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ISOPROSTANES IN ATHEROSCLEROSIS

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Isoprostanes (IP) are a new family of compounds formed during oxidation injury. 8-epi-prostaglandin (PG) $F_{2\alpha}$, a vasoconstrictory and mitogenic substance, is increased in hyperlipidemia in blood and urine as well as at the vascular level in the intima, in particular along foam cells. Similarly, cigarette smoking is associated with an immediate increase in 8-epi-$PGF_{2\alpha}$ and a quick drop after quitting. Also diabetes and even the more a combination of risk factors (for the development of atherosclerosis) results in increased 8-epi-$PGF_{2\alpha}$ in various compartments. Others, such as sex, age, hypertension and obesity were of minor influence. These findings further indicate, that in-vivo oxidation injury as reflected by increased IP may play a relevant role in atherogenesis. IP may serve as useful markers to assess oxidation injury at a local level.

Key words: *Isoprostanes, atherosclerosis, hyperlipoproteinemia, smoking, 8-epi-$PGF_{2\alpha}$, vasoconstriction, oxidation injury.*

INTRODUCTION

Latest since the discovery of the pathogenetic role of oxidized (ox)LDL in the development of vascular disease (1) isoprostanes (IP) formed during oxidation injury (2) gained central interest in atherogenesis. Their formation has been documented during LDL-oxidation and measurement of urinary excretion claimed as a reliable marker (3, 4) for in-vivo oxidation injury. One member of the large new family of compounds, i.e. 8-epi-$PGF_{2\alpha}$ (structure see *Fig. 1*), the substance examined most in detail so far, is a potent vasoconstrictor (5), mitogen and mild proaggregatory agent (6). Abnormal findings on IP have been reported so far almost exclusively on hyperlipidemia and cigarette

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smoking (4, 7). In vascular tissue, an extremely increased 8-epi-$PGF_{2\alpha}$ along foam cells (8) has been described.

It is the aim of this review, to summarize data available on IP, and in particular on 8-epi-$PGF_{2\alpha}$, derived from vascular tissue, blood (plasma and serum) and urine which might be relevant for atherosclerosis.

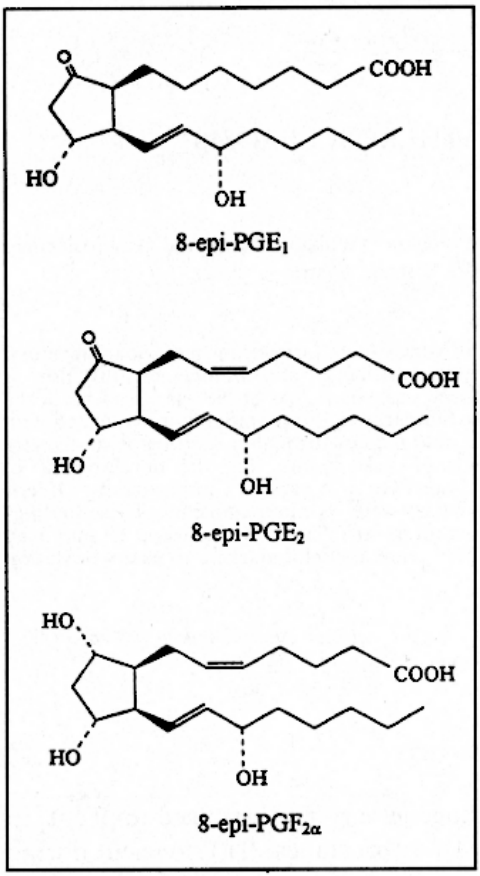

Fig. 1. Chemical structure of 8-epi-$PGF_{2\alpha}$, the most well examined IP at present.

MATERIALS AND METHODS

Vascular tissue

Peripheral vascular tissue was obtained during surgery, coronary vessels from transplant donors (coronary heart disease [CHD], cardiomyopathy and intact vessels). From a total of 1471 patients (and volunteers) — 962 males, 521 females (age range 3—94 years) — blood and/or urine was collected.

Blood withdrawal and processing

If not otherwise stated blood was drawn in the morning after an at least 12 hours overnight fasting period for creatinine and 8-epi-$PGF_{2\alpha}$.

