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Exogenous Arachidonic Acid Metabolism in Platelets from Psoriatic Patients

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Several reports have been published on platelet hyperaggregation in psoriatic patients, which might be related to alterations in arachidonic acid (AA) metabolism by platelets. We have studied the AA metabolism pattern, total eicosanoid formation and biosynthesis rate in platelets from psoriatic patients and from normal subjects after incubation with exogenous AA. We have found no difference in the metabolic pattern of platelets. Total formation of 12-HETE was higher in the control group ($p < 0.01$). The rate of synthesis of cyclo-oxygenase products was higher in the psoriatic group, but only that of thromboxane B_2 was statistically significant ($p < 0.02$). There was a close correlation between thromboxane B_2 formation rate and the lag time of platelet aggregation in response to 1 mM AA ($p < 0.01$). The percentage of aggregation of platelets from psoriatics was significantly higher than that from normal subjects ($p < 0.05$) and the lag time was lower in psoriatic group, but the difference was not statistically significant. ***Key words: Psoriasis; Eicosanoids.***

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Since the original paper from Hammarström et al. in 1975 (1) there have been many reports on arachidonic acid (AA) metabolism in normal and psoriatic skin (2–8). The presence of high concentrations of cyclo-oxygenase products, and especially 5- and 12-lipoxygenase products (mainly 12-hydroxy-6,8,10,14-eicosatetraenoic acid (12-HETE), and leukotriene B_4 (LTB_4) suggests that eicosanoid production by non-epidermal cell types present in psoriatic lesions may be important. Alterations in AA metabolism in polymorphonuclear leukocytes (9–13) and fibroblasts (14) in psoriatic patients have been reported by several authors, but there is some controversy on the cellular origin of eicosanoids present in psoriatic lesions (2–8, 15–18).

Alterations in platelet function have also been found in psoriatic patients. The incidence of occlusive vascular disease has been found to be higher in psoriatic patients than in patients with other dermatological disorders (19). The increased blood viscosity (20) and platelet hyperaggregability (21–23) found in psoriatic patients might point towards some kind of alteration or activation of AA metabolism in platelets. Increased production of 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and 12-HETE, markers of cyclo-oxygenase and 12-lipoxygenase, respectively, has been found in platelets derived from psoriatic patients with extensive disease ($\geq 10\%$ of body surface (21, 24). Increased 12,20-diHETE production has also been found in mixed suspensions of polymorphonuclear leukocytes and platelets derived from psoriatic patients (12).

Platelet activation and production of 12-HETE might play a role in the pathophysiology of psoriasis. We have studied AA metabolic profile, total production and rate of biosynthesis of the main eicosanoids in washed platelet suspensions derived from psoriatic patients and normal controls.

MATERIAL AND METHODS

Patients

Peripheral venous blood was drawn from 8 adult non-smoker and non-diabetic patients (6 male, 2 female, age range 21 to 64) with chronic stable plaque psoriasis involving more than 10% of body surface. Ten healthy non-smoker adult donors, with an age range and sex distribution similar to those of the psoriatic patients, served as controls. None of the subjects studied had received any medication for 2 weeks prior to extraction.

Preparation of platelet suspensions

Peripheral venous blood, anticoagulated with ACD-A, was centrifuged at 200 *g* for 12 min at room temperature. The supernatant (platelet-rich plasma, PRP) was recovered. Washed platelet suspensions were prepared using the method described by DiMinno et al. (25) except that final suspensions were done without albumin. Platelet counts were adjusted to 10^6 cells/μl. $MgCl_2$ was added to a final concentration of 0.5 mM.

Incubation with ^{14}C-AA

1 ml aliquots of platelet suspensions were preincubated at 37°C for 5 minutes. Then, 5 µl ethanol containing a mixture of 0.566 µCi of [^{14}C]arachidonic acid (^{14}C-AA) (Amersham, 56.6 mCi/mmol) and varying amounts of unlabelled AA (Sigma) were added. After incubation at 37°C for different periods of time as required, the reaction was stopped adding 1 ml of methanol at 0°C. The samples were kept in an ice-water bath until centrifugation at 15000 *g* for 2 min. The supernatant was kept at −80°C until analysis.

In order to study the AA metabolic profile, suspensions from 4 controls (3 men, 1 women) and 4 psoriatic patients (3 men and 1 woman) were used. The final AA concentration of the ^{14}C-AA and unlabelled AA mixture was 15 µM and the duration of incubation was 5 min.

Optimal AA concentration to be used in metabolic studies was determined by incubating platelet suspensions for 10 s with ^{14}C-AA and unlabelled AA mixture, final concentrations 15, 50, 100 and 250 µM. Incubation times were chosen following incubation of platelet suspensions for different periods of time with ^{14}C-AA and unlabelled AA mixture, final concentration 50 µM.

