

Short communication

Growth inhibition of fibroblasts from nasal polyps and normal skin by lysine acetylsalicylate

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Some authors have shown that lysine acetylsalicylate (LAS) may help prevent nasal polyp relapses. As some anti-inflammatory drugs have been found to regulate cell growth, we investigated the antiproliferative effect of LAS on fibroblasts derived from nasal polyps. Moreover, we studied the effect of LAS on the growth of fibroblasts derived from normal skin to determine whether the response was similar to that obtained in the above-mentioned cells. Fibroblasts were obtained from tissue samples of nasal polyps from two aspirin-tolerant and two aspirin-intolerant patients, and from the normal skin of a healthy donor. The cells were treated with LAS (20–2000 µg/ml of culture medium). Cell growth and viability were evaluated after 3 and 6 days of culture. LAS had a growth-inhibitory effect on cells independently of their derivation. A reduction in cell growth was seen at the concentrations of LAS tested, which correspond to those used in the local treatment of nasal polyposis.

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Aspirin (ASA), salicylates, or, more generally, non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to interfere with a number of cellular functions and a wide variety of biochemical events which alone or in combination may affect cell growth. In particular, treatment with these drugs reduces protein and nucleic acid synthesis and inhibits proliferation of rat hepatoma and human fibroblast cultures (1, 2). The effect on cell proliferation is reversible, and the order of potency in reduction of cell growth parallels the reported anti-inflammatory activity of the drug (1). This antiproliferative action seems to be the result of blocking cells in the G₁ phase of the cell cycle (2, 3). Moreover, Patriarca et al. have demonstrated that ASA and other salicylates, at physiologic concentrations, markedly inhibit proliferation of phytohemagglutinin-stimulated lymphocytes derived from patients with ASA intolerance (4).

Intranasal treatment with lysine acetylsalicylate (LAS) has been reported to reduce nasal polyp relapses in both ASA-tolerant (ASA-) and ASA-intolerant (ASA+) patients (5, 6). In a recent study,

LAS was shown to reduce nasal polyp recurrence in patients without a prior history of ASA intolerance (7).

From the histopathologic viewpoint, polyps are characterized by squamous metaplasia of the respiratory epithelium (occurring in 25–30% of cases), increase in connective tissue associated with marked edema, dilated capillary and venous channels, and eosinophil and mast-cell infiltrates. As the stroma abnormalities may induce aberrations in the phenotype of epithelium, the present theory is that nasal polyps result from disorders in the stroma, and not in the epithelial component (8).

On the basis of these observations, we investigated the direct action of LAS on the proliferation of fibroblasts derived from nasal polyps of both ASA- and ASA+ patients. Moreover, to determine whether any difference in response to LAS existed between these fibroblasts and others derived from different sources, we studied the action of the drug on the growth of cells obtained from normal skin.

Material and methods

Tissue samples and cells

Nasal polyp tissue samples were obtained, at the time of polypectomy, from two ASA- (DAN/ASA- and CGA/ASA-) and two ASA+ (MAG/ASA+ and SAM/ASA+) patients, aged 26–60 years. We also obtained tissue samples from normal skin (F-1/NS) derived from a healthy donor.

The tissues were removed aseptically and cut into small pieces, and each one of them was placed in a drop of fetal calf serum (FCS) in 35-mm plastic Petri dishes for 24 h. Tissue fragments were kept at 37°C in a 5% humidified environment. Then the medium (Dulbecco's modified Eagle's medium [DMEM], supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 20 mM HEPES buffer) was added. When fibroblasts migrated from tissue to plastic support to form a monolayer, they were harvested, at confluence, by trypsinization; thereafter, they were multiplied in subcultures.

Drug

LAS (Flectadol, Maggioni-Winthrop, Milan, Italy) was directly dissolved in the culture medium to obtain the desired concentrations.

