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Antiproliferative effects of suramin on human cancer cells in vitro and in vivo.

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Abstract

The present experiment was undertaken to study what types of human cancers are responsive to the antiproliferative effects of suramin. The human malignant cells used were as follows: cervical cancer (HeLa), mammary cancer (MCF-7), bladder cancer (EJ), hepatoma (HuH-7, PLC/PRF/5), embryonal carcinoma (PA-1), in vitro transformed fibroblasts (KMST-6, SUSM-1, VA-13), five myeloma cell lines (KMM-1, KMS-5, KMS-11, KMS-12, RPMI 8226), Burkitt's lymphoma (Raji), acute promyelocytic leukemia (HL-60), chronic myelocytic leukemia (K562), Epstein-Barr virus nuclear antigen positive lymphoblastoid cells (KMS-9). The cells were treated with 25 to 100 micrograms/ml suramin for 72h. Proliferation of HuH-7 and two human myeloma cells (KMS-11 and KMS-12) was remarkably inhibited, and that of PA-1, PLC/PRF/5, KMST-6, two other myeloma cell lines (KMM-1 and KMS-5), Raji and HL-60, was moderately inhibited. In order to confirm part of the results obtained from in vitro experiments, in vivo experiments were also undertaken. The growth of HuH-7 cells transplanted subcutaneously into nude mice was significantly suppressed by intravenous injection of suramin. We discussed the possibility that certain types of human cancers, the growth of which seemed to be more or less dependent on polypeptide growth factors, might be sensitive to the antiproliferative effects of suramin.

KEYWORDS: suramin, anticancer drug, human cancers

Antiproliferative Effects of Suramin on Human Cancer Cells *In Vitro* and *In Vivo*

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The present experiment was undertaken to study what types of human cancers are responsive to the antiproliferative effects of suramin. The human malignant cells used were as follows: cervical cancer (HeLa), mammary cancer (MCF-7), bladder cancer (EJ), hepatoma (HuH-7, PLC/PRF/5), embryonal carcinoma (PA-1), *in vitro* transformed fibroblasts (KMST-6, SUSM-1, VA-13), five myeloma cell lines (KMM-1, KMS-5, KMS-11, KMS-12, RPMI 8226), Burkitt's lymphoma (Raji), acute promyelocytic leukemia (HL-60), chronic myelocytic leukemia (K562), Epstein-Barr virus nuclear antigen positive lymphoblastoid cells (KMS-9). The cells were treated with 25 to $100\,\mu$g/ml suramin for 72h. Proliferation of HuH-7 and two human myeloma cells (KMS-11 and KMS-12) was remarkably inhibited, and that of PA-1, PLC/PRF/5, KMST-6, two other myeloma cell lines (KMM-1 and KMS-5), Raji and HL-60, was moderately inhibited. In order to confirm part of the results obtained from *in vitro* experiments, *in vivo* experiments were also undertaken. The growth of HuH-7 cells transplanted subcutaneously into nude mice was significantly suppressed by intravenous injection of suramin. We discussed the possibility that certain types of human cancers, the growth of which seemed to be more or less dependent on polypeptide growth factors, might be sensitive to the antiproliferative effects of suramin.

Key words : suramin, anticancer drug, human cancers

Suramin, a polyanionic polysulphonated naphthylurea, has been used for the treatment of African trypanosomiasis and onchocerciasis since 1924. Recent studies have shown that it also inhibits cell growth by inhibiting the binding of platelet-derived growth factor (PDGF) to its receptors and, furthermore, by dissociation of bound PDGF from its cell surface receptor (1). In fact, suramin efficiently brought about rever-

sion of the simian sarcoma virus-induced transformed phenotype in human and rat fibroblasts by neutralization of the externalized v-sis product (PDGF) (2). Furthermore, suramin not only blocked the rapid intracellular turnover of the PDGF receptor in sis-transformed cells, but also increased the secretion of sis products, which led to reversal of the transformed phenotypes of the cells (3). Recently, suramin has been shown to act as a polypepitide growth factor antagonist for a variety of growth factors such as PDGF,

