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

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Introduction

Human bone marrow stromal cells regulate haemopoiesis by interacting directly with marrow haemopoietic progenitors and/or by releasing cytokines. Lipoxygenase metabolites of arachidonic acid (AA) such as leukotriene B₄ (LTB₄), LTC₄, lipoxin A₄ (LXA₄), LXB₄, 12-hydroxyeicosatetraenoic acid (12-HETE), and 15-HETE are produced by human marrow mononuclear cells. Several of these AA metabolites act on the growth of human myeloid and erythroid progenitors in semi-solid culture medium. 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 [³H]thymidine incorporation in marrow stromal cell cultures. In this study we have assessed the effect of LTB₄, LTC₄, LXA₄, LXB₄, 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 cm² culture flasks in RPMI 1640 with 20% fetal calf serum (FCS) (Gibco, Gergy Pontoise, France), penicillin (100 U/ml) and streptomycin (100 µg/ml) (culture medium) at 37°C in 5% CO₂ in air as previously described. 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.

Cell proliferation

Freshly isolated marrow mononuclear cells (1 × 10⁵) were grown in 100 µl of IMDM with 10% FCS. LTB₄, LTC₄, LXA₄, LXB₄, 12-HETE, 15-HETE (Tebu, Le Perray-en-Yvelines, France), NDGA (Sigma, Saint Charles, MO, USA), LTB₄, LTC₄, LXA₄, LXB₄, 12-HETE, 15-HETE (1 µM) decreased [³H]thymidine incorporation on marrow stromal cell cultures without affecting cell number. Only 12-HETE showed a dose–response effect on [³H]thymidine incorporation. While LTB₄ (1 µM) decreased thymidine incorporation on marrow mononuclear cells, LTC₄, LXA₄, LX₄, 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

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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 \(\mu\)M) 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 \(\mu\)M).

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

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.\(^17\)

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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 triplicate \((n = 6)\)

| Stimuli | Controls | 1 \(\mu\)M | 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 ± 633** | 3151 ± 506 | 3167 ± 520 | 2961 ± 533 | 2905 ± 430 | 2526 ± 319 |
| LXA\(_4\) | 2577 ± 350 | 1776 ± 242** | 2785 ± 364 | 2603 ± 363 | 2795 ± 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.

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 \(\mu\)M). Results \((3 \times 10^3\) cells) are reported as mean ± SEM of four independent experiments in triplicate

| Cell number \((3 \times 10^3)\) | 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.
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.  

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 lipoxigenase 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 lipoxigenase 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, and narrow stromal cell cultures.

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