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Aspirin induces short-chain free fatty acid accumulation in rats

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Abstract

Aspirin is used for the prophylaxis of infarction. A low dose of aspirin is effective for the prophylaxis of myocardial infarction, whereas a higher dose is necessary for that of stroke. Salicylic acid, the in vivo metabolite of aspirin, inhibits the β-oxidation of short-chain fatty acids. Accordingly, drinking water containing 400, 800, or 1200 mg/l aspirin was given to each of eight rats for 30 days to determine the serum short-chain fatty acid levels. Analysis of variance and a post-hoc Fisher's protected least significant differences test revealed significantly increased levels ($P < 0.05$) of monocarboxylic acids, *n*-hexanoate, *n*-octanoate, *n*-decanoate, *n*-dodecanoate, and dicarboxylic acids, adipate (C_6,) and suberate (C_8): 78.7 ± 36.2, 61.1 ± 30.6, 215 ± 151, 47.5 ± 24.0, 3.64 ± 2.09 and 1.71 ± 1.45 μmol/l in the 800 mg/l aspirin group compared to 23.8 ± 12.3, 20.1 ± 9.0, 24.3 ± 12.1, 6.3 ± 5.6, 0.56 ± 0.50 and 0.44 ± 0.25 μmol/l in the control group, respectively. These levels were also increased in the 400 or 1200 mg/l aspirin groups but less so. These findings may help us to understand the aspirin toxicity in Reye's syndrome.

Keywords: Aspirin; Salicylic acid; Infarction; Fatty acid, short-chain, serum; Dicarboxylic acid

1. Introduction

Aspirin is used worldwide as an analgesic, for the prophylaxis of myocardial infarction and cerebral infarction, and for the prevention of occlusion in aortocoronary vein grafts. Aspirin (1.0 g/day) administration reduces the mortality rate in patients who have experienced at least one documented myocardial infarction; however, this use of aspirin has not been recommended because of the increased rate of adverse effects (Aspirin Myocardial Infarction Study Research Group, 1980). Although a low dose of aspirin, 325 mg/day, reduced the risk of myocardial infarction (Steering Committee of the Physicians' Health Study Research Group, 1989), 500 mg/day did not significantly reduce the risk of myocardial infarction or stroke (Peto et al., 1988), whereas 150 mg/day was effective for the prevention of aortocoronary vein graft occlusion (Sanz et al., 1990). A dose of 30 mg/day, however, might be effective, because the inhibition of platelet aggregation caused by the diminished production of thromboxane A_2 is still complete with this dose, whereas the production of prostacyclin, which has an antiaggregation effect, is slightly affected in endothelial cells (Moncada and Vane, 1979; Kallmann et al., 1987). The administration of 75 to 283 mg/day of aspirin reduced the risk of myocardial infarction and stroke (Antiplatelet Trialists' Collaboration, 1994). Aspirin, 325 mg/day has been recommended for the prophylaxis of myocardial infarction and stroke (Matchar et al., 1994; Hart and Harrison, 1996). Many reports concerning the relation of aspirin to the prophylaxis of infarction and stroke have been published, as described above. Patrono and Roth (1996) concluded that 75 to 160 mg/day was optimal for the prophylaxis of myocardial infarction, and that a larger dose of aspirin was necessary for the prophylaxis of stroke. The optimal dose of aspirin for stroke prevention has not been established (Barnett et al., 1995).

The decrease in the incidence of outbreaks of Reye's syndrome in parallel with the decrease in the use of aspirin for viral illnesses suggested that aspirin is associated with the outbreak of this disease (Barrett et al., 1986; Anonymous, 1987; Arrowsmith et al., 1987; Hurwitz et al., 1987). Salicylic acid, an in vivo metabolite of aspirin, is known to affect various mitochondrial functions, e.g., the

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Table 1
Short-chain fatty acid levels in the serum of rats given drinking water with 800 mg/l aspirin for 30 days

	C_3	C_4	isoC$_4$	C_5	isoC$_5$
Normal[a]	8.4 ± 3.0	11.1 ± 4.9	31.9 ± 10.4	4.6 ± 6.1	12.3 ± 6.5
Aspirin[b]	6.8 ± 2.2	9.4 ± 5.3	23.6 ± 12.8	4.0 ± 3.2	8.8 ± 1.4

The data are μmol/l, mean ± S.D. for eight rats per group.
There were non-significant differences (Student's *t*-test) between the non-aspirin control group[a] and the aspirin group[b].
The levels of these fatty acids were determined in each group by the procedure of Bachmann et al. (1979).
Presumed aspirin dose was described in Section 4.
C_3, propionic acid; C_4, butyric acid; isoC$_4$, isobutyric acid; C_5, valeric acid; isoC$_5$, isovaleric acid.

uncoupling of mitochondrial oxidative phosphorylation, the inhibition of adenine nucleotide translocase (Brondy, 1956; Aprille et al., 1986), and the inhibition of fatty acid metabolism, especially the β-oxidation of octanoic acid in mitochondria (Yoshida et al., 1988). We previously reported that the apparent Michaelis constant (K_m) and maximum velocity (V_{max}) of mitochondrial octanoic acid β-oxidation, and the apparent inhibition constant (K_i) of salicylic acid were 1.7 μM and 2.20 nmol/mg protein of mitochondria, and 0.27 mM, respectively. The apparent K_m and V_{max} of mitochondrial octanoic acid activation (octanoyl–CoA ligase activity), and the K_i of salicylic acid were 3.2 μM and 5.4 nmol/mg of mitochondrial protein/min, and 0.13 mM, respectively. Salicylic acid competitively inhibited the octanoic acid β-oxidation and activation. These inhibitory concentrations were not high compared to the in vivo level induced by the administration of the usual aspirin dose.

