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Editorial

# Suramin in the treatment of AIDS: mechanism of action*

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**Summary**

Suramin is a potent inhibitor of the reverse transcriptase (RNA-directed DNA polymerase) of retroviruses, including the HTLV-III/LAV (human T-cell lymphotropic virus type III/lymphadenopathy-associated virus) reverse transcriptase. Although suramin is far from specific as a reverse transcriptase inhibitor and known to interact with a multitude of proteins and enzymes, it is able to suppress the replication and cytopathic effect of HTLV-III/LAV at concentrations which are non-toxic for the host cells and readily attainable in humans. Consequently, suramin is also able to block HTLV-III/LAV replication in patients. The mechanism of action of suramin at the molecular biological level, its mode of transport and accumulation by the infected host cells, and the bases for its rather selective virustatic activity remain, to a large extent, to be elucidated.

AIDS; HTLV-III/LAV; suramin; reverse transcriptase

## Introduction

Suramin is the hexasodium salt of 8,8′-(carbonylbis(imino-3,1-phenylenecarbonylimino(4-methyl-3,1-phenylene)carbonylimino))bis-1,3,5-naphthalenetrisulfonic acid (Fig. 1). It is also known as Antrypol, Bayer 205, Fourneau 309, Germanin, Moranyl, Naganol, and Naphuride. It was originally synthesized at Bayer in 1920 and has ever since been used in the treatment of African trypanosomiasis

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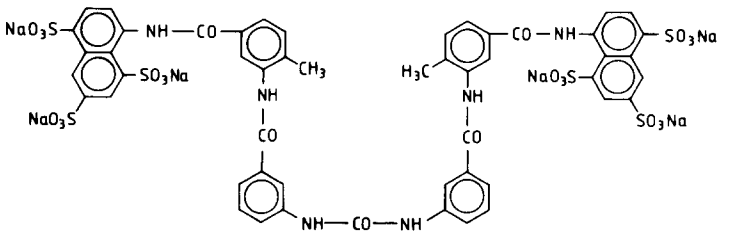


Fig. 1. Formula of suramin.

(sleeping sickness), and since 1950 also in the treatment of onchocerciasis. In 1979 I reported that suramin behaved as a potent inhibitor of the reverse transcriptase of a number of animal retroviruses [6]. Mitsuya et al. [19] confirmed that suramin is inhibitory to the reverse transcriptase of diverse retroviruses including that of HTLV-III/LAV (human T-cell lymphotropic virus type III/lymphadenopathy-associated virus), the causative agent of the acquired immune deficiency syndrome (AIDS). Furthermore, Mitsuya et al. [18,19] demonstrated that suramin blocks the

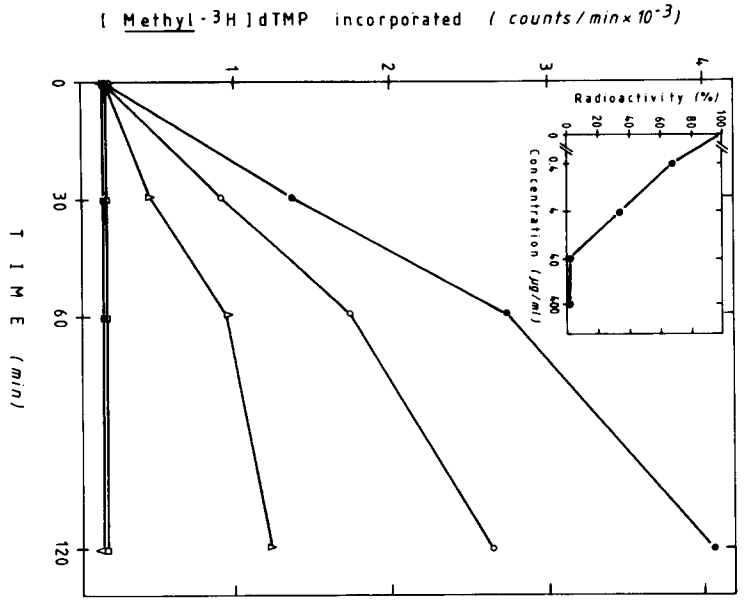


Fig. 2. Effects of different concentrations of suramin on Moloney murine leukemia virus reverse transcriptase. Suramin was added at time zero, and the assay mixtures were incubated for 30, 60 or 120 min. Concentrations of suramin: 400 μg/ml (▽), 40 μg/ml (□), 4 μg/ml (△), 0.4 μg/ml (○) and 0 μg/ml (●). Inset: dose-response effects measured after 60 min incubation. From ref. 6.

