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In vitro Effect of Suramin on Lung Tumour Cells

István Á. Mórocz, Béatrice Lauber, Doris Schmitter and Rolf A. Stahel

In the search for new therapeutic concepts in lung cancer chemotherapy, suramin, a potential anticancer drug which evades multidrug resistance, was tested *in vitro* on 25 lung-derived cell lines, either non-tumorigenic cells, or established cell lines from five different tumour types. Suramin treatment resulted in a time- and dose-dependent decrease in [^{3}H]thymidine incorporation, except in one adenocarcinoma cell line where DNA synthesis was highly stimulated. [^{3}H]Leucine incorporation was less affected, indicating that suramin acted cytostatically rather than cytotoxically. Our results show that suramin affected DNA synthesis of the different types of lung derived cells, including non-tumorigenic and tumour cell lines, to a similar extent.
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INTRODUCTION

SURAMIN HAS a multitude of diverse properties and has been used in the treatment of trypanosomiasis (sleeping disease) since the early 1920s. The antitumoral activity of suramin described a few years ago [1] is either mediated by obstructing extracellular growth factor-receptor interaction [2], or by direct intracellular interference with enzymes essential for growth [3]. Clinical studies show that the antitumoral effect of suramin in a variety of neoplasms is accompanied by severe side-effects [4] due to a plasma half-life of above 50 days [5] and a wide spectrum of biological interactions.

With the exception of small cell lung cancer (SCLC), the results of chemotherapy in inoperable lung neoplasms are marginal, mainly because of primary or secondary multidrug resistance [6]. The cytostatic action of suramin, in particular its interference with growth factors, is mediated by mechanisms which evade multidrug resistance. Lung tumours are known to produce a variety of growth factors [7], some acting in an autocrine manner [8, 9]. Considering these facts, we evaluated whether suramin might be a promising drug for lung cancer treatment by investigating the effect of suramin *in vitro* on

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SCLC, non-small cell lung cancer (NSCLC) including mesothelioma, and lung-derived non-tumorigenic cell lines.

MATERIALS AND METHODS

Cell lines were from our own laboratory unless otherwise stated. SCLC cell lines: SW2 (Dr S.D. Bernal, Boston, USA), OH-1 and OH-3 (Dr S.B. Baylin, Baltimore, USA), NCI-H69 and NCI-H128 (Dr J. Minna, Bethesda, USA); squamous cell carcinoma cell lines: U1752 (Dr J. Bergh, Uppsala, Sweden), HOTZ (Dr P. Groscurth, Zurich, Switzerland), SkMes1 (American Type Culture Collection), ZL7, ZL11 and ZL17; adenocarcinoma cell lines: A23, A125, A427 and A549 (ATCC); large cell lung cancer cell line: SLC-6; mesothelioma cell lines: ZL5, ZL34, ZL55, ZL92, SPC111 and SPC212; the immortalised normal human mesothelial cells MeT5A (Dr J. Lechner, Bethesda, USA) and human lung fibroblasts (Dr P. Groscurth). These cell lines were cultured in RPMI-1640 medium supplemented with 2 mmol/l glutamine and 10% fetal calf serum (FCS). In addition, the immortalised normal human bronchial epithelial-derived cell line BEAS-2B (Dr C. Harris, Bethesda, USA) was grown in a HAM's F12 based medium supplemented with insulin 5 mg/l, transferrin 5 mg/l, hydrocortisone 70 μg/l, vitamin A 0.1 mg/l, triiodo-L-thyronine 650 μg/l, adrenaline 2 mg/l, bovine pituitary extract 30 mg/l, bovine serum albumin 50 mg/l, purified mouse epidermal growth factor (EGF) 5 μg/l, ethanolamine 5 μmol/l and gentamycin 50 mg/l.

Suramin was a gift from Bayer (Leverkusen, Germany). It was prepared as a sterile stock solution of 100 mg/ml in 0.9% NaCl and stored at −20°C.