Cell-growth experiments

Experiments were performed on fibroblasts derived from nasal polyps, subcultured from the third to the seventh passage, and fibroblasts obtained from normal skin, from the seventh to the tenth passage. Fibroblasts were seeded in DMEM, supplemented as previously reported, at an initial density of 50 000 cells/dish in 35-mm Petri dishes. After 24 h, the medium was renewed with fresh medium containing LAS at a concentration ranging from 20 to 2000 µg/ml. In a series of dishes, medium containing the drug was renewed again 3 days later. Control dishes without LAS were run in parallel. Cell growth was evaluated, after 3 and 6 days of exposure to LAS, by counting the cells in a hemocytometer. Experiments were set up in triplicate, and four or more counts were performed on each dish. Cell viability was assessed by Trypan blue stain dye exclusion.

Statistical analysis

In cell-growth experiments, the significance of the differences between treated and untreated fibroblasts was determined by the unpaired two-tailed Student's *t*-test. The significance of the difference in the growth of fibroblasts derived from the five investigated subjects was determined by covariance

analysis. Data from each cell-growth experiment were adjusted on the basis of the correspondent control which was utilized as the covariate.

Linearity of the dose/effect curve was verified by the F test.

Results

Effect of LAS on fibroblasts from nasal polyps of patients tolerant (ASA-) and intolerant (ASA+) of aspirin

The growth of fibroblasts derived from both ASA- and ASA+ patients was not substantially affected by 20 µg/ml LAS (Figs. 1 and 2).

DAN/ASA- cells responded to the action of 200 µg/ml LAS after both 3 and 6 days of treatment, with an inhibition of growth of about 20% with respect to control ($P < 0.001$), while the proliferation of the other cell line (CGA/ASA-) seemed to be influenced only if the medium was changed on day 3 (20% inhibition, $P < 0.01$) (Fig. 1).

The growth of MAG/ASA+ and SAM/ASA+ fibroblasts was reduced by 200 µg/ml LAS (about 30% if compared to control; $P < 0.001$) after 3 and 6 days. When the medium was renewed, the inhibition of cell proliferation remained substantially unchanged (Fig. 2).

The growth of all cell lines derived from nasal polyps was strongly reduced by 2000 µg/ml LAS, reaching an inhibition of about 80% with respect to control ($P < 0.001$) (Figs. 1 and 2).

Effect of LAS on fibroblasts from normal skin

The growth of these cells was reduced by 20 µg/ml LAS only when the medium was renewed on day 3 (20% inhibition, $P < 0.05$) (Fig. 3). Cell growth was slightly inhibited by 200 µg/ml LAS (13%, $P < 0.01$) after both 3 (Fig. 3) and 6 (not shown) days of treatment. Cell numbers were still more reduced when the medium was changed (37%, $P < 0.001$) (Fig. 3).

Maximal growth inhibition was obtained when F-1/NS cells were exposed to 2000 µg/ml LAS. When the medium was renewed on day 3, the reduction in cell numbers reached 78% with respect to control ($P < 0.001$) after 6 days of exposure to the drug.

Effect of LAS on fibroblasts independently of their derivation

The dose/effect curve of LAS on growth of fibroblasts of all the five investigated cell lines exhibited a pattern almost linear; the F test for linearity was highly significant at all the examined times

