Pergamon

Biochemical Pharmacology, Vol. 49, No. 2, pp. 243–248, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00403-X

ENHANCEMENT OF LEUKOTRIENE A_4 BIOSYNTHESIS IN NEUTROPHILS FROM PATIENTS WITH RHEUMATOID ARTHRITIS AFTER A SINGLE GLUCOCORTICOID DOSE

E. THOMAS,* J. L. LEROUX,* F. BLOTMAN,* B. DESCOMPS† and C. CHAVIS†‡

*Service de Réadaptation Fonctionnelle, CHU Lapeyronie, 34059, Montpellier, and †Centre de Recherches INSERM, 70 rue de Navacelles, 34090 Montpellier, France

(*Received* 14 *March* 1994; *accepted* 29 *August* 1994)

Abstract—Human blood polymorphonuclear cells (PMN) from seven patients with active rheumatoid arthritis (RA) were compared for their capacities to produce leukotrienes *ex vivo* before (D0) and 24 hr (D1) after glucocorticoid pulse therapy. The present study shows for the first time that endogenous arachidonic acid metabolism via 5-lipoxygenase pathway is significantly increased after glucocorticoid administration, leading to increased generation of the unstable precursor leukotriene A_4 (LTA_4) followed by predominant non-enzymatic LTA_4 opening and leukotriene B_4 (LTB_4) ω-hydroxylation pathway. These results are unexpected since usually glucocorticoids are usually thought to decrease inflammatory mediator biosynthesis and, moreover, they work to the detriment of the clinical improvement of the patient. The results are discussed in terms of product inactivation and cellular cooperation with monocytes and endothelial cells.

Key words: polymorphonuclear cells; leukotriene A_4 metabolism; RP–HPLC; rheumatoid arthritis; glucocorticoid therapy

Although glucocorticoid pulse therapy has been used with good clinical results for several years in the treatment of RA,§ there is little information available on mechanisms involved in the specific biological effects of this therapy. The effects of glucocorticoids on inflammation and immunity are reflected by alteration of phagocyte cell functions, such as chemotaxis and superoxide anion generation, and by inhibition of the production and release of mediators, as reported by Goldstein *et al.* [1]. There are few reports concerning the effects of glucocorticoid therapy on eicosanoid biosynthesis. Suppression of the lipoxygenase pathway might be restricted *in vivo* to a limited subset of responsive cells (i.e. macrophages), whereas under *in vitro* conditions several cell types may be responsive [2, 3]. We investigated the 5-LO pathway of arachidonic acid metabolism in blood PMN of RA patients with active disease, before and 24 hr after a 250 mg methylprednisolone pulse. Our results clearly demonstrated that there was no marked reduction in the biochemical response of human blood PMN, thus revealing a discrepancy between the clinical improvement of the pathological state and the level of inflammatory mediators biosynthesized.

‡ Corresponding author. Tel. 3367043722; FAX 33675206.

§ Abbreviations: PMN, polymormophonuclear cells; RA, rheumatoid arthritis; 5-LO, 5-lipoxygenase; LTA_4, 5(*S*)-5,6 oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LTB_4, 5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid; NE LTB_4, (non-enzymatic LTB_4), 5(*S*),12(*R*) and 5(*S*),12(*S*)-dihydroxy-6,8,10-*trans*-14-*cis*-eicosatetraenoic acids; 20-OH-LTB_4, 5(*S*),12(*R*), 20-trihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid; E LTB_4, enzymatic LTB_4 (LTB_4 + 20-OH LTB_4); LTC_4, leukotriene C_4; 12-HETE, 12(*S*)-hydroxy-5,8,14-*cis*, 10-*trans*-eicosatetraenoic acid; 5-HETE, 5(*S*)-hydroxy-8,11,14-*cis*,6-*trans*-eicosatetraenoic acid.

MATERIALS AND METHODS

Chemicals. LTB_4 and 20-OH LTB_4, purchased from Euromedex (Strasbourg, France), were from Cascade (Reading, U.K.). Ionophore A23187 was from Sigma Chemical Co. (St Louis, MO, U.S.A.). The solvents were HPLC grade (Carlo Erba). Culture material, PBS with $CaCl_2$ and $MgCl_2$ came from Flow Laboratories (France). RP64966, an inhibitor of LTA_4 hydrolase, was a generous gift from Dr N. Dereu, Rhone Poulenc Rorer Laboratories (Vitry/Seine, France).

