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Effects of lipoxygenase metabolites of arachidonic acid on the growth of human mononuclear marrow cells and marrow stromal cell cultures

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THE effects of various lipoxygenase metabolites of arachidonic acid (AA) were investigated on the growth of freshly isolated human bone marrow mononuclear cells and marrow stromal cell cultures. LTB_4, LXA_4, LXB_4, 12-HETE and 15-HETE (1 μM) decreased [^{3}H]-thymidine incorporation on marrow stromal cell cultures without affecting cell number. Only 12-HETE showed a dose–response effect on [^{3}H]-thymidine incorporation. While LTB_4 (1 μM) decreased thymidine incorporation on marrow mononuclear cells, LTC_4, LXA_4, LXB_4, 12-HETE and 15-HETE had no effect. The lipoxygenase inhibitor NDGA had no effect on both cell types suggesting no role of endogenous lipoxygenase metabolites on cell growth. These results suggest no important role of lipoxygenase metabolites of AA on the proliferation of human marrow mononuclear cells and marrow stromal cell cultures.

Key words: Lipoxin, Hydroxyeicosatetraenoic acid, Leukotriene, Marrow stromal cells, Proliferation

Introduction

Human bone marrow stromal cells regulate haematopoiesis by interacting directly with marrow haematopoietic progenitors and/or by releasing cytokines.[1,2] Lipoxygenase metabolites of arachidonic acid (AA) such as leukotriene B_4 (LTB_4), LTC_4, lipoxin A_4 (LXA_4), LXB_4, 12-hydroxyeicosatetraenoic acid (12-HETE), and 15-HETE are produced by human marrow mononuclear cells.[3,4] Several of these AA metabolites act on the growth of human myeloid and erythroid progenitors in semi-solid culture medium.[5–7] At this time no study has reported the role of AA metabolites on the growth of human marrow stromal cells. These results could be of interest since the lipidic compound platelet-activating factor (PAF) stimulates [^{3}H]-thymidine incorporation in marrow stromal cell cultures.[8] In this study we have assessed the effect of LTB_4, LTC_4, LXA_4, LXB_4, 12-HETE, 15-HETE, and of the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) on the growth of human marrow stromal cell cultures and fresh human mononuclear marrow cells.

Materials and methods

Cell cultures

These experiments were performed according to the Helsinki recommendations. Bone marrow sternal cells were harvested from untreated patients referred for diagnosis. Cells collected by aspiration into heparinized tubes were isolated by separation on a Ficoll gradient (400 × ***g***, 20 min), and washed twice with Hanks's balanced salts solution. Mononuclear marrow cells were used when the cell morphology was normal.

Cultures of human bone marrow stromal cells (mostly fibroblast-like cells) were established from marrow mononuclear cells seeded in 75 cm^2 culture flasks in RPMI 1640 with 20% fetal calf serum (FCS) (Gibco, Cergy Pontoise, France), penicillin (100 U/ml) and streptomycin (100 μg/ml) (culture medium) at 37°C in 5% CO_2 in air as previously described.[9] After one week, non-adherent cells were removed from culture flasks. Adherent cells were grown to confluence for 4–5 weeks with weekly changes of medium and were subcultured after trypsin treatment (0.05% trypsin for 5 min). The cells used in these experiments were at the first passage. In these experimental conditions more than 99.8% of cells were $CD2^-$ and $CD22^-$ indicating the absence of T- and B-cells on the layers and 4% of cells were $CD14^+$ and $CD33^+$ indicating a monocytic/macrophagic lineage.[10,11]

Cell proliferation

Freshly isolated marrow mononuclear cells (1×10^5) were grown in 100 μl of IMDM with 10% FCS. LTB_4, LTC_4, LXA_4, LXB_4, 12-HETE, 15-HETE (Tebu, Le Perray-en-Yvelines, France), NDGA (Sigma, Saint

Table 1. Effects of lipoxygenase AA metabolites on [^{3}H]-thymidine incorporation of human bone marrow stromal cells. Experiments were made with stromal cell cultures from 30 donors. Results (in dpm) are the mean ± SEM from six independent experiments in sixplicate (n = 6)