Aliquots of platelet suspensions from 8 patients and 8 controls were used for the comparative study of eicosanoid production. Platelet suspensions were incubated with ^{14}C-AA and unlabelled AA mixture, final concentration 50 µM. The reaction was stopped after 10 s or 5 min; incubations were performed in duplicate.

Sample analysis

Equipment. High Performance Liquid Chromatography apparatus was consisting of a Kontron 205 gradient programmer, two Kontron T-414 pumps, a Beckman 165 UV-Vis detector and a Beckman 175 radioisotope detector provided with a liquid scintillation cell. Scintillation cocktail was pumped at a flow of 3 ml/min by a Beckman 110 B pump.

Solvents. Acetonitrile, ChromAR HPLC, Mallinckrodt; methanol, HPLC, Scharlau; trifluoroacetic acid, spectroscopy, Merck; triethylamine, Analyticals, Carlo Erba.

Standards. 6-keto-[5,8,9,11,12,14,15(n)-^{3}H]-Prostaglandin $F_{1\alpha}$, 157 Ci/mmol, [5,6,11,12,14,15(n)-^{3}H]-Prostaglandin E_2, 185 Ci/mmol, [5,6,8,9,12,14,15(n)-^{3}H]-Prostaglandin D_2, 193 Ci/mmol, [1-^{14}C] Prostaglandin $F_{2\alpha}$, 60 mCi/mmol, [5,6,8,9,11,12,14,15(n)-^{3}H]-Thromboxane B_2, 120 Ci/mmol, 15(S)-hydroxy-[5,6,8,9,12,14,15(n)-^{3}H]- eicosatetraenoic acid, 285 Ci/mmol, 5(S)-hydroxy[5,6,8,11,12,14,15(n)-^{3}H]-eicosatetraenoic acid, 183 Ci/mmol, and 12(S)-hydroxy-[5,6,8,11,12,14,15(n)-^{3}H]-eicosatetraenoic acid, 119 Ci/mmol, were purchased from Amersham; 12-L-hydroxy-[5,6,8,9,11,12(n)-^{3}H]-5,8,10-heptadecatrienoic acid, 129 Ci/mmol and 20[14,15(n)-^{3}H]-hydroxy-leukotriene B_4, 52 Ci/mmol, were purchased from New England Nuclear.

Chromatographic procedure. Eluents: A) acetonitrile : water at pH 3.4 (with acetic acid) 33 : 67; and B) acetonitrile : water, pH 3.4 9 : 1. After 15 min of equilibration with eluent A, 100 µl samples were injected into the column (Ultrasphere ODS 250×4.6 mm, Beckman). The composition of the mobile phase was 100% A for the first 16 min after injection. Thereafter, the percentage of B was increased linearly to reach 65%, 26 min after injection, and then maintained until minute 40. The column was then washed for 15 min with eluent B. Mobile phase flow rate was 1 ml/min.

When better resolution of HETEs was required, chromatography was performed isocratically with a mixture of methanol : water : triethylamine : trifluoroacetic acid 75 : 25 : 0.05 : 0.1 as the mobile phase.

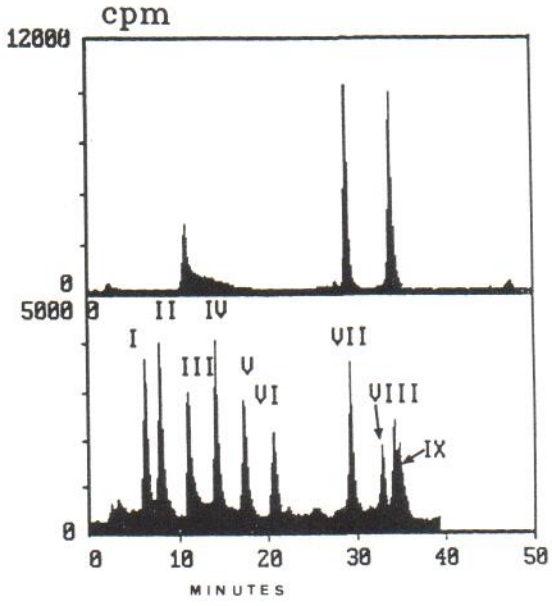

Fig. 1. Typical chromatogram of AA metabolites produced by platelet suspensions (sample above, authentic standards below). ^{3}H-labelled standards: *I*, 6-keto-$PGF_{1\alpha}$; *II*, 20-hydroxy-LTB_4; *III*, TXB_2; *IV*, $PGF_{2\alpha}$; *V*, PGE_2; *VI*, PGD_2; *VII*, HHT; *VIII*, 15-HETE; *IX*, 12- and 5-HETE.

