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Low concentrations of suramin can reduce in vitro infection of human cord blood lymphocytes with HTLV-I during long-term culture

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Summary

In vitro infection of human cord blood lymphocytes (CBL) with human T-cell leukemia/lymphoma virus type I (HTLV-I) was found to be reduced by suramin treatment at a concentration ranging from 10–100 μg/ml. At higher concentrations (500 μg/ml) suramin was toxic to the cells and even resulted in an increased percentage of cells positive for the p19 viral core protein. Suramin treatment at the onset of the CBL coculture with a lethally irradiated HTLV-I donor cell line (MT-2) reduced virus transmission, evaluated as number of $p19^+$ cells, and the consequent amount of integrated provirus in the host genome. The amount of viral RNA transcripts was not reduced in CBL cocultures. On the other hand, suramin affected HTLV-I replication in infected MT-2 cells, when used at a concentration of 50 μg/ml, and this might contribute to the reduced infectivity of suramin-treated MT-2 cells. In addition to its antiviral effects, suramin exerted a modest positive regulation on the natural killing activity of CBL and their early proliferative response in mixed lymphocyte/tumor cell culture.

HTLV-I; Suramin; Human retroviruses; T-lymphocytes

Introduction

Human T-cell leukemia/lymphoma virus type I (HTLV-I) is a retrovirus etiologically associated with a severe form of leukemia in human adults (Gallo, 1985;

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Wong Staal and Gallo, 1985). The virus is highly tropic for the $CD4^+$ lymphocytes, that are transformed after integration of the provirus in their genome. Leukemic cells from patients are usually $CD3^+$, $CD4^+$ and $CD8^-$ (Hattori et al., 1981). These cells also express IL-2 receptors and class II HLA antigen (Waldmann et al., 1984). Cytotoxic and helper T-cell functions are altered after in vitro infection with HTLV-I (De Vecchis et al., 1985; Volkman et al., 1985; Yarchoan et al., 1986) resulting in a general impairment of the immune function (Gallo, 1985).

Infection with HTLV-I can easily be achieved in vitro by coculturing peripheral or cord blood lymphocytes (the last being a very permissive target for the virus) with an HTLV-I donor cell line (Miyoshi et al., 1981). Hence, HTLV-I infection represents a model of great interest for studying the process of retrovirus-induced human leukemogenesis in vitro.

Suramin is an urea-derivative of amino-naphthalene-sulphonic type that can inhibit the reverse transcriptase activity of a number of animal retroviruses (De Clercq, 1979). This multifunctional enzyme is essential for the replicative cycle of retroviruses. As a consequence, the drug was recently tried in the treatment of AIDS, the acquired immunodeficiency syndrome caused by HTLV-III/HIV (Broder et al., 1985; Mitsuya et al., 1984). Plasma levels of suramin over 100 μg/ml resulted in undetectable or substantially diminished virus titers in primary cell cultures from treated patients (Yarchoan and Broder, 1986), but there was no evidence of clinical improvement or immunological reconstitution.

HTLV-III infected $CD4^+$ lymphocytes are destroyed by the cytopathic effect of the virus, new virus particles are released outside the cell and infection is expanded. On the contrary, from HTLV-I infected lymphocytes predominantly monoclonal or oligoclonal cells emerge as immortalized cells (Hahn et al., 1984). In contrast with HTLV-I infected cells lines, that express abundant viral mRNAs, circulating leukemic cells frequently do not express detectable viral mRNA (Hahn et al., 1984). Hence, in the case of HTLV-I infection, the effect of antiviral drugs would be limited to early phase of HTLV-I infection, during the replicative cycle of the virus. In the late phase of the disease, a major role might be played by agents that could modulate the immune response to enhance elimination of transformed cells.

To test the potential inhibiting effect of suramin on in vitro infection with HTLV-I, cord blood mononuclear cells (CBL) were infected by coculturing with a HTLV-I donor line, MT-2 cells, and tested for infection and cell-mediated immune function during a 4 wk culture. Suramin was found to reduce HTLV-I infection by affecting the early phase of virus transmission and integration, but only when used at concentrations less than 100 μg/ml.