Selection of subjects. Seven patients (five women and two men, mean ages 49.6 ± 3.7) were studied (RA group). They presented active rheumatoid arthritis according to the American Rheumatism Association 1987 revised criteria for rheumatoid arthritis. All were positive for rheumatoid factor (>40 UI/mL by nephelometry laser method, Berhing). The mean erythrocyte sedimentation rate (ESR) was 63 mm (range 33–120) for the first hour. They received no glucocorticoid injection for 3 months before the study but all were under non-steroidal anti-inflammatory drug (NSAID) treatment (arylcarboxylic acid molecule family). They were selected to receive glucocorticoid pulse therapy (250 mg intravenously administered methylprednisolone) because inflammatory flare-up of their disease was not able to be controlled. Clinical evolution was assessed by the duration of morning stiffness and the number of painful joints before (D0) and 24 hr after (D1) treatment to estimate clinical improvement and by the physician's and

patient's global assessment. Arachidonic acid metabolites released by PMN were analysed before and 24 hr after the methylprednisolone injection. Seven healthy subjects (HS group, mean ages 37.2 ± 6.5) were studied simultaneously to test whether individual daily variation was observed when leukotriene biosynthesis by PMN was evaluated from one day to another.

Human PMN isolation. Blood cell analysis routinely carried out showed a 15 fold higher amount of PMN versus platelets before or after treatment. PMN were isolated and purified from heparinized blood by centrifugation of samples over discontinuous Percoll gradient as previously described [4]. Briefly a 5 mL volume of 63% percoll in 0.15 M NaCl solution was layered over 5 mL of 72% percoll solution. Whole blood was then layered over the percoll gradient and the tubes were spun at 400 *g* for 20 min at 20°. The upper layer constituted mononuclear cells and platelets. The PMN were recovered in the intermediary layer between the mononuclear cells and the 72% percoll solution. Purity of the PMN suspension was evaluated by microscope observation of the cells after cytocentrifugation and May Grünwald staining. The preparations, at least 95% purity, were greater than 85% viable before and after glucocorticoid injection, as measured by trypan blue exclusion.

Stimulation procedures. Isolated PMN were resuspended in PBS (PBS Dulbecco's, pH 7.4) with Ca^{2+}/Mg^{2+} (final concentrations 2×10^{-3} and 0.5×10^{-3} M, respectively). The cell concentration was adjusted to 10^7 cells/mL. Eicosanoid release was produced by ionophore A23187 stimulation (final concentration 5×10^{-6} M) for 5 min as previously described [5] with or without RP64966. The reaction was stopped by the addition of the same volume of methanol and the samples were stored for at least 24 hr at −20° until reverse phase HPLC analysis (RP–HPLC).

Eicosanoid identification and quantification. RP–HPLC was carried out using two model 510 pumps (Waters), a U6K injector (Waters) and a Lichrospher 100 RP-18 analytical column (150 mm × 3.9 mm, 5 μ particles, Merck). Detection was monitored with a programmable multiwavelength detector model 490 (Waters) and recorded with a C-R3A integrator (Shimadzu). Before injection the samples were centrifuged to pellet the proteins denatured by methanol and aliquots of the supernatants were directly analysed by RP–HPLC without any purification. Leukotrienes were resolved according to the following elution gradient between two solvents A and B and to the following linear flow rate program:

—Solvent A: water 350/acetic acid 1 (v/v) pH 5.6 and solvent B: methanol. The gradient was carried out linearly from 45% A/55% B to 35% A/65% B for 12 min, and then isocratic elution was conducted for 20 min.

—Flow rate: linear flow rate increasing from 0.5 mL/min to 1 mL/min for 12 min and then a constant flow rate for 20 min.

Detection was monitored at 280 nm. Metabolites were identified by comparing their retention times and UV spectra recorded in the stop-flow mode with those of synthetic reference products and quantitated by measuring the areas under the respective peaks as described elsewhere [5].

Statistical analysis. The results presented as ng/10^7 cells or those corresponding to the metabolite ratio were expressed as mean ± SEM (N = 7 for RA patients and N = 7 for HS patients). Statistical differences were determined using Student's *t*-test for paired samples and significance was assumed when $P < 0.05$.

