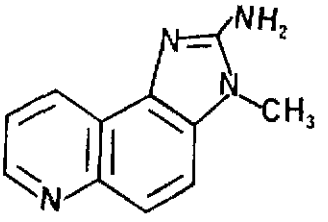
Key words: conjugated linoleic acid (CLA), bacterial mutagenesis, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)

INTRODUCTION

It is evident that dietary factors contribute to human cancer risk (1,2). Diet may serve as a source of carcinogens (and/or their precursors), and modulators which can increase or decrease genotoxic damage (3). Heterocyclic amines produced during the heating of protein-rich foods are considered possible dietary carcinogens (4).

The 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) is a heterocyclic amine mutagen found in the diet (Fig. 1). It has been shown to form at normal household cooking temperature from the condensation of creatinine, amino acid, and/or hexose (5). Activation of IQ for mutagenesis or carcinogenesis requires oxidation of the exocyclic amino group to the corresponding hydroxyamino derivative by cytochrome P450IA2 (6,7), followed by esterification to N-acetoxy metabolites (8).

Conjugated linoleic acid (CLA) is present in meats from ruminant animals at a concentration of about 1 g/kg and in dairy products in amounts ranging from 0.03 to 1.8 g/kg (9,10). CLA is produced by the isomerization of linoleic acid in the rumen of cattle (11). CLA inhibits the initiation of mouse skin carcinogenesis induced by 7,12-dimethylbenz[a] anthracene (DMBA) (12), mouse forestomach neoplasia by benzo[a]pyrene (BP) (13), and rat mammary carcinogenesis by DMBA (14). Other biological activities were evident (15-17).



IQ

Fig. 1. Chemical structure of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ).

The purpose of this study is to investigate the effect of liver S-9 from mice treated with CLA on the activation of IQ for mutagenesis in *Salmonella typhimurium* TA98.

MATERIALS AND METHODS

Materials

IQ was purchased form Toronto Research Chemicals, Inc. (Downsview, Ontario, Canada). BP, DMBA, NADP, glucose-6-phosphate, and dimethylsulfoxide were obtained from Sigma Chemical Co. (St. Louis, MO). Organic solvents (acetonitrile, chloroform, and methanol; all HPLC grade) were obtained from Burdic and Jackson Laboratory (Muskegon, MI). All other

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chemicals used were reagent grade. CLA was synthesized from linoleic acid, derivatized with H_2SO_4/methanol, and analyzed for purity by GC (18). The principal isomers in the mixture were c9,t11 CLA and t10,c12 CLA. The remaining isomers were minor contributors.

Preparation of mice liver S-9 fractions

Seventy-five female ICR mice (Sprague-Dawley, Daegu, Korea), 7 weeks of age, were housed in polycarbonated-plastic cages (5 mice/cage) in a temperature- and humidity-controlled facility and permitted free access to water and food (Chow diet; Daegu, Korea). Two weeks later, the animals were randomized by body weight and divided into three groups (25 mice/group; 5 mice/cage). They were then subjected to one of the treatments. Animals were given (p.o.) 0.1 mL olive oil alone (control), 0.1 mL olive oil plus 0.1 mL linoleic acid, or 0.1 mL olive oil plus 0.1 mL CLA, twice weekly for 4 weeks. At 13 weeks of age, all mice were sacrificed. Liver S-9 fractions were then prepared according to the method of Garner et al. (19), and stored frozen at -70°C until use. Phospholipid fraction of S-9 was separated by the method of Du et al. (20) and the profile of CLA and fatty acid in the phospholipid was determined by the method of Kim et al. (18). Protein content in S-9 was determined by the method of Lowry et al. (21), and was not affected by CLA intubation (Table 1).

***Salmonella* bacterial assay**

Studies on the activation of IQ, BP, and DMBA by liver S-9 from mice treated with olive oil, linoleic acid, or CLA were conducted according to the preincubation method of Lee et al., (22) using *S. typhimurium* TA98. Background tests were also performed by the same method as mentioned above, but without mutagens. No toxicity of mutagens or fatty acids in the ranges tested was observed.

