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Short Research Communication

Serum cholesterol concentration associated with aspirin esterase activity in older people: preliminary data

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Abstract

OBJECTIVE: Metabolism of aspirin (acetylsalicylic acid), commonly used in older people for the prevention of cardiovascular disease, is important to the effectiveness of this drug. Whereas part of aspirin hydrolysis occurs in blood, there is a paucity of information in regards to circulating aspirin esterase activity in various physiological and pathological conditions. High aspirin esterase activity, corresponding to faster aspirin hydrolysis (thus aspirin non-responsiveness), may occur in cardiovascular disease-prone states. The objective of this study was to investigate the effects of cardio-metabolic variables such as cholesterol on serum aspirin esterase activity in older people who participated in an intervention study on physical activity.

METHODS: A total of 18 non-medicated subjects (7 men/11 women, mean age 67.8 years, body mass index = 23.4 ± 3.3 kg/m^2), who completed a 3-month interventional program for a mild-to-moderate increase in physical activity, were analyzed. The body mass index, plasma glucose, serum total cholesterol and aspirin esterase activity were measured in the pre- and post-interventional phases of the study.

RESULTS: During the interventional period, the changes in aspirin esterase activity correlated significantly and positively with those of total cholesterol concentrations ($r = 0.542$, $P = 0.020$; $\beta = 0.609$, $P = 0.035$ in a multiple linear regression analysis after adjusting for all the measured variables).

CONCLUSION: The results suggest that cholesterol metabolism alterations may be associated with aspirin metabolism in older people.

Key words: acetylsalicylic acid; antiplatelet; aspirin resistance; hypercholesterolemia

INTRODUCTION

Aspirin (acetylsalicylic acid) is one of the most frequently used drugs, exploiting its anti-inflammatory and anti-platelet actions for the prevention of cardiovascular disease (1). Especially, for this purpose, older people are commonly medicated with this agent (2). The so-called 'aspirin resistance (or aspirin non-responsiveness) syndrome' is characterized by an incomplete inhibition of platelet function by aspirin (2, 3). It may account for the high variability in effective anti-coagulant doses of aspirin in the population (2, 3). Increased hydrolysis of circulating aspirin, pharmacokinetically corresponding to faster

elimination of the drug from the circulation by transforming it into salicylate and acetate, is thought to participate as a causal factor in the aspirin resistance (4, 5). Although the hepatocyte accounts for most of aspirin catabolism, after the first pass, more than 50 % of the aspirin still gets to the systemic circulation where two distinct aspirin hydrolysis pathways act: a spontaneous pH-dependent hydrolysis and an enzymatic hydrolysis by plasma/serum and erythrocyte esterases (6). The circulating aspirin esterase activities are mainly due to butyrylcholinesterase (BChE, pseudocholinesterase) and, in part, to albumin (6).

Because of higher aspirin esterase activity leading to faster aspirin hydrolysis, the same dose of aspirin in people who metabolize aspirin slowly is more effective than in those who metabolize the drug quickly (4, 5). There is a wide range of rates of aspirin metabolism in the population, and ethnic and individual variations exist (7, 8). Even for a given individual, age-related factors may be also contributory to the range of turnover rates (8). However, despite the universal use of aspirin, the underpinnings of the etiology of aspirin resistance remain unclear as is the putative relative role of peripheral (serum) and central (hepatic) metabolism (4-6, 8).

Recently, a cross-sectional study has reported aspirin esterase activity to be increased in type 2 diabetics and has shown the association to be modulated by circulating lipid metabolism (6). In addition, aspirin resistance has been previously reported in patients with diabetes and cardiovascular disease (4, 5). However, research on variations of aspirin esterase activity in pathological and physiological states, including the influences of therapeutic lifestyle modifications, is very scarce. We set out this study to explore preliminarily the effects of cardio-metabolic variables such as circulating cholesterol on serum aspirin esterase activity among older people who participated in an intervention study on physical activity.

SUBJECTS AND METHODS

A total of 18 community-dwelling Japanese older volunteers (7 men/11women; mean age = 67.8 ± 7.7 years [range = 56-81 years]), who were not current smokers, not on any medication and had not been diagnosed to have cardiovascular, renal, kidney, thyroid and nutritional diseases, were recruited into a 3-month interventional program for health promotion. The program, which focused on the health benefits of a mild-to-moderate increase in physical activity, included monthly explanatory and motivational classes to increase physical activity such as walking and to instruct on the appropriate methods. The Jichi Medical University ethics committee approved the present study and each subject gave informed consent.

The body mass index (BMI) was calculated as the weight divided by the square of the height measured in light indoor clothing without shoes. Serum total cholesterol and plasma glucose were enzymatically determined. Serum aspirin esterase activity was measured kinetically by a modification (optimized in our laboratory) of the procedure described initially by Sorensen (9). The reagent buffer contained Tris-HCl (0.6 mol/L) and $CaCl_2$ (0.4 mol/L) in pH 7.6-7.7. Substrate was 555 mmol/L of acetylsalicylic acid (1g in 10 mL 100 % ethanol was used as the mother solution). Briefly, 15 µL of serum sample was pipetted per well (in duplicate) into a 96 well UV Flat bottom Plate (Thermo 8404). Substrate was extemporaneously diluted 1 in 50 into reagent buffer, and then 200 µL of this final substrate solution was added per well with a multi-channel pipette. The runs were blanked against reagent (to control for spontaneous hydrolysis of acetylsalicylic acid). Plates were placed in a temperature-controlled plate reader SpectraMax UV from Molecular Devices, and samples were kinetically read at 300 nm, 37°C for 15 min, every 57 sec. Data were expressed in nmol of acetylsalicylic acid hydrolyzed per minute and per milliliter (nmol/mL/min). The intra-assay CV (coefficient of variation) is 4% and the inter-assay 5%, respectively.

