Anti-Cancer Drugs, **1**, pp. 59–66

A suramin derivative induces enterocyte-like differentiation of human colon cancer cells without lysosomal storage disorder

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Suramin is a polysulfonated naphthylurea currently investigated for the treatment of advanced malignancy. We have previously reported that suramin was a potent inducer of the differentiation of the human colic adenocarcinoma cell clone HT29-D4 (Fantini *et al., J Biol Chem*, 1989; 264: 10282–10286). In this report, we show that chronic suramin treatment of these cells is associated with a lysosomal storage disease. We have tested five suramin-related compounds for their ability to induce HT29-D4 differentiation and we looked at their action on the lysosomal apparatus. We conclude that one of the derivatives used is a more potent differentiation inducer than suramin, while it does not elicit any perturbation of the lysosomal system.

Key words: Suramin derivatives, lysosomes, differentiation, human colon cancer cells.

Introduction

Suramin is a polysulfonated naphthylurea that has been used for a number of years to treat trypanosomiasis.[1] It was found in 1979 to be an inhibitor of retroviral reverse transcriptase[2] and to prevent the infection of T lymphocytes by human immunodeficiency virus (HIV) *in vitro*.[3] Based on these results and on preliminary clinical data[4] a phase I trial was organized to evaluate suramin in patients with acquired immunodeficiency syndrome (AIDS). Important toxic reactions were reported and the drug was finally abandoned as therapy for AIDS.[5] In parallel, suramin was found to bind tightly a wide range of tumor growth factors *in vitro*,[6–8] and to inhibit proliferation of several tumor cell lines.[9] A new clinical trial was conducted by Stein *et al.*[10] who concluded that suramin had potential as an anti-cancer agent. Recently our group reported for the first time that suramin was a potent inducer of differentiation of human colon cancer cells[11–13] and rat glioma cells.[14] In both cases, the effect of suramin on cell differentiation was observed in serum supplemented media[11,12,14] as well as in serum-free defined media,[13,14] suggesting that the drug acted at the level of autocrine growth factors. In the present work we show that long term suramin treatment of human colon cancer cells (HT29-D4) elicits a perturbation of the lysosomal system. Because this lysosomal storage disorder could account for some of the toxic effects induced by the drug during treatment of humans, we have tested five suramin derivatives for their effect on HT29-D4 cell differentiation and we have studied the lysosomal system of the treated cells by transmission electron microscopy. We report that one of the five suramin-related compounds tested is at least as active as suramin on HT29-D4 enterocytic differentiation while it does not impair the lysosomal system of these cells.

Material and methods

Material

The suramin-related compounds (for chemical structure, see Figure 1) were synthesized at the Pharmazeutisches Institut der Universität (Bonn,