Table 1. Total protein content of liver S-9 fractions of mice treated with olive oil, linoleic acid or CLA[1]

Treatment[2]	Protein content[3] (mg/mL S-9 fraction)
Olive oil (control)	25.2±0.5[4]a
Linoleic acid	26.5±0.1[a]
CLA	26.0±0.4[a]

[1]Mouse liver S-9 fractions were prepared from female ICR mice (25/treatment) given (p.o.) 0.1 mL olive oil alone (control), control plus 0.1 mL linoleic acid, or control plus 0.1 mL CLA, twice weekly, over a period of 4 weeks.
[2]Olive oil, 0.8 mL of olive oil; linoleic acid, 0.8 mL of olive oil plus 0.8 mL of linoleic acid, and CLA, 0.8 mL of olive oil plus 0.8 mL of CLA (p.o.) over a period of 4 weeks.
[3]Protein content of S-9 fractions were determined by the Lowry method (21).
[4]Mean±SD.
The same letters in the column of protein content mean no significant difference at $p<0.05$ by LSD test.

RESULTS

Effect of CLA incorporated in the phospholipid of mouse liver S-9 on IQ activation

The c9,t11 CLA isomer was selectively incorporated into phospholipids of mouse liver S-9 when intubated with CLA containing various isomers (Table 2). Incorporation of the isomer did not produce a gross disturbance of oleic, linoleic, or linolenic acid content in the S-9.

Fig. 2 shows the effect of CLA incorporation into the phospholipid of mouse liver S-9 on IQ activation for mutagenesis. The IQ was held constant at 20 ng/plate, and the S-9 concentration was varied in order to determine the optimal S-9 concentration for IQ activation under these test conditions. Based on these results, 10% S-9 in the cofactor mix was used subsequently.

The S-9 from mice treated with CLA significantly reduced the activation of IQ over the ranges tested, as compared to that from mice treated with olive oil (Fig. 3). The S-9 fraction was held constant at 10% in the cofactor mix, where IQ concentration was varied. At a concentration of 20 ng IQ/plate, the activation of IQ by S-9 from mice treated with CLA was 55% of that by S-9 from mice treated with olive oil (control). However, the activation of IQ by S-9 from mice treated with linoleic acid was 33% greater than that by S-9 from mice treated with olive oil. Such mutagenic effects of CLA and linoleic acid were not seen in the activation of BP and DMBA (Table 3).

To investigate the stability of CLA and the direct involvement of CLA on IQ activation, CLA content was measured during the preincubation of the S-9 from CLA-treated mice at 37°C with IQ and cofactor mix as a function of time (Table 4). The CLA content was not significantly changed during the preincubation over a period of 60 min. These results suggest that the CLA incorporated into the phospholipid of the S-9 fraction is stable, and that the CLA itself does not contribute directly to the inhibitory activity of CLA as seen in Figs. 2 and 3.

Table 2. Fatty acid concentrations (μg/mL) in S-9 fractions 4 weeks after treatment

Treatment[1]	CLA[2]	Oleic acid	Linoleic acid	Linolenic acid
Olive oil (control)	2.1±0.1[3]	80.6±15.2	257.8±43.5	2.1±0.4
Linoleic acid	4.4±0.4[4]	72.1± 3.4	353.0±31.0[4]	1.7±0.2
CLA	53.8±4.2[4]	86.4± 8.0	268.3± 2.3	1.8±0.5

[1]Olive oil, 0.8 mL of olive oil; linoleic acid, 0.8 mL of olive oil plus 0.8 mL of linoleic acid, and CLA, 0.8 mL of olive oil plus 0.8 mL of CLA (p.o.) over a period of 4 weeks.
[2]CLA concentration was determined by GC and c9,t11 CLA isomer was greater than 95% of total CLA.
[3]Mean±SD.
[4]Significantly different from the other values in the same column at $p<0.05$ (LSD test).