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458   Bai *et al.*

epidermal growth factor(EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF-1), interleukin-2 (IL-2) and transforming growth factor-beta (TGFβ) (2-7). Other studies have shown that suramin inhibits reverse transcriptase (8), DNA polymerase α, β, γ, and terminal deoxynucreotidyl transferase (9). Based on these findings, some groups have used suramin in the treatment of patients with renal cancer, adrenocarcinoma, lymphoma and prostatic cancer (10, 11). It has also been shown that suramin inhibits *in vitro* growth of colon adenocarcinoma HT29, cervical carcinoma HeLa and several lymphoma cell lines (9, 12), and that it induces differentiation of HT29 cells into enterocyst-like cells (12). However, which types of cancers are responsive to the cytotoxic effects of suramin remains to be determined. Therefore, the present investigation was undertaken to study the effects of suramin on proliferation of various types of human malignant cells derived from epithelial, mesenchymal and hematopoietic tissues and to

determine which cancers are sensitive to the cytotoxic effects of suramin. We also wished to confirm part of the results obtained from the above *in vitro* experiments by carrying out *in vivo* experiments using a human hepatoma cell line, HuH-7.

Materials and Methods

Cells and cultures. The cells used in the present experiment are shown in Table 1. The cultures were incubated in a CO_2 incubator at 37 °C.

Preparation for suramin. Suramin (Bayer, West Germany, mol. wt. 1492), which was kindly given to us by Dr. Kiyokatsu Tanabe, of the Department of Medical Zoology, Faculty of Medicine, Kagoshima University, was dissolved at 50 mg/ml in distilled sterile water and stored at -20 °C as a stock solution. Before use suramin was diluted with the culture medium. Suramin was used up to 100μg/ml in this study, because, from a clinical point of view, plasma levels of suramin reach about 200μg/ml after the intravenous injection (11).

Table 1   Malignant human cell lines used

Cell lines	Origin	References
Epithelial		
HeLa	Cervical adenocarcinoma	Gey *et al.*: Cancer Res **12**, (1952) 264.
MCF-7	Mammary cancer	Soule *et al.*: JNCI, **51**, (1973) 1409.
EJ	Bladder cancer	Bubenick *et al.*: Int J Cancer **5**, (1970) 310.
HuH-7	Hepatoma	Nakabayashi *et al.*: Cancer Res **42**, (1982) 3858.
PLC/PRF/5	Hepatoma	Catalogue of ATTC cell lines 1985
PA-1	Embryonal cancer	Zeunthen *et al.*: Int J Cancer **25**, (1980) 19.
Fibroblastic		
KMST-6	Co-60 transformed	Namba *et al.*: Int J Cancer **35**, (1985) 275.
SUSM-1	4NQO-transformed	Namba *et al.*: Anticancer Res **8** (1988) 947.
VA-13	SV40-transformed	Girardi *et al.*: Ann Med Exp Biol Fenn **44**, (1966) 242.
Hematopoietic		
KMM-1	Myeloma	Namba *et al.*: In Vitro **25**, (1989) 723.
KMS-5	Myeloma	The same as the above
KMS-11	Myeloma	The same as the above
KMS-12	Myeloma	The same as the above
RPMI 8226	Myeloma	Matsuoka *et al.*: Proc Soc Exp Biol Med **125**, (1967) 1246.
KMS-9	B cells, EBNA[a] (+)	*b*
Raji	Burkitt's lymphoma, EBNA (+)	Pulvertaft *et al.*: Lancet **i**, (1964) 238.
HL-60	Acute promyelocytic leukemia	Collins *et al.*: Nature **270**, (1977) 347.
K562	Chronic myelocytic leukemia	Lozzio *et al.*: Blood **45**, (1975) 321.

a) Epstein-Barr virus nuclear antigen. b) Namba *et al.*: Cultivation of human myeloma cells; in Biotechnology of Mammalian Cells, Umeda *et al.* eds Jpn. Sci. Soc. Press, Tokyo (1987) pp 3-14.

Thus we considered that real concentrations of suramin may be less than $200\,\mu g/ml$ in tumor tissues.

Assessment of cytotoxicity. Cells, with the exception of hematopoietic cells, were suspended in fresh medium by treatment with a 0.2 % solution of trypsin (1:250, Difco Lab., Detroit, MI), and 10^5 cells were seeded into 35 mm plastic culture dishes in 2 ml of culture medium. A synthetic medium, ASF 103 (Ajinomoto, Tokyo), which contains only a small amount of three proteins; 0.1 % bovine serum albumin fraction V, $5\,\mu g/ml$ transferrin and $5\,\mu g/ml$ insulin, was used to eliminate effects of undefined growth stimulatory or inhibitory substances in serum. After about 24 h of incubation in the CO_2 incubator at $37\,^\circ C$, the attached cells in three aliquot dishes were counted. Then suramin was added at the concentrations indicated in Results. After 72 h of suramin addition, the cells were detached from the dishes with the trypsin solution, stained with 0.1 % crystal violet in 0.1 M citric acid, and the number of cells was counted with a hemocytometer.