Serum sampling

Blood was drawn into glass vials. Vials were placed immediately into a water bath at 37°C for exactly 60 minutes. Serum was then removed after a centrifugation step (4°C, 1000 × g, 10 minutes) and stored until determination (no longer than 2 weeks at < -70°C) as described (9). To determine interassay variability, the respective sample was determined several times in several assays. The intraassay variability was determined assaying the same sample several times during the same assay procedure. The interassay variability amounted $3.8 \pm 1.2\%$, the intraassay variability was $1.9 \pm 0.7\%$. Normal value: 150—250 pg/ml ($n = 17$).

Plasma sampling

Blood samples were anticoagulated with 2% EDTA and 1 mg/ml (final blood volume) acetylsalicylic acid (ASA). Immediate centrifugation at 4°C to obtain plasma was done at 1000 × g for 10 minutes. Plasma was removed and stored at < -70°C for not longer than 2 weeks until determination. The interassay variability was $5.5 \pm 1.7\%$, the intraassay variability $2.5 \pm 0.7\%$. Normal value: < 20 pg/ml ($n = 11$).

Urinary sampling

Urine was collected over a period of 24 hours. After assessing total urinary volume, 10 ml aliquots were adjusted to pH 4.0 with formic acid and taken for extraction. The eluate was subjected to silicic acid chromatography and further eluted. This final eluate was dried, recovered in buffer and assayed after dilution. Cross reactivity of the antibody with prostaglandins was $< 2\%$. Values are given in pg 8-epi-$PGF_{2\alpha}$/mg creatinine. The interassay variability was $6.4 \pm 2.3\%$, the intraassay variability $2.7 \pm 0.8\%$. Normal values: 150—250 pg/mg creatinine ($n = 14$).

Statistical analysis

Values are presented as $\bar{x} \pm SD$; calculation for significance was done by means of Student's t-test, regression analysis and ANOVA. A p-value of < 0.01 was considered as significant. The design of experiments was approved by a local Ethical Committee.

RESULTS

Tissue

Vascular 8-epi-$PGF_{2\alpha}$ shows considerable variation by about 1 order of magnitude (61—934 pg/mg) as does PGI_2. In arteries 8-epi-$PGF_{2\alpha}$ is increased in hyperlipidemia (321.0 vs. 225.7 pg/ml; mean), cigarette smoking (336.4 vs. 211.4 pg/ml) and diabetes (357.8 vs. 216.0 pg/ml), while for hypertensive-derived vessels no significant difference can be found (10). A significant correlation

between 8-epi-$PGF_{2\alpha}$ and age ($r = 0.572$; $p < 0.001$) as well as 6-oxo-$PGF_{1\alpha}$ ($r = 0.720$; $p < 0.0001$) can be seen.

Immunohistochemistry confirms these findings (11). The most intensive staining is found in vessels of smokers and diabetics, in particular in the intima along foam cells (8).

In the coronary vascular bed there is a significant association between 8-epi-$PGF_{2\alpha}$ and atherosclerosis. Vascular tissue derived from patients with cardiomyopathy or healthy heart donors revealed much lower 8-epi-$PGF_{2\alpha}$, staining intensity and the area involved both strongly correlated between 8-epi-$PGF_{2\alpha}$ and positivity for anti-ox-LDL. Similar findings we recently obtained from (aortic and pulmonary) heart valves in this group of patients. In umbilical arteries an inverse relation between 8-epi-$PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ can be seen ($r = -0.8509$). There was also a negative correlation between L-arginine and 8-epi-$PGF_{2\alpha}$ ($r = -0.5100$) and a positive between PGI_2 and L-arginine ($r = 0.5785$). Among the risk factors, smoking apparently had the most severe influence ($p < 0.0001$) on the values (12).

Blood

Investigating 8-epi-$PGF_{2\alpha}$ in plasma and serum there is a good correlation ($r = 0.8241$—0.9462). So far, changes seen were always reflected comparably. There was also a good correlation to urinary excretion values ($r = 0.8426$—0.9127 vs. plasma and $r = 0.8523$—0.9264 vs. serum). Especially in older patients with age-related reduced creatinine clearance, the IP-excretion being given as amount/mg creatinine may be overestimated. A detailed investigation concerning this aspect is performed at present in patients with significant impairment in kidney function. In agreement with Helmersson *et al.* (13) no significant seasonal or day to day variation was discovered so far.

