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Original Paper

Intracellular Localisation of Suramin, an Anticancer Drug, in Human Colon Adenocarcinoma Cells: a Study by Quantitative Autoradiography

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Suramin is a polysulphonated naphthylurea currently investigated for the treatment of advanced malignancy. In the present study, we have analysed the uptake and the intracellular localisation of tritiated suramin in human colon adenocarcinoma cells (HT-29-D4), using quantitative autoradiographic techniques at the optical and electron microscopy levels. Our results show that the drug is able to enter both undifferentiated and differentiated HT-29-D4 cells. The process of suramin uptake is time-dependent, and significantly inhibited by the presence of the suramin-binding protein serum albumin in the culture medium of HT-29-D4 cells. Autoradiographic analysis revealed two distinct patterns of intracellular localisation of tritiated suramin labelling, according to the presence or absence of serum albumin. Indeed, in the absence of serum albumin, the labelling of free suramin was distributed over the nucleus, the Golgi apparatus and the mitochondria, while it was restricted to the lysosomal system when suramin was complexed with albumin. These data show that a serum factor, i.e. albumin, influences the biological activity of suramin by determining its intracellular localisation. The presence of suramin in a given compartment may account for specific effects of the drug including mitochondrial hypertrophy, altered gene expression and lysosomal perturbation.

Key words: transmission electron microscopy, quantitative autoradiography, lysosomal storage disorder, mitochondrial hypertrophy, HT-29-D4 cells
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INTRODUCTION

SURAMIN IS a polysulphonated naphthylurea which has been used since 1924 for the treatment of some parasitic diseases including trypanosomiasis and onchocerciasis [1]. In the late 1970s, it was discovered that the drug was a potent inhibitor of reverse transcriptases from several animal retroviruses [2]. Due to this property, suramin was the first molecule evaluated for antiviral therapy in AIDS patients [3]. Unfortunately, despite some clinical response in several patients, the assays were rapidly abandoned because of adverse side-effects [4]. Curiously, it is one of these toxic effects at the level of the adrenal cortex that gave Myers and associates the idea to evaluate suramin in patients with adrenal cortex carcinoma [5]. Seventy years after its synthesis, suramin has thus gained renewed interest for its anti-tumoral properties [6]. However, the clinical improvement achieved by suramin is still associated with undesirable side-effects. Among these side-effects, suramin elicits *in vivo* and *in vitro* a marked perturbation of the lysosomal system which is similar to that observed in patients with inherited mucopolysaccharidosis [7–11]. Interestingly, a similar disorder occurred upon treatment of the human colon cancer cell clone HT-29-D4 with suramin *in vitro* [12]. Thus, this clonal cell line represents a valuable model for studying the lysosomotropic effect of suramin in tumour cells [13]. The suramin-induced lysosomal storage disorder in these cells depends on the presence of serum albumin in the culture medium, and is not observed when the cells are grown in serum-free medium [14].

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Plasma binding can be an important determinant of the pharmacokinetic behaviour of therapeutic drugs [15]. In the case of most drugs, it is albumin which dominates this binding phenomenon. Suramin is known to form stable complexes with serum albumin [16–18], and there is no doubt that the binding of suramin to this protein is an important factor for both the pharmacological effects and the pharmacokinetics of the drug [18]. Among the unsolved questions concerning the interaction of suramin with cells is the problem of suramin entry. Suramin has long been considered to be an impermeant compound, due to its anionic structure and to its analogy with trypan blue [19]. Recent data from our group suggested that suramin was able to enter polarised HT-29-D4 cells in the presence of serum albumin [20]. In the present report, we have studied the time dependency [^{3}H]suramin uptake in both undifferentiated and differentiated HT-29-D4 cells in the presence or absence of albumin. By using quantitative autoradiographic methods at the optical and electron microscopy level, we demonstrate for the first time that suramin does enter the cells, and that serum albumin determines the intracellular localisation of the drug in HT-29-D4 cells.

MATERIALS AND METHODS

Cell culture

HT-29-D4 cells were obtained by cloning the parental HT-29 cell line (ATCC HTB38) by limit dilution techniques [21, 22]. HT-29-D4 cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose and 10% fetal calf serum (FCS). The cells grown under these conditions were undifferentiated [21] and are referred to as HT-29-D4/Glu cells throughout this study. To induce differentiation, HT-29-D4/Glu cells were changed to glucose-free DMEM containing 5 mM galactose and 10% dialysed FCS as previously described [22]. After 12 days of culture in this medium, the cells (referred to as HT-29-D4/Gal cells) formed an epithelial monolayer possessing electrophysiological properties (transepithelial resistance of 200–400 ohm cm^2) [12].

Incubation of HT-29-D4 cells with [^{3}H]suramin

HT-29-D4/Glu or Gal cells were cultured in their respective medium for 12 days, then incubated with 20μCi/ml of uniformly labelled [^{3}H]suramin (Moravek Biochemicals, Inc., 9 Ci/mmol) in serum-free DMEM containing 20 μg/ml cold suramin for 6 days. The suramin-containing medium was changed daily. Under these conditions, suramin inhibited the growth of HT-29-D4 cells by 37% as assessed by counting the cells at the end of suramin incubation. In some experiments, bovine serum albumin (BSA, Sigma, Cell Culture Grade endotoxin-free) was added to the serum-free DMEM at a concentration of 1%. At the end of incubation, cells were processed for light and electron microscopy. For the uptake studies, the cells were incubated with [^{3}H]suramin as described above. At different times (i.e. after 1, 24, 48 and 96 h) the cells were lysed in 0.1 M NaOH and the radioactivity determined in a Beckman β counter.

[^{3}H]suramin specificity

The purity of [^{3}H]suramin was assessed by high performance liquid chromatography as previously described [23], and was found greater than 99%. A 250 × 4.6 mm Spherisorb C18 ODS2 column (Interchim, Montluçon, France) was used throughout. The mobile phase consisted of a mixture of 46% methanol, 5% acetonitrile and 49% of tetrabutylammonium phosphate 5 mM. Binding of [^{3}H]suramin to BSA immobilised on 96-multiwell plates was performed according to Yahi and associates [24]. [^{3}H]suramin binding was specifically displaced by increasing concentrations of unlabelled suramin, with a $K_{0.5}$ of 7.8 μM.

