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AS-101: a modulator of *in vitro* T-cell proliferation

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AS-101 is a tellurate compound originally designed as a drug with cytostatic activity. Nevertheless, *in vivo* it was found to be an immunomodulator agent due to a stimulation of cytokine production. Mitotic index (MI) as an indicator of cytotoxicity and cell proliferation kinetics (CPK) in lymphocytes cultures are parameters used in the evaluation of the antineoplastic activity of drugs, such as mitomycin-C and cisplatin. For this reason, we evaluated the effects of AS-101 upon these two parameters. The results show that AS-101 produces an inhibition of MI in proliferating lymphocytes higher than the inhibition mediated by cisplatin. When CPK was evaluated, AS-101 induced a retardation not related with dose, while cisplatin produced a stepwise inhibition. This effect contrasts with the stimulation observed when AS-101 was added to non-proliferating lymphocytes which was measured as an increased [^{3}H]thymidine incorporation in culture. The results confirm the mode of action of AS-101 as a real modulating agent of cell proliferation.

Key words: AS-101, cell proliferation kinetics, human lymphocytes.

Introduction

AS-101 is an organic tellurate compound originally designed to be used as a cytostatic drug. Therefore, its formula was shaped based on that of the antitumor agent cisplatin, but using tellurium instead of platinum.[1]

When characterized, it was found to be an immunomodulator agent which increased [^{3}H]thymidine incorporation, and stimulated interleukin (IL)-2 production and IL-2 receptor appearance in mononuclear cells.[1–3]

AS-101 also stimulates IL-1 production by spleen cells and peritoneal exudates in mice.[4] For this reason it is considered as a radioprotective agent, since IL-1 has been shown to increase survival in lethaly irradiated mice.[5]

Another effect of AS-101 is to induce the production of colony-stimulating factor (CSF), as well as tumor necrosis factor (TNF) and interferon (IFN)-γ.[1] This effect is enhanced when administered with bryostatin, a natural lactone that activates protein kinase C (PKC).[2]

AS-101 stimulates cytokine production. This induction exerts a protective effect against DNA damaging agents like cyclophosphamide and radiation.[6] When administered in combination with chemotherapy it not only protects against toxic effects of drugs, but also helps in the restoration of bone marrow cellularity after treatment, normalizing hemopoiesis.[6]

In a study made by one of us in 13 patients with advanced cancers (kidney, melanoma, pancreas, colon and mesothelioma), AS-101 administered three times per week in escalating doses of 0.1–3.0 mg/m^2, as an anticancer drug, helped to ameliorate their performance status at one level above the initial, which was maintained over 24 weeks in seven patients. In three patients there were tumor decreases close to 50% (kidney, melanoma and mesothelioma).[7]

The effects on immunological cells and functions suggested that AS-101 might be a useful drug against alterations where there is an impairment of immune cell,[3] proliferation like cancer, systemic lupus erythematosus (SLE), AIDS[8] or immunosuppressive conditions caused by exposure to radiation or chemotherapy.

A mechanism proposed for the action of AS-101 on lymphocytes is that it might efficiently trigger the Ca^{2+} signal required to initiate lymphocyte activation. It has been suggested that AS-101 might generate a second signal, probably the activation of PKC,[2] because when added in combination with phorbol miristate acetate (PMA) or with bryostatin it showed a synergistic effect in mononuclear cell proliferation.

Cell proliferation kinetics (CPK), measured by bromodeoxyuridine (BrdUrd) incorporation into DNA during the S phase of the cell cycle, is a parameter used in the evaluation of cytostatic

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activity of drugs and hormones[9–11] together with mitotic index (MI) as an indicator of cytotoxicity. We have used this system to study the effect of AS-101 on lymphocyte proliferation in whole blood cultures in order to evaluate its mode of action.

Materials and methods

Whole blood cultures

Heparinized blood (0.5 ml) from two healthy subjects (a male and a female) was set in RPMI 1640, supplemented with L-glutamine and non-essential amino acids, in the presence of 32 μM BrdUrd to label cells that synthesize DNA. Lymphocytes were stimulated to grow with 0.2 ml phytohemagglutinine (PHA) (Microlab, Mexico). After 48 h of incubation at 37°C, different concentrations of AS-101 or cisplatin were added to proliferating cultures and incubation went on for an additional 24 h; cisplatin alone was used as a positive control. Concentrations used were chosen previously in a cytotoxicity test to produce a measurable effect on MI as well as in CPK. Cells were harvested as follows: at 70 h colcemid (2 μg/culture) was added to block cells arriving at metaphase; 2 h later cultures were stopped with hypotonic solution (0.075 M KCl) and incubated for 30 min. Material was fixed with Carnoy solution (methanol:acetic acid, 3:1, v/v), and microscope slides were prepared and stained according to the fluorescence plus Giemsa technique[12] to differentiate cells that went through one, two, three or more cell cycles, according to the staining pattern produced by BrdUrd incorporation.