Risk factors

1. Sex: Although different among the subgroups, in some of the subgroups men tend to have higher 8-epi-$PGF_{2\alpha}$, mainly in plasma and urine.
2. Age: In healthy people, there seem to be a trend towards increasing blood and urinary levels with age. The influence of renal function still needs to be assessed concerning 8-epi-$PGF_{2\alpha}$ values in urine.
3. Hyperlipoproteinemia (HLP): Patients with HLP show elevated 8-epi-$PGF_{2\alpha}$ in all the compartments, irrespective of the general risk factors present. Patients with high triglycerides or lipoproteins rich in triglycerides show higher 8-epi-$PGF_{2\alpha}$ as compared to isolated hypercholesterolemia.

Dietary intervention

Dietary intervention using different schemes (9) tends to decrease 8-epi-$PGF_{2\alpha}$, the values, however, in the subgroups mostly were not found to be significant.

Drug treatment

Treatment of patients with different statins clearly results in a decrease in 8-epi-$PGF_{2\alpha}$. Separating the action of the drug itself from severe lipid lowering has not been assessed yet. Furthermore, eventual differences between the various statins (or other lipid lowering drugs) have not been studied yet.

LDL-apheresis

LDL-apheresis is associated with an immediate drop in 8-epi-$PGF_{2\alpha}$ (14). Immediately after the therapeutic session the values are recovering continuously (*Fig. 2*). This decrease in 8-epi-$PGF_{2\alpha}$ is accompanied by a decrease in thiobarbituric acid reactive substances (TBARS), a decreased responsiveness of LDL towards *in vitro* oxidation. Apheresis patients with additional risk factors (smoking for example) exhibit significantly higher values, their kinetics during the therapeutic session and immediately thereafter, however, are identical.

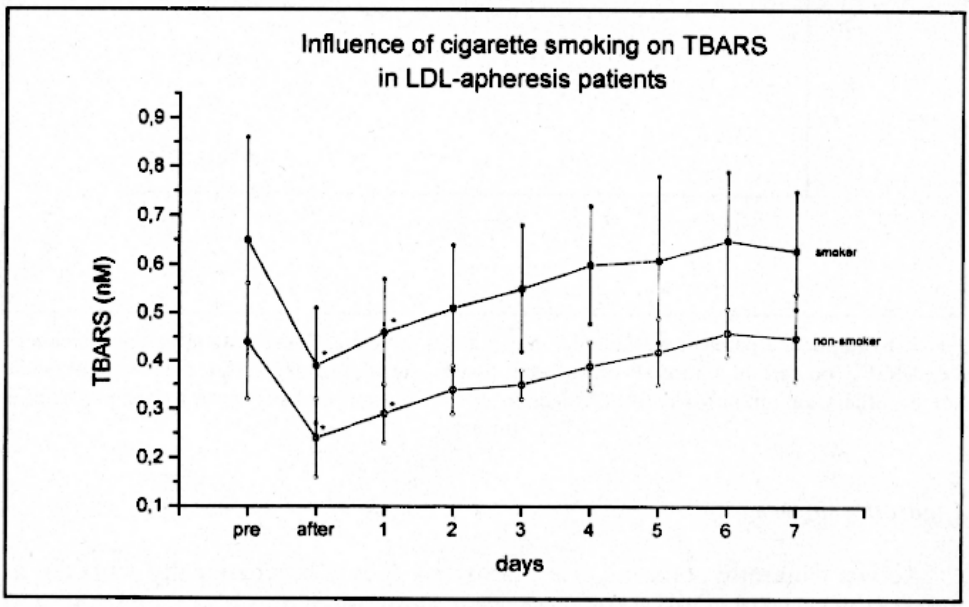

Fig. 2. Thiobarbituric acid reactive substances (TBARS) are diminished to about the half immediately after LDL-apheresis. Thereafter, values continously recover to prevalues. Although exhibiting identical kinetics, there is a significant difference between smokers and non-smokers at all time intervals. * $p < 0.01$ (vs. prevalue).