Materials and Methods

Cell cultures and infection

Human mononuclear cells were collected from heparinized neonatal umbilical cord blood by Ficoll-Hypaque gradients (Pharmacia, Uppsala, Sweden) and cul-

tured in 24-well tissue culture plates or 25 cm^2 flasks (Falcon, Oxnard, U.S.A.) as previously described (D'Onofrio et al., 1988). The culture medium was RPMI 1640 (Gibco, Grand Island, U.S.A.) supplemented with 20% heat-inactivated foetal calf serum (FCS) (Gibco), 20 mM glutamine (Gibco), 100 U/ml penicillin/streptomycin, and 5% purified human interleukin 2 (IL-2) (Cellular Products, Buffalo, U.S.A.) to allow lymphocyte survival during long-term culture. All the human tumor cell lines used were grown in this culture medium (devoid of IL-2).

Freshly isolated CBL or the human recipient tumor lines K562 erythroleukemia (Lozzio and Lozzio, 1975), Molt-4 lymphoma (Minowada et al., 1972) and HL 60 promyelocitic leukemia (Collins et al., 1977) were infected in vitro by coculturing with a HTLV-I producer line, MT-2 (Miyoshi et al., 1981; Yoshida et al., 1982) at 5:1 ratio. MT-2 cells were lethally irradiated (12,000 R, Cesium Gamma Cell 1000, Canada Atomic Energy Ltd., Canada) just before coculturing.

Suramin treatment

Suramin was dissolved in RPMI 1640 at a concentration of 2 mg/ml and aliquots were stored at −20°C. The drug was tested on cell cultures at a concentration of 1,10,50,100 and 500 μg/ml, added only at the onset of the coculture. Alternatively, recipients cells (CBL or tumor cell lines) were pretreated overnight with suramin, then the drug was washed out and cells were replated in the same wells or flasks to keep the originally plated monocyte population. To test a possible direct antiviral effect of suramin on the MT-2 virus donor line, MT-2 cells were pretreated for 1 wk or overnight, before coculturing.

In some experiments, suramin was given every week until the 3rd week of CBL coculture, afterwards the number of viable cells was too low to continue the experiments. As suramin is a very stable molecule, it can accumulate in the culture medium in case of repetitive treatments. To avoid accumulation, the medium was washed out every week and suramin added to fresh medium.

Indirect immunofluorescence for HTLV-I infected cells

The percentage of HTLV-I infected cells was evaluated by indirect immunofluorescence for the p19 virus core protein, as described (D'Onofrio et al., 1988). Monoclonal antibodies to p19 protein were kindly given by M. Robert-Guroff. Positive cells were scored by fluorescence microscopy (Leitz, Wetzlar, F.R.G.). MT-2 cells were nearly 100% positive in each experiment, then they were taken as 100% positive control, while non-infected cells lines were used as negative control. Significance (P) was calculated by χ^2 analysis of percent $p19^+$cells.

DNA and RNA extraction

Genomic DNA was extracted by CBL or MT-2 cells by the standard proteinase K method. RNA was extracted by the guanidine thiocyanate method (Chirgwin et al., 1979).

Dot-blot analysis

Dot-blots for DNA samples were performed according to Kafatos et al. (1979)

on nitrocellulose filter (Schleicher and Schüll, Dassel, F.R.G.). RNA samples were spotted on nitrocellulose filter previously saturated with 20 × SSC, the filters were air dried and baked for 4 h at 80°C.

Hybridization was performed by using a ^{32}P nick-translated probe, the *Sst*I-*Sst*I fragment of the HTLV-I genome derived from the pMT-2 plasmid (kindly given by R.C.Gallo). This fragment is 8.5 kb and accounts for almost the entire HTLV-I genome. Nitrocellulose filters were hybridized in 10× Denhardt's solution, 4× SET and 0.1% SDS as described (D'Onofrio et al., 1988). Kodak XAR-5 films (Kodak Company, Rochester, U.S.A.) were used for autoradiography.