Light microscopy

The cells were fixed *in situ* with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 1 h at 20°C, washed for 10 min in the same buffer with 6.84% saccharose and post-fixed for 1 h at room temperature in 2% osmium tetroxide. After extensive rinses (six times/1 h) in 0.05 M maleate buffer (pH 5.2), the cells were "block stained" with 1.8% uranyl acetate in 0.05 M maleate buffer (pH 4.45) for 1 h at 4°C. Finally, blocks were dehydrated and embedded in Epon. Semi-thin sections were cut perpendicularly to the monolayer at 10 successive levels separated by approximately 20 μm. Sections for each level were collected on to the same slide. These were dipped into Kodak NTB2 emulsion, exposed for 2 months, and developed in Kodak D19b. Sections were stained with toluidine blue prior to examiantion.

Quantitative analysis was performed by visual counting using ×100 objective. The cellular layer was divided into five compartments. The volume density of each compartment was evaluated by stereology using a double square lattice test system C 64 [25] superimposed on the microscope field, and the grain number falling on each compartment was recorded. Microscope fields (one by section; one section by slide; 20 slides) were chosen by systematic sampling along the cellular layer. Compartment volume densities were calculated according to Weibel [25]. The standard deviation attached to each grain density was calculated with the Williams formula [26].

Electron microscopy

The fixation protocol was the same as for light microscopy. Ultrathin vertical sections were collected on collodion-coated slides, and autoradiograms were performed according to Larra and Droz [27], using Ilford L4™ emulsion and Kodak Microdol X™ development after 5 months of exposure. Sections were observed and photographed with a CM 10 PHILIPS electron microscope operated at 80 kV.

Quantitative analysis

Autoradiograms were anlaysed by the circle method [26], which compares localisation of two sets of circles, one centred on actual silver grains (further denoted as "grains"), the other systematically positioned on micrographs (further denoted as "circles"), the latter corresponding to a hypothetical grain set randomly distributed. Circles diameter is adjusted for 50% probability of the source falling within the circle ("HR circle"), 0.56 μm in the present study. Eighteen micrographs (magnification × 9800) per experimental conditions were sytematically sampled at each crossing of the cellular layer with the supporting grid. The cellular layer was divided into seven primary compartments (i.e. nucleus, nucleolus, lysosomes, Golgi apparatus, mitochondria, cytoplasm and extracellular space). All organelles which were not considered as primary compartments were included into the compartment "cytoplasm". This procedure generated in practice 13 junctional compartments (i.e. compartments due to the circle falling on the limit between two or three primary compartments). The compartment "cell membrane" was the

result of the junction between "cytoplasm" and "extracellular space". Overlays printed with 72 systematically arranged circles, and circles of same size centred to each actual grain, were used to attribute circles and grains, respectively, to the defined compartments. Crude specific activities and attached errors were calculated by the Williams method [26], after grouping compartments in order to obtain grain and normalised circle data >5 which is the minimal value needed to perform the chi-square test [26]. For the grouping process, primary compartments were grouped preferably with attached junctional compartments. However, junctional compartments including more than two primary compartments were regrouped in the compartment "cytoplasm + various organelles". A chi-square test between real grains and systematic circles distribution was performed. Results stand between 464 and 1884 (df 8) for all conditions showing clearly a non-random distribution of real grains. In some cases (less than 5%), real grains overlapped, and centres of individual grains could not be easily identified. These figures were considered as simple grains, and the circle was centred on the compound image.

Cell image processing

This method was used to determine the mean spherical volume of mitochondria. Two groups of micrographs corresponding to cells incubated with or without serum albumin were analysed. The negative was placed on the "negatoscope" under a black and white CCD video camera (Panasonic BL 200). The video camera was connected to the black and white screen and simultaneously to the PC computer. The signals generated by the video camera were digitised and the numerical images represent 512 lines by 512 points on 256 pixels. These converted images were analysed using an original computer programme (prepared by Mr C. Gril, Laboratoire de Microscopie Electronique, Université Montpellier II). This programme permitted the application of mathematical algorithms necessary for the extraction of the objects on the plane and the calculation of the sterological parameters. The originality of this programme is that one can use the computer mouse to trace around the object appearing on the screen (in this case, the mitochondria), which allows measurement of

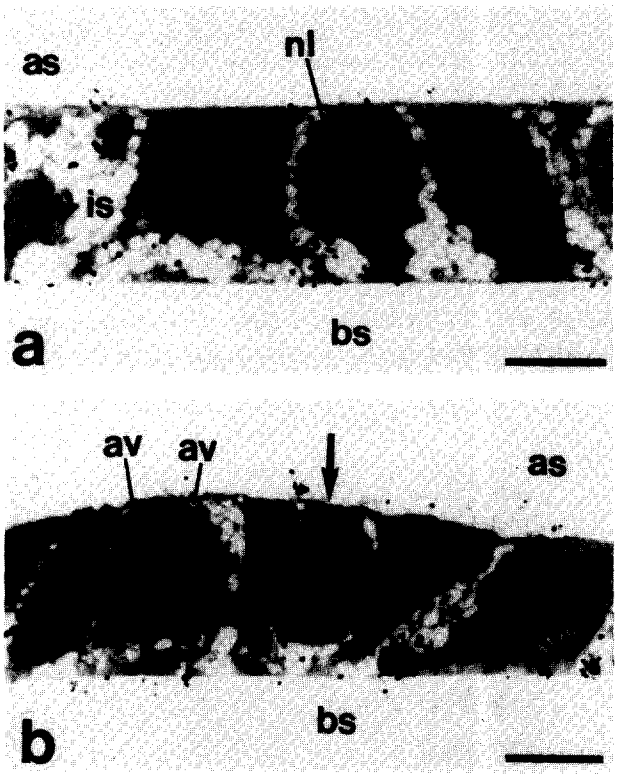

Figure 2. Semi-thin sections of HT-29-D4 cells showing the distribution of silver grains over the cellular layers. HT-29-D4/Gal cells were incubated with [^{3}H]suramin in serum-free DMEM in the absence (a) or presence (b) of serum albumin. Arrow, grains in the apical cell region; av, apical vacuoles; nl, nuclear labelling. Other abbreviations correspond to the cellular layer division into compartments for the quantitative analysis of labelling: bs, basal space (embedding medium under the basal side of the cells); as, apical space (culture medium replaced by the embedding medium); is, intercellular space; c, cytoplasm; n, nucleus. Scale bars, 10 μm.