Reye's syndrome is a serious metabolic disorder of infants and children characterized by mitochondrial injury (Reye et al., 1963; DeDivo, 1978; Brown and Forman, 1982), which is thought to be induced by the accumulation of free fatty acids (Bourgeois et al., 1971; Trauner et al., 1977; Ogburn et al., 1982; Tonsgard and Getz, 1985), probably due to aspirin intake.

We now investigated the effects of aspirin on free fatty acid accumulation in vivo. The results may help to establish the optimal aspirin dose for the prophylaxis of myocardial infarction and stroke.

2. Materials and methods

N,O-bis(trimethylsilyl)-trifluoroacetamide was obtained from Pierce (Rockford, IL, USA). Other chemicals were purchased from Wako (Osaka, Japan).

This experiment was permitted by the ethical board of the Institute of Laboratory Animal Sciences of Kagoshima University. Male Wistar rats each weighing approximately 205 g (8 weeks old) had drinking water containing 400, 800, or 1200 mg/l of aspirin (pH 7.0) (eight rats per group) for 30 days. The rats had ad libitum access to the aspirin water, or (controls) water, and food. Thereafter, blood was collected from the iliac vein, and short-chain fatty acids were determined by the procedure of Bachmann et al. (1979). The other short-chain fatty acids and dicarboxylic acids, adipic, suberic, and sebacic acids were determined by the procedure of Ng et al. (1983), except that a gas chromatograph, GC14A (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and capillary column of CBP1-S25-050 (Shimadzu) was employed. Mandelic acid was used as an internal standard for the second method. The salicylic acid concentration was determined with a fluorescence polarization immunoassay (Jolley et al., 1981) using a TDX™ salicylic acid assay kit (Dainabot, Tokyo, Japan) and an apparatus designed for use with the fluorescence polarization immunoassay, the TDX™ analyzer (Dainabot), according to the supplier's instructions.

Statistical analyses were performed with a Macintosh computer (Apple) using the Statview version 4.5 software (Statview). Student's *t*-test or a one-way analysis of variance (ANOVA) was used and when a significant *F* ratio was found in the ANOVA, a post-hoc Fisher's protected

Table 2
Short-chain fatty acid levels in the serum of rats given drinking water with 400 mg/l, 800 mg/l, or 1200 mg/l aspirin for 30 days

	Monocarboxylic acids				Dicarboxylic acids		
	C_6	C_8	C_{10}	C_{12}	C_6	C_8	C_{10}
Control	23.8 ± 12.3	20.1 ± 9.0	24.3 ± 12.1	6.3 ± 5.6	0.56 ± 0.50	0.44 ± 0.25	0.45 ± 0.32
400 mg/l	55.6 ± 22.0	40.2 ± 28.6	87.9 ± 70.1	35.3 ± 26.2	1.68 ± 1.00	0.73 ± 0.29	1.40 ± 0.54
800 mg/l	78.7 ± 36.2	61.1 ± 30.6	215 ± 151	47.5 ± 24.0	3.64 ± 2.09	1.71 ± 1.45	2.06 ± 1.36
1200 mg/l	54.5 ± 27.1	33.6 ± 15.2	132.6 ± 41.8	27.8 ± 11.8	2.19 ± 1.76	1.24 ± 0.98	1.67 ± 1.85

The data are μmol/l, mean ± S.D. for eight rats per group.
The levels of the fatty acids were determined in each group by the procedure of Ng et al. (1983).
The fatty acid levels were increased in the groups given water containing 400 mg/l, 800 mg/l, or 1200 mg/l of aspirin.
Statistical results are shown in Table 3.
Monocarboxylic acids C_6, *n*-hexanoic acid (*n*-caproic acid); C_8, *n*-octanoic acid (caprylic acid); C_{10}, *n*-decanoic acid (capric acid); C_{12}, *n*-dodecanoic acid (lauric acid); dicarboxylic acids C_6, adipic acid; C_8; suberic acid; C_{10}, sebacic acid.