Side effects

In the majority of those patients showing muscular side effects upon statin treatment irrespective of concomitant CK-elevation there is an increase in 8-epi-$PGF_{2\alpha}$. This increase observed in plasma, serum and urine at the same time (*Fig. 3*) is immediately abolished by stopping the respective drug therapy. No increased prevalence as to one of the statins has been discovered yet. Although an anecdotal report exists that on vitamin E-treatment symptoms were disappearing (15) and older literature indicates that the unselective inhibition of ubiquinone might be involved (for review see 16), this issue still needs to be clarified. Although in the great majority 8-epi-$PGF_{2\alpha}$ decreases during statin therapy, in about 10% of the patients there is, however, an increase even in absence of myopathy or CK-elevation.

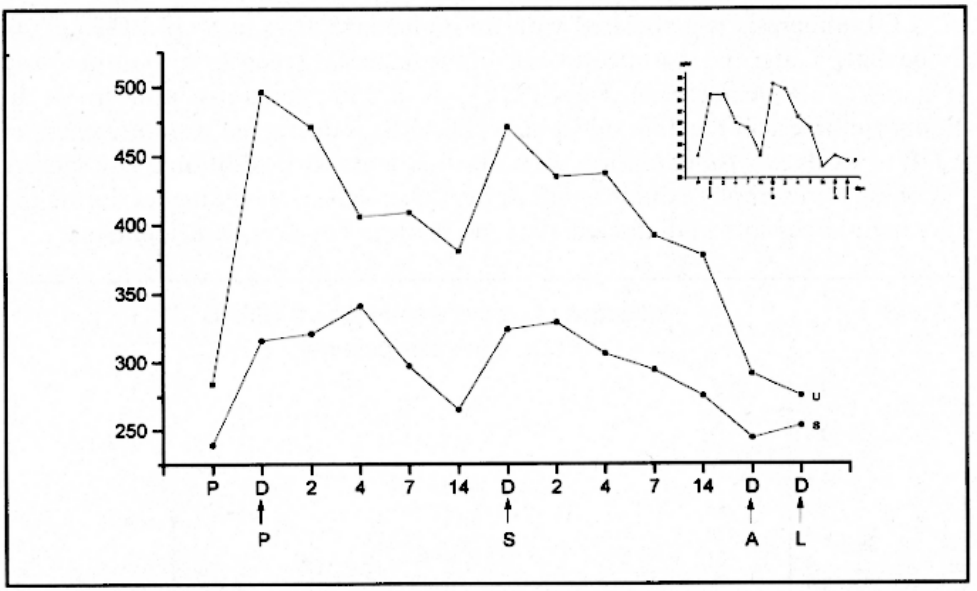

Fig. 3. Example of a pateient with familial hypercholesterolemia. There is an apparent increase of 8-epi-$PGF_{2\alpha}$ on certain statins (P-pravastatin, S-simvastatin, A-atorvastatin, L-lovastatin), while not on others for unknown reason. Values in pg /ml (serum) and plasma (p) pg/mg creatinine (urine).

Cigarette smoking

Active: Cigarette smoking is a major risk factor biochemically working at different levels (17—20). Active cigarette smoking induces a significant and immediate increase in 8-epi-$PGF_{2\alpha}$. *Quiting* smoking (*Fig. 4*) results in a normalization (21, 22) of the respective values after about 2 weeks and a sudden drop, especially during the first days after the *quitting* attempt.

Smoking is the risk factor resulting in the highest 8-epi-$PGF_{2\alpha}$ in vascular tissue, in umbilical arteries of babies born to smoking mothers (12) as well as in coronary (11) or peripheral arteries (10) of patients with clinically manifested atherosclerosis.

Passive: Single passive cigarette smoking is not associated with a significant alteration in 8-epi-$PGF_{2\alpha}$ in any compartment at any time. However, repeated exposure to environmental tobacco smoking induces an increase in 8-epi-$PGF_{2\alpha}$ (unpublished data).