infectivity and cytopathic effect of HTLV-III/LAV at concentrations that do not adversely affect the immune function of the host cells and are clinically attainable in humans. In fact, a virustatic effect was obtained when suramin was administered to patients with HTLV-III/LAV infection at such a dosage regimen that plasma drug concentrations were achieved which inhibited HTLV-III/LAV replication in cell culture [3,5].

## Inhibition of reverse transcriptase

Suramin is a potent inhibitor of the RNA-directed DNA polymerase (reverse transcriptase) of several retroviruses, i.e. Moloney murine leukemia virus (Fig. 2). It causes a 50% inhibition of the reverse transcriptase activity at a concentration of about 1 μg/ml (0.7 μM). Yet, the effect of suramin on reverse transcriptase is *not* specific. Other DNA polymerases, viz. DNA polymerase α and DNA primase are also strongly inhibited by suramin [4,21], whereas DNA polymerases β and γ and terminal deoxynucleotidyl transferase (TdT) are relatively resistant to the drug

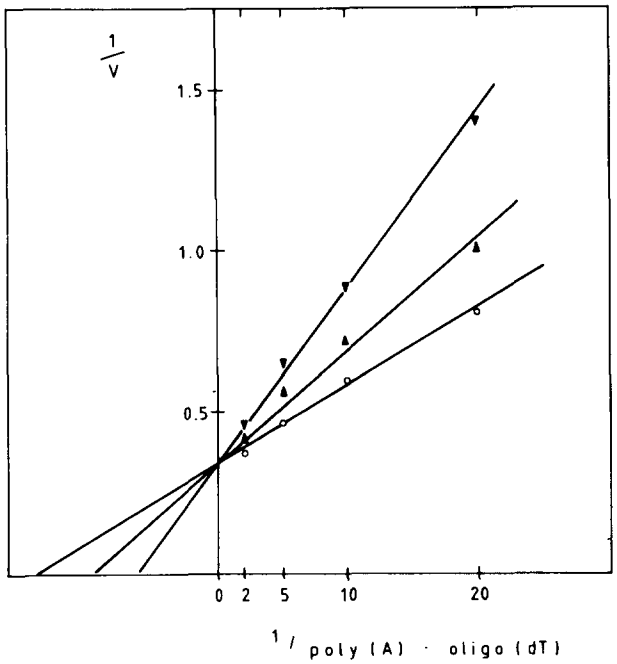


Fig. 3. Double-reciprocal plot for the kinetics of inhibition of avian myeloblastosis virus reverse transcriptase by suramin. Concentrations of suramin: 0.3 μg/ml (▼), 0.1 μg/ml (▲) or 0 μg/ml (○). Concentration of poly(A) in μM, oligo(dT) at 1/4 concentration of poly(A). V = cpm × $10^{-3}$. Time of incubation: 15 min. From ref. 6.

[21]. The inhibition of reverse transcriptase by suramin is competitive with respect to the template-primer, i.e. poly(A)·poly(dT) for the avian myeloblastosis virus reverse transcriptase (Fig. 3). This suggests that the drug interacts with the template-primer binding site of the enzyme. Through its naphthalenetrisulfonic acid groups, suramin may bind to basic amino acid residues at or near the active center of the reverse transcriptase.

