Advances in Brief

Suramin Increases p53 Protein Levels but Does Not Activate the p53-dependent G₁ Checkpoint

Steven P. Howard, Sonia J. Park, Luke Hughes-Davies, C. Norman Coleman, and Brendan D. Price

Abstract

Suramin is an antineoplastic agent which has a cytoprotective effect on both normal and tumor-derived cells. We have investigated whether the induction of growth arrest by suramin requires the p53 protein, a tumor suppressor gene product involved in the initiation of growth arrest following DNA damage. Activation of the p53 protein by genotoxic agents causes increased p53 protein levels and p53-dependent transcription of the p21 gene. The p21 protein then inhibits cyclin-dependent kinases, initiating G₁ arrest. Exposure of NIH-3T3 cells to suramin caused a rapid (1-2 h) increase in the level of p53-DNA-binding activity. Flow cytometric analysis indicated that suramin arrested NIH-3T3 cells in G₀-G₁. However, suramin did not increase the p53-dependent transcription of the p21 gene or inhibit cyclin-dependent kinase 2 kinase activity. If NIH-3T3 cells were exposed to radiation or suramin plus radiation, p21 mRNA levels were increased and cyclin-dependent kinase 2 kinase activity was inhibited, indicating that suramin does not block the cells’ ability to increase p21 levels. To determine whether the G₀-G₁ arrest induced by suramin required p53, NIH-3T3 cells transfected with a dominant negative mutant p53 gene do not undergo radiation-induced G₁ arrest, but instead proceed on to G₂ before arresting (22, 23). The induction of G₁ arrest by p53 may afford the cell time to repair DNA damage before proceeding to DNA synthesis and cell division (18-21). These cell cycle checkpoints allow the cell to monitor the integrity of the genome before committing to either DNA synthesis or cell division (18-21). It is now clear that the G₁ checkpoint is controlled by the wtp53 protein (22-26). Irradiation of human cells induces G₁ arrest (19, 20) and increases the level of wtp53 protein in the cell (22, 24-26). Cells which have a mutation or deletion in the p53 gene do not undergo radiation-induced G₁ arrest, but instead proceed on to G₂ before arresting (22, 23). The induction of G₁ arrest by p53 may afford the cell time to repair DNA damage before proceeding to DNA synthesis and cell division (21). The failure of cells with wt p53 to undergo G₁ arrest may cause point mutations and chromosomal deletions to be incorporated into the genome during DNA replication and division (18, 22, 23). The loss of wt p53 function in human malignancies may therefore be a key step in the progression of human cancer.

We have previously shown that a range of chemotherapeutic agents are able to activate the p53 response and initiate effect on tumor cells (3-6). The mechanism by which suramin exerts its antineoplastic effects is poorly understood, in part because of the wide range of cellular processes that it inhibits. For example, suramin antagonizes the mitogenic actions of epidermal growth factor (7), platelet-derived growth factor (8), insulin-like growth factor I (9), and serum (7), leading to cell cycle arrest in vitro. In HeLa cells, suramin inhibits both DNA synthesis and DNA polymerase activity (10). Mitochondrial toxicity following exposure to suramin disrupts the cellular energy balance (11). Suramin can competitively inhibit PKC, an enzyme intimately involved in the regulation of cellular signaling events (12, 13). Suramin can also alter the metabolism of phosphoinositides (14) and may block increases in cellular Ca²⁺ following growth factor stimulation of cells (15). Another report indicates that suramin may inhibit topoisomerase II, which is required for the replication of DNA (16).