Assay for cell-mediated cytotoxicity of CBL

The natural killer (NK) activity of freshly isolated CBL against NK-susceptible K562 target cells was tested under routine conditions in a 4-h ^{51}Cr-release assay, as described (D'Onofrio et al., 1988). CBL were treated overnight or for 1 wk with 1,10,50,100 and 500 μg/ml suramin and compared to untreated controls.

Natural and antigen-specific cellular cytotoxicity of CBL cocultured with MT-2 cells were also tested on day 7 by ^{51}Cr-release of labelled K562 or MT-2 target cells in a 4-h assay. For antigen-specific cellular cytotoxicity, CBL were challenged on day 0 with MT-2 infecting cells at 5:1 ratio (optimal infective ratio for MT-2) and 40:1 ratio (optimal sensitizing ratio to generate allospecific cytotoxic T lymphocytes, i.e. CTL) and tested on day 7 against the same challenging cell line. The effect of suramin on the killing capacity of CBL primed with MT-2 was tested only at standard infecting ratio of 5:1.

Percent specific lysis was calculated according to the formula (Herberman et al., 1974):

$$\% \text{ specific lysis} = \frac{(\text{sample cpm} - \text{autologous control cpm})}{\text{total cpm}} \times 100$$

'Autologous control' is the total release of target cells incubated with non-labelled target cells as effectors. Cytotoxicity was evaluated at a graded Effector/Target cell ratio (E/T = 100:1, 50:1, 25:1, 12.5:1).

Dose-response curves were obtained by plotting the percentages of specific ^{51}Cr-release of E/T ratios (Thorn and Henney, 1976) and the number of killed cells (KC) per million effector cells was calculated. Significance (P) was calculated by regression test analysis.

Assay for the proliferative response of CBL in mixed lymphocyte/tumor culture

The [^3H]thymidine incorporation in blast lymphocytes was evaluated in an 18 h assay (Tood et al., 1980). CBL were plated in 96-well microtiter plates (2×10^5/well) in IL-2 enriched medium and infected by coculturing with irradiated MT-2 cells. Blastogenesis was tested on day 0,2,4,6,8. [*Methyl*-^3H]thymidine (Amersham Int., Amersham, U.K.) was used at the concentration of 1 μCi/well and cells were harvested by Microtiter Cell Harvester (Titertek 530, Flow Laboratories, Ir-

vine, U.K.). Samples were counted in a beta-counter (LKB, Bromma, Sweden) and mean cpm of quadruplicate groups were compared by *t* test analysis.

Results

Effect of suramin on in vitro infection of CBL

Suramin was able to reduce the extent of HTLV-I infection of CBL in vitro when used at a low concentration (1 to 100 μg/ml), without affecting cell viability. On the contrary, higher concentrations even increased the degree of infection (500 μg/ml) in terms of percentage of $p19^+$ CBL (Table 1). This concentration was toxic to CBL since the total cell number was reduced during culture time. However, the relative percentage of viable cells was higher than in samples treated, with low concentration of suramin (data not shown).

TABLE 1

Effect of suramin cotreatment or overnight pretreatment of CBL on HTLV-I infection in vitro, expressed in terms of percent $p19^+$ CBL.

