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BRIEF COMMUNICATION

Aspirin Toxicity in Chicks Given Diets Deficient in Linoleic Acid

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MURAI, A., M. FURUSE AND J. OKUMURA. *Aspirin toxicity in chicks given diets deficient in linoleic acid.* PHARMACOL BIOCHEM BEHAV **48**(4) 1047–1051, 1994. – The toxicity of dietary aspirin on growth rate and lipid metabolism was investigated under linoleic acid (LA; 18 : 2n-6) deficient conditions. One-week-old chicks were given diets containing 0 or 2% LA with or without 0.4% aspirin, until 4 weeks of age. Growth was severely depressed by dietary aspirin when chicks were given the LA-free diet. The liver was enlarged by both the aspirin and LA deficiency. The aspirin treatment induced a significant increase of 18 : 0 and arachidonic acid (20 : 4n-6) and a decrease of 18 : 1n-9 in the liver. In chicks fed LA-free diets, the ratio of 20 : 3n-9/20 : 4n-6, which was used as an indicator of LA deficiency, was suppressed by aspirin treatment. In conclusion, the present results suggest that aspirin toxicity is altered by dietary LA concentrations.

Chick Aspirin Linoleic acid Growth Liver enlargement Fatty acid composition

LINOLEIC acid (LA, 18 : 2n-6) and other n-6 poly unsaturated (PUFA) fatty acids are essential fatty acids. LA is metabolized to dihomo-gamma-linolenic acid (DGLA; 20 : 3n-6) via gamma-linolenic acid (18 : 3n-6) and finally to arachidonic acid (AA; 20 : 4n-6). Eicosanoids, which act as local hormones, are synthesized from n-6 PUFA such as DGLA and AA. Aspirin had been used as a relief for headaches for almost a century before anyone had any idea of how it worked. Now, it is well known that aspirin inhibits eicosanoid biosynthesis by irreversible acetylation of cyclooxygenase (18). The use of aspirin frequently causes poisoning or serious intoxication. Furthermore, there is a possibility that renal or hepatic insufficiency, hypothrombinemia, and other bleeding disorders are enhanced by the presence of aspirin (14). In man, the use of aspirin is contraindicated in children and adolescents with febrile viral illness because of the risk of Reye's syndrome, which is characterized by an encephalopathy and fatty infiltration of viscera (7). It has been suggested that eicosanoids synthesized from n-6 PUFA may be involved in the etiology of encephalopathy in chicks (5).

The symptoms of LA deficiency in growing chicks are retarded growth and enlarged liver accompanied by lipid accumulation (13,16,17). Moreover, fat metabolism is influenced by LA deficiency. In particular, there is increased hepatic 5, 8, 11-eicosatrienoic acid (20 : 3n-9) (16) and plasma cholesterol concentration (8). However, it is not known whether these phenomena are directly induced by the deficiency of LA itself or by the deficiency of LA metabolites such as eicosanoids. If eicosanoids are important factors, aspirin treatment may give rise to more serious disorders under LA or other n-6 PUFA deficient conditions in the growing stage.

The present study was conducted to examine whether the toxicities of dietary aspirin on growth rate and lipid metabolism in growing chicks are altered by dietary LA concentrations.

METHOD

Subjects and Housing

Day-old Single Comb White Leghorn male chicks were purchased from a local supplier (Hattori Hatchery Co. Ltd., Nagoya, Japan). The animals were fed an LA-free semipurified diet for 7 days to reduce the LA reserve in their body (Table 1). The detail of diets is explained in the following section. At this stage, chicks were housed in stainless steel metabolism cages in a constant temperature (30 ± 1°C) with artificial continuous lighting.

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TABLE 1
COMPOSITION OF EXPERIMENTAL DIETS (g/kg)

Ingredients	Linoleic Acid Level 0	20
Glyceryl tricaprylate	26	–
Safflower oil	–	26
Casein	220	
Mineral mixture*	58.46	
Vitamin mixture†	2	
Choline chloride	1.5	
Inositol	1	
L-Methionine	1.1	
L-Arginine · HCl	5.3	
Glycine	7.1	
Cellulose	100	
Potato starch	377.39	
Sucrose	200	
Antioxidant‡	0.15	
Total	1000	

*Contained 20.7 g $CaHPO_4 \cdot 2H_2O$, 14.8 g $CaCO_3$, 10 g K_2HPO_4, 3 g KCl, 6 g NaCl, 3 g $MgSO_4$, 0.5 g $FeSO_4 \cdot 7H_2O$, 0.35 g $MnSO_4 \cdot 5H_2O$, 2.6 mg KI, 40 mg $CuSO_4 \cdot 5H_2O$, 62 mg ZnO, 1.7 mg $Na_2MoO_4 \cdot 2H_2O$, 0.4 mg Na_2SeO_3, 0.93 mg $CoCl_2$.