Before this treatment scheme was applied, another scheme was tried where cells were treated with AS-101 at the onset of cultures and without PHA stimulation. It did not work, as will be discussed later.

MI was scored as the number of mitotic figures in 2000 cells. Percentage of proliferation in the presence of either drug was calculated according to the formula:

$$\% \text{ of proliferation} = 100(MI_{ob}/MI_{cntl}),$$

where MI_{ob} is the MI found with a drug concentration and MI_{cntrl} is the MI in cultures with no treatment.

CPK was evaluated in the first 100 metaphases, by counting the proportions of cells in the first (M1), second (M2) and third (M3) mitotic divisions.

Molar concentrations of AS-101 tested were: 3.23×10^{-7}, 3.23×10^{-6} and 3.23×10^{-5} M.

Molar concentrations of cisplatin tested were: 3.4×10^{-5}, 8.5×10^{-5} and 2×10^{-4} M.

Chi-square and Student's *t*-test were used for statistical analysis.

Microplate cultures

Peripheral blood mononuclear cells (3×10^4 per well) were plated, treated with 50 μl of the corresponding drug concentration and incubated for 18–19 h at 37°C in a humidified atmosphere with 5% CO_2. At that time cells were labelled with 0.1 μCi/well [^{3}H]thymidine (6.7 Ci/mmol) and incubated for an additional 8 h.

Cells were harvested on glass fiber filters, dried and counts measured in a scintillator counter. Percentage of proliferation was calculated according to the formula:

$$\% \text{ of proliferation} = 100 \ (\text{c.p.m.}_{ob}/\text{c.p.m.}_{cntl})$$

where c.p.m._{ob} is the [^{3}H]thymidine incorporation measured in drug treated wells and c.p.m._{cntl} is the incorporation found in controls.

Results and discussion

When AS-101 was administered at the initiation of whole blood cultures (without PHA stimulation), all doses were toxic, no stimulation was observed and erythrocytes were affected, apparently by hemolisis. An erythrocyte antisickling effect by tellurite was described by Kurantsin *et al.*[13] An effect of AS-101 on this cell type in culture was observed, even when administered after 48 h in PHA-stimulated cultures. Although no adverse *in vivo* action related to this matter has been reported, its effect should be monitored in case long-term treatments are planned.

When AS-101 was added in proliferating cells it produced MI inhibition, which increased with the dose (Figure 1), and a delay in CPK, which was not dose related ($p < 0.025$) (Figure 2). The cells received AS-101 after 48 h of PHA stimulation, when PKC activation had already occurred[14] and the cells were in active proliferation. The inhibition could be due to the induction of a negative feedback of PKC in these cells when there is a sustained activation, because AS-101 did not prevent lymphocytes from dividing once (according to the amount of M1 found at 72 h, Figure 2), but rather

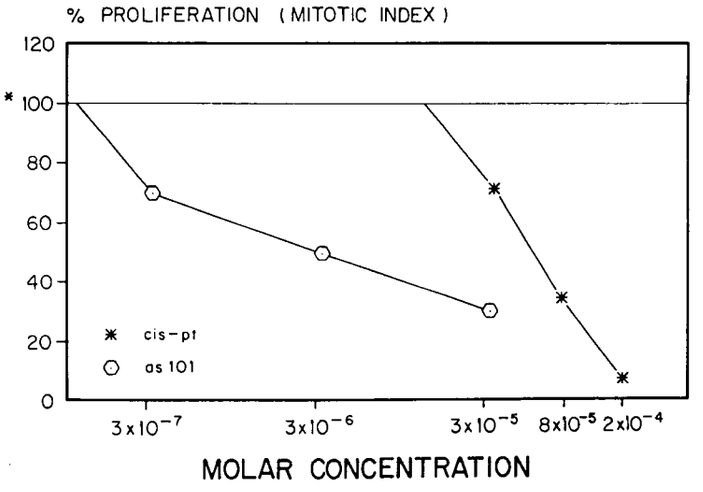

Figure 1. Effect of cisplatin and AS-101 on lymphocytes from cultures stimulated with PHA. Lower doses of AS-101 were necessary to produce similar MI inhibition. *Control taken as 100%.

