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Age-Related Increased ^{14}C-Arachidonic Acid Uptake by Platelets in Normal Subjects

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Arachidonic acid uptake activity (pmol/10^8 platelets min) measured in platelets obtained from normal subjects was significantly higher in over-forty year old (3.53 ± 0.38) than in under-forty year old subjects (2.33 ± 0.12). No significant correlations were found between the arachidonic acid uptake activity and fasting plasma glucose, total cholesterol, HDL-cholesterol or triglyceride levels. The arachidonic acid uptake activity was significantly reduced by the presence of indomethacin in platelets obtained from both under and over-forty year old subjects, and by the presence of nordihydroguaiaretic acid in platelets obtained from over-forty year old subjects. In conclusion, these data show that arachidonate uptake activity by platelets increased with age. This increase was abolished when platelets were incubated in the presence of inhibitors of the arachidonic acid metabolism.

Key words: Platelets, Arachidonic acid, Age.

It has been postulated that platelets adhering to the vessel wall and aggregating with each other could represent one of the initial events in the development of atherosclerosis (8). Since thromboxane A2 (Tx A2) plays an important role in the process of platelet aggregation (5), a relative or absolute increase in thromboxane synthesis might be a crucial step in the development of atherosclerosis (7). It is generally accepted that the Tx A2 synthesis rate is regulated at the step of liberation of arachidonate from platelet membrane phospholipids (5); therefore, an increase in Tx A2 production may be a consequence of an increase in arachidonate content in platelet phospholipids. In fact, the arachidonic acid uptake activity and its incorporation to phospholipids have been reported to increase in diabetic patients (6), and these modifications were considered as one possible cause of the increased Tx A2 synthesis in diabetes (9).

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The present report is an investigation of the influence of age on the arachidonate uptake activity in platelets from normal subjects and its relationship with plasma total cholesterol, triglycerides and HDL-cholesterol levels. Since arachidonate incorporated to platelets may be metabolized via the cyclooxygenase or lipooxygenase pathways, the arachidonate uptake activity has been investigated when platelets were incubated in the presence of indomethacin (INDO) and/or nordihydroguaiaretic acid (NDG), specific inhibitors of cyclooxygenase (7) or lipooxygenase (11) activity, respectively.

Materials and Methods

A group of 34 normal subjects, with an 18-70 year age range, with no known history of diabetes, hypertension or atherosclerotic complications were used in this study. No subject had taken aspirin or any other drugs which might alter arachidonate metabolism during the previous week.

A blood 9 mL sample was collected from an antecubital vein in overnight fasted subjects by using a plastic syringe with 1 mL EDTA (4.5 mmol/l). Plasma was obtained by blood centrifugation at 2500 r.p.m. for 10 minutes at room temperature.

The arachidonate uptake activity of platelets was measured by the method of TAKAHASHI *et al.* (9) with minor modifications. Platelet-rich plasma was obtained by centrifugation of the venous blood at 500 r.p.m. for 10 minutes at room temperature. The final platelet concentration in the platelet-rich plasma was evaluated by counting in a Neubauer chamber. Only platelet numbers ranging from $1.5 \cdot 10^8$ to $5 \cdot 10^8$ cells per mL platelet-rich plasma were used. A suspension was prepared of 1 µL ^{14}C-arachidonic acid (specific activity 56.6 Ci/mol, concentration 50 µCi/mL, Amersham) and 12.5 µL of Tris buffer (100 mM, pH 9.0), and added to 250 µL of platelet-rich plasma. Incubation was carried out at 30 °C for 60 min and was terminated by centrifugation at 2000 r.p.m. for 10 min at 4 °C. The supernatant was discarded and the pellet was gently washed twice with 200 mL of cold NaCl 0.9 %, followed by centrifugation at 2000 r.p.m. for 10 min at 4 °C. The supernatants were discarded and the final pellet was counted by liquid scintillation spectrometry.

Platelets obtained from 6 under-forty and 6 over-forty year old subjects were incubated as described, but in both the absence and presence of 0.2 mM INDO or 0.1 mM NDG in the incubation medium.

In 12 under-forty and 8 over-forty year old subjects, plama levels of glucose, triglycerides, total cholesterol and HDL-cholesterol were assayed enzymatically with kits from Boehringer-Mannheim, (glucose oxidase method, GOP-POD method and CHOD-POD method).

Results are presented as mean ± SEM. Statistical analysis was performed by using Mann-Whitney Analysis test. Relations between variables were analyzed by linear regresion analysis (least squares method).

Results

The ^{14}C-arachidonic acid uptake by platelets obtained from over-forty year old subjects was significantly higher than that measured in platelets obtained from under-forty year old subjects (fig. 1).