†Contained 15 mg calcium pantothenate, 6 mg riboflavin, 4 mg pyridoxine hydrochloride, 40 mg nicotinic acid, 1.5 mg folic acid, 0.2 mg biotin, 0.02 mg cyanocobalamin, 3 mg thiamin hydrochloride, 200 ICU vitamin D_3, 0.5 mg vitamin K_3, and 1.930 g glucose. The DL-α-tocopheryl acetate (10 IU) and retinyl acetate (1700 IU) were dissolved in the glyceryl tricaprylate and safflower oil.

‡6-Ethoxy-2, 2, 4-trimethyl-1, 2-dihydroxyquinoline.

Procedure

On day 7, chicks were weighed and distributed into four groups of five chicks each, so that mean body weights were as uniform as possible. The composition of the diets is presented in Table 1. Casein and cellulose in the diet were treated with hot methanol to remove lipid components. The LA level was adjusted by addition of safflower oil (Rinoru Oil Mills Co. Ltd., Tokyo, Japan), and in the LA-free diet glyceryl tricaprylate (Kao Co., Wakayama, Japan) was substituted for the safflower oil. The safflower oil was composed of the following fatty acids: 14 : 0, 0.1%; 16 : 0, 6.5%; 18 : 0, 2.3%; 18 : 1n-9, 13.1%; 18 : 2n-6, 77.1%; 18 : 3n-3, 0.7%; 20 : 1n-9, 0.1% and 22 : 0, 0.2%. Aspirin (acetylsalicylic acid) was purchased from Sigma Chemical Co. (St. Louis, MO), and supplemented to each diet at 0 or 0.4%. All chicks were given diets and water ad lib for 3 weeks. Body weight and food consumption were measured weekly. On the final day, all chicks were fasted for 4 h and blood samples were obtained from the jugular vein. The collected blood was centrifuged at 3000 rpm for 20 min and the serum was removed and stored at −80°C. After blood sampling, birds were killed by decapitation. The livers were immediately removed and weighed, and stored at −20°C until the measurement of fatty acid composition.

Measurement of Serum Cholesterol Concentration and Liver Fatty Acid Composition

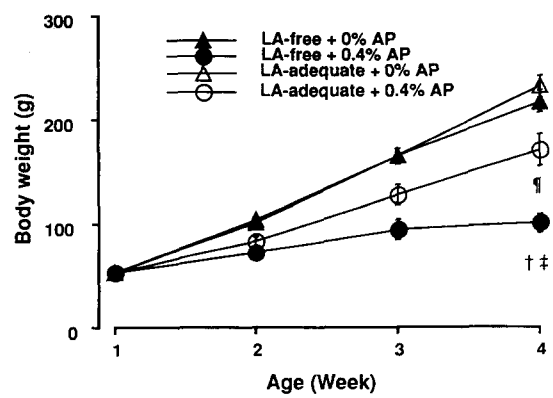
The concentration of serum cholesterol was determined using a kit (Cholesterol C-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Liver samples were weighed, and dehydrated by mixing with 1–2 g sodium sulfate anhydrous, followed by extraction with chloroform : methanol (2 : 1, v/v) solvent. The total lipid extract was filtered and dried under a gentle stream of nitrogen, and the total lipid was redissolved in 2.8% sodium methylate. The solution was incubated at 45°C for 2 h and mixed with hexane. A fraction of the hexane was used for the determination of fatty acid composition. The composition of fatty acid methyl ester was determined by using a gas chromatography (GC-14A, Shimadzu Co., Kyoto, Japan) fitted with a 25 m × 0.25 mm i.d. PEG-20M capillary column (Gasukuro Kogyo, Inc., Tokyo, Japan) and a data analytical system (CR-4A, Shimadzu Co., Kyoto, Japan).