This wide range of biological targets for suramin indicates that the antineoplastic activity of the drug may be exerted at multiple cellular sites and may vary according to cell type. The exact mechanism by which suramin exerts its antiproliferative and antineoplastic effects is therefore unclear. We have recently demonstrated that suramin sensitizes human prostatic epithelium to radiation-induced DNA damage in a manner independent of cellular proliferation (17). This suggests that suramin may be interacting with the DNA damage detection pathways in the cell. In the present study, to further evaluate the interactions between DNA damage and suramin, we sought to examine the mechanism by which suramin induces cellular growth arrest. Many chemotherapeutic agents target the DNA of tumor cells, causing lesions in the genomic DNA. Cells respond to DNA damage by pausing at the G₁ or G₂ stages (18-20). These cell cycle checkpoints allow the cell to monitor the integrity of the genomic DNA before committing to either DNA synthesis or cell division (18-21). It is now clear that the G₁ checkpoint is controlled by the wtp53 protein (22-26). Irradiation of human cells induces G₁ arrest (19, 20) and increases the level of wtp53 protein in the cell (22, 24-26). Cells which have a mutation or deletion in the p53 gene do not undergo radiation-induced G₁ arrest, but instead proceed on to G₂ before arresting (22, 23). The induction of G₁ arrest by p53 may afford the cell time to repair DNA damage before proceeding to DNA synthesis and cell division (21). The failure of cells with wt p53 to undergo G₁ arrest may cause point mutations and chromosomal deletions to be incorporated into the genome during DNA replication and division (18, 22, 23). The loss of wt p53 function in human malignancies may therefore be a key step in the progression of human cancer.

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growth arrest (25). In the current study, we investigated whether suramin’s ability to induce growth arrest was mediated by the p53 protein. The data indicate that suramin activates the p53 response and induces G1 arrest in NIH-3T3 cells, but that the growth arrest mediated by suramin does not require functional p53. In addition, the activation of p53 by suramin does not lead to the activation of the normal down stream effectors of the p53 protein.

Materials and Methods

Cells. NIH-3T3 mouse fibroblasts were cultured in DMEM with 10% calf serum and antibiotics (Hyclone, Los Angeles, CA). Cells were used when 80% confluent. Suramin (Mobay Chemical Corporation, New York, NY) was prepared fresh each day in culture medium. Radiation was delivered using a 137Cs irradiator at a dose rate of 100 eGy/min.

Electrophoretic Gel Mobility Shift Assays. The EMSA was carried out as previously described (26). EMSA reactions contained 32P-p53 oligonucleotide (0.5 ng, GGACACGGCCGCGCCATGTCC; Ref. 27), nuclear extract (20 μg), and sonicated salmon sperm DNA (0.2 μg; Sigma Chemical, St. Louis, MO) in 25 μl (final volume) binding buffer [20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.6), 20% glycerol, 10 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 1 mM DTT, and 1 mM phenylmethylsulfonyl fluoride]. Antibody pAb421 (Oncogene Science, Manhasset, NY) was used at 100 ng/assay. Binding assays were incubated for 20 min at 25°C and separated on 4% nondenaturing polyacrylamide gels as previously described (26).

p53 Mutant Cell Lines. NIH-3T3 cells were transfected by calcium phosphate precipitation with 5 μg pC53-SCX3 (containing a valine to alanine substitution at codon 143; Ref. 28) or wild type p53 cDNA. Cells were trypsinized, replated at 1:5-1:20 dilution, and exposed to G418 (250 μg/ml) for 12 days. Surviving colonies were trypsinized, pooled, and tested for expression of mutant p53 using the 2-kb CR1 probe as previously described (26).

Immunoprecipitation and Western Blot Analysis. Preparation of cell extracts and immunoprecipitation (with p53 antibody Ab421) were carried out as previously described (26). For Western blotting, cell extracts (5 μg; prepared as in Ref. 26) were separated by SDS-PAGE, transferred to Immobilon polyvinylidene difluoride membranes (Millipore Corp., Bedford, MA), and blocked in 10% dried milk. p53 was detected with p53 antibody pAb421. Antigens were detected using a coupled biotin-avidin immunoenzyme method (Vector Laboratories, Burlingame, CA).

Cell Cycle Distributions. Flow cytometric analysis of total DNA content and the simultaneous detection of BrdUrd incorporation is described elsewhere (29). NIH-3T3 cells were pulse labeled with BrdUrd (10 μM for 20 min), trypsinized, and resuspended in PBS containing 10% serum. Cells were collected by centrifugation, resuspended in PBS, and fixed with 70% ethanol/0.5% Tween 20. Nuclei were isolated by centrifugation, and the DNA was denatured by digesting for 30 min with pepsin (0.04% in 0.1 M HCl) followed by incubation with 2 M HCl at 37°C for 30 min. Nuclei were washed in sodium borate (0.1 M), resuspended in PBS containing goat serum (0.1%)/Tween 20 (0.5%), then incubated with anti-BrdUrd monoclonal antibody (Becton Dickinson, Mountain View, CA) for 30 min at 37°C. Nuclei were washed with PBS-TB (PBS containing 0.1% BSA and 0.5% Tween 20) and incubated with FITC-conjugated antimouse IgG for 30 min at 37°C. Nuclei were washed in PBS-TB and resuspended in PBS-TB containing 10 μg/ml propidium iodide and 10 μg/ml RNase. Flow cytometric analyses of the nuclei were performed using a FACScan (Becton Dickinson); nuclei were excited at 488 nm. Gated data were acquired and analyzed using LYYSIS II software (Becton Dickinson).