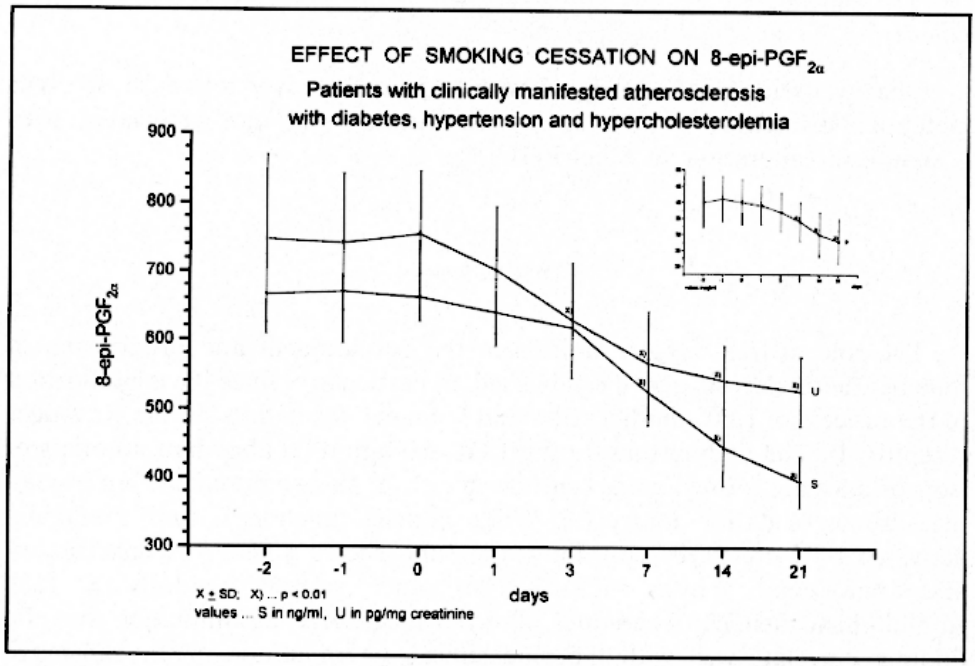

Fig. 4. Quiting smoking causes an immediate drop in 8-epi-$PGF_{2\alpha}$ in all the compartments examined, becoming significant after only a few days. Patients with clustering of risk factors exhibit extremely high values as compared to controls (see material and methods).

Hypertension

Hypertension alone is not associated with any significant change in 8-epi-$PGF_{2\alpha}$. Concomitant drug treatment in particular with ACE-inhibitors and some calcium antagonists (23) revealed values ranging somewhat below the ones found in normal controls. This indicates that maybe some of these compounds may exert an antioxidative action as it has been proposed using a different methodological approach. Liberation of PGI_2 by these drugs which has an antioxidative action (24) may be one of the factors.

Diabetes

Diabetes is associated with a severe oxidation injury and significantly increased 8-epi-$PGF_{2\alpha}$ (25). Additional risk factors are inducing a further increase of the respective values. Patients with an impaired glucose tolerance show a very wide range of levels from normal to elevated values normally found in manifested disease, the mean value, however, due to the large variation not becoming significant.

Obesity

Obesity itself, if not associated with impaired glucose tolerance or other metabolic disorders — such as hyperlipidemia — is not associated with a significant alteration in 8-epi-$PGF_{2\alpha}$.

DISCUSSION

The role of free oxygen radicals in the development and progression of human atherosclerosis is well established, in particularly since the identification of the process of LDL-modification and foam cell formation via the scavenger receptor (1). The finding that during LDL-oxidation (2) abundant amounts of isoprostanes are formed gains central interest for this compound as an in-vivo measure of oxidation injury (3). While platelet function is only minimally activated by 8-epi-$PGF_{2\alpha}$ (6), this compound shows potent vasoconstrictory and mitogenic activity (2, 7, 26) and releases endothelin (26). Immunohistochemical techniques allow to localize IP accumulation and the findings correlate well with the biochemical determination in the respective segment. The fact that LDL are carriers for lipid peroxides (27) and they inhibited PGI_2 formation while in parallel 8-epi-$PGF_{2\alpha}$ increases, nicely reflects the local role of free oxigen radicals. These data further show that increased oxidation injury is associated with a great many of risk factors. They also demonstrate that rapid normalization upon removal of the respective factor occurs, apparently independent of the duration before as in people quitting cigarette smoking (21), for example.

CONCLUDING REMARKS

Our findings demonstrate that IP reflecting in-vivo oxidation injury are particularly increased in hyperlipidemia, cigarette smoking and diabetes. An increase during statin therapy in certain cases indicates a central role of

oxidation injury in drug-induced side effects such as myopathy and CK elevation.

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