RESULTS

Formation of LTA_4 metabolites by human PMN from RA patients

The endogenous arachidonic acid 5-LO pathway of human PMN was investigated by eicosanoid synthesis under stimulation conditions by Ca^{2+} ionophore A23187 for 5 min. Before corticotherapy, PMN from RA subjects released into the culture medium 20-OH LTB_4, immunoreactive LTB_4, the two $\Delta 6$-*trans* isomers of LTB_4 occurring as a result of the non-enzymatic opening of LTA_4 (non-enzymatic LTB_4, i.e. NE LTB_4) and 5-HETE (data not shown). No detectable amounts of leukotriene C_4 (LTC_4) or 12-HETE were observed. These results are in agreement with leukotriene release by human blood PMN as previously described [6–8]. The

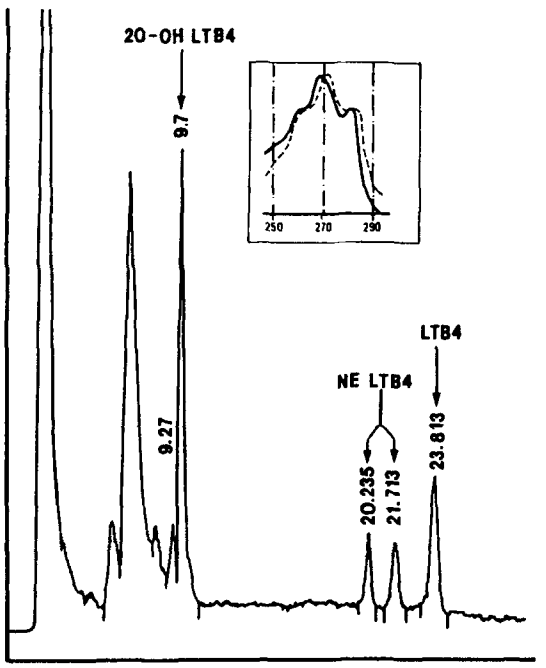

Fig. 1. RP–HPLC profile (detected at 280 nm) of leukotrienes biosynthesized by human PMN from subjects with RA after incubation with calcium ionophore A23187 (final concentration 5×10^{-6} M) at 37° for 5 min. The chromatography conditions are described in Materials and Methods. Retention times of the authentic reference products are represented by arrows. Insert: UV spectra of NE LTB_4 (filled line) and LTB_4 (dotted line) recorded in stop-flow mode (λ_{max} 268 nm and 270 nm, respectively).

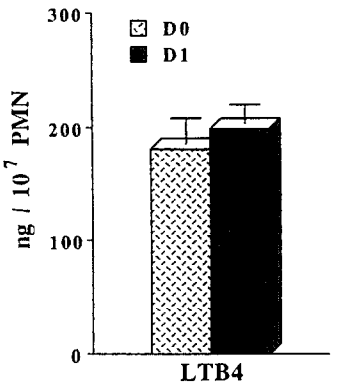

Fig. 2. LTB_4 biosynthesized *in vitro* by human PMN from subjects with RA before (D0) and 24 hr (D1) after 250 mg methylprednisolone pulse (experimental conditions are described in Materials and Methods). Results presented in ng/10^7 cells are expressed as mean ± SEM (N = 7). No significant difference was observed.

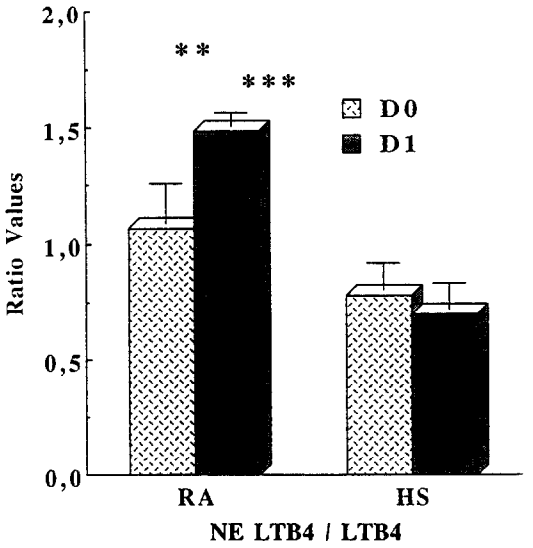

Fig. 4. NE LTB_4/LTB_4 ratio at D0 and D1. Results are expressed as mean + SEM. Significant increases were observed for RA at D0 and D1 (**P < 0.05) and between RA and HS (***P < 0.02).

elution profile, detected at 280 nm under our experimental conditions, with a characteristic equal peak area for the two NE LTB_4 isomers, is shown in Fig. 1. Retention times for 20-OH LTB_4, NE LTB_4 and LTB_4 were 9.7 min, 20.2 and 21.7 min and 23.8 min, respectively. Characteristic conjugated triene UV spectra showed maximum absorption at 268 nm with shoulders at 258 and 280 nm for the Δ6-*trans* isomers, as described elsewere, with a 2 nm shift as compared to that of LTB_4 [9, 10].