cdk2 Kinase Assays. NIH-3T3 cells were solubilized in 400 μl lysis buffer [50 mM Tris (pH 7.4), 0.15 M NaCl, 0.5% NP40, 2 mM EDTA, 20 μg/ml aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride, 50 mM NaN3, and 100 μM sodium o-vanadate], and cell extracts were prepared as described elsewhere (30, 31). Cell extract (150 μg) and cdk2 antibody M-2 (2 μg; Santa Cruz Biotech, Santa Cruz, CA) were incubated in lysis buffer (500-μl final volume) for 2 h at 4°C. Immune complexes were collected on protein A-Sepharose beads and washed in lysis buffer, followed by two washes in 0.5 ml kinase buffer [50 mM Tris (pH 7.6, 10 mM MgCl2, and 1 mM DTT)]. The remaining liquid was removed with a hypodermic needle and 35 μl kinase buffer containing ATP (0.7 μM), [γ-32p]ATP (10 μCi), and histone H1 (2 μg) were added. After 20 min at 37°C, reactions were terminated, and the phosphorylated histone H1 substrate was separated using SDS-PAGE. Histone H1 bands were cut from the polyacrylamide gel, and the amount of radioactive incorporation was assessed by liquid scintillation counting.

Northern Blot Analysis. Total cellular RNA was prepared using the method of Chirgwin et al. (32). RNA (10 μg) was separated on 1% agarose/2.2 M formaldehyde gels, transferred to nylon membranes, and probed as previously described (33) using an oligonucleotide complementary to amino acids 111-120 (TCAGACCAAGATGCAAGACAGCGACAA) of the mouse p21 cDNA clone (34), which specifically detects the 2-kb p21 mRNA. Equal loading was confirmed by measuring the concentration of RNA (absorbance, 260 nm) and by staining the agarose gels with ethidium bromide and assessing the intensity of the 18S and 28S RNA bands. Membranes were washed in 0.2 × SSC (33) at 56°C for 30 min.

Results

Many chemotherapeutic agents increase cellular p53 protein levels, which in turn induces a p53-dependent G1 arrest (22, 23). Accordingly, we investigated whether the antineoplastic agent suramin may exert its antiproliferative effects through modulation of cellular p53 levels. Murine NIH-3T3 fibroblasts were used since they have a well-defined cell cycle and possess wt/p53 alleles (25, 26). Asynchronously growing NIH-3T3 cells were exposed to suramin, and the levels of p53-DNA-binding activity were assessed using an EMSA. Low levels of p53-DNA-binding activity were detected in untreated NIH-3T3 cells (Fig. 1A, Lane 0). Cells exposed to <100 μg/ml suramin showed no significant increase in p53-DNA-binding activity.
Fig. 1 Suramin increases the level of p53-DNA-binding activity in NIH-3T3 cells. A, NIH-3T3 cells were exposed to increasing concentrations of suramin (50–800 μg/ml) for 2 h. Cells were harvested, and nuclear extracts were prepared for EMSA as described in "Materials and Methods." Control cells (○) were not treated with suramin. B, NIH-3T3 cells were incubated with suramin (200 μg/ml) for 1 to 12 h, followed by preparation of nuclear extracts and analysis using an EMSA. Cells at time 0 (○) were not exposed to suramin.

However, suramin concentrations above 200 μg/ml increased the levels of p53-DNA-binding activity, with maximum activation occurring at 800 μg/ml. Above 800 μg/ml, suramin begins to exhibit toxicity toward NIH-3T3 cells. Subsequent experiments were carried out at 200 μg/ml suramin. In Fig. 1B, NIH-3T3 cells were exposed to suramin for the indicated times. Increased p53-DNA-binding activity can be detected after a 1-h exposure to suramin. Maximum activation occurred by 4–6 h, and this was sustained for at least 10–12 h during continuous exposure to suramin. Fig. 1 demonstrates that suramin induces a rapid sustained increase in the levels of p53-DNA-binding activity in NIH-3T3 cells. In NIH-3T3 cells, increased p53-DNA-binding activity can also be detected when the cells are exposed to DNA-damaging agents, including X-ray irradiation (26).