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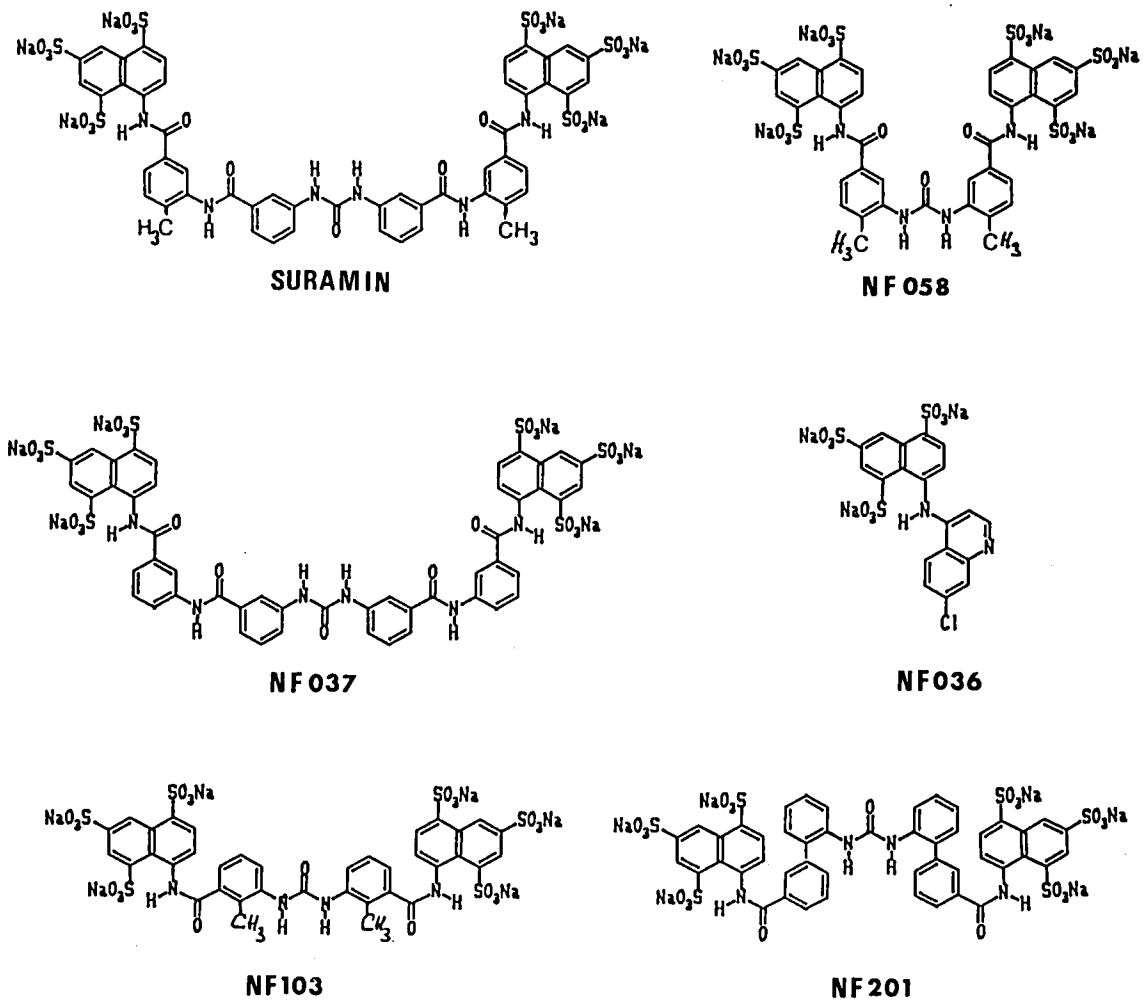

Figure 1. Chemical structure of suramin and of the suramin-related compounds investigated.

FRG) according to Jentsch *et al.*[15] They were prepared as sterile solutions of 10 mg/ml in distilled water and stored at −45°C.

Cell culture

HT29-D4 cells (passage 5) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose and 10% fetal calf serum (FCS). The suramin-related compounds were added to the medium from the second day of culture and the cells were cultured for 15 further days in the presence of the tested drug. In all cases the culture medium was changed every day.

Transmission electron microscopy

The cells were fixed *in situ* with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for 1 h, washed 10 min in the same buffer with 6.84% saccharose, post-fixed in 1% osmium tetroxide, then dehydrated in ethanol and embedded in Epon. Sections were cut perpendicularly to the plane of the cell monolayer and observed with a Jeol 100 C electron microscope.