Cells were plated in 150 μl culture medium in 96-well microtitre plates (3×10^3 cells/well). 24 h after plating (day 0), cells were treated by a single dose (10 μl) of suramin of specified concentrations. [^{3}H]Thymidine and [^{3}H]leucine incorporation was determined at indicated time points during a 6-h pulse in presence of 37 kBq/well thymidine or leucine. Cells cultured for longer than 5 days were fed on day 6 by adding 100 μl fresh medium. The experiments were performed in quadruplicate and repeated at least twice.

RESULTS

Time dependency

With a single dose of suramin (300 μg/ml) DNA synthesis was reduced 2 days after treatment, it further decreased until day 4 and stabilised thereafter at a negligible level (Fig. 1). In untreated cultures [^{3}H]thymidine incorporation peaked at day 2 and plateaued thereafter at a lower level, possibly owing to cell confluency and medium depletion. In the presence of suramin, protein synthesis remained at the basal level of day 0 throughout the 8 days of observation, although compared to untreated controls [^{3}H]leucine incorporation was reduced from day 2 onwards.

Dose dependency

The effect of different suramin concentrations was determined in each of the 25 cell lines at day 2, when untreated cells were still in the exponential growth phase. Six profiles of [^{3}H]thymidine incorporation are depicted in Fig. 2, showing representative response patterns of the different cell types. Most profiles show typical dose-dependent growth inhibitions after suramin treatment. Following treatment with 30 μg/ml, no substantial effect on DNA synthesis was observed, whereas a significant reduction of [^{3}H]thymidine incorporation was measured after treatment with 100 μg/ml suramin. In the presence of 300 μg/ml suramin, which corresponds to the highest clinically tolerated serum level [10], DNA synthesis reached < 30% of untreated controls in 24/25 cell lines; no striking differences regarding the responsiveness to suramin were observed between the different classes of cells, including the non-tumorigenic cells.

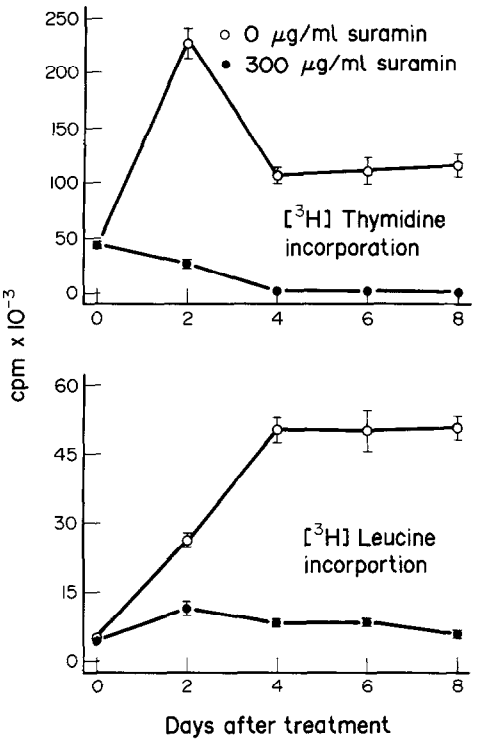

Fig. 1. Time-dependent effect of suramin on DNA synthesis ([^{3}H]thymidine incorporation) and protein synthesis ([^{3}H]leucine incorporation) of the mesothelioma cell line ZL5. Data represent mean of quadruplicates of one representative experiment: incorporated radioactivity (cpm with S.D.).