In Fig. 2, we investigated whether the levels of p53 detected after exposure to suramin were similar to those seen following X-ray irradiation. p53 levels were monitored by measuring the rate of [35S]methionine incorporation into immunoprecipitable p53. In Fig. 2A, low levels of methionine-labeled p53 were detected in control cells (Lane C). Both suramin-treated (Fig. 2A, Lane S) and irradiated cells (Fig. 2A, Lane XR) showed increased levels of methionine-labeled p53 protein, although suramin treatment yielded a much larger accumulation of 35S-labeled p53. The effects of suramin and radiation appeared to be additive, since irradiation of suramin-treated cells increased the labeling of the p53 protein above that seen with suramin alone (Fig. 2A, Lane S + XR). In Fig. 2B, the levels of p53-DNA-binding activity detected after suramin treatment (Lane S) are greater than those detected following irradiation (Lane XR). When suramin-treated cells were irradiated (Fig. 2B, Lane S + XR), the level of p53-DNA-binding activity was greater than that seen with suramin alone (Fig. 2B, Lane S). Fig. 2 indicates that suramin is an effective activator of the p53 response, giving rise to higher cellular levels of p53 than those seen after irradiation. In addition, the effects of suramin and radiation appeared to be additive.

Increased p53 levels transcriptionally activate the p21 gene, which encodes a specific inhibitor of cdk kinases (30, 34, 35). Since cdk2 kinase activity is required for entry into S-phase (36), inhibition by the p21 protein leads to G1 arrest. Because suramin induces p53 and causes growth arrest (7–9), we investigated whether the activation of p53 by suramin leads to the inhibition of cdk2 kinase and increased transcription of the p21 gene. The effect of suramin on cdk2 kinase activity was measured by immunoprecipitation of cdk2 and monitoring the ability of cdk2 to phosphorylate histone H1. In Fig. 3, NIH-3T3 cells were irradiated, and the rate of histone H1 phosphorylation was followed over the next 6 h (○). cdk2 kinase activity was rapidly decreased following irradiation, reaching a minimum at 1–2 h, followed by a slow recovery to normal levels by 6 h. In contrast, cells exposed to suramin showed only a small decline in cdk2 kinase activity during a 6-h suramin exposure (◇), despite the induction of p53 within 1 h of suramin addition (Fig. 1B). To ensure that the effects of suramin were not due to a nonspecific effect such as inhibition of transcription or translation, cells were incubated with suramin, and then irradiated (Fig. 3, △). Cells exposed to suramin plus radiation exhibited a prompt
decrease in cdk2 activity, reaching a minimum value 1 h after treatment, which was virtually indistinguishable from that seen with radiation alone (Fig. 3, ○). This indicates that suramin does not block the ability of radiation to inhibit cdk2 kinase.

In Fig. 4, we investigated whether suramin was able to increase the levels of p21 mRNA. NIH-3T3 cells were exposed to suramin for the indicated times, and the amount of p21 mRNA was measured using Northern blotting. In Fig. 4A, cells exposed to suramin showed no increase in p21 mRNA levels during a 4-h incubation, which is in agreement with the data indicating that cdk2 kinase activity is unaffected by suramin (Fig. 3). If the length of incubation with suramin was extended for up to 12 h, no accumulation of p21 mRNA was observed (data not shown). In contrast, irradiated cells (Fig. 4A, 5 Gy) had increased p21 mRNA levels at 1 h and 2 h after irradiation, and these had returned to basal levels by 4 h. These changes in the levels of p21 mRNA are consistent with the rapid inhibition and recovery of cdk2 kinase activity following irradiation (Fig. 3). When cells were treated with suramin and then irradiated (Fig. 4, Sur + 5 Gy), p21 mRNA levels still increased, although there was a slight delay in the peak of p21 mRNA accumulation compared to radiation alone. Because suramin-treated cells still respond to radiation by induction of the p21 mRNA and cdk2 inhibition, it is unlikely that suramin’s effect is due to inhibition of the transcriptional or translational apparatus.