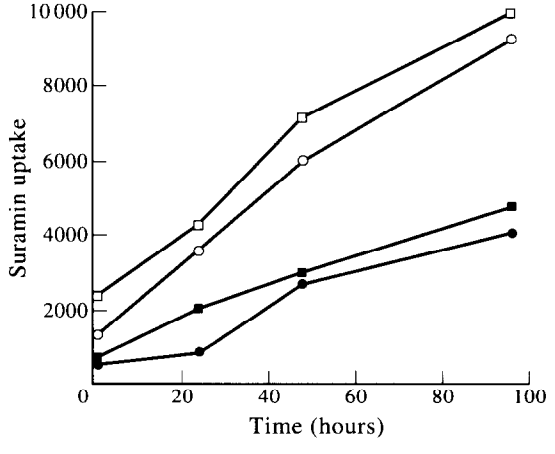

Figure 1. Uptake of [^{3}H]suramin by HT-29-D4/Glu (□, ■) and Gal cells (○, ●) in the presence (■, ●) or absence (□, ○) of 1% serum albumin in serum-free DMEM. Suramin uptake is expressed as cpm/10^6 cells. The cell number was determined by counting the cells of triplicate wells in a Malassez chamber. Values are the mean of two experiments performed in triplicate. Standard errors were always below 5% of mean values.

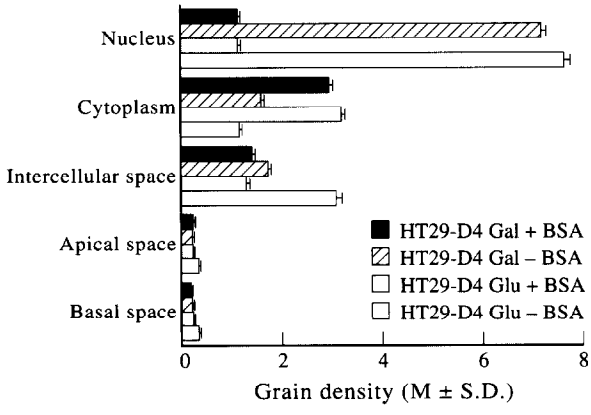

Figure 3. Grain densities of [^{3}H]suramin in the different cellular compartments of HT-29-D4/Gal and HT-29-D4/Glu cells. Grain densities are the ratio of real grain on systematic points falling on each compartment. The densities in apical and basal spaces are very low and correspond to the background.

the periphery and the surface. The computer then calculates the original dimensions and the volume adapted to the chosen model (here, spherical volume). The stored data from the computer were then subjected to the statistical analysis applied in this study.

RESULTS

Kinetics of suramin uptake by HT-29-D4 cells

The pharmacokinetics of suramin uptake in HT-29-D4 cells was studied using [^{3}H]suramin. The results showed that the drug was able to enter similarly undifferentiated and differentiated HT-29-D4 cells (Figure 1). In both cases, the process of suramin uptake was time-dependent and it was markedly decreased in the presence of serum albumin.

Light microscopy

Undifferentiated (HT-29-D4/Glu) or terminally differentiated cells (HT-29-D4/Gal) were incubated with [^{3}H]suramin in the presence or absence of serum albumin in serum-free medium. After 96 h, the cells were fixed and processed for autoradiography. In the presence of serum albumin, the labelling was mainly observed in the cytoplasm of both cell types. The grains were generally found within vacuoles or in the vicinity of these vacuoles (Figure 2b). In contrast, when serum albumin was not added to the culture medium, the labelling was mostly observed over the nucleus (Figure 2a). A quantitative analysis of the distribution of grains in these experiments is presented in Figure 3. This quantitation confirmed that the labelling was mainly cytoplasmic in the pres-