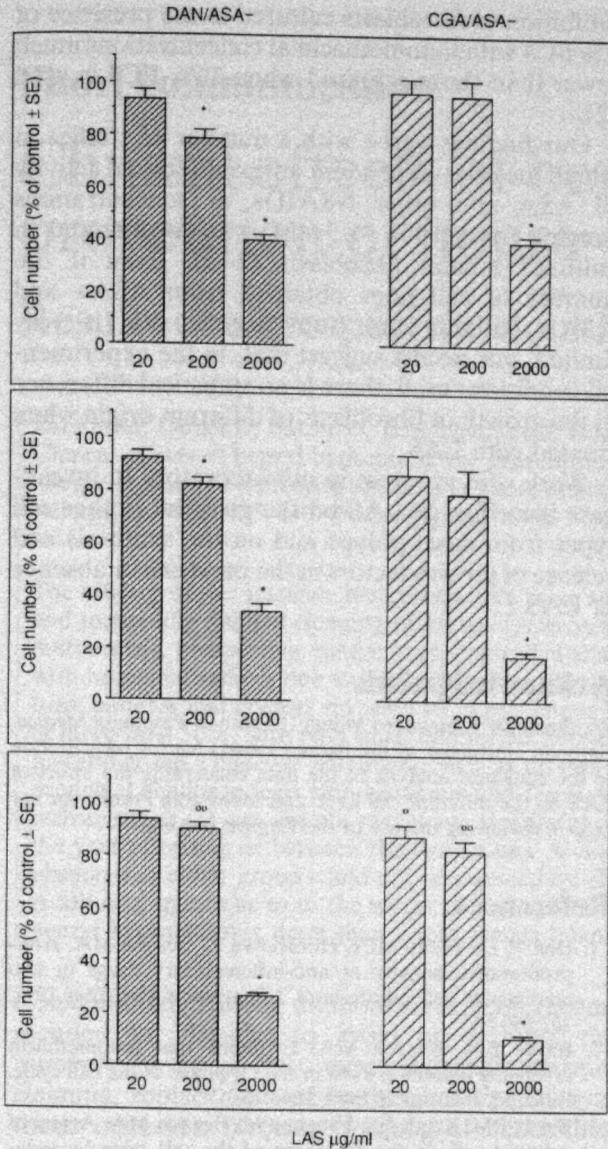


Fig. 1. Effect of LAS on growth of DAN/ASA- and CGA/ASA- cells, after 3 (upper panel) and 6 days (middle panel) of treatment. Lower panel shows antiproliferative effect after 6 days, when medium containing drug was renewed on day 3. Columns represent mean of six observations from two different experiments; bars are SE. ° $P<0.05$; °° $P<0.01$; * $P<0.001$.

($P<0.001$). After correction for the covariate, the inhibitory effect of the different LAS concentrations accounted for almost all the residual variance. No statistical difference was observed in the rate of growth of fibroblasts in either ASA+ or ASA- patients, or from normal skin.

Cell viability

Cell viability was higher than 85% in all the experiments performed and in all conditions of treatment.

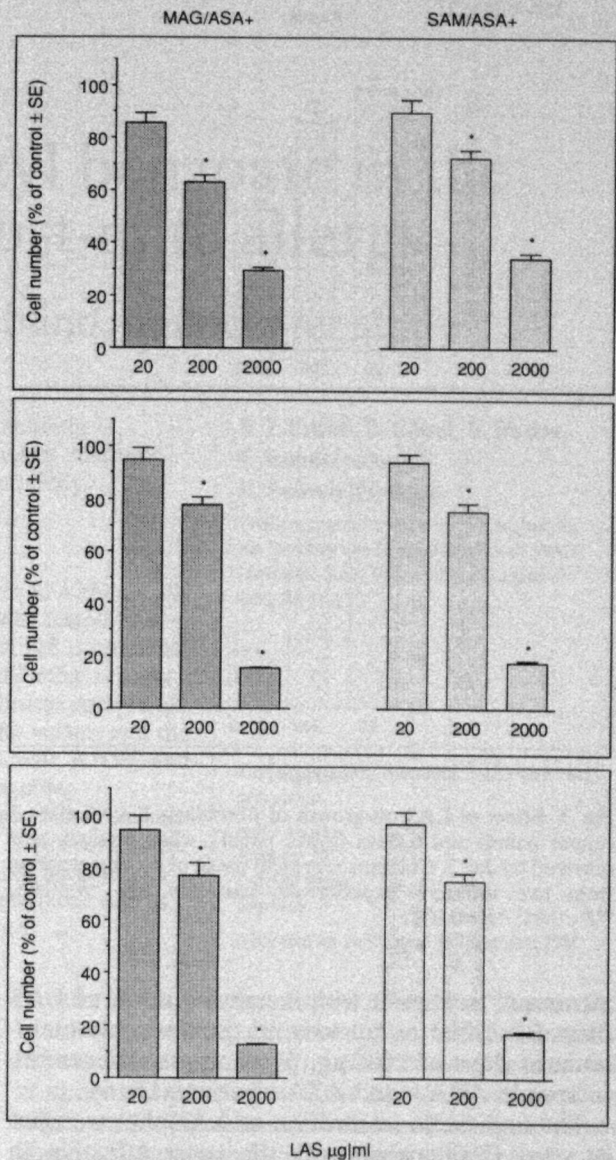


Fig. 2. Effect of LAS on growth of MAG/ASA+ and SAM/ASA+ cell lines, after 3 (upper panel) and 6 days (middle panel) of treatment. Lower panel shows effect of LAS after 6 days of exposure when medium was changed on day 3. Columns represent mean of six observations from two different experiments; bars are SE. * $P<0.001$.