To determine whether the induction of p53 by suramin is required for the initiation of growth arrest by suramin, NIH-3T3 cells were stably transfected with a mtp53 gene as described in “Materials and Methods.” Transforming mtp53 is dominant over endogenous wtp53 and results in functional inactivation of the cellular p53 response and loss of the DNA damage-induced G1 arrest (22, 23, 24). In Fig. 4B, NIH-3T3 cells (NIH), NIH-3T3 cells bearing only the neomycin selection plasmid (NNC) and NIH-3T3 cells transfected with a mtp53 expression vector (NMP) were analyzed using Western blotting to detect p53 protein. Only the NMP cells, transfected with the mtp53 expression vector, displayed significant levels of the Mr 53,000 p53 protein. These NMP and NIH cells were then compared to determine their response to DNA damage. In Table 1, the effect of radiation on the cell cycle distribution of asynchronously growing NIH-3T3 cells was determined. Irradiation increased the percentage of NIH-3T3 cells in G2-M, indicating a DNA damage-induced G2 block. In addition, there was a decrease in the percentage of S-phase cells, but no decrease in the number of cells in G1, indicating that the cells were also arrested in G1. NMP cells, expressing mtp53, had a larger percentage of cells in

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**Fig. 3** cdk2 kinase activity in cells exposed to radiation and suramin. NIH-3T3 cell extracts were immunoprecipitated with anti-cdk2 antibody. The washed immune complexes were then allowed to phosphor-ylate histone H1, and the amount of radioactive phosphate incorporation was measured as described in “Materials and Methods.” Results are expressed as cpm/50 µg protein/20 min. ○, suramin (200 µg/ml); ●, irradiated (6 Gy); △, suramin plus radiation.

**Fig. 4** Northern blot analysis of p21 mRNA levels in NIH-3T3 cells and Western blot analysis of transfected NIH-3T3 cells. A, NIH-3T3 cells were either exposed to suramin (Sur; 200 µg/ml), X-ray radiation (5 Gy), or both of these agents simultaneously (Sur + 5 Gy). RNA was prepared at the indicated time (in h), separated on agarose gels, transferred to nylon membranes, and probed for p21 mRNA levels as described in “Materials and Methods.” B, NIH-3T3 cells were transfected with pCMVneo or pC53-SCX3 (mtp53), as described in “Materials and Methods,” and stable cell lines were prepared. Extracts from equal numbers of cells were prepared, separated by SDS-PAGE, and transferred to Immobilon membranes. Western blot analysis was carried out using Ab421 to detect the mtp53 protein. NIH, normal NIH-3T3 cells; NNC, NIH-3T3 cells bearing the neomycin selection marker; NMP, NIH-3T3 cells bearing a mtp53 gene.
the S-phase than NIH cells, but fewer cells in G1. After irradiation of NMP cells, there was an accumulation of cells in G2-M, indicating that the G2-M block, which is independent of p53, remained intact (Table 1, NMP). However, the percentage of NMP cells in G1 and the S-phase was decreased after irradiation, indicating that NMP cells were continuing to progress from G1 into the S-phase. The absence of a G1 block in NMP cells is consistent with the absence of functional p53 protein in NMP cells. Therefore, the NMP cells have lost the G1 checkpoint, as has been demonstrated previously for other cell types expressing mtp53 proteins (22, 23). To determine whether the induction of p53 by suramin could induce growth arrest, asynchronously growing NIH-3T3 cells were treated with suramin. The position of the cells in the cell cycle was then determined by pulse labeling with BrdUrd followed by staining with propidium iodide as described in "Materials and Methods." In untreated NIH-3T3 cells (Fig. 5A), the percentage of cells in G1, S-phase, and G2-M remained relatively constant over the 13 h time course of the experiment. In Fig. 5B, NIH-3T3 cells continuously exposed to suramin began to accumulate in G0 or G1 after 3–4 h of exposure. We refer to this as G0-G1 arrest since we have not determined the precise point in the cell cycle that suramin arrests cells. The percentage of cells in G0-G1 became increasingly more pronounced with increasing length of exposure to suramin until a majority (approximately 75%) was arrested in G0-G1 by 13 h. Suramin appeared to cause a selective G0-G1 arrest, since cells did not accumulate in either S-phase or G2-M. Similar results were obtained when NNC cells (bearing only the neomycin selection marker) were exposed to suramin (data not shown). To determine whether suramin-induced growth arrest was dependent on wtp53, NMP cells, which lack the p53-dependent G1 checkpoint (Table 1), were examined to determine whether suramin would induce growth arrest. In untreated cultures of NMP cells, the percentage of cells in G1, S-phase, and G2-M phase were unchanged throughout the experiment, although the NMP cells had a higher percentage of cells in G1 than the parental NIH-3T3 cell line (Fig. 5C). When NMP cells were exposed to suramin, there was an increase in the percentage of cells in G1 (Fig. 5D), with at least 85% in G0-G1 after 13-h exposure to suramin, which was similar to that seen in suramin-treated NIH-3T3 cells (Fig. 5B). This indicates that suramin-induced growth arrest is independent of wtp53.