## Inhibition of cytopathic effect of HTLV-III/LAV

The influence of suramin on the cytopathic effect of HTLV-III/LAV has been determined in ATH8 cells, which represent an immortalized OKT4$^+$ T-cell clone obtained by cloning a normal tetanus toxoid-specific T-cell line in the presence of human T-cell lymphotropic virus type I (HTLV-I)-producing MJ-tumor cells [17]; ATH8 cells grow rapidly (in the presence of interleukin-2) and are exquisitely sensitive to the cytopathic effect of HTLV-III/LAV. When infected at a multiplicity of $5 \times 10^3$ virions per cell, and incubated for 7–8 days, almost 100% of the ATH8 cells die (Fig. 4). In the presence of suramin, however, ATH8 cells are protected against the cytopathic effect of HTLV-III/LAV. At 10 and 25 μg/ml only partial protection is noted, but at a concentration of 50 μg/ml and higher suramin completely protects ATH8 cells against the cytopathic effect of HTLV-III/LAV, thereby enabling the cells to survive and grow. At 50 μg/ml, suramin does not impair the growth of normal, uninfected ATH8 cells (Fig. 4). Neither does suramin inhibit the immunological reactivities, including the antigen- or mitogen-induced proliferative response of the target immune T-cells at concentrations up to 50 μg/ml [18]. Only at relatively high concentrations (100 μg/ml and higher) suramin inhibits normal cell growth (Fig. 4) or immune reactivity [18].

Thus, suramin does not inhibit normal cell growth, viability and functions at concentration levels (i.e. 50 μg/ml) that completely block the cytopathogenicity of HTLV-III/LAV. The inhibitory effect of suramin on the cytopathogenicity of

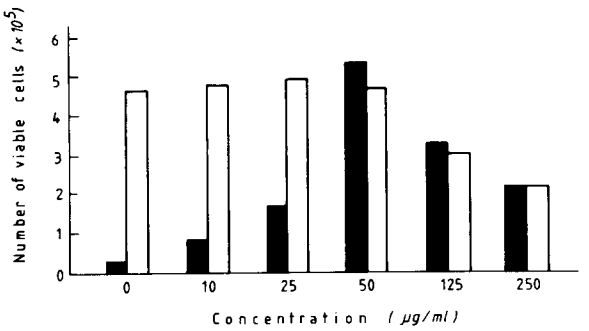


Fig. 4. Inhibition of cytopathogenicity of HTLV-III/LAV for ATH8 cells by suramin. The number of viable cells was measured after an incubation period of 7 days in the presence of varying concentrations of suramin. □ : mock-infected ATH8 cells; ■ : HTLV-III/LAV-infected ATH8 cells. From references 1 and 2.

HTLV-III/LAV most probably results from an inhibition of the virus replicative cycle, since, concomitantly with the viral cytopathogenicity, expression of the viral core (*gag*) p24 protein is also completely suppressed at a suramin concentration of 50 μg/ml [1].

## Specificity

Suramin is known to combine with proteins of all kinds, including albumin, globulins, fibrinogen and histones. It is inhibitory to a wide variety of enzymes, i.e. hyaluronidase, urease, hexokinase, fumarase, succinic dehydrogenase, β-glucuronidase (and other lysosomal enzymes), ATPases, enzymes requiring ATP, lysozyme, thrombin, kallikrein, plasmin, chymotrypsin and several enzymes of the complement system. How these effects relate to the antiparasitic action of suramin is not at all clear [13]. According to Fairlamb and Bowman [12], at least part of the trypanocidal action of suramin may be attributed to an inhibition of glycolysis and ATP production. Suramin would inhibit two key enzymes in glycolysis, i.e. glycerol-3-phospate oxidase and $NAD^+$-dependent glycerol-3-phosphate dehydrogenase [12].

Considering the multitude of proteins and enzymes with which suramin can interact, how would it be able to achieve any specificity in its antiviral action, particularly against HTLV-III/LAV? Secondly, how could the specific activity of suramin against HTLV-III/LAV be rationalized in terms of an inhibitory effect at the reverse transcriptase level, if it is correct that, as mentioned above, cellular DNA polymerases, i.e. DNA polymerase α [4,21], are inhibited equally well by the drug as the virus-associated reverse transcriptase? As a working hypothesis one may assume that suramin is taken up by endocytosis [8]. To this end, the drug should be bound first to a putative carrier protein. Once within the cell, the endocytic vesicles containing the suramin-protein complex may fuse with lysosomes, which would result in the formation of secondary lysosomes, the degradation of the protein carrier molecule and the release of free suramin into the cytoplasm. To ascertain the validity of this hypothesis, the transport and accumulation in infected cells should be monitored with radiolabeled drug. This has, to the best of my knowledge, not yet been accomplished.