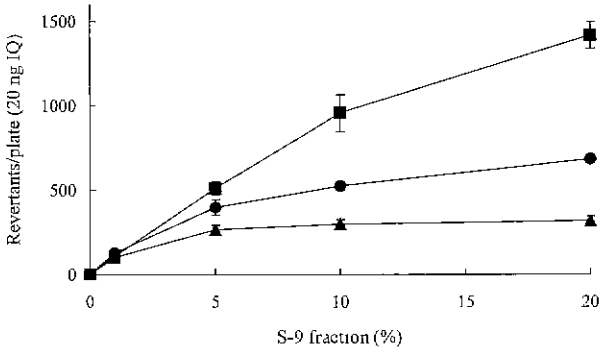

Fig. 2. Effect of the concentrations (%) of S-9 fractions in cofactor mix on the activation of IQ (20 ng/plate) for mutagenesis to *S. typhimurium* TA98. S-9 fractions were prepared from livers of mice treated with linoleic acid (closed squares), oleic acid (closed circles), or CLA (closed triangles).

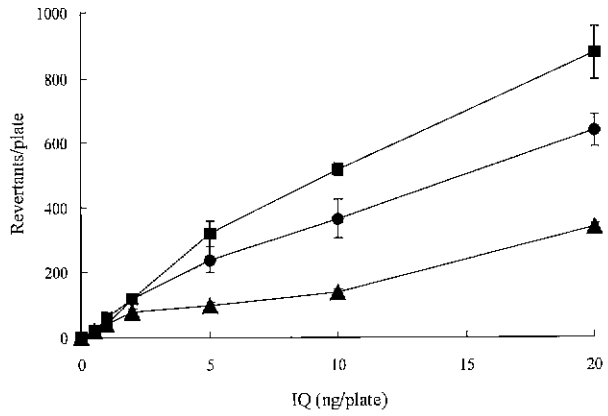

Fig. 3. Effect of mouse liver S-9 fractions on the activation of IQ for mutagenesis to *S. typhimurium* TA98. S-9 was prepared from mice treated with linoleic acid (closed squares), olive oil (closed circles), or CLA (closed triangles).

Table 3. Effect of mouse liver S-9 on the mutagenecity of BP and DMBA for *S. typhimurium* TA98

Mutagens μg/plate[1]		S-9 fraction from[2]		
		Olive oil (control)	Linolcic acid	CLA
BP	0	0	0	0
	2	68±4[3]	70±5	69±3
	5	125±15	155±7	115±8
	10	164±11	158±8	160±7
	20	145±16	130±7	123±9
DMBA	0	0	0	0
	50	26±1	27±2	25±2
	100	38±2	35±3	33±4
	200	35±3	28±2	30±2
	500	24±4	20±2	21±2

[1]Amount of mutagens used per plate.
[2]Mouse liver S-9 fractions were prepared from female ICR mice (25/treatment) given (p.o.) 0.1 mL olive oil alone (control), control plus 0.1 mL linoleic acid, or control plus 0.1 mL CLA, twice weekly, over a period of 4 weeks.
[3]Mean of revertants/plate±SD.

Table 4. Effect of preincubation process on the CLA stability of S-9 from mice treated with CLA[1]

Preincubation time (min)	c9,t11 CLA (μg/mg protein)
0	2.37±0.62[2]a
10	1.94±0.62[a]
30	1.90±0.11[a]
60	1.91±0.04[a]

[1]The c9,t11 CLA was determined by GC method (18).
[2]Mean±SD.
Same Letters in the column of CLA content mean no significant difference at $p<0.05$ by LSD test.