Culture time	1 wk		2 wk		3 wk		4 wk	
	%p19	*P*	%p19	*P*	%p19	*P*	%p19	*P*
CBL + MT-2	14.09	–	12.48	–	7.99	–	6.22	–
A:								
suramin 1 μg/ml	14.09	NS	10.16	NS	10.55	NS	3.04	<0.05
suramin 10 μg/ml	11.49	NS	10.71	NS	8.33	NS	3.48	NS
suramin 50 μg/ml	7.61	<0.05	14.12	NS	7.33	NS	4.01	NS
suramin 100 μg/ml	9.87	<0.05	13.04	NS	5.10	NS	4.96	NS
suramin 500 μg/ml	11.21	NS	10.08	NS	6.89	NS	7.47	NS
B:								
suramin 1 μg/ml	10.00	<0.01	6.28	<0.01	5.12	NS	4.15	NS
suramin 10 μg/ml	9.82	<0.01	6.86	<0.01	5.71	NS	4.72	NS
suramin 50μg/ml	10.25	<0.05	5.56	<0.01	4.48	<0.05	4.16	NS
suramin 100 μg/ml	8.84	<0.01	6.37	<0.01	3.16	<0.01	4.11	NS
suramin 500 μg/ml	27.56	<0.01	20.77	<0.01	4.73	<0.01	4.20	NS
C:								
suramin 1 μg/ml	10.01	<0.01	10.5	NS	4.55	<0.05	n.d.	–
suramin 10 μg/ml	9.82	<0.01	8.3	<0.05	3.52	<0.01	n.d.	–
suramin 50 μg/ml	10.25	<0.05	7.02	<0.01	1.92	<0.01	n.d.	–
suramin 100 μg/ml	8.84	<0.01	7.25	<0.01	6.71	<0.01	n.d.	–
suramin 500 μg/ml	27.56	<0.01	41.20	<0.01	10.51	NS	n.d.	–

CBL were cocultured with lethally irradiated MT-2 cells at 5:1 ratio. Culture medium was supplemented with 5% natural IL-2. For scheme A: suramin-pretreated CBL were washed twice with medium before coculturing. For scheme B: suramin was given only at the onset of coculture. For scheme C: suramin was given every week when renewing the culture medium. Percent $p19^+$ CBL were scored by fluorescence microscopy after indirect staining. Significance (*P*) was calculated by χ^2 analysis. n.d.: not done, because of very few viable cells. NS: not significant.

TABLE 2

Effect of suramin on in vitro infection of human tumor cell lines (Molt-4, HL-60 and K562) with HTLV-I.

Suramin treatment (μg/ml)	Molt-4		HL-60		K562	
	$p19^+$	*P*	$p19^+$	*P*	$p19^+$	*P*
–	18.92%	–	11.06%	–	12.23%	–
10	8.86%	<0.01	8.29%	<0.05	9.73%	NS
50	13.97%	<0.05	9.12%	NS	11.33%	NS
100	14.18%	<0.05	7.63%	<0.01	9.48%	NS
500	7.62%	<0.01	7.30%	<0.01	5.26%	<0.01

Recipient cells were infected by coculturing with lethally irradiated MT-2 virus-donor cells. Suramin was added at the concentration of 10, 50, 100 and 500 μg/ml at the onset of coculturing. $p19^+$ cells were scored by immunofluorescence microscopy. Significance (*P*) was calculated by χ^2 analysis of percent $p19^+$ cells.

The treatment was effective both when the drug was given only once at the onset of the coculture, or when it was given every week during culture time. This second treatment schedule was more effective than the first one in reducing the percentage of $p19^+$ CBL, but cell viability was affected during culture time. Pretreatment of CBL with suramin before infection to test a potential drug-mediated conditioning of CBL resulted in a slight, if any, reduction of the number of $p19^+$ CBL.

Effect of suramin on in vitro infection of human tumor cell lines

Human tumor lines K562, Molt-4 and HL60 were infected by coculturing with irradiated MT-2 cells, and $p19^+$ cells were scored 5 days p.i. All three cell lines were susceptible to HTLV-I infection in vitro. The virus was cytophatic to recipient cells and their viability was reduced to 40–50% after 1 wk of coculture, approaching total cell death after 2 wk. Infection of suramin-treated Molt-4 and HL60 cells was significantly lower than in controls, whereas K562 cells were not affected (Table 2). Suramin was effective at low (10 to 100 μg/ml) as well as high concentrations (500 μg/ml), and no toxic effect was observed, cell viability being higher than that of untreated controls, with the exception of a minimal toxicity for HL60

Fig. 1. Dot-blot analysis for viral RNA transcripts in MT-2 virus-producer cell line after treatment with increasing concentrations of suramin. A: untreated MT-2 cells; B.C.D.: suramin-treated MT-2 cells (50, 100, 500 μg/ml, respectively).

cells after 500 μg/ml treatment. As for CBL cultures, suramin pretreatment of recipient tumor cells before coculturing did not affect the subsequent degree of infection (data not shown). The ability of suramin to interfere with virus replication and/or transmission was confirmed by the finding that MT-2 cells, pretreated for 1 wk with suramin, irradiated and cocultured with CBL, were less infective (50% inhibition) than non-treated MT-2 cells (data not shown).