If suramin is indeed taken up by the cells through endocytosis and finally released as such in the cytoplasm, it would have the opportunity to first hit the reverse transcriptase (within the cytoplasm of the virus-infected cell) before gaining access to the nucleus and the cellular DNA polymerases. Thus, because of compartmental reasons, suramin may be preferentially inhibitory to the reverse transcriptase in vivo (within the virus-infected cell), notwithstanding the lack of specificity of suramin towards the isolated enzyme. It is also possible that upon isolation and purification of the enzyme, some factors are removed which make the reverse transcriptase more sensitive to inhibition by suramin and which, again, may explain why in vivo (infected cell) the reverse transcriptase is more vulnerable to the inhibitory effect of suramin than are the cellular DNA polymerases. Whether these

hypotheses are correct remains, once more, to be proven by experimental evidence.

## Target(s) for the antiviral action

The elucidation of the mode of action of suramin could to a great extent benefit from the study of drug-resistant HTLV-III/LAV mutants. Whether and how frequently such drug-resistant mutants may emerge upon the clinical use of suramin is unknown. However, attempts should be made in the laboratory to obtain suramin-resistant mutants, i.e. upon repeated passage of HTLV-III/LAV in the presence of the drug.

As matters stand, suramin is assumed to act at the reverse transcriptase level, and hence to block the replicative cycle of HTLV-III/LAV by preventing the early transcription of 'genomic' viral RNA to 'plasmidic' viral DNA (Fig. 5). There are, however, various other steps in the virus replicative cycle that could serve as targets, and an inhibitory effect at one particular level (i.e. reverse transcriptase) does not preclude the possibility of additional effects at other levels (i.e. virus adsorption or penetration). Although there is no evidence for an inhibitory effect of suramin on the adsorption of HTLV-III/LAV to the host cell, this possibility should not be dismissed. Indeed, polyanionic substances, such as polyacrylic acid, polymethacrylic acid, polyvinyl sulfate, dextran sulfate and polyphloroglucinol phosphate, are all known to interfere with the virus adsorption process [9,10], and, being an hexasulfonic acid derivative, suramin might also affect virus adsorption to the cells. Such a mode of action may enable suramin to curtail virus spread from one cell to another.

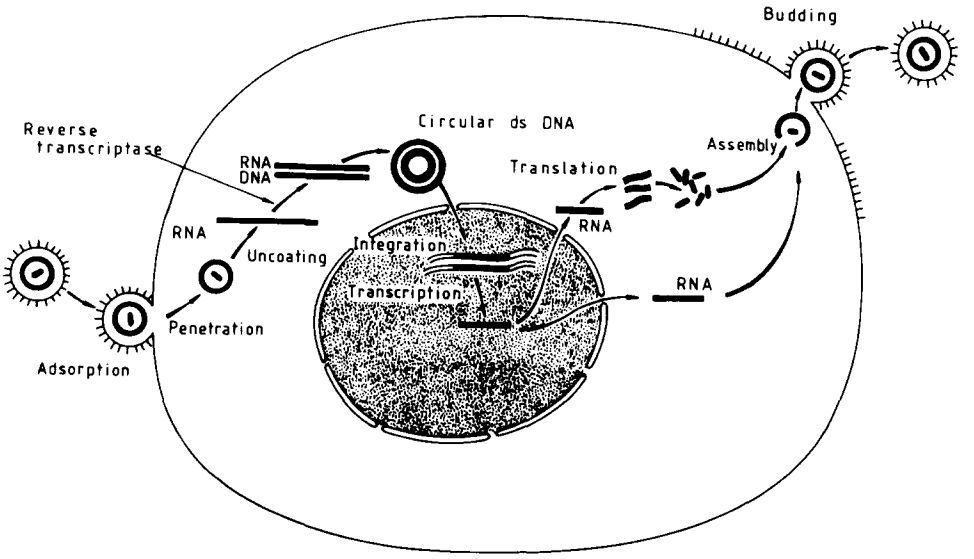


Fig. 5. Replicative cycle of retroviruses.