Effect of free CLA on IQ activation

The effect of adding CLA methylesters directly to S-9 from mice treated with olive oil was investigated (Table 5). IQ and the S-9 were held constant, respectively, at 20 ng per plate and 10% in cofactor mix, and the concentration of CLA methylester was varied. The CLA methylester substantially reduced the activation of IQ for mutagenesis. Similar reductions were also seen by oleic or linoleic acid methylesters. It is noteworthy that CLA or linoleic acid methylester substantially reduced the activation of BP and DMBA, not of 2-nitrofluorene, a direct acting mutagen (data not shown). These results indicate that the polyunsatured fatty acids tested reduce the activation for bacterial mutagenesis of mutagens which require metabolic activation by S-9, and are consistent with the results of Hayatsu et al. (23), who observed that polyunsaturated fatty acids including oleic acid inhibited the mutagenesis of IQ.

DISCUSSION

This study showed that the c9,t11 CLA isomer, when incorporated into the phospholipids of mice liver S-9, effectively reduced the activation of IQ. By contrast, the activation of BP and DMBA (Table 3) was unaffected. These results established that the c9,t11 CLA isomer incorporated into mouse liver microsomal membrane selectively reduced the activation of IQ, possibly affecting cytochrome P450 isozymes responsible for activating IQ. The fact that methylester of CLA, linoleic, or oleic acid added to the control S-9 inhibited the activation

Table 5. Effect of the addition of fatty acid methyl esters to control S-9 on the activation of IQ for mutagenesis to *S. typhimurium* TA98

Concentration (μg/plate)[1]	Fatty acid methylester		
	Oleic acid	Linoleic acid	CLA
0	389± 7[2]a	398± 9[a]	401± 6[a]
10	302±14[b]	271±11[b]	255± 8[b]
20	271±13[b]	283±15[b]	249± 9[b]
50	280± 6[b]	255± 8[b]	261±18[b]
100	291±11[b]	240±13[b]	255±14[b]

[1]Concentration of fatty acid methylesters (CLA, linoleic acid, and oleic acid) directly added to the control S-9 prepared from mice treated with olive oil.
[2]Mean of revertants/plate±SD.
Same letters in the column of the mean of revertant numbers/plate represent no significant difference at $p<0.05$ by LSD test.

of all three mutagens (IQ, BP and DMBA) to a lesser extent could be, in part, due to the physical interference of enzymes with mutagens for activation by the fatty acid, not due to true inhibition of specific cytochrome P450 isozymes. This study did not reveal direct evidence that the c9,t11 CLA incorporated in phospholipids of liver S-9 inhibits the activity of specific cytochrome P450 isozymes for the activation of mutagens, but it could be deduced from literature related to the activation of IQ, DMBA or BP by liver S-9 and the data obtained from our study.

IQ, BP, and DMBA are activated by microsomal cytochrome P450 enzymes to intermediates that are mutagenic to *S. typhimurium* TA98 (24). The liver is considered to be a main organ for the activation of these mutagens. Among several families of cytochrome P450 in animal or human liver, cytochrome P450I family is known to catalyze the activations (6,7,25). IQ was found to be activated through hydoxylation of the exocyclic amino group, N-hydroxy-3-methylimidazo[4,5-f]quinoline, mainly by cytochrome P450IA2, a high-spin form of cytochrome P450I (6). By contrast, BP and DMBA were activated through the activation of cytochrome P450IA1, a low-spin form of cytochrome P450I (26,27). Hence, the implication of our results is that the CLA incorporated into the microsomal membrane of S-9 inhibits the activation of mouse liver cytochrome P450IA2 responsible for the activation of IQ, but not the cytochrome P450IA1 responsible for the activation of BP and DMBA.

Many heterocyclic amines produced during the cooking of protein-rich food at elevated temperatures are carcinogenic in test animals (28). Given the suggestion that they may be linked to human cancer (1,2), our finding suggests that CLA seems to reduce the mutagenecity of a heterocyclic amine. The current study expands the effect of CLA on cytochrome P450- mediated carcinogenesis/mutagenesis activation.

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