For the cytotoxicity assay of hematopoietic cells, 5×10^4 cells/well in 1 ml ASF 103 medium were seeded into 24-multiwell plates with suramin at the concentrations indicated in Results. After 72 h of incubation, the number of cells were counted by the above-mentioned method. Growth inhibition was expressed as the percentage of cells surviving after treatment of the cells with suramin, with survival in the control condition representing 100 %. Triplicate dishes or wells were used at each assay point, and at least two experiments were carried out with the same cell line.

Assessment of the effect of interleukin-6 (IL-6) on myeloma cell growth. Cells were seeded into 24-multiwell plates (5×10^4 cells/well) in 1 ml RPMI-1640 medium, and $20\,U/ml$ IL-6 was added to the cultures. After 48 h of incubation, the cells were labeled with 1 $\mu Ci/ml$ ^3H-thymidine (Amersham, $5\,Ci/mmol$) for 30 min, and then the radioactivity in 6 % cold trichloroacetic acid insoluble cell fractions was counted and compared with that of the cultures untreated with IL-6.

In vivo experiments. Six-week-old Balb nu/nu mice were subcutaneously inoculated with 10^7 human hepatoma cells (HuH-7). Two days later intravenous administration of suramin ($30\,mg/kg$ body weight) was begun. The mice of the control group were injected with phosphate buffered saline (pH 7.4) which was used as a solvent of suramin. Treatment was carried out twice a week for four weeks. Thereafter the tumors formed were removed, and their weights were compared with those of tumors in the control group. The results were analyzed using Student's t test.

Results

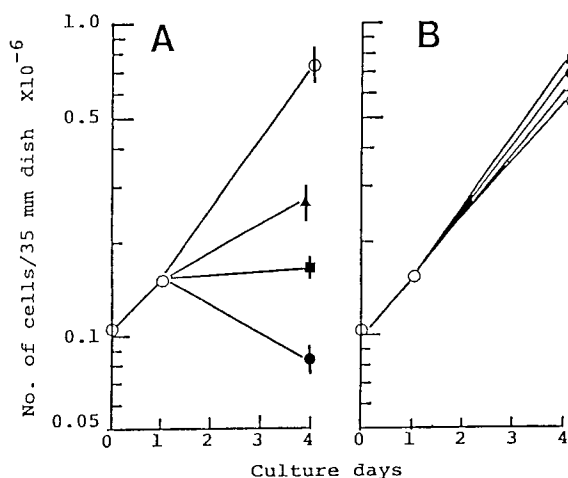Fig. 1 shows two examples of the effects of suramin on cell growth; *i.e.*, effective and

Fig. 1   Effects of suramin on cell proliferation of hepatoma (A; HuH-7) and mammary carcinoma (B; MCF-7). ○, control; ▲, 25 $\mu g/ml$; ■, $50\,\mu g/ml$; ●, $100\,\mu g/ml$ suramin. The drug was added to the culture on day 1. Each point represents the mean of triplicate cultures. Bars indicate standard errors.
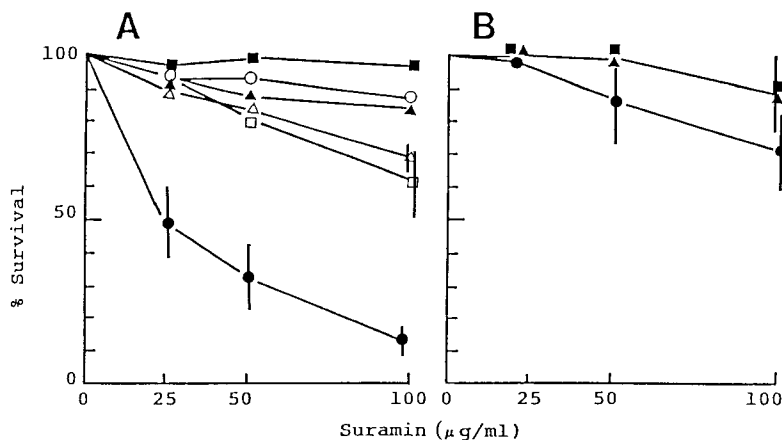
Bai *et al.*

