Suramin enters and accumulates in low pH intracellular compartments of v-sis-transformed NIH 3T3 cells

Shuan Shian Huang, Han A. Koh, Jung San Huang*

E.A. Doisy Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, 1402 South Grand Blvd., St. Louis, MO 63104, USA

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Abstract Using acridine orange as a reporter compound, we demonstrate that suramin enters and accumulates in low pH intracellular compartments (endosomes, lysosomes, and trans-Golgi complex) of normal and v-sis-transformed NIH 3T3 cells. The concentration of suramin in these acidic compartments is estimated to be > 150 μM, higher than the concentration known to completely inhibit interaction of the platelet-derived growth factor (PDGF) receptor and v-sis gene product. These results support the hypothesis that suramin reverses the transformed phenotype of v-sis-transformed cells by entering the cell via endocytosis and blocking interaction of the v-sis gene product and PDGF receptor in intracellular organelles.

Key words: Suramin; Acidic intracellular compartment; Acridine orange; Cellular entry; v-sis-transformed cell

1. Introduction

Suramin is an antiparasitic, antiviral and anticancer agent [1–10]. It inhibits the activities of many cytoplasmic and nuclear enzymes in vitro [11–14], but the significance of these effects in the mode of suramin action remains uncertain due to the fact of very poor permeability of suramin in the plasma membrane [1]. Suramin inhibits interactions between growth factors and their receptors [15–25] and reverses the transformed phenotype of v-sis-transformed cells [17]. Suramin was reported to reverse the transformed phenotype of v-sis-transformed cells by blocking the v-sis gene product/PDGF receptor interactions at the cell surface [26,27]. In previous reports [28,29], we showed that suramin treatment of v-sis-transformed cells inhibited intracellular turnover of both platelet-derived growth factor (PDGF) receptor and v-sis gene product by blocking their interaction in the endoplasmic reticulum (ER) and Golgi complex. Together with other observations, these results led us to suggest that suramin reverses the transformed phenotype of v-sis-transformed cells by entering the cell via endocytosis and inhibiting the interaction of the PDGF receptor and v-sis gene product in intracellular organelles such as trans-Golgi complex [28,29]. To further define the intracellular action of suramin, we examined the subcellular localization of suramin following treatment of cells with this drug. Using acridine orange as a reporter, we demonstrate here that suramin accumulates in the acidic intracellular compartments following treatment of acridine orange-preloaded v-sis-transformed NIH 3T3 cells with suramin. These results are consistent with the hypothesis that suramin blocks the intracellular interaction of the v-sis gene product and PDGF receptor in v-sis-transformed cells [28,29].

2. Materials and methods

2.1. Materials

Suramin was obtained from FBA Pharmaceuticals (West Haven, CT). Acridine orange was obtained from Sigma Chemical Company (St. Louis, MO). 4-Methylumbelliferyl (4-MU) β-D-glucuronide was obtained from Research Products International (Elk Grove, IL), v-sis-transformed (simian sarcoma virus-transformed) NIH 3T3 and NRK (normal rat kidney) cells were kindly provided by Drs. Stuart A. Aaronson and Keith C. Robbins, National Cancer Institute. v-sis-transformed NIH 3T3, v-sis-transformed NRK, NIH 3T3, Swiss mouse 3T3 and NRK cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum.

2.2. Measurement of acridine orange fluorescence in the presence of various concentrations of suramin

The reaction mixture contained 1 μM acridine orange and various concentrations of suramin as indicated in 0.2 M sodium phosphate buffer (pH 7.4), 0.2 M sodium phosphate buffer (pH 6.5), or 0.2 M sodium acetate buffer (pH 5.0). The fluorescence emission of the reaction mixture was scanned from 500 nm to 575 nm using the excitation wavelength at 480 nm.

2.3. Suramin treatment of acridine orange-preloaded cells

Cells were grown on coverslips for one day in DMEM containing 10% fetal calf serum. Cells were washed with serum-free DMEM and incubated with 1 μM acridine orange in serum-free DMEM at 37°C for 30 min. The acridine orange-preloaded cells were washed and treated with various concentrations of suramin at 37°C for the indicated time periods. The cells were then viewed and photographed with a Leitz Orthoplan fluorescence microscope.

2.4. Measurement of β-glucuronidase activity in the medium of suramin-treated v-sis-transformed NIH 3T3 cells

Cells grown on 35 mm Petri dishes were treated with and without 21 μM suramin in serum-free DMEM at 37°C for 1 h. The β-glucuronidase activity in the conditioned medium was assayed using 4-methylumbelliferyl β-D-glucuronide as substrate according to the procedure of Brot et al. [30].