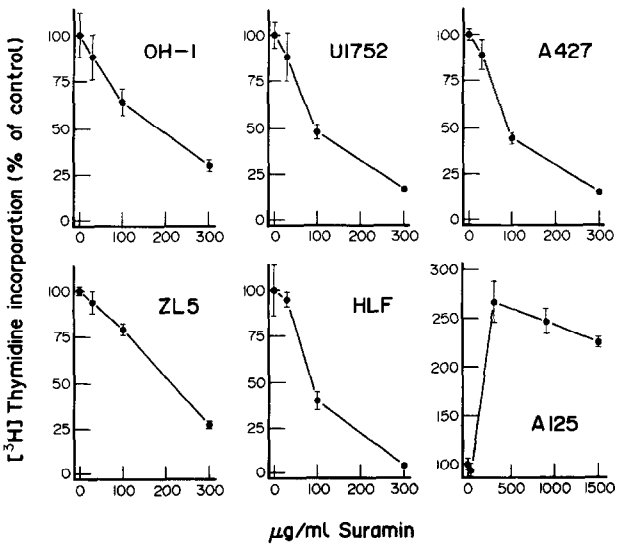

Fig. 2. Dose-dependent effect of suramin on six lung-derived cell lines measured by [^{3}H]thymidine incorporation 2 days after treatment. Small cell lung cancer cell line: OH-1; squamous cell carcinoma cell line: U1752; mesothelioma cell line: ZL5; human lung fibroblasts: HLF; and adenocarcinoma cell lines: A427 and A125. [^{3}H]Thymidine incorporation: 100% = cpm (untreated cells); % of control = cpm (treated cells)/cpm (untreated cells). Data represent mean with S.D. of quadruplicates of one representative experiment.

As a notable exception, the adenocarcinoma cell line A125 was growth stimulated by suramin (Fig. 2), when examined under the same conditions. [^{3}H]Thymidine incorporation increased with suramin concentrations up to 300 μg/ml and slowly decreased thereafter; at the highest suramin concentration tested (1500 μg/ml), the level of DNA synthesis was still > 2 times higher than in untreated controls. Interestingly, in the absence of FCS, suramin had an inhibitory effect on DNA synthesis in A125 cells (data not shown).

DISCUSSION

With the aim of potentially circumventing the problem of multiple drug resistance in the treatment of lung cancer, we have studied the *in vitro* effect of suramin on a large number of different types of cell lines derived from lung neoplasms. Suramin treatment resulted in a marked inhibition of [^{3}H]thymidine incorporation in 21 lung tumour-derived and three non-tumorigenic cell lines. Thus, our results confirm the data of Bergh [11], who observed a growth inhibitory effect of suramin on 4/6 NSCLC. The antineoplastic action of suramin was not restricted to malignant cells; the immortalised cell lines MeT5A and BEAS-2B as well as normal human lung fibroblasts were affected to a similar extent. This observation is unfortunate, but not surprising, since growth of these cells is also regulated by growth factors present in the culture medium. Considering the important reduction of DNA synthesis, together with the stabilising effect of suramin on protein synthesis, our results are in agreement with other reports suggesting that suramin preferentially affects the mitogenic pathway and induces cell-differentiation [12]; also that suramin effects are reversible [13].

In contrast to the overall response pattern, the adenocarcinoma cell line A125 showed a significant increase in DNA synthesis following suramin treatment, although A125 cells are responsive to exogenous growth factors [9]. Slight increases in cell numbers after suramin treatment have been reported in rat prostate cancer cells [14], where maximal stimulation was observed with 143 μg/ml suramin. In the case of a renal carcinoma cell line, suramin reversed the growth inhibitory effect of TGF-β [15]. A125 cells may be similarly sensitive to an inhibitory activity present in FCS, which could preferentially interact with suramin. This hypothesis would also be an explanation for the growth inhibitory effect of suramin on A125 cells, in the absence of serum.

This is the first study evaluating the effect of suramin on the growth of a large number of different types of lung-derived cell lines. The generally observed cytostatic effect of suramin confirms our hypothesis and previous reports [1, 11–13]. As a notable exception the adenocarcinoma cell line A125 is highly growth stimulated by suramin *in vitro*, thereby alluding to a novel potential danger of suramin when tested as an anticancer drug. Our results indicate that suramin affects most lung-derived cell lines, including non-tumorigenic cells, in a similar way and does not act selectively on a particular type of lung tumour.

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