Stimuli	Controls	1 μM	100 nM	10 nM	1 nM	100 pM	10 pM
LTB_4	2625 ± 522	2142 ± 542**	2788 ± 467	3008 ± 736	2831 ± 673	2632 ± 605	2718 ± 547
LTC_4	2950 ± 550	3132 ± 638	3151 ± 506	3167 ± 520	2961 ± 533	2905 ± 430	2526 ± 319
LXA_4	2577 ± 350	1776 ± 242**	2785 ± 364	2603 ± 363	2785 ± 417	2785 ± 417	2570 ± 393
LXB_4	2508 ± 399	1730 ± 283**	2634 ± 421	2221 ± 327	2541 ± 382	2017 ± 367	2575 ± 479
12-HETE	2413 ± 225	524 ± 44**	1577 ± 183**	1731 ± 180**	1944 ± 208*	1789 ± 196*	2057 ± 243
15-HETE	1859 ± 172	1170 ± 111**	1753 ± 213	1642 ± 190	1699 ± 195	1532 ± 179	2398 ± 280

**P < 0.001, *P < 0.01 (Wilcoxon test) compared with control values.

Quentin Fallavier, France) or the appropriate vehicle (10 μl 2% of human serum albumin) were added at the start of the culture. Trypsinized marrow stromal cells (1×10^4 per well) were plated for 24 hours in 96-well plates in 100 μl of culture medium. Adherent cells were washed with HBSS and 200 μl of serum-free medium was added to each well for 2 days. Adherent cells were reactivated with 100 μl of RPMI 1640 with 5% FCS. The various compounds were added immediately after reactivation. After 60 h of growth, all cultures (in sixplicate (n = 6) samples) were pulsed for 12 h with 1 μCi/ml [^{3}H]-thymidine (Amersham, Les Ulis, France) and the cells were harvested using a Skatron cell harvester. Results (in dpm) were compared by Wilcoxon test.

In a separate set of experiments, human marrow stromal cells (in triplicate samples) were harvested after trypsin treatment (0.05% trypsin for 5 min at 37°C) and counted by using a haemocytometer. Results (in cell number) were compared by Mann–Whitney U-test.

Results

All these experiments were done with bone marrow cells from 47 different donors. Previous experiments showed that FCS increased in a dose-dependent manner [^{3}H]-thymidine incorporation by stromal cells with 5% as suboptimal FCS concentration.[2]

As reported in Table 1, the addition of LTB_4, LXA_4, LXB_4, 12-HETE and 15-HETE (1 μM) significantly (P < 0.001) decreased [^{3}H]-thymidine incorporation by cells cultured with 5% FCS. LTB_4, LXA_4, LXB_4, 12-HETE and 15-HETE inhibited by 19 ± 8%, 24 ± 10%, 22 ± 6%, 74 ± 6% and 37 ± 4% [^{3}H]-thymidine incorporation, respectively. Except for 12-HETE, no dose–response curve was found. LTC_4 had no effect on thymidine incorporation. As reported in Table 2, despite a significant effect on [^{3}H]-thymidine incorporation no significant effect was found on the number of marrow stromal cells after 3 days of growth with the different AA metabolites (1 μM). Finally the lipoxygenase inhibitor NDGA (1 μM) had no significant (P > 0.05, six independent experiments) effect on the [^{3}H]-thymidine incorporation of stromal cell cultures (3493 ± 1871 dpm *vs* 2664 ± 1372 dpm for NDGA-treated cells and control cells, respectively).

As reported in Table 3, only LTB_4 had a significant effect on the [^{3}H]-thymidine incorporation of freshly isolated human marrow mononuclear cells. NDGA (1 μM) had no effect (P > 0.05, four experiments) on their thymidine incorporation (90326 ± 9262 dpm vs. 81712 ± 8532 dpm for NDGA-treated cells and control cells, respectively).