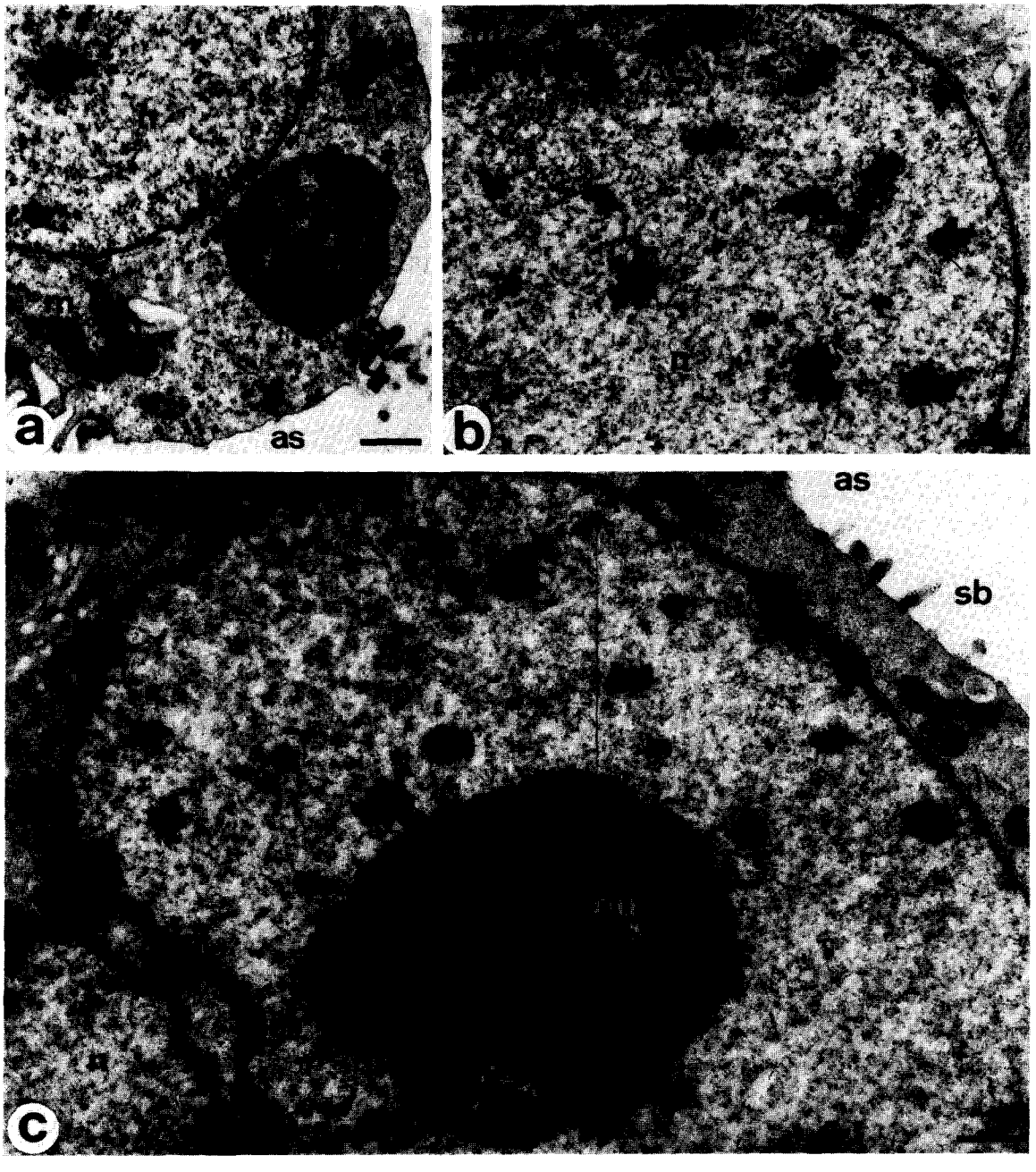

Figure 4. (a–c).

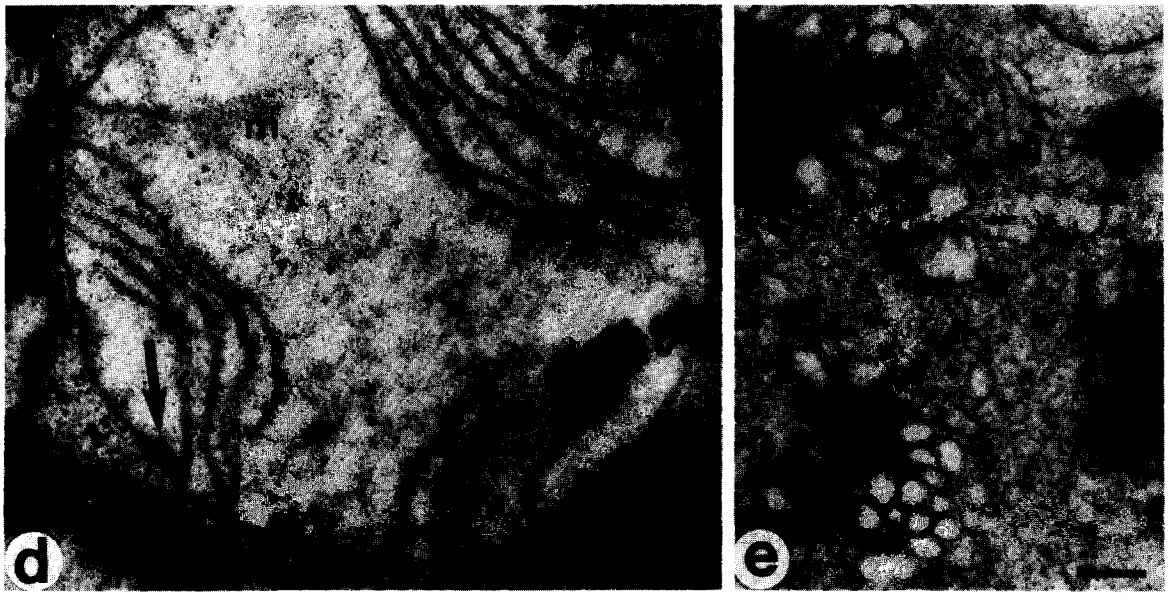

Figure 4. Electron microscopic autoradiographs of HT-29-D4/Glu (a, b) and Gal cells (c, d, e) fixed 6 days after the beginning of incubation with [^{3}H]suramin in serum-free DMEM. Most grains are located directly over the nucleus (b, c), the nucleolus (c), the mitochondria (a, d) and the Golgi apparatus (e). In mitochondria, the labelling was found in the matrix and in the membrane (a, d). Note that the presence of grains in the mitochondria is associated with a hypertrophy of this organelle (a, d). In addition, some mitochondria present electron dense granules (arrows) in the matrix. m, mitochondria; n, nucleus; nu, nucleolus; g, Golgi apparatus; er, endoplasmic reticulum; is, intercellular space; as, apical space; sb, striated border. Scale bars: 0.5 μm (a, b, d), 0.3 μm (c), 0.2 μm (e).

ence of serum albumin, and nuclear in its absence. Interestingly, the state of differentiation of the cells did not significantly modify the subcellular distribution of [^{3}H]suramin.