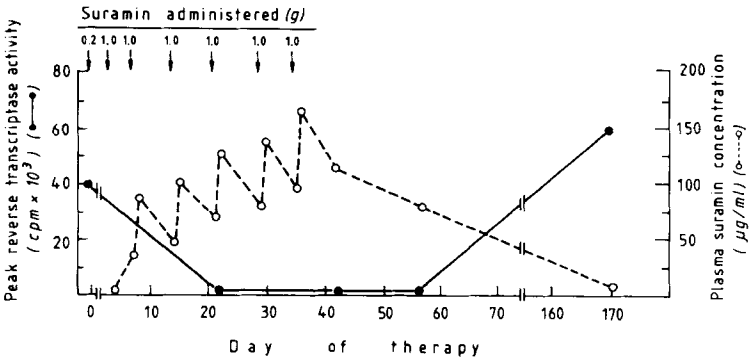


Fig. 6. Peak reverse transcriptase activity in cultures of peripheral blood lymphocytes and plasma suramin concentrations in a patient who had detectable HTLV-III/LAV virus before the start of suramin therapy. From ref. 3.

## Virustatic effect in patients

The fact that suramin combines with a great variety of proteins points, on the one hand, to its lack of specificity, but, on the other hand, binding of suramin to plasma proteins (at least 99.7%, according to Collins et al. [5]) prevents suramin from being eliminated prematurely and permits the compound to attain sufficiently high blood levels with a dosage regimen of one injection of 1 g suramin per week [3,5]. Suramin clearly accumulates during such 5-week courses of 1 g a week (Fig. 6). A plasma drug level of 50 μg/ml is reached after the second injection of 1 g suramin, and, as the drug accumulates, plasma drug levels well above 100 μg/ml are attained upon the last dose of the full therapy course. In keeping with the cell culture data, showing complete protection of ATH8 cells by suramin against HTLV-III/LAV infection at a drug concentration of 50 μg/ml (Fig. 4), virus replication (in peripheral blood lymphocytes of patients treated with suramin) becomes undetectable as soon as a plasma drug level of 50 μg/ml is attained (Fig. 6). Thus, suramin appears to be virustatic in patients at concentration levels which are also virustatic in cell culture. A single dose of 1 g suramin per week suffices to maintain its virustatic effect in patients, which, again, correlates nicely with the time course of its antiviral activity in cell culture (at least 7 days). However, the inhibitory effect of suramin on HTLV-III/LAV replication appears to be reversible, since virus can be detected again following cessation of suramin therapy [3].

## Structurally related analogues

Suramin is not unique in its action as an inhibitor of HTLV-III/LAV replication or reverse transcriptase. Anionic dyes, such as Evans Blue, Direct Yellow 50, Congo Red and Chicago Sky Blue (Fig. 7), are at least as potent, if not more potent, inhibitors of the retrovirus-associated reverse transcriptase as suramin [1].