Effect of suramin on HTLV-I amplification and expression in MT-2 cells

The antiviral effect of low concentration of suramin on HTLV-I infection in vitro appeared not to be mediated by a reduction of provirus genome amplification of MT-2 cells (data not shown). On the contrary, a significant reduction of viral RNA transcripts was found when the drug was added to MT-2 cells for 1 week at the concentration of 50 μg/ml (Fig.1).

Effect of suramin on HTLV-I integration and expression in infected CBL

The reduced percentage of $p19^+$ cells among suramin-treated CBL cocultured with irradiated MT-2 cells (Table 1) corresponded to a lower amount of integrated HTLV-I provirus in infected CBL (Fig. 2). Low concentrations of suramin were more efficient than higher concentrations in preventing HTLV-I transmission and integration. Viral RNA transcription was found not to be inhibited by suramin treatment (Fig. 3).

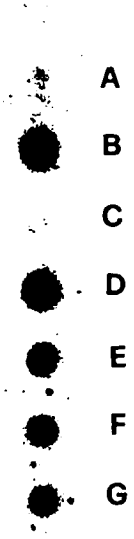

Fig. 2. Dot-blot analysis for HTLV-I provirus integrated in the host CBL genome after 2 wk of coculture with irradiated MT-2 cells. A: negative control (K562); B: positive control, i.e. untreated MT-2 cells; C: CBL control; D: CBL cocultured with MT-2 cells; E,F,G: CBL cocultured with MT-2 cells and cotreated with 50, 100 and 500 μg/ml of suramin, respectively.

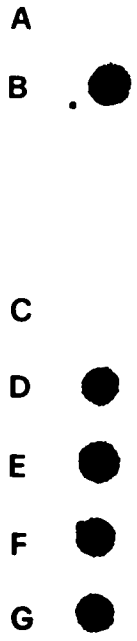

Fig. 3. Dot-blot analysis for viral RNA transcripts in CBL cocultured with irradiated MT-2 cells. RNA was extracted from CBL 2 weeks after the onset of the coculture. A: negative control (K562); B: positive control, i.e. untreated MT-2 cells; C: CBL control; D: CBL cocultured with MT-2 cells; E, F, G: CBL cocultured with MT-2 cells and cotreated with 50, 100 and 500 μg/ml of suramin, respectively.

TABLE 3

Effect of suramin treatment on the cell-mediated cytotoxicity of freshly isolated CBL (panel A) and CBL cultured for 1 wk in IL-2 (20 U/ml) enriched medium (panel B).

Effector cells	Suramin treatment (μg/ml)	A: day 0				B: day 7			
		Target cells: K562		Target cells: MT-2		Target cells: K562		Target cells: MT-2	
		KC	*P*	KC	*P*	KC	*P*	KC	*P*
CBL	–	1891.1	(–)	330.5	(–)	5994.0	(–)	4783.7	(–)
CBL	1	2213.9	(<0.01)	375.0	(n.d.)	6298.1	(<0.01)	3834.8	(<0.01)
CBL	10	2129.7	(<0.05)	415.5	(n.d.)	5547.1	(NS)	3695.3	(<0.01)
CBL	50	2300.0	(<0.01)	352.0	(n.d.)	5543.9	(NS)	3853.1	(<0.01)
CBL	100	2102.2	(<0.01)	310.0	(n.d.)	5836.7	(NS)	3144.0	(<0.01)
CBL	500	2270.3	(NS)	312.3	(n.d.)	340.3	(<0.01)	319.0	(<0.01)

Suramin was added as overnight treatment (Panel A) or maintained in the culture medium during the week (panel B). Number of killed target cells (KC) per million of effector cells, calculated by plotting the percent specific lysis ± SE of 4 replicate samples at graded effector/target cell ratio (100:1, 50:1, 25:1, 12.5:1) in a 4 h ^{51}Cr-release assay. Probability (*P*) was calculated by regression analysis; NS: not significant; n.d.: not detectable. In the absence of IL-2 in the medium, on day 7 CBL killed 518 K562 targets and 158 MT-2 target cells.