Electron microscopy

In the absence of serum albumin, the autoradiographic reaction of [^{3}H]suramin was almost exclusively located over the nucleus, the nucleolus, the mitochondria and the Golgi apparatus (Figure 4). The pattern of [^{3}H]suramin labelling was similar in HT-29-D4/Glu and Gal cells. In the case of mitochondria, the labelling was not only found over the matrix but also over the membranes (Figure 4d). The presence of suramin in the nucleolus and chromatin did not induce an ultrastructural alteration of these compartments. In contrast, its presence in mitochondria was consistently associated with mitochondrial hypertrophy (Figure 4a,d). In addition, electron-dense granules were frequently observed in these organelles (Figure 4d).

In the presence of serum albumin, the labelling was almost exclusively restricted to the lysosomal compartment of both HT-29-D4/Glu (Figure 5b,c) and HT-29-D4/Gal cells (Figure 5a). More precisely, the grains were found over lysosomes (Figure 5b,c), heterolysosomes (Figure 5e) and lamellar and multivesicular bodies (Figure 5d). Virtually no labelling was found over any other compartment including the nucleus (Figure 5a). The lysosomal accumulation of suramin in the presence of serum albumin induced a perturbation of the lysosomal system. These ultrastructural alterations consisted in the occurrence of lamellar bodies otherwise rarely found in HT-29-D4/Glu cells and an increase of these structures in HT-29-D4/Gal cells.

The circle method [26] was used to more precisely and statistically identify the structures associated with the autoradiographic grains. The results obtained are shown in Figure 6. From this analysis, it should be noted that, in presence of serum albumin, the labelling previously referred to as "cytoplasmic" was almost exclusively lysosomal in both cell types. In the absence of serum albumin, the cytoplasmic labelling could be assigned principally to the Golgi apparatus and the mitochondria, while the nuclear labelling was mainly nucleolar.

Mitochondrial hypertrophy estimated by image processing

A cell imaging method was developed to quantify the mitochondrial hypertrophy induced by free suramin. In both cell types (i.e. HT-29-D4/Glu and Gal cells), the mean spherical volume of the mitochondria was $3.75 \pm 2.79 \mu m^3 (n=77)$ and $0.07 \pm 0.06 \mu m^3 (n=127)$ in the absence and presence of serum albumin, respectively. This corresponded to a 50-fold increase of mitochondrial volume in suramin-treated cells. Since the standard deviation varied according to the mean, we performed a Student t-test on log transformed data using the STATISTIX programme. The t-test with equal variance showed that the two-sample average values were significantly different ($P < 0.0001$). We also performed a two-sample t-test directly on the original data. As above, the t-test with unequal variance showed that the two sample values were significantly different ($P < 0.0001$). Therefore, the increase of mitochondrial volume observed in cells incubated with free suramin was statistically significant.

Controls

No detectable radioactivity was present in any of the four glutaraldehyde/osmium fixed tissue blocks prepared from both HT-29-D4 cells incubated for 6 days with a solution containing free ^{3}H. HT-29-D4 cells which were briefly exposed (5 min) to [^{3}H]suramin, showed no cell-associated radioactivity above background.