3. Results and discussion

Acridine orange is a non-invasive fluorescent compound which has been used to examine the luminal pH of intracellular organelles in many cell types including fibroblasts [31]. It accumulates in low pH intracellular compartments such as endosomes, lysosomes, trans-Golgi complex and exocytic vesicles [31]. The accumulation of acridine orange is dependent on the luminal pH of these acidic compartments. The more acidic the compartments are, the more acridine orange accumulates [31]. The concentration of acridine orange determines the color of fluorescence emitted [31,32]. An orange-red color is seen in vesicles of low pH such as lysosomes and late endosomes, whereas a yellow to orange-red color is seen in

*Corresponding author. Fax: +1 (314) 577-8156.
E-mail: huangjs@wpogate.slu.edu

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organelles of high pH (pH 6.0–6.5) such as trans-Golgi complex and exocytic vesicles (Fig. 1) [31].

Acridine orange is a weak base and polyaromatic compound and complexes with suramin possibly through stacking and electrostatic interactions. To examine this interaction, we investigated the effect of various concentrations of suramin on the fluorescence of acridine orange. As shown in Fig. 2A, increasing concentrations of suramin progressively diminished the intensity of fluorescence of acridine orange. The maximal effect of suramin was observed at a molar ratio of ~1:3 (suramin:acridine orange) in the range of pH 5.0 to 7.4 (Fig. 2B). This molar ratio of suramin-acridine orange complex, which is constant at least up to 800 μM of acridine orange, is essentially identical with that reported for the complex of suramin and 4′,4″-bis (1,4,5,6-tetrahydro-2-pyrimidinyl) terephthalanilide (a weak-base aromatic compound analogous to acridine orange) [33]. These results show that acridine orange can be used as a reporter compound for probing the entry of suramin into acidic intracellular compartments.

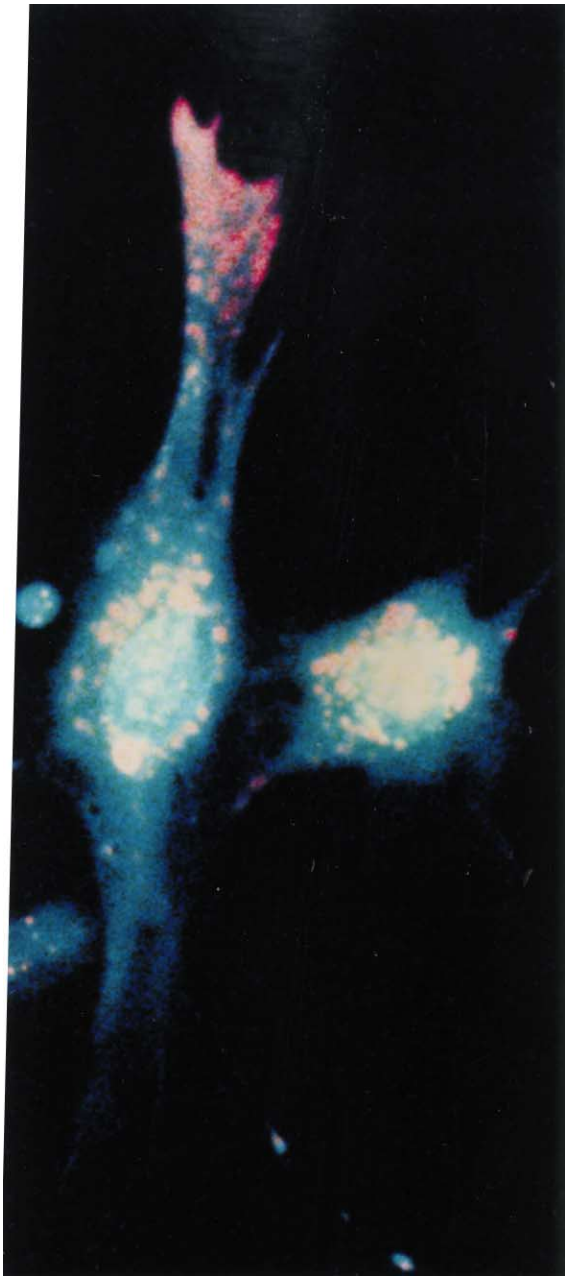

Fig. 1. Acridine orange fluorescence in normal NIH 3T3 cells. Normal NIH 3T3 cells were treated with 1 μM acridine orange as described in the text. The bright orange-red fluorescence at the distal ends represents lysosomes and late endosomes. The yellow to orange-red fluorescence at the perinuclear region represents exocytic vesicles and trans-Golgi complex [31,32].