Statistical Procedure

Data were analyzed by two-way analysis of variance (ANOVA) having LA (two levels) and aspirin (two levels) as crossed factors and differences between means were assessed by Student's *t*-test. Statistical analysis was done using a commercially available statistical package (19).

RESULTS

The influence of dietary aspirin on the growth of chicks fed LA-free or LA-adequate diet is shown in Fig. 1. ANOVA revealed that the only significant interaction between dietary LA and aspirin level occurred at 4 weeks of age. Without aspirin treatment, no significant difference was observed in body weight between two dietary levels of LA over the experimental period. With the aspirin treatment, however, the growth of chicks fed the LA-free diet was severely retarded and that of chicks fed the LA-adequate diet was mildly depressed. This implies that growth depression in chicks given a large amount of dietary aspirin was reinforced by the LA deficiency.



$F_{LA}(1, 16)$	$= 2.3^{NS}$	$= 4.0^{NS}$	$=13.6^{*}$
$F_{Aspirin}(1, 16)$	$=30.2^{***}$	$=39.8^{***}$	$=58.5^{***}$
$F_{Interaction}(1, 16)$	$= 0.8^{NS}$	$= 3.7^{NS}$	$= 5.7^{*}$

FIG. 1. Effect of dietary LA and aspirin on body weight of chicks. The diets were given ad lib for 3 weeks. In each week, data was analyzed using two-way ANOVA. Number of birds used was five per treatment. Significantly different from LA-adequate diet with aspirin treatment (†) and LA-free diet without aspirin treatment (‡) at $p < 0.001$. Significantly different from LA-adequate diet without aspirin treatment (¶) at $p < 0.01$. Significance levels: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS, not significant.

TABLE 2

EFFECT OF DIETARY ASPIRIN ON FOOD INTAKE, LIVER WEIGHT, AND SERUM CHOLESTEROL IN CHICKS FED LINOLEIC ACID (LA)-FREE OR -ADEQUATE DIET

LA	Aspirin (%)	Food Intake (g/day)	Liver Weight (g/kg body weight)	Serum Cholesterol (mg/100ml)
Free	0	24.6 ± 0.6	31.6 ± 1.5	166 ± 6
	0.4	13.9 ± 0.8*	37.6 ± 2.6	161 ± 26
Adequate	0	25.2 ± 0.9	24.5 ± 1.2	126 ± 11
	0.4	20.7 ± 1.0†‡	26.0 ± 0.9	115 ± 8
F_{LA}	(1, 16)	19.1§	31.6§	8.3#
$F_{Aspirin}$	(1, 16)	80.3§	5.0#	6.3^{NS}
$F_{Interaction}$	(1, 16)	13.0¶	1.8^{NS}	0.1^{NS}

Data were analyzed using two-way ANOVA. Difference between means was assessed by Student's *t*-test. Number of birds was five per treatment.

Values are means ± SEM.

*Significantly different from LA-free diet without aspirin treatment at $p < 0.001$.

†Significantly different from LA-free diet with aspirin treatment at $p < 0.001$.

‡Significantly different from LA-adequate diet without aspirin treatment at $p < 0.01$.

Significance levels: §$p < 0.001$; #$p < 0.05$; ¶$p < 0.01$; NS, not significant.

TABLE 3

EFFECT OF DIETARY ASPIRIN ON FATTY ACID COMPOSITION OF LIVER LIPIDS IN CHICKS FED LINOLEIC ACID (LA)-FREE OR -ADEQUATE DIET