Data are shown as the mean ± standard deviation or the median [interquartile range]. Wilcoxon test was used to compare the pre- and post-interventional values of respective measured variables. The level changes were calculated by subtracting the pre-interventional values from the post-intervention values. A single linear regression analysis (Pearson's rank correlation test) and a multiple linear regression analysis adjusted for confounders were used to evaluate the correlations between the variables. The log-transformed values of aspirin esterase were used in these correlation analyses because of the skewed distribution. A value of $P < 0.05$ was considered to be significant.

RESULTS

The intervention on physical activity reduced weakly but significantly the BMI levels (23.4 ± 3.3 kg/m^2 at baseline to 23.1 ± 3.0 in post-intervention, $P = 0.037$). The averaged levels of other measured variables between pre- and post-intervention were unchanged at statistical significant levels: 5.7 ± 1.1 to 5.8 ± 1.1 mmol/L in total cholesterol, 5.6 ± 0.8 to 5.5 ± 1.1 mmol/L in glucose, 41.4 [36.0-50.0] to 39.8 [34.6-48.1] nmol/mL/min in aspirin esterase activity. At baseline

(pre-intervention), aspirin esterase activity levels were correlated significantly and inversely to age (r = -0.569, P = 0.014). After adjustment for sex, aspirin esterase had a significant positive correlation with age (r = -0.548, P = 0.020), but the significance of this correlation was lost after adjusting for sex and BMI (r = -0.406, P = 0.113). The results were not affected by adjustment for total cholesterol and glucose levels.

During the intervention period, the changes in aspirin esterase activity levels were correlated significantly and positively with those of total cholesterol concentrations (Table 1). Moreover, both variables showed a significantly and independently positive correlation after the adjustments for age (r = 0.534, P = 0.021), age and sex (r = 0.475, P = 0.035) as well as age, sex and the changes in BMI (r = 0.618, P = 0.018). The results on the significant, independent and positive correlation between total cholesterol and aspirin esterase were not affected by adjustment for all the measured variables (Table 1).

Table 1: The correlation between the change in the levels (Δ values) of aspirin esterase and the other variables during an intervention study period.

Variables	r (P value)	β (P value)
For Δ aspirin esterase (nmol/mL/min)		
Age (years)	-0.276 (0.268)	-0.389 (0.100)
Sex (men)	0.352 (0.152)	0.306 (0.173)
Δ Body mass index (kg/m^2)	-0.154 (0.542)	0.295 (0.269)
Δ Total cholesterol (mmol/L)	0.542 (0.020)*	0.609 (0.035)*
Δ Glucose (mmol/L)	0.249 (0.320)	0.022 (0.922)

r: correlation coefficient of single linear regression analysis, β: correlation coefficient of multiple linear regression analysis adjusted for all the listed variables. Age in the pre-intervention was used as age-variable. The level changes (Δ values) were calculated by subtracting the pre-interventional values from the post-interventional values. Aspirin esterase is tested after a log-transformation. Significance level: * P < 0.05.

DISCUSSION

The present study's main finding is that the changes in serum total cholesterol concentrations are clearly associated with those of aspirin esterase activity during a period of physical activity modification in an older population. This finding suggests cholesterol metabolism alterations may be important in mediation of aspirin metabolism among older people. A recent report has shown that greater aspirin hydrolysis can be associated with decreased cholesterol levels of high-density lipoprotein in type 2 diabetics (6). Although our present results are not strictly comparable to those of this previous report since that evidence was obtained in diabetics, both studies potentially seem to bring our attention to the notion that aspirin hydrolysis rates may be linked to cholesterol metabolism (6). Whereas the molecular mechanisms underlying the modulation of circulating aspirin esterase activity remain to be elucidated, our data showing its significant correlations with the changes in cholesterol concentrations may open new avenues for exploration of aspirin metabolism. Our present results of improvement of aspirin effect with decreased total cholesterol may partly account for non-effectiveness of aspirin in cardiovascular disease among patients with concurrent increases of total cholesterol levels (4, 5); thus, emphasizing the clinical importance of control of total cholesterol with a mild-to-moderate increase of physical activity among such patients.

While age-related reductions in the activities of several enzymes are known (2), data regarding the correlation of age to aspirin esterase are inconsistent (2, 10-12). Some studies reported a decrease of aspirin esterase in unhealthy, frail older subjects (10-12). Our present results at baseline only showed an inverse correlation of age to aspirin esterase activity, but the present study population was not frail, and BMI was also a confounder of this correlation. BMI is a surrogate clinical marker for various common clinical syndromes; thus, more research is needed to ascertain its role on the effects of aging on aspirin metabolism.

This study had the limitation of a small sample size and a short-term interventional period. In addition, we did not measure circulating albumin and BChE. Nevertheless, albumin has a very small contribution to the whole circulating aspirin esterase activity as compared to that of BChE (8), and our study population did not include subjects with malnutrition nor there were any with nutritional modifications during the interventional period. More studies with larger sample sizes and longer follow-up as well as including BChE measurement and nutritional assessment will be considered in the future.

In summary, we found a significant and positive association of changes in circulating total cholesterol with those of serum aspirin esterase activity in an older population that participated in a physical activity modification program. Although aspirin metabolism is probably multi-factorial, the association with cholesterol metabolism has merits for further investigations.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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