Fig. 2   Survival (%) of cells treated with suramin for 3-4 days. Each point represents the mean of three experiments. In these experiments three dishes were used for each point. Bars indicate standard errors. A; epithelial cancers: ●, HuH-7; □, PA-1; △, PLC/PRF/5; ▲, HeLa; ○, EJ; ■, MCF-7. B; malignantly transformed fibroblasts; ●, KMST-6; ■, SUSM-1; ▲, VA-13
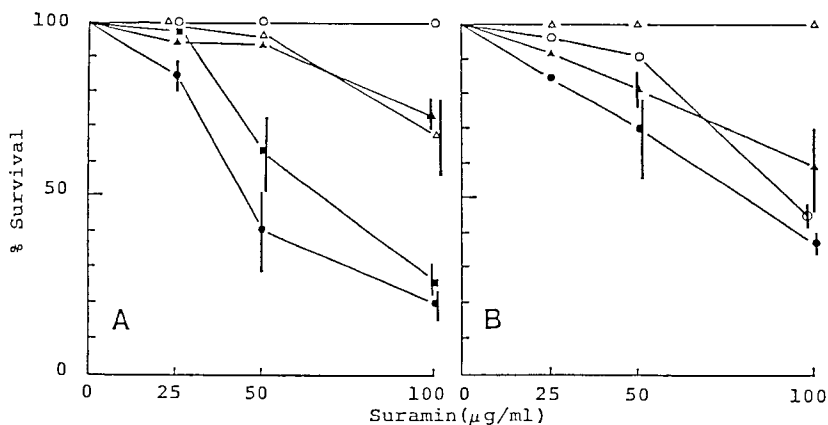

Fig. 3   Effects of suramin on cell growth of human myeloma and other hematopoietic malignant cells. The data shown represent the average of two experiments, each performed in triplicate. Bars indicate standard errors. A; Myeloma: ▲, KMM-1; △, KMS-5; ■, KMS-11; ●, KMS-12; ○, RPMI 8226. B; Other hematopoietic malignant cells: ●, KMS-9; ▲, Raji; ○, HL-60; △, K562

ineffective cases. The proliferation of human hepatoma HuH-7 cells was dose-dependently inhibited by suramin. Cell growth was stopped by treatment with 50 μg/ml suramin and considerable cell death occurred when the cells were exposed to 100 μg/ml suramin for three days.

On the other hand, suramin had no inhibitory effects on the cell proliferation of human mammary carcinoma MCF-7 cells when administered at concentrations up to $100 \mu g/ml$ suramin.

Effects of suramin on proliferation of various types of human cancers, with the exception of the malignant hematopoietic cells, were studied with the defined medium, ASF 103, to eliminate serum effects. As summarized in Fig. 2, the cell growth of HuH-7 cells was the most remarkably inhibited, whereas the cell growth of an undifferentiated hepatoma cell line, PLC/PRF/5, was not so significantly subdued as that of HuH-7 cells. The teratocarcinoma cell line (PA-1) was moderately sensitive to antiproliferative effects of suramin, but three other epithelial cell lines; i.e., HeLa, EJ, and MCF-7 were resistant to the cytotoxicity of suramin at concentrations up to $100 \mu g/ml$. Among three mesenchymal malignant cell lines, only KMST-6, which was transformed in culture by ^{60}Co irradiation, was slightly responsive to the antiproliferative effects of suramin. Although all these experiments were carried out using a synthetic culture medium without serum, there were no significant differences in the results even when a culture medium containing 10 % fetal calf serum was used (data not shown).

Then we investigated the effect of suramin on hematopoietic cell lines. As shown in Fig. 3, prominent inhibition of the cell growth was noticed in two myeloma cell lines, KMS-11 and -12, but another myeloma cell line, RPMI 8226, did not show any decrease in the cell growth up to $100 \mu g/ml$ concentration of suramin. The cell proliferation was moderately inhibited in two other myeloma cell lines, KMM-1 and KMS-5. No correlation, however, was observed between the decreased cell growth and the differentiated state of myeloma cells, because, as reported elsewhere (13), KMM-1 cells are morphologically and functionally more differentiated than KMS-5 cells. Interestingly, as shown in Fig. 4, myeloma cells, whose growth was enhanced by IL-6, were sensitive to the growth inhibitory effects of suramin. As for other hematopoietic cell lines (Raji, KMS-9 and HL-60), their growth was moderately inhibited, but no growth inhibition was noticed in K562 cells.

