PLASMA THROMBOXANE B_2 AND LEUKOTRIENE B_4 LEVELS IN PATIENTS WITH CORONARY ATHEROSCLEROSIS

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SUMMARY: Plasma TXB_2 and LB_4 levels of 17 patients with coronary atherosclerosis were studied prior to bypass surgery. Also correlations between plasma TXB_2, LB_4 levels and other biochemical parameters including glucose, urea, ALT, AST, LDH, uric acid, cholesterol, triglyceride, platelet count, were investigated in patients with coronary atherosclerosis.

Patients with coronary atherosclerosis had increased TXB_2 plasma levels as compared with those of control subjects. In contrast, no statistically significant differences were noted for LB_4 between control subjects and coronary atherosclerotic patients. TXB_2 levels were positively related to serum triglyceride contents and were inversely related to platelet counts and serum LDH levels. In addition, plasma TXB_2 levels were positively correlated to plasma LB_4 levels. In the present study, serum concentrations of glucose, urea, ALT, AST, uric acid and cholesterol, were not correlated to plasma levels of TXB_2 and LB_4 .

Key Words: Thromboxane B_2 , leucotriene B_4.

INTRODUCTION

Current concepts of atherogenesis based on animal and human investigations indicate prostaglandins (PG) and thromboxanes (TX) as key factors in atherosclerotic lesions. Thromboxane A_2, derived from PGH_2 in platelets by thromboxane synthetase enzyme, induces platelet aggregation and has a vasoconstricting effect (21). Several studies in patients with coronary arteriosclerosis obliterans and myocardial infarction have recently demonstrated elevated levels of TXB_2 (12,18). On the other hand it has been reported that specific inhibitors of thromboxane synthesis increase coronary blood flow, decrease myocardial lactate production and plasma creatine kinase activity (21,22).

Although the function of prostaglandins in coronary atherosclerosis has been investigated extensively, the potential role of leukotrienes have not been defined. Leukotrienes are derived from arachidonic acid like thromboxanes. Arachidonic acid is derived from phospholipids in plasma membranes and is a substrate which synthesizes PG_2 compounds and leukotrienes. Arachidonic acid is the precursor of the 2-series of prostaglandins, prostacyclin and the thromboxanes via the cyclooxygenase pathway: via the lipoxygenase pathway, it is the precursor of the leukotrienes. Arachidonic acid is present in the diet and can also be synthesized from dietary linolenic acid. Arachidonic acid is stored in cell walls esterified in phospholipids and may be released upon demand by the action of phospholipases. The free arachidonic acid may then be shunted into the prostaglandin and thromboxane pathway by

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cyclooxygenase or into the hydroxy fatty acid pathway by lipoxygenase. It is known that leukotrienes can cause increased capillary permeability and are powerful chemotactic agents for leukocytes and are also powerful broncho-constrictors (3,7,10). There is some indication that a transient vasoconstrictive effect of the leucotrienes may be involved in coronary insufficiency and angina.

The aim of the present study was to investigate the cyclooxygenase pathway which is the precursor of prostaglandins and thromboxanes and the lipoxygenase pathway which is the precursor of the leukotrienes in patients with coronary atherosclerosis. Therefore in these patients, we have investigated the plasma profiles of TXB_2 which is a stable metabolite of TXA_2 and LB_4. We also tried to correlate TXB_2 and LB_4 levels with glucose, urea, ALT, AST, LDH, uric acid, cholesterol, triglyceride and platelet count in these patients.

MATERIALS AND METHODS

In this study, 17 patients with coronary atherosclerosis who were undergoing bypass surgery, 35 to 65 years old, have been chosen. There have been no illness besides coronary atherosclerosis in these patients. They had no symptomatic or prior cerebrovascular disease and they were free of diabetes mellitus. 17 healthy volunteers who had been chosen from hospital personnel served as control subjects. Plasma LB_4 levels measured in 8 patients with coronary atherosclerosis and 8 healthy volunteers.

