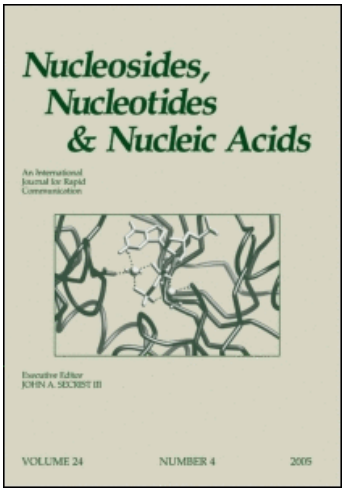

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PURINE CATABOLISM IN ADVANCED CAROTID ARTERY PLAQUE

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□ *This study was carried out on carotid artery plaque and plasma of 50 patients. We analyzed uric acid, hypoxanthine, xanthine, and allantoin levels to verify if enzymatic purine degradation occurs in advanced carotid plaque; we also determined free radicals and sulphydryl groups to check if there is a correlation between oxidant status and purine catabolism. Comparing plaque and plasma we found higher levels of free radicals, hypoxanthine, xanthine, and a decrease of some oxidant protectors, such as sulphydryl groups and uric acid, in plaque. We also observed a very important phenomenon in plaque, the presence of allantoin due to chemical oxidation of uric acid, since humans do not have the enzyme uricase. The hypothetical elevated activity of xanthine oxidase in atherosclerosis could be reduced by specific therapies using its inhibitors, such as oxypurinol or allopurinol.*

Keywords Xanthine oxidase; Purine compounds; Free radicals; Sulphydryl groups

INTRODUCTION

The common risk factors for atherosclerosis, such as hypercholesterolemia, hypertension, diabetes, and smoking increase the production of reactive oxygen species (ROS) by endothelial, vascular smooth muscle, and advential cells. ROS starts processes involved in atherogenesis through several important enzyme systems, including xanthine oxidase, NADH/NADPH oxidase, and nitric oxide synthetase.[1] Xanthine oxidase (XO) is a molybdoenzyme catalyzing the oxidation of hypoxanthine

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(Hx) and xanthine (X) in purine catabolism. XO exists in 2 convertible forms, one form reduces NAD^+, while the other prefers molecular oxygen, leading to the production of both O_2^- and H_2O_2. XO produces urate (UA), which has an important biological role for its antioxidant properties.

Endothelial dysfunction has an important role in the physiopathology of human vascular disease, where an alteration of enzymatic pathways may generate a broad variety of ROS and consequent intermediates able to cause further endothelial injury or loss of the barrier function. The endothelial cells of arterial wall are continuously exposed to changes of oxygen pressure and high free radical formation, which can cause further development through lesion progress. Already in 1981 Granger and colleagues[2] hypothesized that XO-generated-ROS cause ischaemic bowel injury due to ATP catabolism during hypoxia and increased electron acceptor availability on reperfusion. The increased XO activity, enhanced 5′-nucleotidase and AMP deaminase, lead to accumulation of free purine bases and their catabolic products.[3,4] In this study we measured in plaque UA, Hx, X, allantoin (ALL) levels to verify if enzymatic purine activity continues in advanced carotid artery plaque; we analyzed also free radicals (FR) and sulphydryl (SH) groups to check if there was a correlation between oxidant status and purine catabolism. The same parameters also were determined in plasma of patients.

MATERIALS AND METHODS

Plasma and carotid plaque were obtained from 50 patients (29 males and 21 females), aged 60–88. Clinical parameters of atherosclerotic subjects were: cardiopatic ischaemia (9 males, 6 females), diabetes (10 males, 7 females), hypertension (19 males, 18 females), dislipidemia (6 males, 8 females), smokers (3 males, 2 females), and former smokers (8 males, 1 female).

We measured UA, Hx, X, ALL, FR levels and SH-groups in plaque and in corresponding plasma. Plaque were frozen with liquid nitrogen and pulverized (3 cycles of 1 minute) with homogenizer (Dismembrator, Braun AG, Melsungen, Germany). The plaque powder (20 mg) was solubilized with 1 ml of double distilled water and, after centrifugation, the supernatant was used for assay of UA, Hx, X, ALL[5] levels and for determination of SH-groups,[6] while precipitates were used for determination of FR (Kit d-ROMs Diacron, tissue-specific).

The same methods as above were used to determine UA, Hx, X, ALL levels and SH-groups in plasma, while FR was determined with plasma-specific kit d-ROMs Diacron.

TABLE 1 Levels of Free Radicals, Sulphydryl Groups, Uric Acid, Xanthine, Hypoxanthine, and Allantoin

Parameters	Plaque n = 50 (μmoles/g)	Plasma n = 50 (μmoles/ml)
FR	503 ± 348	4.67 ± 1.15
SH	1.59 ± 0.39	0.326 ± 0.083
UA	0.061 ± 0.041	0.33 ± 0.10
X	0.263 ± 0.112	0.0013 ± 0.0009
Hx	0.077 ± 0.053	0.0058 ± 0.0038
ALL	0.86 ± 0.15	n.d

All data were expressed as mean and standard deviation.

RESULTS

We analyzed FR, SH-groups, UA, X, Hx, and ALL levels in plaque and in corresponding plasma of 50 patients with atherosclerosis, as reported in Table 1.

Comparing plaque and plasma there was, in plaque, a substantial increase of FR, Hx, X, SH levels, and a decrease of an oxidant protector, such as UA; we also observed the presence of ALL phenomena possibly due to chemical oxidation of UA, since humans do not have the enzyme uricase. The plaque concentration of UA is similar to that reported in literature and significantly higher than control arteries,[7] but lower than plasma level.

DISCUSSION

The association between atherosclerosis, XO and its products has been suggested as "response-to-injury" hypothesis by clinical and basis science reports. To explore the pathological significance of purine catabolism in advanced plaque, we tested purine catabolites (Hx, X, UA), FR, SH-groups, and the possible presence of ALL, in plaque and plasma of atherosclerotic subjects.

The results of this study show an increased purine catabolism, an abnormal response regarding oxidative phenomena and XO activity presence in plaque, which could lead to further tissue damage at distant districts, where endogenous XO level or stress oxidative activity are low: these phenomena could indicate which also in advanced plaque there are dynamic processes.

In conclusion, the hypothetical elevated activity of XO in this pathology could be reduced by specific therapies using its inhibitors, such as oxypurinol or allopurinol, which are just in use, with positive effects, in hypercholesterolemic patients.[8]

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