Discussion

The results demonstrate that suramin induces a sustained increase in wtp53 protein levels in NIH-3T3 cells. However, the induction of p53 by suramin does not lead to the activation of the downstream effectors of p53. Suramin treatment of NIH-3T3 cells did not increase the level of p21 mRNA or cause inhibition of cdk2 kinase, although suramin caused higher levels of p53 accumulation than ionizing radiation (Fig. 2). When NIH-3T3 cells were exposed to suramin, they became arrested in G0-G1, with >75% being in this phase within 13 h of suramin addition. If wtp53 function was abolished by overexpression of mtp53 protein, the ability of suramin to induce G0-G1 arrest was unaffected. This is the first report, to our knowledge, of an antineoplastic agent that can increase p53 protein levels but does not activate the associated downstream effectors.

When cells are exposed to DNA damage, including ionizing radiation, p53 levels are increased, and the p53 protein transcriptionally activates the p21 gene (30, 34, 35). The p21 gene product binds to and inhibits cdk2 kinase, causing the cells to arrest at the G1-S boundary (35). Suramin-induced p53 exhibited DNA-binding activity in the in vitro EMSA assay, which was indistinguishable from radiation-induced p53 (Figs. 1 and 2). However, suramin did not increase p21 mRNA levels or inhibit cdk2 kinase activity (Figs. 3 and 4). Suramin has a wide range of biochemical effects on cells, including alterations in the cellular energy balance (11) and inhibition of topoisomerase II, an enzyme involved in the unwinding of DNA (16). Inhibitors of topoisomerase II can stabilize the cleaveable complex formed between topoisomerase II and DNA, leading to the formation of single-strand breaks (25, 37), which are thought to be the signal for p53 activation (25). Suramin inhibition of topoisomerase II does not lead to the formation of a cleaveable complex (16), suggesting that suramin does not induce p53 through topoisomerase II-induced strand breaks. This does not exclude the possibility that suramin may cause DNA damage by a separate

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* Asynchronously growing NIH and NMP cells were either untreated (control) or irradiated (5 Gy). Two-hundred min after irradiation, cells were collected and analyzed using flow cytometry to determine the cell cycle distribution. The percentage of cells in each phase is indicated.
Suramin Induces the p53 Protein

in cell cycle distribution (7–9). In G0-arrested murine fibroblast cells, the p53 levels are minimal, but increase as cells progress through G1 and S-phase, before decreasing during G2-M (38).

However, increased p53 levels can be detected within 1 h of suramin addition, which precedes the onset of G0-G1 arrest (Figs. 1 and 5). In addition, serum starvation of NIH-3T3 cells, which arrests the cells in G0, does not increase the p53 levels (data not shown), indicating that the changes in the p53 levels seen after suramin treatment cannot be accounted for by changes in cell cycle distribution.

Analysis of the cell cycle distribution of suramin-treated NIH-3T3 cells indicates that the cells are arrested in G0 or G1. Studies in the LNCaP (39) and DU-145 (40) human prostate cell lines and in primary cultures of normal human prostatic epithelium (17) indicate that suramin causes G1-G0 arrest. However, suramin treatment of the MCF-7 human breast cancer cell line (41) and the PC-3 human prostate cell line (42) appears to result in a selective G2-M arrest. In addition, the magnitude of the observed growth arrest could be modulated by manipulating components of the culture medium (42). Hence, suramin can cause cell cycle arrest at either G1-G0 or G2-M, depending on the cell type and growth conditions.