To confirm part of the results obtained from *in vitro* experiments, *in vivo* experiments were also carried out. We subcutaneously inoculated HuH-7 cells into nude mice and 2 days later began to treat them with suramin. After four weeks of the treatment, the mean and standard error of tumor weight in the control and treated mice were 2.51 ± 0.35 and $0.67 \pm 0.11 g$, respectively (*t*-test; $p < 0.05$). These results indicated that the treatment with suramin significantly reduced the growth of HuH-7 cells (Fig. 5).
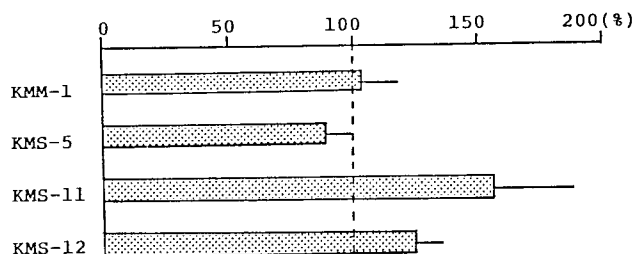
Fig. 4   Effects of interleukin 6 on DNA synthesis of human myeloma cells. The data shown represent the average of two experiments, each performed in triplicate. The values of the controls were taken as 100 %. Bars indicate standard errors.
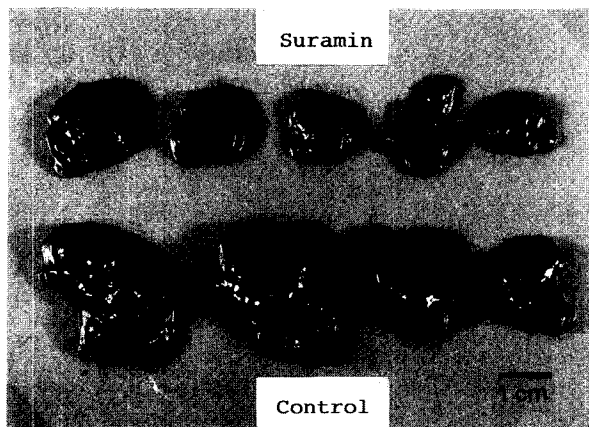
Bai *et al.*

Fig. 5   Inhibitory effects of suramin on growth of hepatoma cells (HuH-7).

Discussion

We investigated the effects of suramin on the cell proliferation of various types of human malignant cells *in vitro* and *in vivo*. Especially, we wished to determine which types of cancers are sensitive to the cytotoxic effects of suramin. Among nine epithelial and mesenchymal malignant cell lines, HuH-7 cell line was the most sensitive to the cytotoxic effects of suramin and three other cell lines were moderately sensitive. HuH-7 hepatoma cells exhibit various differentiated funtions in culture. In addition, since they produce PDGF and EGF in culture medium (14), their growth may be partly dependent upon these growth factors via autocrine loops. These facts might explain that HuH-7 cells have been the most responsive to the cytotoxic effect of suramin.

These findings led us to investigate whether or not more differentiated cells or growth factor responsive cells might be sensitive to the cytotoxic effects of suramin. Therefore, we decided to investigate the effects of suramin on human myeloma cells, since myeloma cells are derived from the differentiated stage of B-cells and some of these cell lines are responsive to the growth stimulatory effects of IL-6. As a result, the myeloma cell lines which were responsive to the stimulatory effects of IL-6 were more sensitive to the cytotoxicity of suramin than the non-responsive myeloma cell lines. However, myeloma cell lines which have been cultured for more than a decade, such as KMM-1, KMS-5 and RPMI-8226, were less responsive to suramin than myeloma cell lines cultured for a shorter period of time. The former cell lines may have become less growth factor dependent in the course of the long period of culturing. These findings indicate that myeloma cells in patients may be more appropriate for treatment with suramin. Taken together, the present results demonstrates that in some types of human malignant cells, the growth of which is more or less dependent upon growth factors, their growth will be suppressed by suramin.

The question arises as to whether or not *in vitro* experiments are applicable to treatment of patients with cancers. The tumor growth in nude mice injected with HuH-7 hepatoma cells was significantly inhibited by i.v. administration of suramin. Although we did not undertake animal experiments using the cell lines other than HuH-7, our present results indicate the possibility that

some data obtained under *in vitro* conditions will positively correlate with *in vivo* ones.

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