		Fatty Acid			
LA	Aspirin (%)	18 : 0	18 : 1n-9	18 : 2n-6	18 : 3n-6
Free	0	14.66 ± 0.43	50.64 ± 1.24	1.49 ± 0.23	0.12 ± 0.04
	0.4	20.88 ± 2.36	39.09 ± 4.80	2.78 ± 0.40	0.07 ± 0.02
Adequate	0	20.59 ± 0.47	12.28 ± 0.91	29.88 ± 1.69	0.83 ± 0.18
	0.4	24.20 ± 1.35	9.08 ± 0.49	27.87 ± 1.23	0.52 ± 0.04
F_{LA}	(1, 14)	12.1*	226†	661†	45.3†
$F_{Aspirin}$	(1, 14)	13.7‡	10.5‡	0.1^{NS}	4.4^{NS}
$F_{Interaction}$	(1, 14)	1.0^{NS}	3.4^{NS}	2.5^{NS}	2.2^{NS}
		Fatty Acid			
LA	Aspirin (%)	20 : 3n-6	20 : 4n-6	20 : 3n-9	20 : 3n-9/20 : 4n-6
Free	0	0.59 ± 0.35	1.39 ± 0.08	7.28 ± 1.24	5.17 ± 0.64
	0.4	0.74 ± 0.14	5.36 ± 2.02	7.82 ± 1.56	1.76 ± 0.30[a]
Adequate	0	1.15 ± 0.22	11.97 ± 2.20	0.93 ± 0.11	0.08 ± 0.005[b]
	0.4	1.13 ± 0.20	16.34 ± 0.52	1.21 ± 0.05	0.07 ± 0.004[c]
F_{LA}	(1, 14)	3.4^{NS}	64.2†	43.3†	77.6†
$F_{Aspirin}$	(1, 14)	0.1^{NS}	9.6‡	0.2^{NS}	19.8†
$F_{Interaction}$	(1, 14)	0.1^{NS}	$<0.1^{NS}$	$<0.1^{NS}$	19.6†

Data were analyzed using two-way ANOVA. Difference between means was assessed by Student's *t*-test. Number of birds was five per treatment. Number of birds in linoleic acid-free diet with 0.4% aspirin and linoleic acid-adequate diet without aspirin was four due to one missing value.

Values are means ± SEM.

[a]Significantly different from LA-free diet without aspirin treatment at $p < 0.001$.

[b]Significantly different from LA-free diet with aspirin treatment at $p < 0.001$.

[c]Significantly different from LA-free diet without aspirin treatment at $p < 0.01$.

Significance levels: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$; NS, not significant.

Food intake, liver weight and serum cholesterol concentration of chicks are given in Table 2. There was no significant interaction between LA and aspirin level in liver weight and serum cholesterol concentration, although a significant interaction was observed in food intake. The values for food intake showed similar tendencies to those observed in body weight (Fig. 1). Liver weights were significantly increased by both the LA-free diet and the aspirin treatment. Serum cholesterol concentration of chicks fed the LA-adequate diet was significantly lower than that of chicken fed the LA-free diet, although there was no significant difference with aspirin treatment.

The fatty acid composition of the liver lipids is shown in Table 3. No significant interactions between LA and aspirin level were found in liver fatty acid levels, although the interaction was significant for the 20 : 3n-9/20 : 4n-6 ratio. The fatty acid content of n-6 PUFA in chicks fed the LA-free diet was significantly lower than that of chicks fed the LA-adequate diet. In contrast, n-9 unsaturated fatty acids were increased by the LA-free diet. The aspirin treatment induced a significant increase of 18 : 0 and AA, but 18 : 1n-9 was decreased. In chicks fed the LA-free diet, the ratio of 20 : 3n-9/20 : 4n-6 was significantly decreased by the aspirin treatment; however, the ratio under LA-adequate conditions was not affected by the aspirin treatment.

DISCUSSION

It is well known that aspirin inhibits the cyclooxygenase enzyme and, thus, inhibits the synthesis of prostaglandin G, prostaglandin H, and all that follows. Therefore, aspirin is still the most widely prescribed analgestic-antipyretic and anti-inflammatory agent. Hart (10) suggested that lethal doses of aspirin for mice and rats are 1.1 and 1.5 g/kg body weight, respectively, when it was given once through the stomach tube. Temple (21) also assessed the severity of salicylate intoxication in humans and suggested that a dose more than 0.5 g/kg body weight would cause lethal toxic reactions. In the present study, chicks were given a large amount of aspirin, ranging from 0.7 to 0.8 g/day/kg body weight (on the basis of average body weight and food intake). These aspirin levels were not lethal, because all chicks were alive at the end of the experiment. We did not measure the eicosanoids concentration in the present study. However, it is likely that the activity of cyclooxygenase was fully inhibited by aspirin; Bruckner et al. (4) reported that the serum concentration of thromboxane B_2, which is transformed from thromboxane A_2, in chicks fed a diet containing 0.1% aspirin was dramatically reduced compared to chicks on a control diet (from 176 to 0.34 ng/ml serum).