Data processing. Identification of eicosanoids formed was done by coelution with authentic standards. Chromatographic peaks were integrated by means of 171 Radioisotope Detector Chromatographics Software (Beckman) in an IBM-PC XT 286 coupled to the scintillation detector. Quantitative results are expressed as nmoles of ^{14}C-AA converted into each peak.

Platelet aggregation in vitro in response to AA. In vitro aggregation, in response to 1 mM sodium arachidonate (Sigma), was performed in an automatic aggregometer (Aggrecoder PA-3210, Menarini). PRP aliquots from 8 patients and 8 controls were adjusted with platelet-poor plasma (PPP) to $2.5–3\times10^5$ platelets/µl. Transmittance was recorded for 5 min after addition of the inductor. Response was quantified as the maximum percentage of change in relative transmittance and the latency time before the onset of transmittance change.

Statistics. Statistical significance was assessed using two-tailed Student's *t*-test. A *p*-value below 0.05 was considered significant.

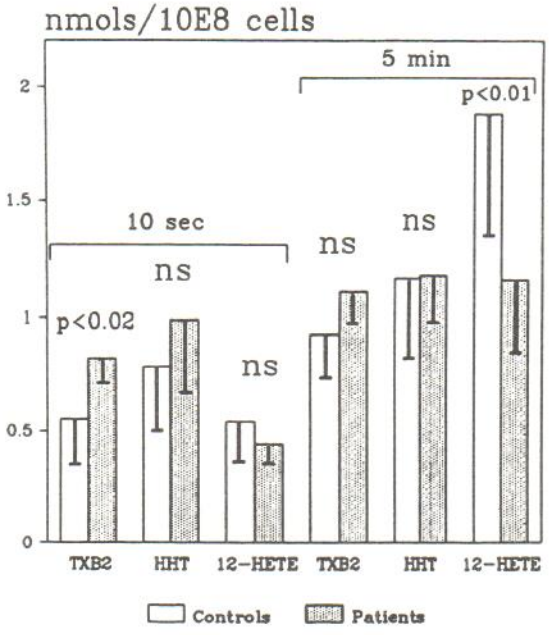

Fig. 2. Production of TXB_2, HHT and 12-HETE by washed platelets from patients and controls after incubation with 50 μM AA for 10 s or 5 min. $n=8$, mean ± S.D.

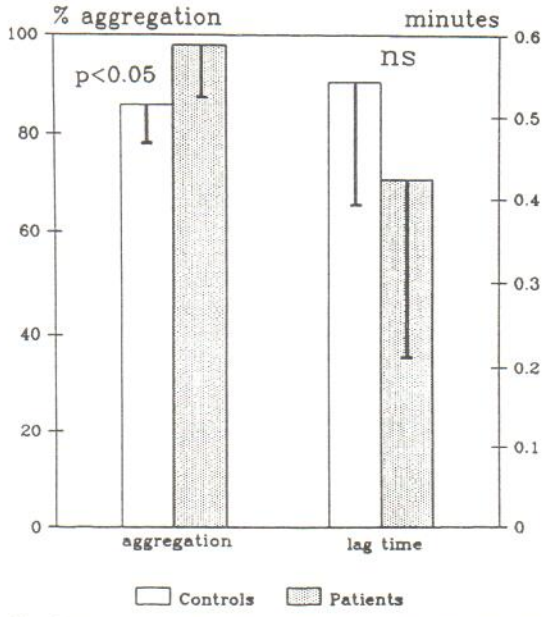

Fig. 3. Percentage of PRP aggregation (*left*) and lag time (*right*) in response to 1 mM sodium arachidonate. $n=8$, mean ± S.D.

RESULTS

Fig. 1 shows the typical metabolic profile of platelets. Three main peaks can be observed, coeluting with TXB_2, HHT and 12-HETE standards. In some cases (2 psoriatic patients and 1 control) minor peaks, corresponding to PGE_2 and PGD_2, also appeared. Metabolic profiles of platelets from psoriatic patients and controls did not differ.

A plateau of prostanoid and 12-HETE production was reached for concentrations of AA ≥ 50 μM. Biosynthetic rate at 10 s of incubation with 50 μM AA approached linearity, whereas production was maximal at incubation times of 5 min (data not shown). Incubation times of 10 s and 5 min were chosen in order to appreciate any differences that could exist in biosynthetic rate and total production of the three main eicosanoids, respectively.