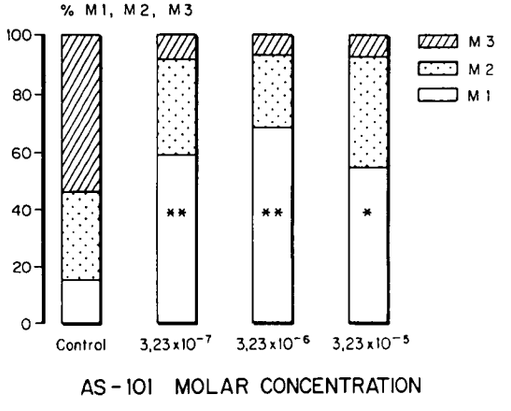

Figure 2. Proportions of cells that divided once (M1), twice (M2) or thrice (M3) during the culture were significantly altered by AS-101 with respect to control without treatment. Nevertheless inhibition was not related to the dose. $^{*}p < 0.025$; $^{**}p < 0.01$.

impaired or delayed successive cell divisions. In other studies the drug has been tested for its effect on non-cycling cells where proliferation stimulation was found.[1]

Cytotoxicity or inhibition of MI by AS-101 was shown to be more potent than cisplatin since smaller concentrations of AS-101 were needed to lower MI to similar levels (Figure 1). However, if CPK is analyzed, AS-101 showed a unique action which was detected at the lowest concentration used (3×10^{-7} M) and sustained at the same magnitude for the higher concentrations (3×10^{-6} and 3×10^{-5} M). The proportion of M1 cells increased, affecting only the proportion of M3 cells (Figure 2). Cisplatin, in turn, showed a stepwise retardation of CPK, which was reflected as a gradual accumulation of cells in M1 and M2 until the proportions of M1, M2 and M3 cells were almost the same (Figure 3).

Due to its stimulating effect on non-proliferative cells, Sredni *et al.*[1,2] proposed AS-101 to be a modulating agent. Since we found an inhibitory effect on lymphocyte cultures, to confirm our observations and make them comparable to those of Sredni *et al.*, we tested AS-101 on lymphocyte microcultures in 96-well plates and measured its effect on [^{3}H]thymidine incorporation, using

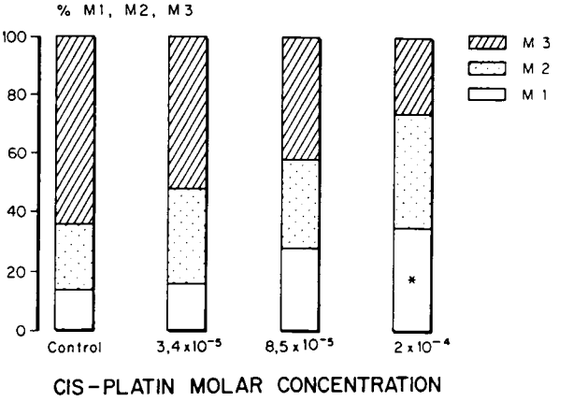

Figure 3. Cisplatin also altered the proportions of M1, M2 and M3 cells with respect to control cultures. In this case the effect increases with the dose. $^{*}p < 0.05$.

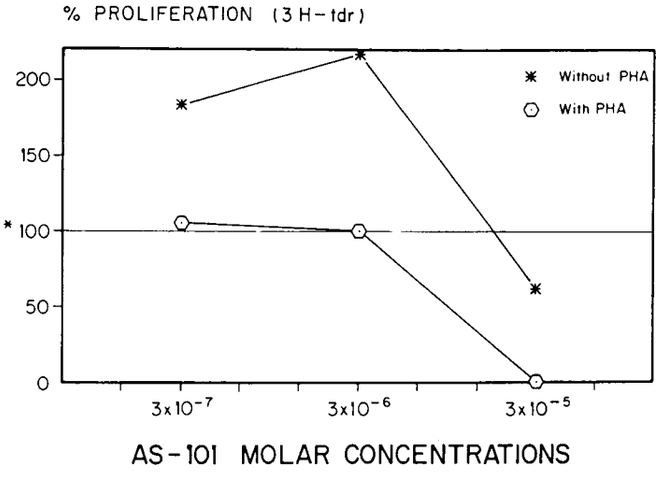

Figure 4. AS-101 induced [^{3}H]thymidine incorporation in lymphocytes not stimulated with PHA, whereas in a stimulated culture it did not alter incorporation and in the higher dose it induced inhibition, which was also found in non-stimulated cells. *Control taken as 100%.

comparable doses. We found a stimulation effect without PHA, as Sredni *et al.* did, and no effect or inhibition when the mitogen was present (Figure 4).

This mode of action of AS-101 on lymphocytes confirms that this drug has a real modulating effect on cell growth, since it induces division in non-stimulated cells and inhibits mitosis and DNA synthesis when the cells are proliferating.

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