The LA deficiency symptoms of growing chicks is characterized by growth depression and enlarged liver. However, these symptoms do not appear over short periods. Long-term feeding (more than 8 weeks) of an LA-deficient diet (2,11), or rearing hens under an LA-deficient condition (11,17), is necessary for the appearance of the symptoms. These facts suggest that newly hatched chicks contain adequate amounts of LA in their body. In turn, adequate amounts of eicosanoids can be synthesized for a long time after hatching, even though an LA-deficient diet is supplied. This idea is supported by the present result. No significant difference in growth was observed between LA-adequate and LA-free diets when aspirin was not added to the diet. On the other hand, both growth and food intake of chicks fed the LA-free diet were severely and rapidly depressed by the aspirin treatment compared with the LA-adequate counterparts. The typical clinical features of moderate overdose consist of nausea, vomiting, and epigastric discomfort (14). With a large overdose, these symptoms may be followed by pulmonary oedema, convulsions, and coma with severe dehydration and ketosis. Brenes and Jensen (3) reported that a commercial diet supplemented with 0.2% aspirin reduced food intake in broiler chicks. However, the difference in food intake between LA-free and LA-adequate diets treated with aspirin could not be explained by this drug effect alone. The toxicity of aspirin is, therefore, increased in LA-deficient conditions. The reduced food intake by aspirin could be caused by the inhibition of eicosanoid synthesis.

Aspirin can produce various hepatic metabolic disorders. Histopathologic changes of salicylate intoxication may stimulate Reye's syndrome, which is characterized by the large fatty droplets and mitochondrial changes in the liver (9,22). On the other hand, the n-6 PUFA-deficient chicks exhibited liver enlargement accompanied by lipid accumulation (13,16,17). The liver weights obtained here showed no significant interaction between dietary LA and aspirin level, but the aspirin treatment elicited 19% augmentation in chicks fed LA-free diet against 6.1% augmentation in chicks fed LA-adequate diet. AA is more effective than LA in curing hepatic triglyceride accumulation in n-6 PUFA-deficient rats (20), which indicates that AA itself or metabolites from AA would prevent liver enlargement accompanied with lipid accumulation. The reason for the enlarged liver in aspirin-treated chicks is unclear. There are two possibilities. First, DGLA, or AA metabolites such as eicosanoids, might control the liver weight. Second, various toxicities of aspirin might induce liver enlargement. Aspirin also reduces lipogenesis by partially blocking conversion of acetate to fatty acids (14) and also inhibits epinephrine-stimulated lipolysis in fat cells (14). These effects lead to increased entry and enhanced oxidation of fatty acids in muscle, liver, and other tissues, and to decreased plasma concentrations of cholesterol (14). Davison and Mandel (6) reported that the hypocholesterolemic effect of aspirin may be related to the reduction of nonesterified fatty acids. The addition of adequate amounts of n-6 PUFA to the diet lowers serum cholesterol levels (1,8,15). In the present study, however, cholesterol lowering was observed in response to LA deficiency but not to the addition of aspirin to the diet.

Increased concentrations of AA in the liver were observed by the aspirin treatment, which may be attributable to inhibition of the synthesis of eicosanoids from AA in the liver, and accelerated accumulation of AA. The ratio of 20 : 3n-9/20 : 4n-6 under aspirin treatment would, therefore, be decreased compared to the control. Biochemical diagnosis of essential fatty acid deficiency is most often based on analysis of the quantity or ratio of 20 : 3n-9 relative to 20 : 4n-6 (12). Ratios of greater than 0.4 and 0.1 have been considered consistent with normal essential fatty acids deficiency in rats (12) and chicks (16), respectively. The results show that the ratio of 20 : 3n-9/20 : 4n-6 was changed by the aspirin treatment and, consequently, the index of the LA deficiency was lower in the severest LA-deficient condition (LA-free diet with aspirin) than in the mild deficient condition (LA-free diet without aspirin). This suggests that the ratio of 20 : 3n-9/20 : 4n-6 is not be a proper index of LA deficiency in conditions such as aspirin treatment.

In conclusion, aspirin toxicities can be modified by dietary LA condition, and are increased in LA deficiency.

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