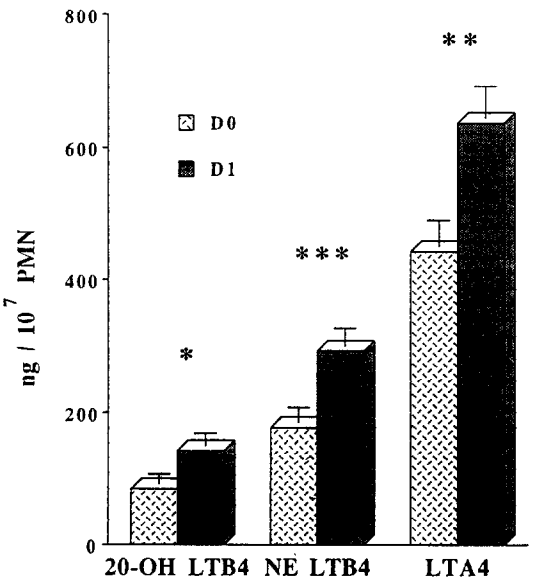

Fig. 3. Leukotriene metabolites and leukotriene precursor: 20-OH LTB_4 NE LTB_4 isomers and LTA_4 released *in vitro* by human PMN from patients with RA, before (D0) and 24 hr after (D1) 250 mg methylprednisolone pulse (experimental conditions described in Materials and Methods). Results presented in ng/10^7 cells are expressed as mean ± SEM (N = 7). Significant increases were observed *P < 0.015 **P < 0.010 ***P < 0.005.

Before corticotherapy. Significant LTB_4 levels (180.4 ± 22.3 ng) were released by the PMN of RA patients. Surprisingly, NE LTB_4 levels (177.8 ± 22.9 ng) were the same as the LTB_4 levels, with a NE LTB_4/LTB_4 level of 1.07 ± 0.18. This was unexpected since levels of these metabolites are usually approximately two fold lower than those of LTB_4 up to 5 min incubation, as previously reported for various other subjects [6] as well as for the HS group included in the study to investigate possible individual daily variation (Fig. 4).

After glucocorticoid pulse. No significant differences were observed in LTB_4 release: 198.3 ± 15.8 ng versus 180.4 ± 22.3 ng (Fig. 2). The results for 20-OH LTB_4 and NE LTB_4 are given in Fig. 3. 20-OH LTB_4 and NE-LTB_4 levels were significantly enhanced the day after treatment: 144.3 ± 16.4 ng versus 85.1 ± 12.9 ng (P < 0.015) and 293.9 ± 24.4 versus 177.8 ± 22.9 ng (P < 0.005), respectively. This rise was in agreement with a simultaneous increase in the precursor LTA_4 as reported in Fig. 3: 637.0 + 48.6 ng versus 443.4 ± 40.4 ng (P < 0.010). Moreover, the NE LTB_4/LTB_4 ratio was significantly increased to 1.48 ± 0.06 (P < 0.040), as shown in Fig. 4. This result is similar to that obtained when PMN were stimulated by ionophore in the presence of the inhibitor of LTA_4 hydrolase RP64966 (10^{-6} M): the 3.15 ± 0.16 ratio was more than four fold higher than the 0.74 ± 0.17 obtained without inhibitor. The increased generation of leukotrienes and 5-HETE (data not shown) by PMN after prednisolone injection revealed marked 5-LO activation.

Comparison of PMN LTA_4 biosynthesis between

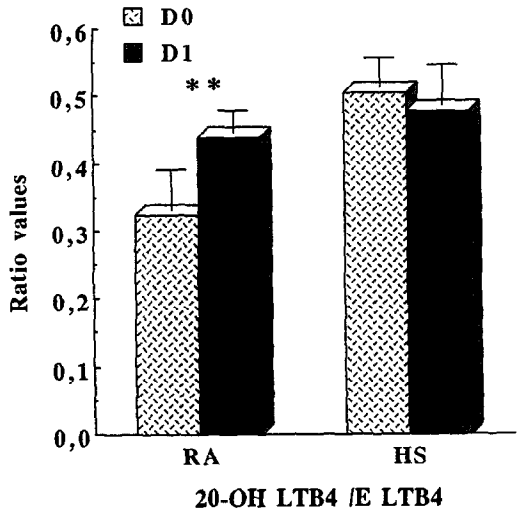

Fig. 5. 20-OH LTB_4/E LTB_4 ratio at D0 and D1 (E LTB_4 = 20-OH LTB_4 + LTB_4). Results are expressed as means ± SEM. Significant increases were observed for RA at D0 and D1 (**$P < 0.05$).