The arachidonate uptake activity measured in platelets that were incubated either in the presence or in the absence of 0.2 mM INDO and/or 0.1 mM NDG in the incubation medium are shown in fig. 2. The arachidonate uptake activity in

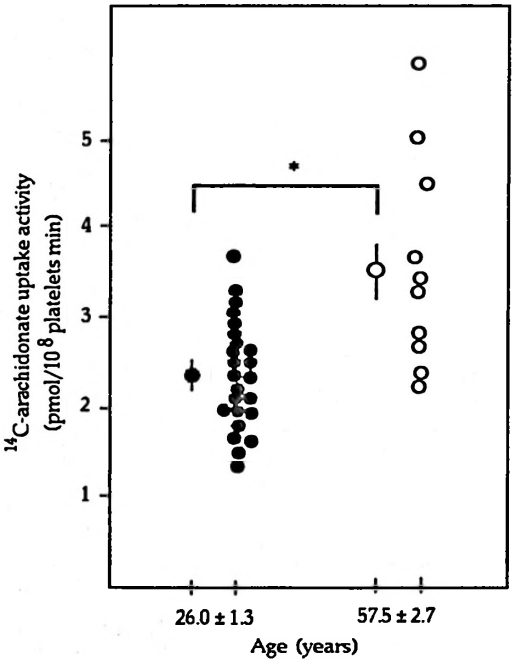

Fig. 1. *The ^{14}C-arachidonate uptake activity in platelets obtained from over-forty, and under-forty year old subjects.*
• Over forty-year old subjects 3.5 ± 0.38 pmol/10^8 platelets min. o Under forty-year old subjects 2.33 ± 0.12 pmol/10^8 platelets min.

platelets fom under-forty year old subjects was not significantly reduced in the presence of NDG (2.60 ± 2.1 % inhibition), but was significantly reduced in the presence of indomethacin (15.16 ± 5.3 % inhibition) and INDO plus NDG (18.80 ± 8.2 % inhibition). The arachidonic acid uptake activity in platelets from over-forty year old subjects was significantly reduced in the presence of both indomethacin (30.0 ± 4.9 % inhibition) and nordihydroguaiaretic acid (24.30 ± 5.7 % inhibition), and was further reduced in the presence of the two substances together (35.40 ± 4.1 % inhibition).

Plasma levels of glucose, triglyceride, total cholesterol, HDL-cholesterol and the total cholesterol/HDL-cholesterol rate were significantly increased in the over-forty year old subjects (table I).

Table I. *Plasma levels (mg/dL) of glucose, triglycerides, total cholesterol, HDL-cholesterol, the total cholesterol/HDL-cholesterol rate assayed in under-forty and over-forty year old subjects.*

	Under (n=12)	Over (n=8)
Glucose	87.6 ± 1.0	91.1 ± 2.3
Triglycerides	87.0 ± 9.2	140 ± 35
Total cholesterol	157 ± 6.2	196 ± 7.2*
HDL-cholesterol	44.0 ± 3.5	36.0 ± 2.5
Tot chol/HDL chol	3.8 ± 1.0	5.6 ± 2.0*

*P < 0.05

No significant correlations were found between the arachidonate uptake activity and the glucose, triglyceride, total cholesterol or HDL-cholesterol plasma levels.

Discussion

The measurement of arachidonate uptake activity by platelets incubated *in vitro* has been proposed as a straightforward and reliable method of evaluating platelets metabolic activity (9). The method described by that author has been used, with some modifications, to study the uptake of ^{14}C-arachidonic acid in platelets incubated *in vitro* from subjects over and under forty years of age in plasma samples obtained with EDTA. The cyclooxygenase and lipooxygenase enzymes are active under these conditions, no restrictions existing, therefore, for the metabolic utilization of the arachidonic acid taken up by the platelets. There is a rise in platelet uptake of arachidonate as a function of age (fig. 1). This effect is coherent with the well-known fact that aging is a major determining factor of platelet function (4). Investigators reporting this type of study have systematically interpreted this uptake of arachidonate as