Blood samples were obtained under the same basic conditions in all cases. Samples were drawn from the midstream of antecubital vein by careful venipuncture after a period of stasis of less than 10 seconds. Stasis was removed. The first 2 ml of blood were discarded and the subsequent 5 ml were collected in EDTA and indomethacin containing tubes. After immediate centrifugation (3000 rpm for 10 minutes, at 4°C), the plasma was separated and kept in the deep freeze (at -70°C) until further processing. These techniques minimize blood platelet release of PG material.

Liquid phase extraction procedure had been chosen for the extraction of TXB_2 and LB_4 (1,6,13). Plasma samples were acidified to pH 3.5 and extracted twice with ethyl acetate. The combined ethyl acetate extract was evaporated under

Table 1: Plasma levels of TXB_2 and LB_4 in control subject and patients with coronary atherosclerosis.

	Control Subject	Coronary Atherosclerosis	
TXB_2	6.87 ± 2.24	89 ± 136	p<0.05
LB_4	60.5 ± 17.5	58.5 ± 17.9	p>0.05

Table 2: Correlation coefficients between plasma levels of TXB_2 and LB_4 and other factors, glucose, urea, ALT, AST, LDH, uric acid, cholesterol, triglyceride and platelet count in patients with coronary atherosclerosis.

	TXB_2	LB_4
Glucose	-0.059	0.249
Urea	0.190	0.012
ALT	-0.030	-0.117
AST	-0.108	-0.188
LDH	-0.517	-0.166
Uric acid	0.011	0.236
Cholesterole	0.064	0.253
Triglyceride	0.528	0.314
Platelet count	-0.637	-0.247
TXB_2	-	0.565

Figure 1: The relationship between the plasma levels of TXB_2 and triglyceride in patients with coronary atherosclerosis.

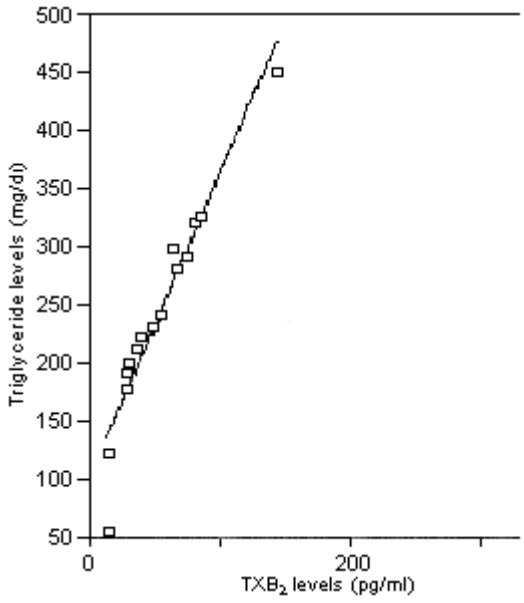
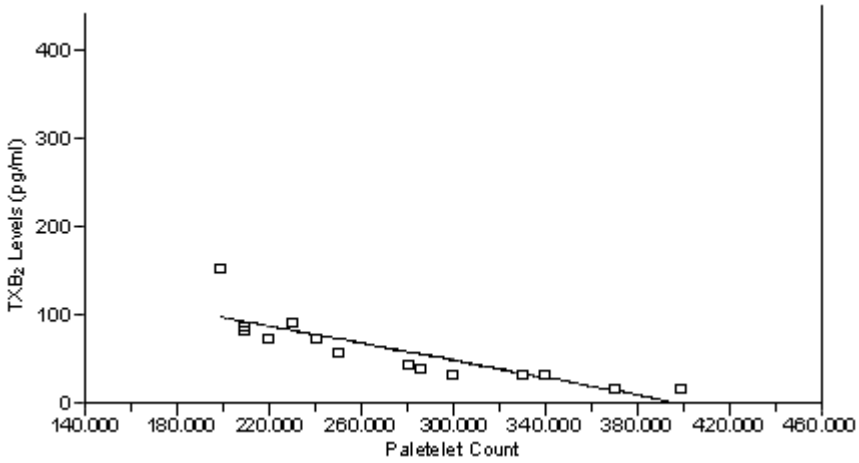


Figure 2: The relationship between the plasma levels of TXB_2 and platelet count in patient with coronary atherosclerosis.



nitrogen, lyophilized and then RIA applied on these extracts. Results were expressed as pictograms per milliliter of plasma.