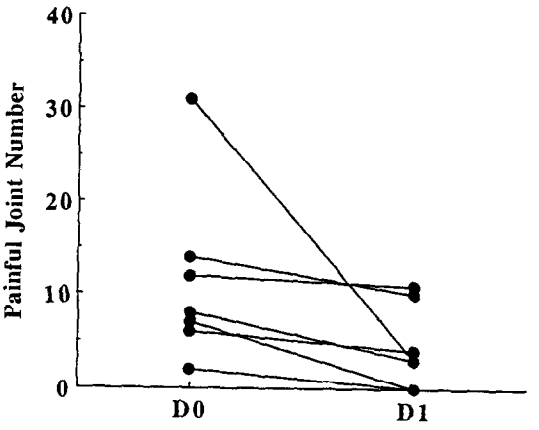

Fig. 6. Individual clinical evolution as measured by painful joint count. Mean values at D0 and D1: 15 ± 3 and 5 ± 2, respectively (***$P < 0.01$).

RA and HS. Eicosanoid biosynthesis was studied under the same experimental conditions in a group of healthy subjects to investigate whether the effects observed in PMN from RA after glucocorticoid administration were not simply due to a daily variation in cellular responsiveness or preparation. The estimation of NE LTB_4 was expressed as its ratio versus immunoreactive LTB_4 (Fig. 4) and 20-OH LTB_4 synthesis was expressed as its ratio versus total enzymatically produced LTB_4 (E LTB_4 = 20-OH LTB_4 + LTB_4) (Fig. 5). NE LTB_4 was significantly increased after pulse therapy: 1.480 ± 0.062 versus 1.060 ± 0.183 ($P < 0.040$) whereas in HS PMN the ratio remained strictly independent of the day of sampling: 0.774 ± 0.117 versus 0.697 ± 0.0110 (not significant). The same result was obtained with 20-OH LTB_4: 0.323 ± 0.060 versus 0.440 ± 0.030 ($P < 0.050$) whereas in HS PMN no significant difference was observed.

Clinical data

Clinical improvement was assessed 24 hr after pulse therapy. Statistically significant improvements were observed in the painful joint count (5 ± 2 versus 15 ± 3, $P < 0.010$) and duration of morning stiffness (7 ± 3 min versus 68 ± 8 min, $P < 0.003$) as reported in Figs 6 and 7. Only the swollen joint count did not improve significantly (data not shown). Because each patient served as his or her own control for the biochemical studies, there was no placebo control.

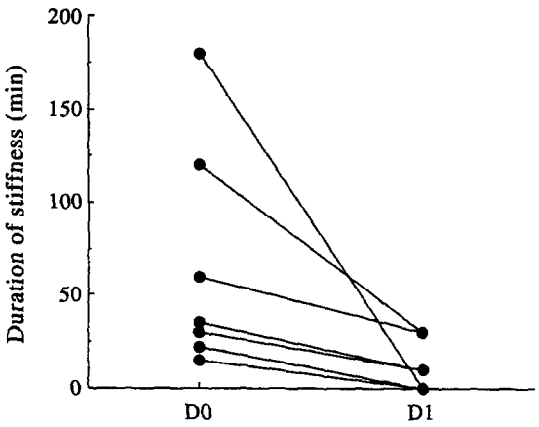

Fig. 7. Individual clinical evolution as measured by duration of morning stiffness expressed in minutes. Mean values ± SEM at D0 and D1: 68 ± 8 and 7 ± 3 min, respectively (***$P < 0.003$).

DISCUSSION

We investigated eicosanoid generation from endogenous arachidonic acid via 5-LO pathway in human PMN stimulated by ionophore A23187. The ionophore of divalent cation is not cell type specific but is a useful tool for the generation of individual eicosanoids in amounts that facilitate their isolation and structural determination by physical methods. Sample aliquots were directly injected onto the analytical RP–HPLC column without any solid phase extraction, concentration or derivatization. The samples only had to be deproteinized and centrifuged, thus minimizing double bond isomerization and loss and deterioration of eicosanoids. We were therefore able to assess that LTB_4 isomers did not result from LTB_4 isomerization provided by a chemical experimental procedure but rather from the non-enzymatic opening of LTA_4 producing equal amounts of the Δ6-*trans* isomers 12(*R*) and 12(*S*). Moreover, the chromatographic elution system was specially appropriate for the study of LTA_4 metabolites since

it allowed us to resolve 20-OH LTB_4 away from the solvent peak to obtain precise quantification.