Fig. 2 shows TXB_2, HHT and 12-HETE production by platelets derived from psoriatic patients and controls, after incubation with 50 μM AA for 10 s or 5 min. No differences could be found as regards total production of cyclo-oxygenase products. Total 12-HETE production by controls was higher and statistically significant ($p<0.01$). Following 10 s of incubation, the biosynthetic rate of cyclo-oxygenase products tended to be higher in platelets derived from psoriatic patients, although only TXB_2 production was significantly higher ($p<0.02$). 12-HETE production rate was slightly higher in the control group, though the difference was not statistically significant.

Fig. 3 shows the maximal aggregation percentages and lag times of platelet aggregations in samples from patients and controls. Platelet aggregation was higher in the group of psoriatic patients ($p<0.05$). Lag times tended to be shorter in this group, but the difference was not statistically significant. Fig. 4 shows the correlation between lag times and TXB_2 production in samples incubated for 10 s ($p<0.01$). There was no correlation between lag times and TXB_2 total production after 5 min of incubation. There was no correlation between the production of TXB_2 after incubation for 10 s or 5 min and the percentage of maximal aggregation.

DISCUSSION

We did not find any qualitative difference between platelets from psoriatic patients and controls as regards AA metabolic pattern. A significant finding was the higher production rate of cyclo-oxygenase pro-

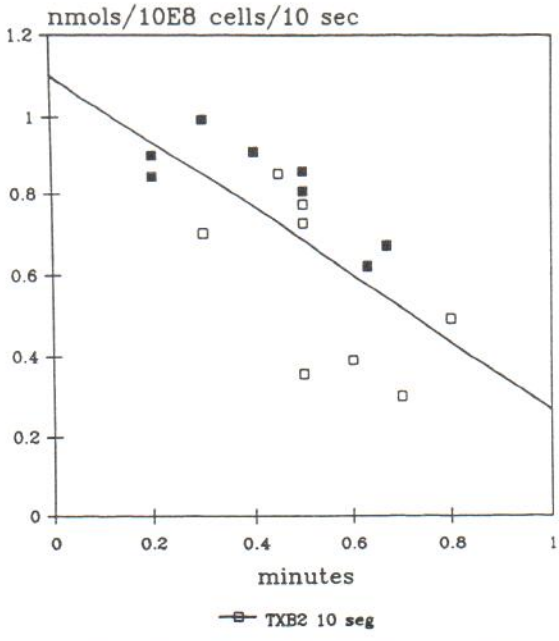

Fig. 4. Correlation between TXB_2 formation by washed platelets incubated with 50 μM AA for 10 s and lag time of PRP aggregation in response to 1 mM sodium arachidonate ($p<0.01$). ■, Patients; □, controls.

ducts in platelets from psoriatic patients, which has not been previously reported, to our knowledge. A correlation was found between TXB_2 production rate and lag times of PRP aggregation in response to 1 mM AA, which tended to be shorter in psoriatic patients, although the difference was not statistically significant, possibly because of small sample size and/or the high concentration of AA we used. 12-HETE production after incubation for 5 min was lower in samples obtained from psoriatic patients ($p<0.01$). This finding might be explained by an increase in cyclo-oxygenase activity, which would leave less substrate available (less than 3% of initial AA was recovered after 1 min of incubation) for 12-lipoxygenase, the activity of which outlives that of cyclo-oxygenase following activation (data not shown).

Our results differ from those of Kragballe & Fallon (21, 24) who found an increased production of 12-HETE and HHT, following stimulation with ionophore A23187, by platelets from patients with extensive psoriasis. Ionophore A23187 causes release of sterified AA through increased availability of Ca^{2+} to phospholipases. Greater production of 12-HETE and HHT by platelets from psoriatic patients might be due to an increased response in AA release following that stimulus, which does not necessarily imply an increase in platelet cyclo-oxygenase and/or 12-lipoxygenase activity in those patients. The increment in production of 12-HETE could be more pronounced than that of HHT, as found by those authors, due to differences in substrate availability at the incubation times employed (15 min). Following prolonged incubation times, if AA concentrations remained high, the balance of production of AA metabolites through cyclo-oxygenase and 12-lipoxygenase pathways could favour the latter.

Our results are consistent with those of other authors (21, 23) as regards platelet hyperaggregability in psoriatic patients. This hyperaggregability could be accounted for in part by an increased release of AA sterified in platelet plasma membrane in response to platelet activators, as well as by an increase in cyclo-oxygenase activity, which would yield more thromboxane A_2 available. In the aggregation studies reported herein, we have evaluated only the response to 1 mM AA. We are now performing additional studies in order to observe the response of platelets from psoriatic patients to other aggregating agents (such as ADP or collagen), and to evaluate the dependence of the hyperaggregability in psoriatic patients on the metabolism of AA.

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