Student's t test for paired data and the regression analysis were employed for the statistical analysis of the results.

RESULTS

Thromboxane B_2 levels of coronary atherosclerosis were found higher than TXB_2 levels of control subjects. In contrast, no statistically significant difference was noted for LB_4 between control subjects and coronary atherosclerotic patients (Table 1).

It was found that TXB_2 was positively related to serum triglyceride content (Table 1, Figure 1). In addition, plasma TXB_2 levels were found inversely related to platelet count and serum LDH levels (Figures 2 and 3). Although no statistically significant difference was noted for LB_4 between control subjects and coronary atherosclerotic patients, plasma TXB_2 levels were positively related to plasma LB_4 levels (Figure 4). In the present study, other factors, glucose, urea, ALT, AST, uric acid and cholesterol were found not to be correlated to plasma levels of TXB_2 and LB_4 (Table 2).

DISCUSSION

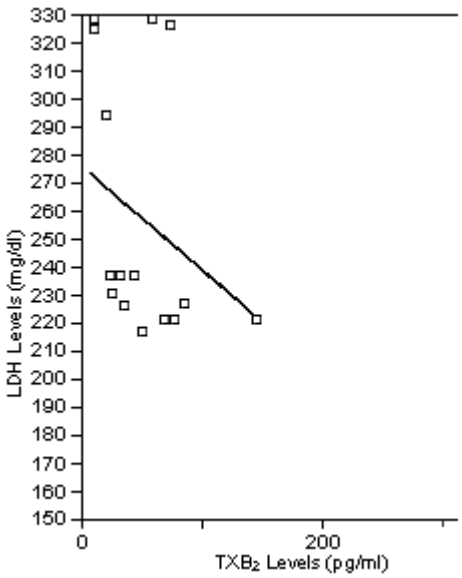
Although the studies about levels of prostaglandins and thromboxanes in cardiovascular diseases are numerous, most of them are experimental studies. The rapid metabolism of prostaglandins in organs such as the lungs need to be discussed when assessing the biologic relevance of PG plasma levels. On the other hand, the continuous secretion of prostaglandins tend to establish the basic levels of prostaglandins in a given disease (14).

It was examined that the levels of TXB_2, 6-keto-PGF_1, PGE_2, PGF_2 and PGA_1 in arteriosclerosis obliterans and was detected abnormally high levels of TXB_2 and PGE_2 and increased ratio between TXB_2 and 6-keto-PGF_1 (18). The relation between prostaglandin levels and clinical state and or pathological findings were also investigated. Friedrich *et. al.* (12) measured 6-keto-PGF_1 and TXB_2 in venous blood of patients with myocardial infarction on the first, third, and seventh days and observed elevated TXB_2 and 6-keto-PGF_1 levels.

It was demonstrated that dietary supplementation with n-3 polyunsaturated fatty acids could be regarded as beneficial for the prevention and treatment of atherosclerosis and thrombosis and also of chronic inflammatory diseases like Rheumatoid arthritis and psoriasis (1992).

De Caterina *et. al.* (11) evaluated the hypothesis that LB_4 a potent chemotactic and endothelium permeabilizing autocoid, may be one of the factors influencing the progress of the atherosclerotic lesions. They found that there was no relationship between concomi-

Figure 3: The relationship between the plasma levels of TXB_2 and LDH in patients with coronary atherosclerosis.



tantly measured prostacyclin production (mainly endothelial), while a correlation was observed between LB_4 levels and the degree of white cell infiltration of the tissue(11).