PMN from patients with active RA biosynthesized *in vitro* LTB_4, 20-OH LTB_4 and Δ6-*trans* LTB_4 isomers without any detectable amounts of LTC_4. We show for the first time that the amounts of the two Δ6-*trans* LTB_4 isomers occurring from non-enzymatic LTA_4 opening were as high as immunoreactive LTB_4 levels in these patients (Fig. 4). This contrasts with the results reported for various other subjects [6], suggesting that in RA patients the ability of PMN to release high *in vitro* levels of NE LTB_4 is relevant to this particular pathology. Moreover, 24 hr after glucocorticoid administration, the PMN produced two-fold greater amounts of 20-OH LTB_4 and NE LTB_4 while LTB_4 levels remained unchanged. These results cannot simply be explained by increased LTA_4 synthesis, since the metabolic pattern was different and cannot account for the enhancement of 20-OH LTB_4 biosynthesis which occurred enzymatically. Moreover LTA_4 at D1 cannot be considered as "suicide substrate" for LTA_4 hydrolase since recent works [11] have demonstrated that loss of enzymatic activity accompanying LTB_4 formation was proportional to substrate concentration and that a 50% loss in hydrolase activity corresponded with the formation of 10.3 ± 2.1 μM LTB_4. The amounts of enzymatically produced LTB_4 at D1, 332.6 ± 21.5 ng/mL, i.e. 0.95 ± 0.06 μM, are too low to account for hydrolase inhibition. The data cannot be interpreted in terms of a cellular cooperation between platelets and PMN: even though some platelets could have adhered to PMN in spite of gradient isolation, the platelet/PMN ratio is too low to account for the enhancement of LTA_4 metabolite levels. A 20 platelet/PMN ratio is required to significantly modify arachidonic acid metabolism and leads, in these conditions, to the enhancement of 12-HETE biosynthesis [12] and not to increased LTA_4 production. The presence of platelets would also lead to LTC_4 biosynthesis by the transcellular metabolism of LTA_4 released by PMN [13], especially when significant amounts of LTA_4 have not been metabolized by LTA_4 hydrolase. There was no evidence of detectable amounts 12-HETE and LTC_4 in our samples, so the nature of the products more likely indicated a deviation towards the non-enzymatic pathway (as observed when the cells were incubated in the presence of the LTA_4 hydrolase inhibitor) and towards LTB_4 catabolism. These two modification steps thus led to inflammatory mediator inactivation and seemed to function as a detoxification-like process [14–16]. Since high amounts of Δ6-*trans* LTB_4 isomer (0.6 mM) were released under our experimental conditions, it would be interesting to investigate in future studies whether the triene reductase pathway is involved when PMN incubation is extended to 30 min as reported by Powell *et al.* [17].

From the data reported in Figs 4 and 5, it appears that the NE LTB_4 and 20-OH LTB_4 level increase at D1 in PMN from RA patients may not be explained by an individual daily variation which leads us to conclude that metabolite level enhancement is relevant to glucocorticoid action on cells. This 5-LO activation after glucocorticoid therapy was unexpected since it is generally accepted that anti-inflammatory drugs reduce inflammatory mediator production. Thus, glucocorticoids would be expected to inhibit arachidonic acid metabolite biosynthesis. In 1990, Sebaldt *et al.* [2] described a strong inhibitory effect of corticosteroid administration on eicosanoid biosynthesis by human alveolar macrophages and human whole blood cells *ex vivo* [2, 3]. More recently De Catarina *et al.* [18] described human alveolar macrophages as the most likely cell targets for glucocorticoid-induced eicosanoid inhibition and lipocortin expression. Nevertheless, Freeland *et al.* [19] reported that glucocorticoids failed to inhibit neutrophil LTB_4 production induced by ionophore A23187 but did not mention a significant modification in 5-LO activity since they did not measure LTA_4 production. Schleimer *et al.* [20] reported a small non-significant enhancement of LTB_4 generation *in vitro* by PMN after 24 hr dexamethasone exposure. However, LTB_4 was monitored by RIA and this technique did not take into account Δ6-*trans* isomers and the 30% LTB_4 antibody crosslinking with 20-OH LTB_4. These two limiting parameters might explain the low increase in LTB_4. Our study demonstrated, for the first time, the unexpected increase of LTA_4 metabolites, i.e. an enhanced pro-inflammatory state in spite of the clinical improvement in the physiological state of the patients after a single glucocorticoid dose administration as reported in Figs 6 and 7. These unusual findings are in agreement with reduced LTA_4 hydrolase activity and with increased LTB_4 catabolism subsequent to corticoid pulse. There are few data concerning the *in vitro* action of glucocorticoids on blood PMN. After *in vitro* exposure to dexamethasone, blood monocytes are able to release chemotactic factors [21, 22] and the number of interleukin-1 receptors increase in human PMN [23]. Tsai *et al.* [24] reported that human blood monocytes cultured with phytohemaglutinin produced a factor which enhanced PMN LTB_4 production. Rheumatoid arthritis modulates blood mononuclear cell cytokine secretion [25] and in numerous diseases, vascular endothelial cells are now considered to be active inflammatory cells rather than a biological barrier to body fluids [26]. The priming of glucocorticoids on PMN might be explained by potential cellular interactions with monocytes and/or with vascular endothelial cells since several authors have reported evidence for a lipoxygenase dependent mechanism in neutrophil adherence to endothelial cells [27–30]. It will then be of interest to proceed with this work in order to investigate the mechanisms of cell to cell interactions which could be involved in glucocorticoid action on arachidonic acid metabolism in human blood neutrophils and provide a link between neutrophil lipid mediators and cell mediated immunity in the context of RA.