Discussion

Studies have reported the positive or negative effects of lipoxygenase metabolites of AA on cell proliferation. Thus, 12-HETE and 15-HETE stimulate [^{3}H]-thymidine incorporation in mammary tumour cells,[12] and endothelial cells,[13] but inhibit it in neuroblastoma cell cultures.[14] LTB_4 and LTC_4 stimulate DNA synthesis in cultured arterial smooth muscle cells,[15] while 12-HETE decreases the growth of aortic smooth muscle cells.[16] These latter results are of interest since bone marrow stromal cells share numerous phenotypic similarities with vascular smooth muscle cells.[17]

The growth of fresh marrow mononuclear cells is not affected by lipoxygenase metabolites of AA.

Table 2. Effects of lipoxygenase metabolites of AA on human marrow stromal cell number. Cell number was determined after 3 days of growth with AA metabolites (1 μM). Results (in 1×10^3 cells) are reported as mean ± SEM of four independent experiments in triplicate

	Cell number ($\times 10^3$)	Statistical significance
Controls	6.6 ± 0.5	
LTB_4	4.9 ± 0.7	P = 0.38
LXA_4	6.3 ± 0.7	P = 0.77
LXB_4	5.0 ± 0.5	P = 0.14
12-HETE	5.6 ± 0.7	P = 0.53
15-HETE	5.6 ± 0.6	P = 0.46

Statistical significance was determined by Mann–Whitney U-test as compared with control values.

Table 3. Effects of lipoxygenase AA metabolites on [^{3}H]-thymidine incorporation of freshly isolated human mononuclear bone marrow cells. Experiments were made with marrow cells from 14 donors. Results (in dpm) are the mean ± SEM from four independent experiments in sixplicate (n = 6)

Stimuli	Controls	1 μM	100 nM	10 nM	1 nM	100 pM
LTB_4	58499 ± 9276	46780 ± 11243*	61661 ± 13177	66545 ± 11617	66075 ± 11169	63568 ± 10476
LTC_4	69415 ± 6558	64311 ± 7881	74968 ± 8451	70270 ± 7475	73999 ± 10340	71075 ± 8278
LXA_4	57227 ± 12448	54848 ± 13025	54362 ± 11939	64290 ± 15259	58318 ± 13514	51295 ± 11268
LXB_4	100218 ± 13485	95781 ± 12384	94208 ± 12468	87635 ± 11725	83321 ± 11639	80401 ± 12070
12-HETE	73871 ± 10048	69283 ± 10318	73732 ± 8534	73157 ± 10490	73050 ± 9269	76918 ± 10998
15-HETE	77168 ± 9008	73956 ± 8742	75191 ± 8449	80799 ± 10372	81043 ± 8788	77516 ± 8635

*$P < 0.01$ Wilcoxon test.

Although statistically significant, the small decrease of incorporation of thymidine with LTB_4 brings some doubts on its physiological meaning. Micromolar concentrations of LTB_4, LXA_4, LXB_4 and 15-HETE decrease [^{3}H]-thymidine incorporation in human bone marrow stromal cell cultures. However the fact that no dose–response curve was found and that no effect was documented on cell number cast some doubts on the physiological meaning of the observed effects on thymidine incorporation. In contrast to the other AA metabolites, 12-HETE inhibits in a dose-dependent manner thymidine incorporation in marrow stromal cell cultures. However no effect was found on cell counts. An explanation might be that [^{3}H]-thymidine incorporation is not only an index of cell proliferation but may also reflect intracellular events other than cell division such as diffusion of DNA precursors.[18] Another explanation for these results might be that only a small percentage of cells were proliferating and that cell counts were not sensitive enough to detect changes.

Taken together our results suggest no important role of exogenous lipoxygenase metabolites of AA in the growth of human bone marrow stromal cells and mononuclear marrow cells *in vitro*. Moreover results with NDGA suggest that endogenous lipoxygenase metabolites had no role on FCS-induced cell growth. These results markedly differ from data showing that PAF stimulates [^{3}H]-thymidine incorporation in freshly isolated human mononuclear marrow cells,[19] and marrow stromal cell cultures.[8]

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