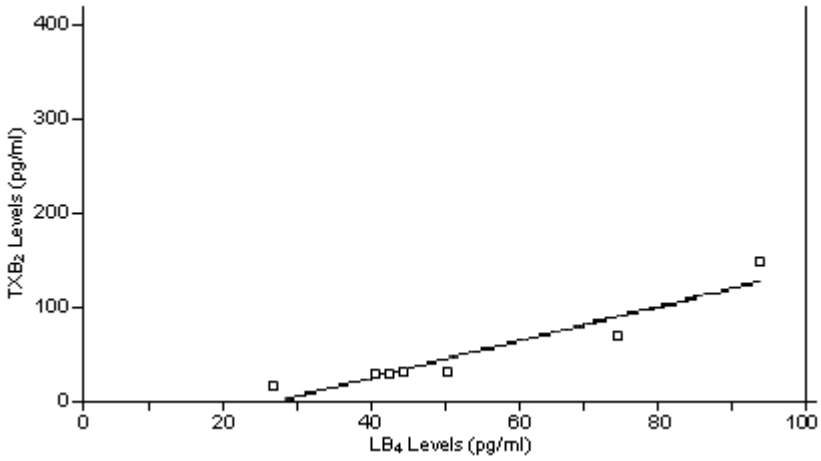
In this study, no statistically significant difference was noted for LB_4 between control subjects and coronary atherosclerotic patients. It was demonstrated that LC_4 and LD_4 were more potent than LB_4 in increasing vascular permeability and causing vasoconstriction (5,9,22). Further research concerning LC_4 and LD_4 in patients with coronary atherosclerosis will most likely help to define the contribution of leukotrienes to coronary atherosclerosis.

In the present study, although no statistically significant difference was noted for LB_4 between control subjects and coronary atherosclerotic patients, plasma TXB_2 levels were positively related to plasma LB_4 levels. This may be possible since both of them are derivatives of arachidonic acid.

Also, it was found that TXB_2 levels were inversely related to the platelet counts. The finding of a lower platelet count concomitant with the increased platelet TXB_2 production suggests that this may be the consequence of increased platelet activity. This is thought to occur because of the potent proaggregatory activity of TXA_2 (20).

It has been reported that patients with ischemic heart disease (IHD) may sometimes exhibit platelet activation in peripheral venous plasma but do not present any significant difference between aortic and coronary venous TXB_2 levels (25). On the basis of these reports and our results, we suggest that both clinical features and the extension of atherosclerosis may increase the biological signs of platelet activation in peripheral venous plasma.

Figure 4: The relationship between the plasma levels of TXB_2 and LB_4 in patients with coronary atherosclerosis.



In the present study, TXB_2 levels were not related to other parameters without triglyceride and LDH levels. The rise of TXB_2 with triglyceride plasma content may help to understand why the bleeding time and platelet survival are reduced in type IV hyperlypoproteinemia (8,17). In contrast, we have observed no relation between TXB_2 and cholesterolemia. But it must be kept in mind that diabetes was an exclusion criterion and that cholesterol results were normal in our patients.

Stragliotto *et. al.* (26) investigated same functions of monocytes from 20 type II a hypercholesterolemic and five homozygous familial hypercholesterolemic patients. Monocytes from the hypercholesterolemic patients contained as much cholesterol and formed as much TB_2 in response to N-formyl-methionyl-leucyl phenylalanine or calcium ionosphere A23187 as those from normal individuals. In contrast, the generation of prostaglandin E_2 and 6-keto PGF_1 in response to these agonists was 1,5-3 times normal, and that of LB_4 was 40-60% of the normal value ($p<0.05$ for all).

In this study, it was found that TXB_2 levels were inversely related to LDH levels. Normally, it was excepted that TXB_2 levels should have been positively correlated to serum LDH levels. Because coronary ischemia and tissue hypoxia followed by coronary atherosclerosis would initiate anaerobic glycolysis and lactate derivation from pyruvate which is catalyzed by LDH, would increase.

It was observed that specific inhibitors of thromboxane synthesis increase coronary blood flow, decrease myocardial lactate production and plasma creatine kinase activity and protect rabbits against sudden death induced by arachidonic acid (17,21,22). Perhaps no relationship would have been uncovered if an investigation would be carried out comparing normal and pathological LDH levels in atherosclerotic patients.

In the present study, drugs which were given to the patients were not noticed. Since it is impossible to control all the variable influencing prostaglandin levels, the results may be false. Jouve *et. al.* (18) observed elevated TXB_2 levels in coronary atherosclerotic patients who were free of drugs known to influence either metabolic values or the arachidonic acid cascade for at least 1 week before the investigation.

It therefore appears that studies on prostaglandins and leukotrienes in cardiovascular diseases are not sufficiently investigated. Therefore more detailed studies are needed to evaluate the possible role of these substances in cardiovascular diseases.

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