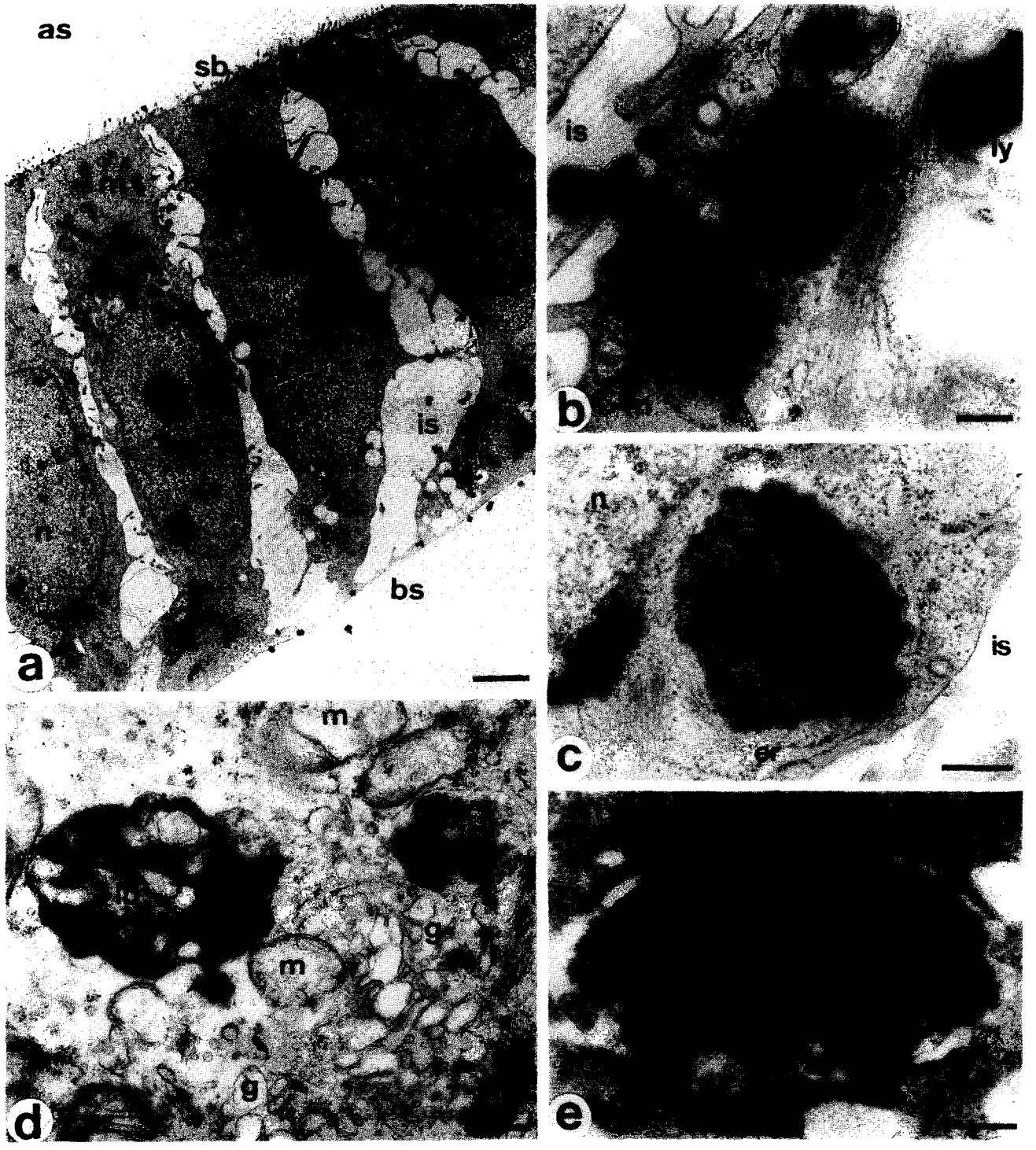

Figure 5. Electron microscopic autoradiographs of HT-29-D4/Glu (b, c, e) and Gal cells (a, d) fixed 6 days after the beginning of [^{3}H]suramin incubation in serum-free DMEM containing 1% BSA. nu, nucleolus; er, endoplasmic reticulum; is, intercellular space; ly, lysosomes; lb, lamellar bodies; mvb, multivesicular bodies; sb, striated border; bs, basal space; as, apical space; g, Golgi apparatus; m, mitochondria; n, nucleus. Arrows show accumulation of silver grains over the lysosomal system. Scale bars: 3 μm (a), 0.1 μm (b), 0.2 μm (c), 0.5 μm (d), 0.3 μm (e).

DISCUSSION

In this study, we present for the first time morphological evidence of the uptake of [^{3}H]suramin by cultured cells at the ultrastructural level. The radioactivity found in HT-29-D4 cells following suramin uptake was most likely associated with the drug, since it was previously shown not to be metabolised by the cells [28]. A further assessment of this point is given by experiments showing that the radioactivity extracted from HT-29-D4 cells incubated for 96 h with [^{3}H]suramin co-eluted with standard suramin in HPLC (data not shown). Based on these data, one can reasonably assume that the radioactivity detected in HT-29-D4 cells following incubation

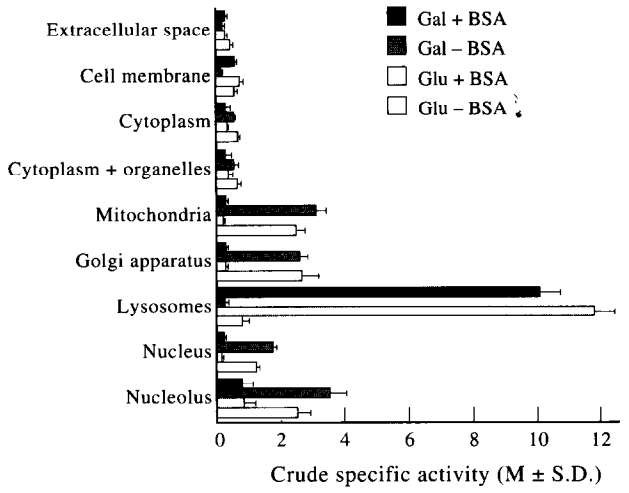

Figure 6. Crude specific activity of [^{3}H]suramin in the different cellular compartments of HT-29-D4/Gal and HT-29-D4/Glu cells. The crude specific activity in the extracellular space corresponds to the background.

with [^{3}H]suramin is actually associated with tritiated drug. Moreover, the tritiated analogue of suramin used throughout this study was found to bind to serum albumin with the same affinity as unlabelled suramin [18].

The autoradiographic data allowed a quantitative evaluation of the intracellular distribution of the drug in undifferentiated and differentiated HT-29-D4 cells, in the presence or absence of serum albumin in serum-free culture medium. One of the main results of this study is that suramin was indifferently taken up by HT-29-D4 cells whatever their differentiation state. Therefore, the mechanisms of suramin entry into these cells are not controlled by cell differentiation. The other major point is that the presence of serum albumin determines the intracellular localisation of suramin in HT-29-D4 cells. Indeed, in the absence of the suramin-binding protein, the drug is distributed over the Golgi apparatus, the mitochondria and the nucleus. In contrast, when serum albumin is added to the culture medium, the drug is mostly found in lysosomes. Clearly, the absence of albumin in one of our experimental conditions does not reflect a normal physiological environment. However, the data obtained do show an interesting localisation of suramin, especially in the nucleus. In addition, one should note that the drug is usually not totally bound to serum albumin during suramin therapy [1]. Therefore, it can be speculated that two distinct intracellular pathways of suramin uptake may actually co-exist *in vivo*, depending on the degree of complexing of the drug with albumin.

The presence of suramin in mitochondria is consistent with a recent report suggesting that suramin may affect mitochondrial functions [29]. At the molecular level, the drug has been shown to inhibit the oxidation of succinate and the synthesis of ATP in intact submitochondrial particles [30]. Suramin may therefore directly inhibit these enzymatic complexes and perturbate the respiratory activity of mitochondria. At the ultrastructural level, our observation of suramin-associated mitochondrial hypertrophy is in agreement with a recent report describing a similar disorder in suramin-treated patients [31].

The accumulation of suramin in the nucleolus and the chromatin of HT-29-D4 cells may be related to another facet of suramin activity in tumoral cells [32]. The drug is a potent inhibitor of RNA polymerase [1] and DNA topoisomerase II [33]. This is compatible with an action of the drug at the level of gene expression.

The concentration of suramin in the lysosomal system of HT-29-D4 cells in the presence of serum albumin is not surprising. Suramin inhibits the activity of several lysosomal hydrolases [34–36]. Therefore, the accumulation of [^{3}H]suramin in the lysosomal system of HT-29-D4 cells may account for the lysosomal storage disorder induced by the drug in the presence of serum albumin. On the basis of the results shown in this study and of previous reports by our group [20,37] it is possible to draw a sequence of the events leading to this disorder: (i) suramin, complexed with albumin, enters the cells by endocytosis; (ii) the drug accumulates in lysosomes, and (iii) inhibits the activity of lysosomal enzymes, which induces an accumulation of secondary lysosomes and lamellar bodies in the cytoplasm of the cells. This sequence may occur in every cell type able to endocyte suramin–albumin complexes.