NK-activity and cell-mediated cytotoxicity of suramin-treated CBL

To verify whether suramin exerted some positive regulation of cell-mediated immune function, in addition to its direct antiviral effect on HTLV-I, both non-infected and HTLV-I infected CBL were tested for their cytotoxic capacity against tumor target cells under the effect of increasing concentrations of suramin. Different schemes of challenging of CBL were followed: a) overnight pretreatment of CBL with increasing concentration of suramin and testing for natural killing against K562 cells (Table 3A); b) treatment of CBL with suramin for 1 wk (one single dose of suramin on day 0) and testing on day 7 for cytotoxicity against a natural killing sensitive target, K562 cells (anomalous killing), or against MT-2 (HTLV-I producer) cells (Table 3B); c) infection of CBL on day 0 by coculturing with irradiated MT-2 cells at standard infecting ratio of 5:1 and testing on day 7 for cytotoxicity against K562 and MT-2 cells (Table 4); d) internal control for CBL cytotoxicity on day 7 was performed by priming on day 0 with MT-2 cells at standard ratio (40:1) for eliciting a cytotoxic response against allogenic target-cells (CTL-like) and comparing with the cytotoxic activity of CBL primed at standard infecting ratio of 5:1. From these experiments it was clear that the cytotoxic response of cocultured CBL against different target cells is extremely reduced in comparison with controls and this occurred in spite of IL-2 supply in the culture medium, that was able to activate naturally cytotoxic cells for human tumor targets (Grimm et al., 1982) only in normal CBL. Suramin further decreased the low NK activity of cocultured CBL (Table 4). The drug had no toxic effect (up to 100 μg/ml) on the natural cytotoxic capacity of non-infected CBL (Table 3B), after slightly increasing their NK activity on day 0 (Table 3A). In contrast, the cell-mediated cytotoxicity of normal CBL against MT-2 cells was significantly reduced by suramin treatment (Table 3B).

It is noteworthy that the poor killing capacity of CBL during coculturing is not related to the high infecting ratio (5:1) in comparison with priming ratio (40:1) for

TABLE 4

Effect of suramin treatment on the cell-mediated cytotoxicity of CBL, cocultured with lethally irradiated MT-2 cells in IL-2 enriched medium and tested on day 7.

Effector cells	Suramin treatment (μg/ml)	IL-2 (U/ml)	Target cells: K562 KC	*P*	*P*	Target cells: MT-2 KC	*P*	*P*
CBL	–	20	4022.9	–		1864.1	(–)	
CBL + MT-2	–	20	2396.0	(<0.01)	(–)	228.2	(<0.01)	(–)
CBL + MT-2	1	20	1770.9		(<0.05)	335.2		(NS)
CBL + MT-2	10	20	2108.9		(NS)	510.0		(<0.01)
CBL + MT-2	50	20	1979.1		(<0.01)	444.9		(NS)
CBL + MT-2	100	20	1078.7		(<0.01)	452.2		(NS)
CBL + MT-2	500	20	373.7		(<0.01)	n.d.		(–)

Suramin was added on day 0 at the onset of the coculturing. Number of killed target cells (KC) per million of effector cells calculated by plotting the percent specific lysis ± SE of 4 replicate samples at graded effector/target cell ratio (from 100:1 to 12.5:) in a 4-h ^{51}Cr-release assay. Probability (*P*) was calculated by regression analysis; NS: not significant.