Table 3
Summary of the significance of differences (P value) in the serum short-chain fatty acid levels in control and aspirin groups

	Monocarboxylic acids				Dicarboxylic acids		
	C_6	C_8	C_{10}	C_{12}	C_6	C_8	C_{10}
ANOVA	0.0046	0.044	0.0021	0.0068	0.0045	0.021	0.095
Control and 400m g/l[a]	0.027	0.16	0.17	0.0068	0.16	0.53	
Control and 800m g/l[b]	0.0004	0.0063	0.0002	0.0004	0.0005	0.0043	
Control and 1200m g/l[c]	0.032	0.35	0.024	0.038	0.046	0.064	
400 and 800 mg/l[d]	0.089	0.13	0.0070	0.23	0.015	0.015	

Statistical analyses were performed with a one-way analysis of variance (ANOVA).
A significant F ratio ($P < 0.05$) was found for serum monocarboxylic acid levels of C_{6-12} and dicarboxylic acid levels of $C_{6,8}$; accordingly, a post-hoc Fisher's PLSD test was performed on each variable.
Significant differences were found between the control group and the groups given water with 400 mg/l[a], 800 mg/l[b] or 1200 mg/l[c] aspirin, or 400 mg/l and 800 mg/l[d] (underlined).
There were no significant differences in all fatty acid levels between groups given 400 mg/l or 800 mg/l and 1200 mg/l (not shown).

least significant differences (PLSD) test was performed on each variable. Significance was set at $P < 0.05$.

3. Results

The administration of 800 mg/l aspirin induced no accumulation of serum (C_{3-5}) short-chain fatty acids (Table 1). However, the administration of 400, 800, or 1200 mg/l aspirin induced the accumulation of serum (C_{6-12}) short-chain monocarboxylic and ($C_{6,8}$) dicarboxylic acids as shown by ANOVA, and there was no significant difference in sebacic acid level (C_{10}) (Tables 2 and 3). The standard deviation (S.D.) of fatty acid levels was large in the control group, but was greater in the aspirin groups. Post-hoc Fisher's PLSD test revealed that 800 mg/l aspirin administration significantly increased the serum fatty acid levels of monocarboxylic (C_{6-12}) and dicarboxylic ($C_{6,8}$) acids and 400 or 1200 mg/l aspirin administration also increased these levels but less than did 800 mg/l. The salicylic acid levels in the rats' serum were 0.29 ± 0.10 mM in the group given 400 mg/l aspirin ($n = 8$, mean $\pm$ S.D.), 0.50 ± 0.14 mM in the group given 800 mg/l, and 0.59 ± 0.14 mM in the 1200 mg/l group.

The rats' body weights were not increased significantly in each group (ANOVA) (Table 4). However, the increase in body weight of the rats kept with water containing 1200 mg/l of aspirin was less than that of rats given 800 mg/l, because of the bitter taste of water containing 1200 mg/l aspirin. Therefore, the fatty acid increase in the 1200 mg/l group was smaller than that of the group given 800 mg/l despite the salicylic acid level of 0.59 ± 0.14 mM.

Table 4
Body weights of control and treated rats before and after the administration of aspirin for 30 days

	Before	After
Control	206 ± 5	333 ± 9
400 mg/l	202 ± 7	347 ± 15
800 mg/l	203 ± 5	365 ± 14
1200 mg/l	203 ± 4	347 ± 30

The data are means $\pm$ S.D. for eight rats per group.
The weights were not significantly increased (ANOVA, $P = 0.077$).

4. Discussion

Rats were kept with drinking water containing 400 or 800 mg/l of aspirin. Since the rats drank 35–40 ml/day of water, the aspirin intake per day was estimated to be 14–16 and 28–32 mg/rat (calculated from the mean body weight of rats, 275 and 284 g); that is, 51–58 and 99–113 mg/kg of body weight. This dose is moderately large compared to that administered to humans; however, the salicylic acid levels in the rats' serum were not high.

The serum fatty acid levels were variable even in the control group. Serum fatty acid levels depend on the diet and fasting state, but in this experiment, the rats were fed and killed under the same diet conditions. Therefore, aspirin administration may have been responsible for the large standard deviation of fatty acid levels.

Salicylic acid (an in vivo metabolite of aspirin) competitively inhibits mitochondrial β-oxidation in short-chain fatty acid degradation (Yoshida et al., 1988). The short-chain fatty acids of dicarboxylic acids are biologically active and may be responsible for the manifestation of the signs and symptoms of Reye's syndrome (Tonsgard and Getz, 1985), but those of octanoic, decanoic and dodecanoic acids are not active (Okita, 1986; Singh et al., 1989). The block of mitochondrial β-oxidation induced the activation of microsomal ω-oxidation (Kundu et al., 1991; Mortensen, 1992), resulting in a high level of dicarboxylic acids. Whether this level is toxic is unknown, but when infectious diseases such as Dengue fever (Malewitcz et al., 1981) and viral infection cause an abnormal fatty acid metabolism, dicarboxylic acids may reach the toxic level. The murine adenovirus type I (MAV-I) infection of CB-17 severe combined immunodeficiency (SCID) mice (which

are homozygous for a SCID mutation) induces hepatic histopathologic and ultrastructural features that are strikingly similar to human Reye's syndrome (Pirofski et al., 1991). This result suggests that viral infection induces abnormal fatty acid metabolism. Both aspirin administration and infection may promote the increase of serum dicarboxylic acid levels.

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