In conclusion, our data demonstrate that an external factor, i.e. serum albumin, is able to influence the activity of a drug by determining its intracellular localisation. Suramin exerts a wide range of biological effects with certainly several mechanisms of action [38]. In some cellular systems, including the HT-29-D4 cell clone, the drug disrupts autocrine loops of growth stimulation by interfering with the binding of growth factors to their receptors [39–49]. This intriguing property of suramin is inherent to the presence of sulphonyl groups able to interact with basic amino acid residues of several growth factors [41]. As a consequence, suramin may be able to control the balance between proliferation and differentiation of cancer cells by acting at the extracellular level [40,46]. Indeed, under our experimental conditions (i.e. a chronic treatment with 20 μg/ml of suramin), the drug inhibited the growth of HT-29-D4 cells by 37%. This concentration of suramin was also sufficient to induce some morphological alterations in mitochondria and lysosomes. The results of the present study demonstrate for the first time that suramin is able to enter cancer cells by at least two independent mechanisms leading to different intracellular localisations. The presence of suramin in a given intracellular compartment may account for specific effects of the drug including mitochondrial hypertrophy, altered gene expression and lysosomal perturbation.

1. Hawking F. Suramin: with special reference to onchocerciasis. *Adv Pharmacol Chemother* 1978, **15**, 289–322.
2. De Clerq E. Suramin: a potent inhibitor of the reverse transcriptase of RNA tumor viruses. *Cancer Lett* 1979, **8**, 9–22.
3. Mitsuya H, Popovic M, Yarchoan R, *et al.* Suramin protection of T cells *in vitro* against infectivity and cytopathic effect of HTLV-III. *Science* 1984, **226**, 172–174.
4. Cheson BD, Levine AM, Mildvan D, *et al.* Suramin therapy in AIDS and related disorders. Report of the US suramin working group. *J Am Med Assoc* 1987, **258**, 1347–1351.
5. Stein CA, La Rocca RV, Thomas R, *et al.* Suramin: an anticancer drug with a unique mechanism of action. *J Clin Oncol* 1989, **7**, 499–508.
6. Myers C, Cooper M, Stein C, *et al.* Suramin: a novel growth factor antagonist with activity in hormone-refractory metastatic prostate cancer. *J Clin Oncol* 1992, **10**, 881–889.
7. Gritli A, Septier D, Goldberg M. Suramin-induced mucopolysaccharidosis in rat incisor. *Cell Tissue Res* 1993, **273**, 53–64.
8. Christensen B, Lullmann-Rauch R. On the alcianophilia of the drug suramin used as a tool for inducing experimental mucopolysaccharidosis. *Histochemistry* 1988, **89**, 365–367.