Discussion

Our results show that LAS has a dose-dependent antiproliferative effect on all the cell lines tested, independently of the origin of fibroblasts. The behavior of fibroblasts, taken from polyps belonging to ASA+ and ASA- patients and from normal skin, almost overlapped.

The direct effect of LAS on cell proliferation, even if not specific, supports the clinical results obtained by some authors in preventing relapses of nasal polyps. In fact, Patriarca et al. showed that

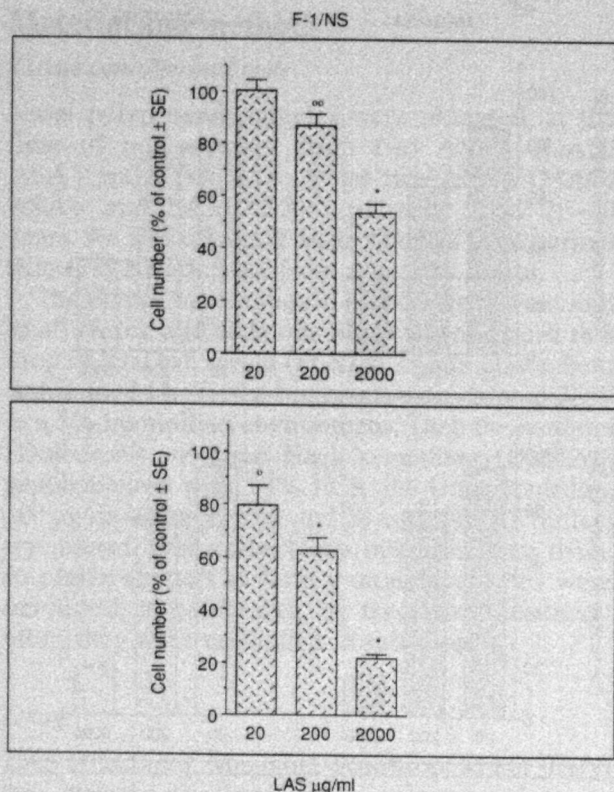


Fig. 3. Effect of LAS on growth of fibroblasts F-1/NS after 3 (upper panel) and 6 days (lower panel), when medium was renewed on day 3. Columns represent mean of six observations from two different experiments; bars are SE. ^o $P < 0.05$; ^{oo} $P < 0.01$; ^{*} $P < 0.001$.

intranasal treatment with increasing doses of LAS from 20 to 200 µg, up to a maximal weekly maintenance dose of 2000 µg, prevents or reduces the relapse in ASA- and ASA+ patients (5, 6).

The highest concentration of LAS that we used *in vitro* (2000 µg/ml) was the most effective in inhibiting the growth of all fibroblasts. Nevertheless, cell viability was high at all the drug concentrations tested. This suggests that LAS has a cytostatic effect, in agreement with the report by Bayer et al., who found that the addition of indomethacin to fibroblast cultures blocks the cells in the G₁ phase of the cell cycle (2, 3).

The culture conditions used in our experiments might explain the slight response of our cells to low concentrations of LAS. In fact, culture medium was supplemented with 10% FCS, which presumably contains factors antagonizing the inhibitory action of LAS. Some authors reported a marked growth

inhibition of fibroblasts cultured in the presence of 2% FCS with indomethacin at concentrations much lower than those required when 10% FCS is used (2).

Our findings agree with a number of studies in which the dose-dependent antiproliferative activity of ASA and other NSAIDs, at concentrations ranging from 0.001 to 5 mM, was documented in cultured human fibroblasts (1-3). Even if the number of cell lines obtained from ASA+ and ASA- patients and from normal skin is very limited, our results suggest that, in the experimental conditions used, there is no statistical difference in the growth of fibroblasts of different origin when treated with LAS.

Work is in progress in our laboratory to investigate the effect of LAS on the growth of other cell types from nasal polyps and on the synthesis and release of growth factors in the presence or absence of LAS.

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