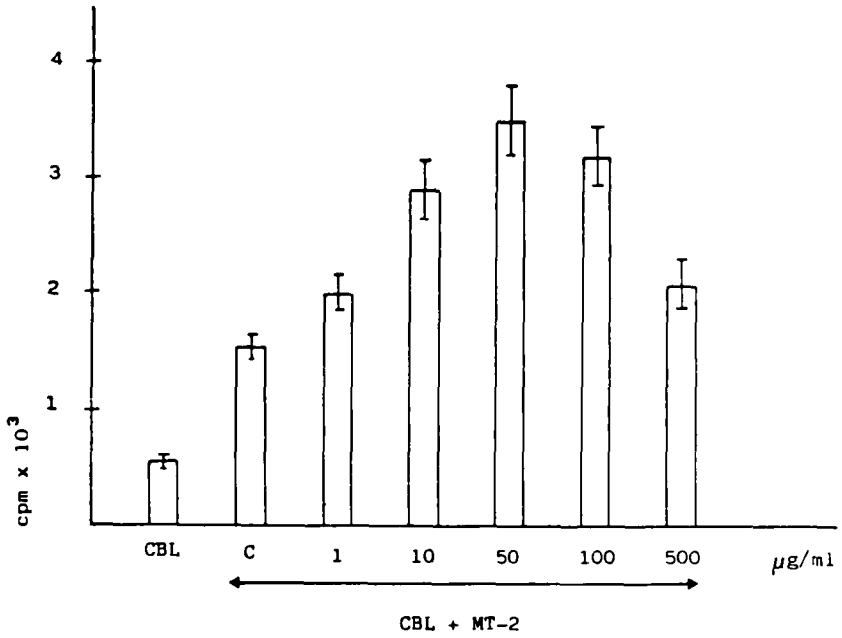

Fig. 4. [^{3}H]Thymidine incorporation (18 h assay) of freshly isolated CBL cocultured with lethally irradiated MT-2 cells (5:1 ratio) and tested after overnight treatment with increasing concentrations of suramin. (C: untreated cocultured CBL.)

CTL. In fact, CBL primed on day 0 with MT-2 cells at 40:1 ratio were equally poorly cytotoxic against MT-2 targets as CBL primed at 5:1 ratio (data not shown).

Proliferative response of CBL in mixed-lymphocyte/tumor culture

Blastogenesis of CBL during the 1st week of coculture, evaluated in terms of [^{3}H]thymidine incorporation, was very modest when compared with non-infected cells. Normal CBL did not proliferate in the absence of IL-2 in the culture medium, but they did so when IL-2 was supplied, as in routine culture conditions. However, CBL cocultured with MT-2 cells incorporated minimal amount of thymidine, although IL-2 was present in the medium.

Suramin was found to boost the very early phase of the proliferative response of CBL. In fact, a dose-dependent enhancement of [^{3}H]thymidine incorporation was observed only at the onset of the coculture (Fig. 4).

Discussion

Evidence is here provided that cotreatment of CBL/MT-2 cells with low concentration of suramin (10–100 μg/ml) can reduce HTLV-I infection in vitro without affecting cell viability and proliferation. Higher concentration (500 μg/ml) was

moderately toxic to recipient CBL and even resulted in an increased degree of target cell infection. In this case, few CBL seemed to survive after 4 wk culture, but most of these cells were $p19^+$ and showed a good viability, as if suramin treatment would have favored the growth of an infected subpopulation of CBL. When given at a concentration up to 100 μg/ml, suramin was able to inhibit HTLV-I infection both when immunocompetent cells, i.e. CBL, or non-immunocompetent human tumor lines were used as recipient cell, although the degree of inhibition varied with different cell types. The inhibitory effect of low concentrations of suramin appeared to be mostly due to its direct antiviral activity. This might well result from multiple mechanisms, including the inhibition of the viral reverse transcriptase and binding with other regulatory proteins of the virus or host cell.