Acknowledgement—The authors thank Dr N. Dereu, Rhone Poulenc Rorer Laboratories (Vitry/Seine, France) for the generous gift of LTA_4 hydrolase inhibitor RP64966.

REFERENCES

1. Goldstein RA, Bowen DL and Fauci AS, Adrenal

corticosteroids. In: *Inflammation: Basic Principles and Clinical Correlates*, 2nd Edn (Eds. Gallin JI, Goldstein IM and Snyderman R), pp. 1061–1081. Raven Press, New York, 1992.
2. Sebaldt RJ, Sheller JS, Oates JR, Roberts LJ and Fitzgerald GA, Inhibition of eicosanoid biosynthesis by glucocorticoids in humans. *Proc Natl Acad Sci USA* **87**: 6974–6978, 1990.
3. Sebaldt RJ, Yang XH, Adachi JD and Bensen W, Pulse glucocorticosteroid (GCS) therapy in R.A. patients inhibits whole blood leukotriene B_4 biosynthesis *ex vivo*. *Arthritis Rheum* **36** (suppl): F269, 1993.
4. Radeau T, Chavis C, Damon M, Michel FB, Crastes de Paulet A and Godard Ph, Enhanced arachidonic acid metabolism and human neutrophil migration in asthma. *Prostaglandins Leuk Essent Fatty Acids* **41**: 131–138, 1990.
5. Chavis C, Godard Ph, Crastes de Paulet A and Damon M, Formation of lipoxins and leukotrienes by human alveolar macrophages incubated with 15(*S*)-HETE: a model for cellular cooperation between macrophages and airway epithelial cells. *Eicosanoids* **5**: 203–211, 1992.
6. Radeau T, Chavis C, Godard Ph, Michel FB, Crastes de Paulet A and Damon M, Arachidonate 5-lipoxygenase metabolism in human neutrophils from patients with asthma: *in vitro* effect of nedocromil sodium. *Int Arch Allergy Appl Immunol* **97**: 209–215, 1992.
7. Borgeat P and Samuelsson B, Arachidonic acid metabolism in polymorphonuclear leukocytes: unstable intermediate in formation of dihydroxy acids. *Proc Natl Acad Sci USA* **76**: 2148–2153, 1979.
8. Borgeat P and Samuelsson B, Arachidonic acid metabolism in polymorphonuclear leukocytes: effect of ionophore. *J Biol Chem* **256**: 11828–11831, 1980.
9. Powell WS, Novel pathway for the metabolism of 6-*trans*-leukotriene B_4 by human polymorphonuclear leukocytes. *Biochem Biophys Res Commun* **136**: 707–712, 1986.
10. Brewer O and Hammarstrom S, Enzymatic conversion of leukotriene B_4 to 6-*trans*-leukotriene B_4 by rat kidney homogenates. *Biochem Biophys Res Commun* **142**: 667–673, 1987.
11. Orning L, Jones DA and Fitzpatrick FA. Mechanism-based inactivation of leukotriene A_4 hydrolase during leukotriene B_4 formation by human erythrocytes. *J Biol Chem* **265**: 14911–14916, 1990.
12. Tremoli E, Interactions between platelets and neutrophils: quantitative changes of hydroxyacid formation. In: *9th International Conference on Prostaglandins and Related Compounds, Florence, Italy*, Abstract book: 6, Raven Press, New York, 1994.
13. Maclouf JA and Murphy R, Transcellular metabolism of neutrophil-derived leukotriene A_4 by human platelets. *J Biol Chem* **263**: 174–181, 1988.
14. Shak S and Goldstein IM, ω-Oxidation is the major pathway for the catabolism of leukotriene B_4 in human polymorphonuclear leukocytes. *J Biol Chem* **259**: 10181–10187, 1984.
15. Maurice PDL, Camp RDR and Allen BR, The metabolism of leukotriene B_4 by peripheral blood polymorphonuclear leukocytes in psoriasis. *Prostaglandins* **33**: 807–817, 1987.