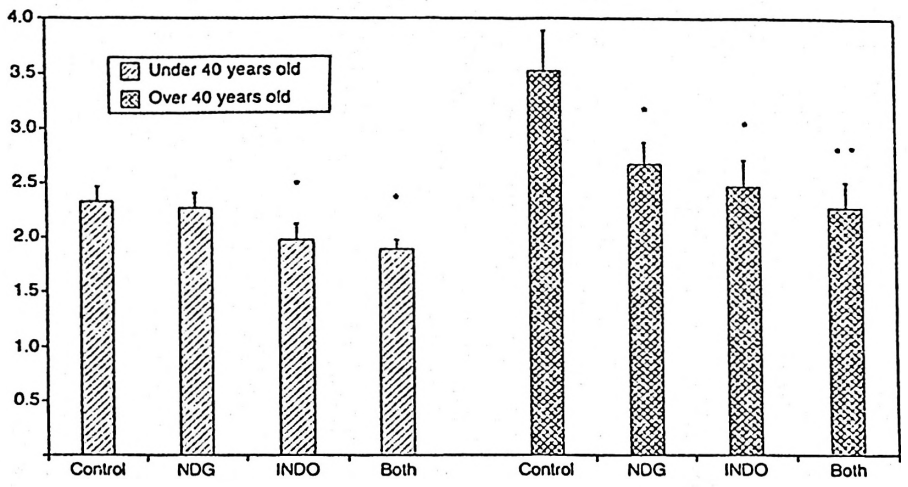

Fig. 2. *The ^{14}C-arachidonate uptake activity in platelets obtained from under-forty and over-forty year old subjects (n = 6 per group), either in the presence or in the absence of 0.2 mM indomethacin (INDO) and/or 0.1 mM nordihydroguaiaretic acid (NDG) in the incubation medium.* (*p < 0.05, **p < 0.01).

a mechanism that contributes to the hyperproductiveness of metabolites, considering exclusively the cyclooxygenase pathway (9). This pathway exhibits maximal and half-maximal activity at lower levels of substrate concentration than the lipooxygenase pathway (1, 2). Therefore the arachidonic acid uptake activity by platelets has been studied under conditions specifically inhibiting both cyclooxygenase and lipooxygenase.

The present study shows that when platelets were incubated in the presence of 0.2 mM INDO, an inhibitor of cyclooxygenase (7), the arachidonic acid uptake activity was significantly reduced, whereas when platelets were incubated in the presence of 0.1 mM NDG, a well known inhibitor of lipooxygenase activity (11), only arachidonic acid uptake activity measured in platelets from over-forty year old subjects was significantly reduced. It is interesting to note that the presence of either indomethacin or nordihydroguaiaretic acid reduced the increased arachidonic acid uptake activity in platelets from over-forty year old subjects to values identical to those measured in platelets from under-forty year old subjects when they were incubated in the absence of inhibitors.

While the study of arachidonic acid metabolism is a subject of current interest for many investigators, the determination of epithelial cell biosynthesis and degradation of arachidonate metabolites being a critical step towards understanding the biological function of epithelial tissues in general (3), no references have been found to the study of arachidonic acid uptake activity in normal subjects in the presence or the absence of inhibitors of the major enzymatic pathways capable of fatty acid oxygenation (cyclooxygenase and lipooxygenase). At any rate, our results do not differ from the traditional view concerning alterations in arachidonic acid metabolism in platelets. It seems to be a well-proven fact that both leukotrienes and hydroperoxides (products of the lipooxygenase pathway) can stimulate cyclooxygenase activity, and thus give rise secon-

darily to the metabolite hyperproduction of this pathway (10).

Plasma lipid, lipoprotein and platelet abnormalities are well established to be predictive of atherosclerosis development and coronary heart disease (8). In the present study, as well as observing an increased arachidonic acid uptake as a function of age, we found a significant increase in plasma lipids and a significant reduction in plasma HDL-cholesterol levels in the group of over-forty year old subjects, all of which predisposes to the development of atherosclerosis. The reduction, by using specific inhibitors, of arachidonate uptake by platelets in subjects over forty years in age could slow down the development of this pathological process.

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J. CANAL, E. DOMÍNGUEZ, M. D. TORRES, J. E. CAMPILLO y C. PÉREZ. *Relación entre la edad y la captación de C^{14}-ácido araquidónico por plaquetas en sujetos normales.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (1), 37-42, 1995.

La captación de ácido araquidónico (pmol/10^8 plaquetas min) medido en plaquetas de sujetos normales, es significativamente mayor en personas mayores de 40 años (3,53 ± 0,38), que en personas menores de 40 (2,33 ± 0,12). No se encuentra correlación significativa entre la captación de araquidónico y los niveles plasmáticos de glucosa, colesterol total, HDL-colesterol o triglicéridos. La captación de ácido araquidónico se reduce significativamente en presencia de indometacina en las plaquetas de ambos grupos. En presencia de ácido nordihidroguaiarético, esta disminución aparece únicamente en las de sujetos mayores de 40 años. Estos datos muestran que la captación de ácido araquidónico por plaquetas aumenta con la edad, y que este incremento queda abolido en plaquetas incubadas en presencia de inhibidores del metabolismo del ácido araquidónico.

Palabras clave: Acido araquidónico, Plaquetas, Edad.

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