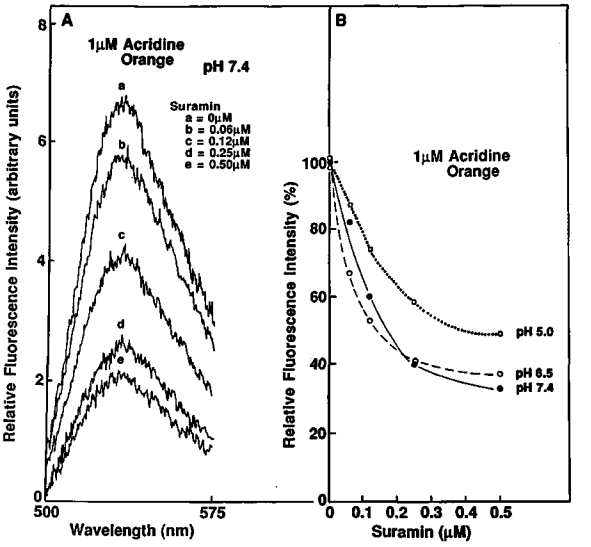

Fig. 2. Fluorescence spectra of acridine orange in the presence of various concentrations of suramin (A) and pH effect of suramin-quenching of acridine orange fluorescence (B). A: The reaction mixture contained 1 μM of acridine orange and various concentrations of suramin in 0.2 M sodium phosphate buffer (pH 7.4). The fluorescence emission of the reaction mixture was scanned from 500 nm to 575 nm using the excitation wavelength at 480 nm. The fluorescence spectrum of acridine orange revealed a peak at 530 nm. B: The reaction mixture contained 1 μM acridine orange and various concentrations of suramin in 0.2 sodium phosphate buffer, pH 7.4, 0.2 M sodium phosphate buffer, pH 6.5 or 0.2 M sodium acetate buffer, pH 5.0. The relative fluorescence intensity of the reaction mixture was measured at 530 nm of fluorescence emission. The relative fluorescence intensity of the reaction mixture without suramin was taken as 100%. Increasing concentrations of suramin diminished the fluorescence of 1 μM acridine orange, reaching a maximal at a ratio of ~1:3 (suramin:acridine orange).

To examine the subcellular localization of suramin, NIH 3T3 cells were preloaded with 1 μM of acridine orange at 37°C for 30 min. The acridine orange-preloaded cells were then treated with various concentrations of suramin at 37°C. After 1 h, the cells were then examined with a fluorescence microscope. As shown in Fig. 3, the control cells (without suramin) showed yellow to orange-red fluorescence. However, suramin treatment resulted in the disappearance of yellow to orange-red fluorescence in these cells. At 21 μM concentration, suramin almost completely abolished the yellow to or-

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Fig. 4. Reversibility of the suramin-quenching effect on the distinct fluorescence in acidic intracellular compartments of acridine orange-preloaded v-sis-transformed NIH 3T3 cells. v-sis-transformed NIH 3T3 cells were incubated with 1 μM acridine orange in DMEM at 37°C for 30 min. The acridine orange-loaded cells were then treated without (A) or with (B) 21 μM suramin in DMEM at 37°C for 1 h. The cells were then incubated in DMEM without suramin at 37°C for 1.5 h (C) and finally further incubated with 1 μM acridine orange in DMEM at 37°C for 30 min (D).