Suramin is known to inhibit the reverse transcriptase activity of a number of animal retroviruses (De Clercq, 1979), including the HTLV-III enzyme (Chandra et al., 1985). This multifunctional enzyme is essential for the replicative cycle of retroviruses. Thus, it is very attractive to inhibit this cycle by blocking the enzyme with specific inhibitors. To this repect, suramin is a very active agent, although its effect is not very specific (Chandra et al., 1985; De Clercq, 1987). The biochemical similarities between the HTLV-I (Rho et al., 1981) and HTLV-III (Mitsuya et al., 1984) reverse transcriptases suggest that this might be one of the mechanisms by which suramin inhibits HTLV-I infection. In addition to its effect on reverse transcriptase, suramin is known to combine with a variety of cellular proteins, including histones, cytosomal enzymes, ATPases and several enzymes of the complement system (De Clercq, 1987). Inhibition of HTLV-I transmission might well involve some of these cellular proteins. Very recently, the polyanion suramin was shown to bind to the polycation platelet-derived growth factor (PDFG), thus blocking the binding to its physiological receptor (Hosang, 1985). In our experiments, suramin had no effect on the IL-2 dependent activation of normal CBL for natural killer activity up to 100 μg/ml (Table 3B), thus suggesting that no blocking of IL-2 receptor occurred. It is also likely that suramin could affect HTLV-I transmission by interfering with adsorption of virus particles to recipient cells, as many polyanionic substances can alter this process (De Somer et al., 1968a, b). This might in part explain the reduced number of $p19^+$ cells and the low amount of integrated HTLV-I provirus. This effect would be additional to the possible inhibitory role of suramin on virus reverse transcriptase and further prevent HTLV-I integration. Evidence that the virus replicative cycle was impaired by suramin was also provided by the 50–60% inhibition of virus transmission, that was found when MT-2 infecting cells were pretreated with suramin (100 μg/ml) for 1 wk (data not shown).

Suramin did not reduce the amount of viral RNA transcripts in infected CBL (Fig. 3), suggesting that, at least in these cells, this step of viral replication is not affected by the drug. Moreover, transcription of HTLV-I RNA was not inhibited in MT-2 cells by a concentration higher than 50 μg/ml (Fig. 2). The finding that at a lower dose suramin interfered with this process suggests the possibility that a consistent percentage of MT-2 cells were sensitive to the drug at this concentration. It is conceivable that concentrations over 50 μg/ml might select cell clones in which HTLV-I transcription is suramin-resistant.

The fact that suramin was effective also when given only once at the onset of CBL/MT-2 coculture prompted us to verify whether this agent might have a role in modulating the immune repsonse of CBL, possibly increasing the immune surveillance against HTLV-I infection. By testing for cell-mediated cytotoxicity, it was found that overnight pretreatment of CBL with suramin (up to 100 μg/ml) could moderately but significantly boost the NK activity (Table 3A). This effect of suramin has to be underlined considering that LGL with NK activity have recently been shown to afford protection of PBL against infection with HTLV-I (Macchi et al., 1987; Ruscetti et al 1986). On the contrary, the alloantigen-elicited cell-mediated cytotoxicity of CBL, cocultured with virus-donor MT-2 cells, against 'stimulator' MT-2 target cells declined early after HTLV-I infection and suramin was not capable of antagonizing this immunodepression.

A boosting effect of suramin was also evident on the very early step of CBL blastogenesis in mixed culture with MT-2 tumor cells and resulted in a dose-dependent increase of [^{3}H]thymidine incorporation of CBL within 24 h from the onset of the coculture.

The overall picture of experimental results shows that suramin could counteract HTLV-I infection in vitro when used at low concentrations (below 100 μg/ml), without any detectable toxicity on cell cultures. In addition, the finding that higher concentrations (500 μg/ml) could in some cases increase the infectivity of HTLV-I underlines the importance of the dose and pharmacokinetics of this drug in the clinical situation. Considering that suramin is a very stable molecule and that its body clearance is very slow, with a plasma half-life ranging between 44 and 54 days (Collins et al., 1986), it should be necessary to keep its dose not exceeding the optimal concentration, to avoid both cellular toxicity and enhancement of infection. At low concentration, suramin appeared to be virustatic for HTLV-I, in accordance with previous reports on HTLV-III (Broder et al., 1985; Mitsuya et al., 1984) and significantly reduced in vitro transmission of HTLV-I both in CBL and in human tumor lines (Molt-4 and HL60). However, virus transmission was impaired to a greater extent in the case of CBL, possibly depending on the positive regulation on NK function and the proliferative response against tumor cells mediated by suramin in addition to its antiviral effects.

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