9. Rees S, Constantopoulos G, Brady RO. The suramin-treated rat as a model of mucopolysaccharidosis: reversibility of biochemical and morphological changes in liver. *Virchows Arch (Cell Pathol)* 1986, **51**, 235–245.
10. Constantopoulos G, Rees S, Barranger JA, Brady RO. Animal model of human disease. Suramin-induced storage disease mucopolysaccharidosis. *Am J Pathol* 1983, **113**, 266–268.
11. Rees S, Constantopoulos G, Barranger JA, Brady RO. Organomegaly and histopathology in an animal model of mucopolysaccharidosis induced by suramin. *Naunyn-Schmiedeberg's Arch Pharmacol* 1982, **319**, 262–270.
12. Baghdiguian S, Verrier B, Marvaldi J, Fantini J. Kinetics of biochemical, electrophysiological and morphological events (including lysosomal disorder) during the course of suramin-induced differentiation of the human colon cancer cell clone HT29-D4. *Int J Cancer* 1991, **49**, 608–615.
13. Baghdiguian S, Verrier B, Marvaldi J, Fantini J. Short-term suramin treatment followed by the removal of the drug induces terminal differentiation of HT29-D4 cells. *J Cell Physiol* 1992, **150**, 168–174.
14. Baghdiguian S, Verrier B, Roccabianca M, *et al.* Vectorial release of carcinoembryonic antigen induced by IFN-γ in human colon cancer cells cultured in serum free medium. *Eur J Cancer* 1991, **27**, 599–604.
15. Bos OJM, Fischer MJE, Wilting J, Janssen LHM. Drug-binding and other physiochemical properties of large tryptic and large peptic fragment of human serum albumin. *Biochim Biophys Acta* 1988, **953**, 37–47.
16. Bos OJM, Vansterkenburg ELM, Boon JPCI, *et al.* Location and characterization of the suramin binding sites of human serum albumin. *Biochem Pharmacol* 1990, **40**, 1595–1599.
17. Vansterkenburg ELM, Wilting J, Janssen LHM. Influence of pH on the binding of suramin to human serum albumin. *Biochem Pharmacol* 1989, **38**, 3029–3035.
18. Muller WE, Wollert U. Spectroscopic studies on the complex formation of suramin with bovine and human serum albumin. *Biochim Biophys Acta* 1976, **427**, 465–480.
19. Windholz M. *The Merck Index, 10th edn.* Rahway, New Jersey, Merck and Co, 1983.
20. Baghdiguian S, Boudier JA, Verrier B, *et al.* Suramin elicits a lysosomal storage disorder in HT29-D4 cells by a mechanism of polarized endocytosis. *CR Acad Sci Paris* 1992, **314 (III)**, 67–73.
21. Rabenandrasana C, Baghdiguian S, Roccabianca M, *et al.* The concentration of glucose in the culture medium determines the effect of suramin on the growth and differentiation of the human colonic adenocarcinoma cell clone HT29-D4. *Cancer Lett* 1990, **53**, 109–115.
22. Fantini J, Abadie B, Tirard B, *et al.* Spontaneous and induced dome formation by two clonal cell populations derived from a human adenocarcinoma cell line. *J Cell Sci* 1986, **83**, 235–249.
23. Fantini J, Guo XJ, Marvaldi J, Rougon G. Suramin inhibits proliferation of rat glioma cells and alters N-CAM cell surface expression. *Int J Cancer* 1990, **45**, 554–561.
24. Yahi N, Sabatier JM, Nickel P, *et al.* Suramin inhibits binding of the V3 region of HIV-1 envelope glyocprotein gp 120 to galactosylceramide, the receptor for HIV-1 gp 120 on human colon epithelial cells. *J Biol Chem* 1994, **269**, 24349–24353.
25. Weibel ER. Sterological methods. *Practical Methods for Biological Morphometry, Vol. 1.* London, Academic Press, 1979.
26. Williams MA. Quantitative methods in electron microscopy . In Glauert AM, ed. *Practical Methods in Electron Microscopy, Vol. 6.* Amsterdam, North-Holland, 1977.
27. Larra F, Droz B. Techniques radioautographiques et leur application au renouvellement des constituants cellulaires. *J Microsc (Paris)* 1970, **9**, 845–880.
28. Armand JP, Cvitkovic E. Suramin, a new therapeutic concept. *Eur J Cancer* 1990, **26**, 417–419.
29. Rago RP, Mitchen J, Cheng AL, *et al.* Disruption of cellular energy balance by suramin in intact human prostatic carcinoma cells, a likely antiproliferative mechanism. *Cancer Res* 1991, **51**, 6629–6635.
30. Calcaterra NB, Vicario LR, Roveri OA. Inhibition by suramin of mitochondrial ATP synthesis. *Biochem Pharmacol* 1988, **37**, 2521–2527.
31. Rago RP, Miles JM, Sufit RL, *et al.* Suramin-induced weakness from hypophosphatemia and mitochondrial myopathy. Association of suramin with mitochondrial toxicity in humans. *Cancer* 1994, **73**, 1954–1959.
32. Yamazaki H, Dilworth A, Myers CE, Sinha BK. Suramin inhibits DNA damage in human prostate cancer cells treated with topoisomerase inhibitors *in vitro*. *Prostate* 1993, **23**, 25–36.
33. Bojanowski K, Lelievre S, Markovits J, *et al.* Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in chinese hamster fibrosarcoma cells. *Proc Natl Acad Sci USA* 1992, **89**, 3025–3029.
34. Constantopoulos G, Rees S, Cragg BG, *et al.* Effect of suramin on the activities of degradative enzymes of sphingolipids in rats. *Res Commun Chem Path Pharm* 1981, **32**, 87–97.
35. Constantopoulos G, Rees S, Cragg BG, *et al.* Differential inhibition of β-hexosaminidase A and β-hexosaminidase B by suramin. *Biochem Biophys Res Commun* 1981, **101**, 1345–1349.
36. Constantopoulos G, Rees S, Cragg BG, *et al.* Experimental model for mucopolysaccharidosis: suramin-induced glycosaminoglycan and sphingolipid accumulation in the rat. *Proc Natl Acad Sci USA* 1980, 77, 3700–3704.
37. Baghdiguian S, Boudier JA, Boudier JL, Fantini J. Autoradiographic localization of tritiated suramin in polarized human colon adenocarcinoma cells. *Cancer Lett* 1993, **75**, 151–156.
38. Stein CA. Suramin: a novel antineoplastic agent with multiple potential mechanisms of action. *Cancer Res* 1993, **53**, 2239–2248.
39. Tsutsumi K, Kitagawa N, Niwa M, *et al.* Effect of suramin on ^{125}I-insulin like growth factor-1 binding to human meningiomas and on proliferation of meningioma cells. *J Neurosurg* 1994, **80**, 502–509.
40. Baghdiguian S, Verrier B, Gerard C, Fantini J. Insulin like growth factor I is an autocrine regulator of human colon cancer cell differentiation and growth. *Cancer Lett* 1992, **62**, 23–33.
41. Middaugh CR, Mach H, Burke CJ, *et al.* Nature of the interaction of growth factors with suramin. *Biochemistry* 1992, **31**, 9016–9024.
42. Minniti CP, Maggi M, Helman LJ. Suramin inhibits the growth of human rhabdomyosarcoma by interrupting the insulin-like growth factor II autocrine growth loop. *Cancer Res* 1992, **52**, 1830–1835.
43. Kopp R, Pfeiffer A. Suramin alters phosphoinositide synthesis and inhibits growth factor receptor binding in HT-29 cells. *Cancer Res* 1990, **50**, 6490–6496.
44. Mills GB, Zhang N, May C, *et al.* Suramin prevents binding of interleukin 2 to its cell surface receptor: a possible mechanism for immunosuppression. *Cancer Res* 1990, **50**, 3036–3042.
45. Pollack M, Richard M. Suramin blockade of insulin like growth factor I-stimulated proliferation of human osteosarcoma cells. *J Natl Cancer Inst* 1990, **82**, 1349–1352.
46. Fantini J, Rognoni JB, Roccabianca M, *et al.* Suramin inhibits cell growth and glycolytic activity and triggers differentiation of human colic adenocarcinoma cell clone HT29-D4. *J Biol Chem* 1989, **264**, 10282–10286.
47. Moscatelli D, Quarto N. Transformation of NIH 3T3 cells with basic fibroblast growth factor of the hst/K-*fgf* oncogene causes down-regulation of the fibroblast growth factor receptor: reversal of morphological transformation and restoration of receptor number by suramin. *J Cell Biol* 1989, **109**, 2519–2527.
48. Coffey RJ, Leof EB, Shipley GD, Moses HL. Suramin inhibition of growth factor receptor binding and mitogenicity in AKR-2B cells. *J Cell Physiol* 1987, **132**, 143–148.
49. Hosang M. Suramin binds to platelet-derived growth factor and inhibits its biological activity. *J Cell Biochem* 1985, **29**, 265–273.

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