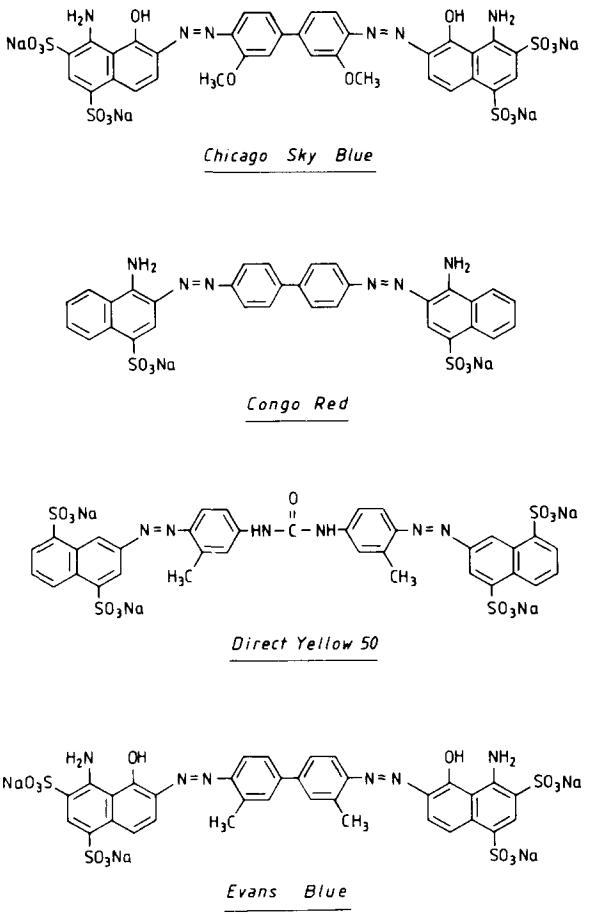


Fig. 7. Anionic dyes, structurally related to suramin.

Evans Blue confers a complete protection of ATH8 cells against the cytopathic effect of HTLV-III/LAV at a concentration of 25 μg/ml, while not being toxic for the uninfected host cells at concentrations up to 100 μg/ml [1,2]. The selectivity margin (ratio of cytotoxic concentration to antiviral concentration) displayed by Evans Blue is similar to that of suramin, and it is likely that these compounds share many other properties, including a common mechanism of action. An inhibitory effect at the reverse transcriptase level may be one, but not necessarily the sole, reason for the activity of this class of compounds against HTLV-III/LAV.

## Other inhibitors of HTLV-III/LAV

Suramin was the first compound to be described as an inhibitor of HTLV-

III/LAV replication [19]. Its future as a chemotherapeutic drug for the treatment of AIDS or AIDS-related complex (ARC) will depend on a number of factors, especially the ultimate clinical response of the patients. This issue can only be assessed by appropriately controlled long-term studies and, possibly, long-term maintenance therapy may be required to obtain favorable clinical responses.

In the mean time, several other inhibitors of HTLV-III/LAV replication have been described [7], viz. aurintricarboxylic acid [2], ribavirin [15], phosphonoformate [22,23], human interferon αA [14], 3′-azido-2′,3′-dideoxythymidine [20,24], various 2′,3′-dideoxynucleosides such as 2′,3′-dideoxycytidine [16]; and ammonium-21-tungsto-9-antimoniate (HPA-23), which is inhibitory to HTLV-III/LAV reverse transcriptase [11]. However, ribavirin and HPA-23 are negatively selective in that they inhibit HTLV-III/LAV replication at concentrations well above the cytotoxicity threshold [1]. Phosphonoformate and aurintricarboxylic acid are, like suramin, selective inhibitors of HTLV-III/LAV [1,2]. Their inhibitory concentrations for HTLV-III/LAV fall in the same concentration range as that of suramin.

3′-Azido-2′,3′-dideoxythymidine (AZT) and 2′,3′-dideoxycytidine (ddCyd) completely protect ATH8 cells against the cytopathic effect of HTLV-III/LAV at a concentration of 1 μM and 0.5 μM, respectively [16,20]; that is at concentrations which are about 50-fold lower than those required to inhibit normal cell growth. Thus, AZT, ddCyd, and probably other 2′,3′-dideoxynucleoside analogues as well, appear to be more potent anti-HTLV-III/LAV agents than suramin (for which 35 μM is required to effect a complete protection of ATH8 cells against HTLV-III/LAV). The safety margin offered by AZT and ddCyd also seems to be larger than that of suramin (50 as compared to 5).

Considering the complexity of the HTLV-III/LAV-lymphocyte interactions involved in the pathogenesis of AIDS which may be inhibited by the drugs to a varying extent, it may not seem justified to extrapolate from the relative selectivity indexes in cell culture which compounds would offer the greatest benefit in AIDS patients. This becomes all the more difficult when different classes of compounds are compared, i.e. polyanionic derivatives versus nucleoside analogues, which differ fundamentally not only in their mechanism of action at the molecular biologic level but also in their pharmacokinetics and toxicity profile.

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