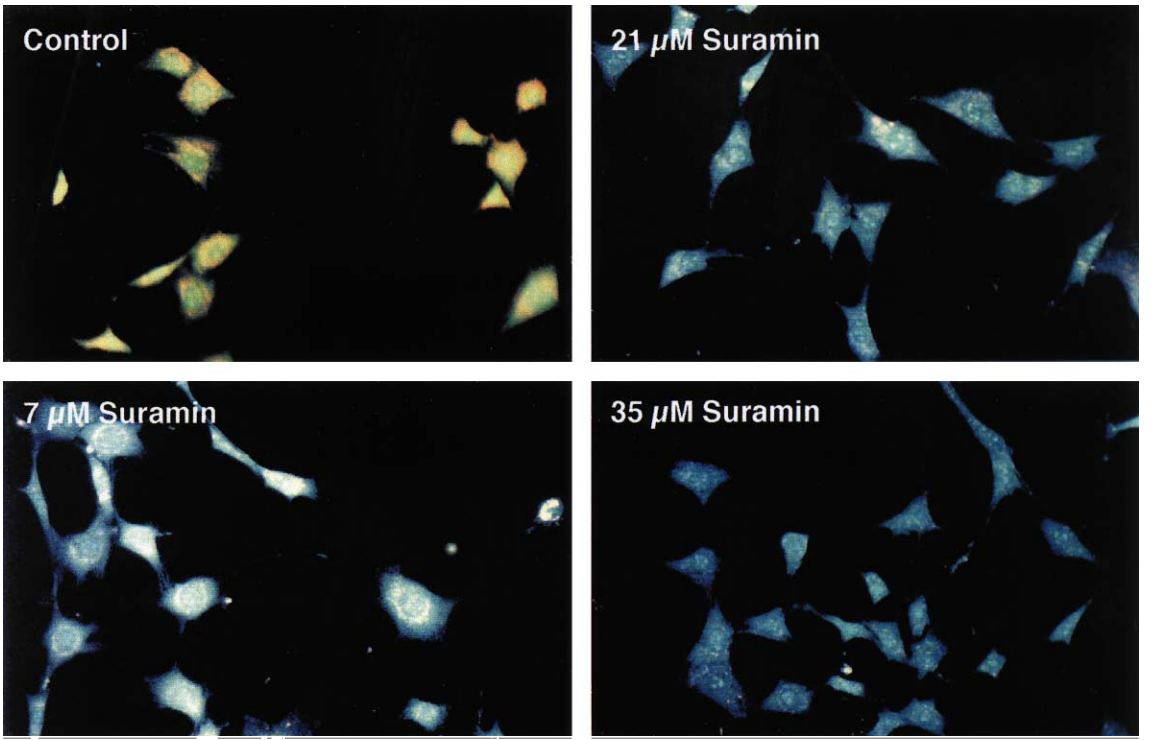
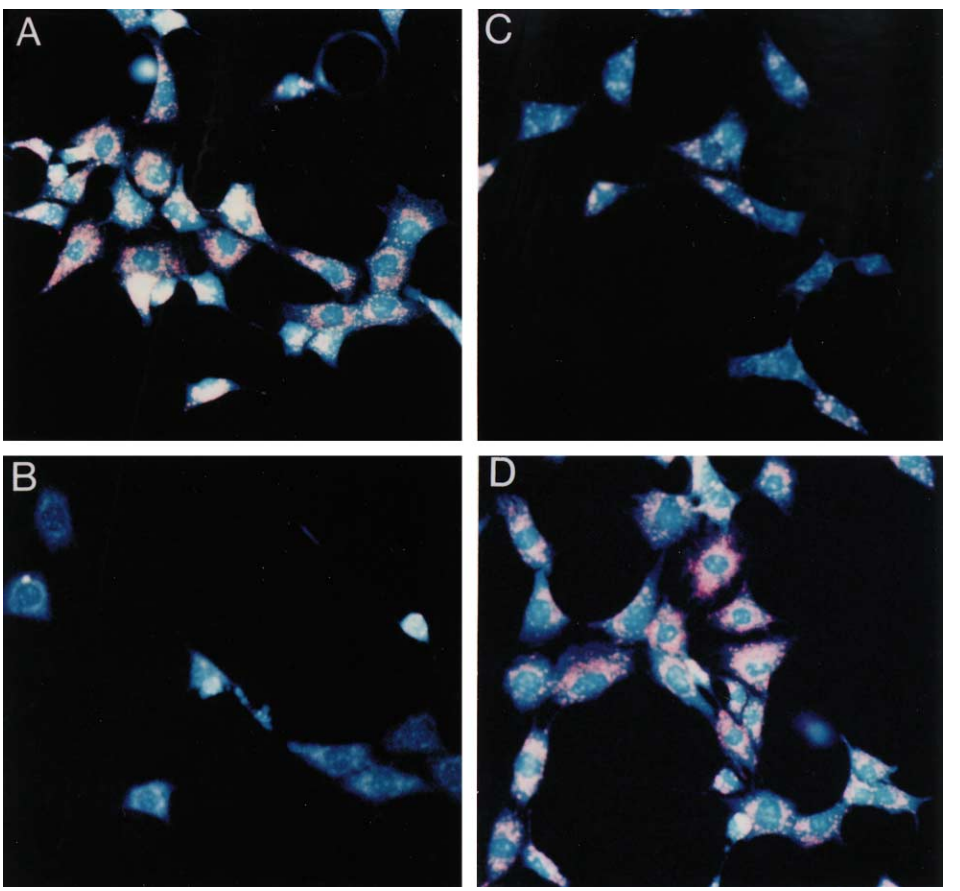