16. Brom J, Konig W, Stunong S, Raulf M and Koller M. Characterization of leukotriene B_4-omega hydroxylase activity within human polymorphonuclear granulocytes. *Scand J Immunol* **25**: 283–294, 1987.
17. Powell WS and Gravelle F, Metabolism of 6-*trans* isomers of leukotriene B_4 to dihydro products by human polymorphonuclear leukocytes. *J Biol Chem* **263**: 2170–2177, 1988.
18. De Catarina R, Sicari R, Giannessi D, Paggiaro PL, Paoletti P, Lazzerini G, Bernini W, Solito E and Parente L, Macrophage-specific eicosanoid synthesis inhibition and lipocortin-1 induction by glucocorticoids. *J Appl Physiol* **75**: 2368–2375, 1993.
19. Freeland HS, Pipkorn U, Schleimer RP, Bascom R, Lichtenstein LN, Naclerio RM and Peters SP, Leukotriene B_4 as a mediator of early and late reactions to antigens in humans: the effect of systemic glucocorticoid treatment *in vivo*. *J Allergy Clin Immunol* **83**: 634–642, 1989.
20. Schleimer RP, Freeland HS, Peters SP, Brown KE and Derse CP, An assessment of the effects of glucocorticoids on degranulation, chemotaxis, binding to vascular endothelium and formation of leukotriene B_4 by purified human neutrophils. *J Pharmacol Exp Ther* **250**: 598–605, 1989.
21. Chettibi S, Lawrence AJ and Stevenson RD, A factor released by monocytes in the presence of dexamethasone stimulates neutrophil locomotion. *Br J Pharmacol* **108**: 248–254, 1993.
22. Li YS, Shyy YJ, Wright JG, Valente AJ, Cornill JF and Kollattuduki PE, The expression of monocyte chemotactic protein (MCP-1) in human vascular endothelium *in vitro* and *in vivo*. *Mol Cell Biochem* **126**: 61–68, 1993.
23. Shieh JH, Peterson RHF and Moore AS, Cytokines and dexamethasone modulation of IL-1 receptors on human neutrophils *in vitro*. *J Immunol* **150**: 3515–3524, 1993.
24. Tsai JJ, Cromwell O, Maestrelli P, O'Heir R, Moobel R and Kay AB. Leukotriene release enhancing factor. *J Immunol* **142**: 1661–1668, 1989.
25. Ruschen S, Stellberg W and Warnatz H. Kinetics of cytokine secretion by mononuclear cells of the blood of rheumatoid arthritis patients are different from those of healthy controls. *Clin Exp Immunol* **89**: 32–37, 1992.
26. Lassalle P, Delneste Y, Gosset P and Tonnel AB. Endothelial cells and asthma. In: *New Concept in Asthma* (Eds. Tarayre JP, Vargaftig B and Carilla E), pp. 200–208. Macmillan Press, London, 1993.
27. Sud'ina GF, Galinka SI, Barsky OA and Margolis LB, Adhesive interactions of neutrophils and leukotriene biosynthesis. *FEBS Lett* **336**: 201–204, 1993.
28. Palmblad J, Lerner R and Larson SH, Signal transduction mechanisms for leukotriene B_4 induced hyperadhesiveness of endothelial cells for neutrophils. *J Immunol* **152**: 262–26, 1994.
29. Palmblad J, Lindström P and Lerner R. Leukotriene B_4 induced hyperadhesiveness of endothelial cells for neutrophils. *Biochem Biophys Res Commun* **166**: 848–851, 1990.
30. Tonnesen NG, Anderson DC, Springer TA, Knedier A, Avdi N and Henson PM, Adherence of neutrophils to cultured human microvascular endothelial cells. *J Clin Invest* **83**: 637–646.