Suramin can antagonize the action of growth factors which are required for NIH-3T3 cells to progress through the cell cycle, including platelet-derived growth factor (1, 7–9). The onset of G1-G0 arrest by suramin may reflect the loss of growth factor action in these cultures, causing the cells to remain in G0.

Suramin also arrested NMP cells (expressing mtpt53) in G1-G0, indicating that the growth arrest properties of suramin are independent of p53 status. Suramin therefore exerts its cytostatic effects independently of its ability to induce the p53 protein. p21 is transcriptionally induced by the p53 gene product and mediates the observed G1 arrest associated with DNA-damaging agents (30, 34, 35). Other cyclin-dependent kinase inhibitors function independently of the p53 suppressor gene. The p27 protein participates in the growth arrest following serum deprivation, contact inhibition, or exposure to transforming growth factor β (43, 44). p15, p16, p18, and p19 (45–48) induce cell cycle arrest by competing with D-type cyclins for binding to the appropriate cyclin-dependent kinase. All of these p53-independent pathways of cell cycle regulation represent potential target sites for suramin-induced growth arrest. Interestingly, suramin does not appear to inhibit either DNA synthesis or mitosis in NIH-3T3 cells, since suramin-treated cells already in S-phase progressed through mitosis before arresting in G1-G0 (Fig. 5).

This is in contrast to studies showing that suramin can inhibit the activity of DNA polymerases (10). The most likely explanation for suramin-induced growth arrest is the blockade of growth factor action, preventing the cells from reinitiating cell cycle progression. The increase in p53-DNA-binding activity seen with suramin may therefore have an alternative function than the activation of p21 transcription and initiation of G1 arrest. The p53 gene product can initiate the apoptotic cell death seen after radiation exposure (49). Suramin can potentiate radiation-induced apoptosis (3), suggesting a possible interaction with other p53 target genes involved in the apoptotic pathway.

Irradiation of suramin-treated cells increased p21 mRNA levels and inhibited cdk2 activity, indicating that the p53-dependent G1 checkpoint is still intact after suramin treatment.
This observation may be clinically important since the integration of cytostatic agents such as suramin with fractionated radiation therapy is currently being evaluated (50). In both in vitro (51) and animal solid tumor systems (52), the loss of a functional p53 response has been shown to disrupt an important apoptosis pathway, resulting in refractoriness to standard antineoplastic therapies. In prostate cell lines, prior treatment with suramin decreased radiation-induced cell death, whereas exposure to suramin after radiation increased the cytotoxic effects of radiation (3). These results are in direct contrast to our own observations using cultured normal human prostatic epithelium (17). In this model system, pretreatment with suramin resulted in G1 arrest and increased radiosensitivity. The reasons for these discrepancies are unclear. However, these data suggest that a combination of the cytostatic effect of suramin with radiation could potentially yield a beneficial therapeutic effect in cancer therapy.

Why suramin induces a transcriptionally inactive p53 protein is unclear. DNA damage causes G1 and G2 arrest in cells (20, 22). Suramin does not appear to activate the G1 or G2 checkpoints, suggesting that DNA damage is not the primary signal for suramin induction of p53. One potential mechanism by which suramin may increase p53 could involve modulation of PKC activity. We have previously shown that activation of PKC by phorbol esters blocks the increase in p53 protein seen after DNA damage (26), and it has been demonstrated that suramin can inhibit PKC (12, 13, 53). Inhibition of PKC by suramin may relieve the inhibitory effects of this kinase on p53 levels, causing accumulation of the p53 protein. Suramin may therefore allow accumulation of p53 in the cell by an indirect mechanism that does not involve DNA damage. Alternatively, the activation of p53 following DNA damage involves a number of steps, including stabilization of the p53 protein (25, 26), and may include alterations in the phosphorylation status of p53. Many of the kinases responsible for p53 phosphorylation are regulated by DNA damage, including cdk2 kinase (31, 54), mitogen-activated protein kinase (55), and the double-strand DNA kinase (56). Suramin may fail to activate these kinase pathways, resulting in accumulation of p53 in a form unable to activate transcription of the p21 gene. Suramin may provide important functional information on the regulation of the p53 protein levels and the activation of p53-dependent transcription.

Acknowledgments

We thank Dr. Roy Tishler for his helpful discussions and thoughtful review of the manuscript.

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Suramin increases p53 protein levels but does not activate the p53-dependent G1 checkpoint.

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