Fig. 3. Disappearance of the distinct fluorescence in acidic intracellular compartments of acridine orange-preloaded NIH 3T3 cells following treatment with suramin. NIH 3T3 cells were incubated with 1 μM acridine orange in DMEM at 37°C for 30 min. After washing, the acridine orange-loaded cells were treated with 0, 7, 21 and 35 μM of suramin in DMEM at 37°C for 1 h. The cells were then washed and photographed under a Leitz Orthoplan fluorescence microscope. The black background shown in the control (0 μM suramin) is due to color filtration, which was used to enhance the yellow to orange-red color.



ange-red fluorescence. It is of interest to note that this optimal concentration of suramin (21 μM) was very close to the concentration of suramin (25 μM) required to block the turnover of the PDGF receptor in the Golgi complex of v-sis-transformed cells [28,29]. Similar results were also observed in v-sis-transformed NIH 3T3, NRK (normal rat kidney), v-sis-transformed NRK, and Swiss mouse 3T3 cells.

The concentration of acridine orange in acidic intracellular compartments is believed to exceed 500 μM at which yellow to orange-red fluorescence is observed [31,32]. Since suramin quenches the fluorescence of acridine orange by forming a 3:1 (mole:mole) complex (Fig. 2B) and since the treatment of cells with suramin results in virtually complete abolishment of yellow to orange-red fluorescence in the acidic intracellular compartments (Fig. 3), it is estimated that the minimal concentration of suramin in these acidic compartments is >150 μM. This exceeds the concentration required for complete inhibition of the interaction between the cell-surface PDGF receptor and v-sis gene product (or PDGF) [15,16].

In order to exclude the possibility that the observed alteration in fluorescence might be due to the suramin toxicity (damage to organelles), we examined the reversibility of the suramin effect. v-sis-transformed NIH 3T3 cells preloaded with acridine orange as described above, were treated with or without 21 μM suramin. After 1 h at 37°C, suramin was removed. The cells were then incubated with medium in the absence of suramin for 1.5 h and finally reloaded with 1 μM acridine orange. As illustrated in Fig. 4B, suramin treatment abolished the orange-red fluorescence when compared with control (Fig. 4A). It is of importance to note that suramin treatment did not affect the intensity of blue fluorescence in the cytoplasm. After a 1.5 h chase in the medium without suramin, the orange-red fluorescence gradually reappeared (Fig. 4C). Reloading of acridine orange completely restored the orange-red fluorescence in these cells (Fig. 4D). These results suggest that the suramin effect is reversible and not mediated by cellular toxicity.

As previously described, the accumulation of acridine orange in intracellular organelles is dependent on their luminal pH. Suramin might raise the pH of these acidic compartments by depletion of ATP or by inhibition of the proton pump without direct entry into these organelles. We investigated this possibility by measuring the release of the lysosomal enzyme β-glucuronidase into the medium. Elevation of the pH of acidic organelles is known to cause newly synthesized lysosomal enzymes to be rerouted from the lysosomal to a secretory pathway [34]. Suramin treatment did not augment the concentration of β-glucuronidase in the medium (data not shown), suggesting that suramin did not affect the luminal pH of acidic intracellular compartments.

Suramin appears to be unable to enter cells by directly passing through hydrophobic plasma membranes because it possesses strongly hydrophilic polysulfonate groups [1]. This is supported by the observation that suramin treatment did not alter the intensity of blue fluorescence in the cytoplasm (Fig. 4). Because of its polysulfonate and polyaromatic groups, suramin can bind through electrostatic and hydrophobic interactions to a variety of proteins including membrane proteins. This suggests that suramin possibly enters the cell through endocytosis mediated by clathrin-coated and/or non-clathrin-coated vesicles following its binding to plasma membrane. Suramin is able to accumulate in the acidic intracellular compartments to reach >150 μM. At >150 μM, suramin is capable of blocking the interaction of the PDGF receptor and v-sis gene product in the trans-Golgi complex as previously reported [28,29].

This communication provides the first semiquantitative measurement of suramin accumulation in acidic intracellular compartments. These results pave the way for examination of the real-time kinetics of suramin entry into cells by optical (video camera) methodology.

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