Results

After 17 days of culture in DMEM supplemented with 10% FCS (standard medium), HT29-D4 cells

formed a poorly organized multilayer (Figure 2A). When suramin was added at a concentration of 100 μg/ml from the second day of culture, the cell growth was strongly reduced and the cells formed a monolayer in which cell boundaries appeared very bright at the level of optical microscope (Figure 2E). This change in HT29-D4 morphology induced by suramin was dose-dependent. Indeed when treated with suramin 10 μg/ml, the cells grew as multilayers just like they did when cultured in the absence of the drug (Figure 2B). The effects of suramin derivatives (tested at the concentration

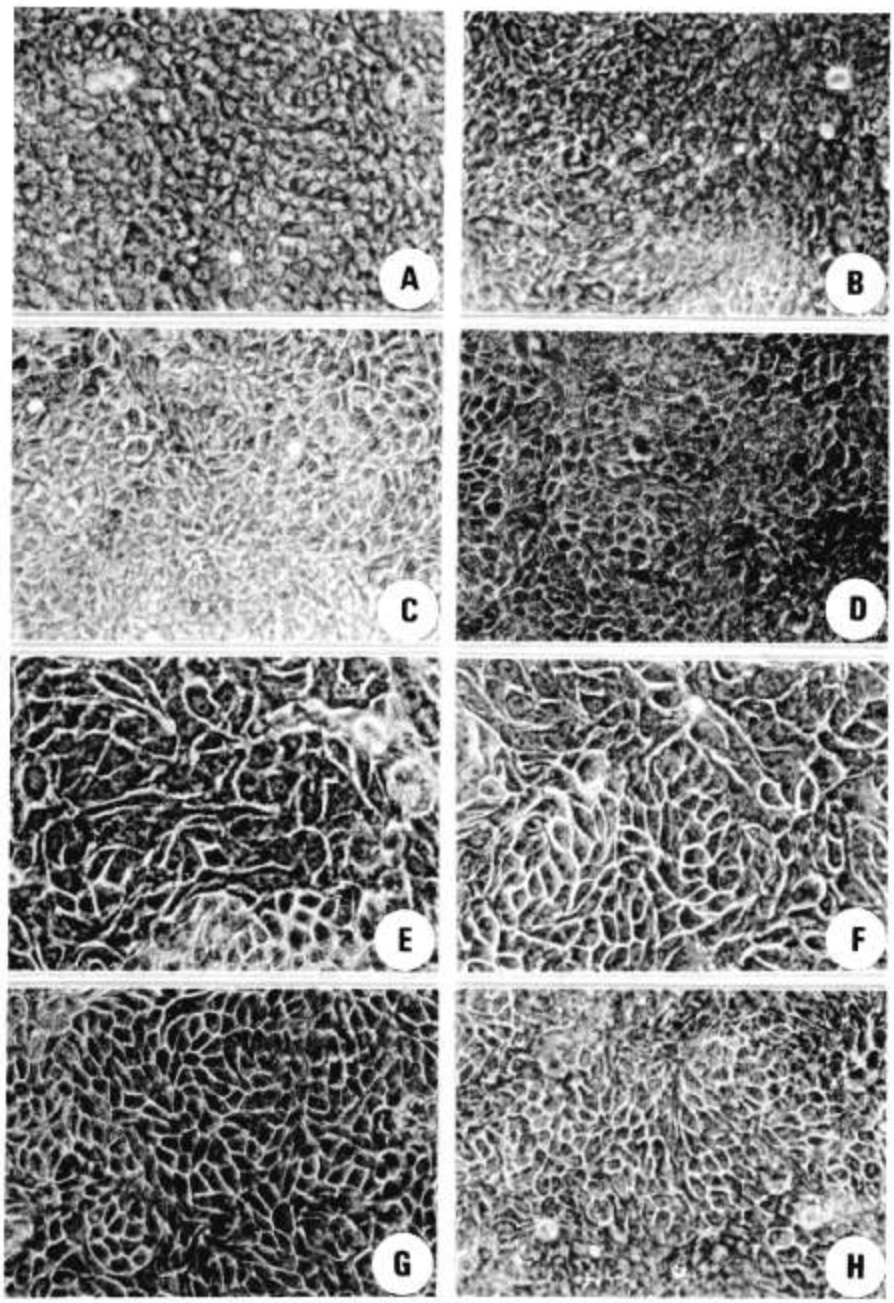

Figure 2. Phase-contrast micrographs of HT29-D4 cells treated by different suramin derivatives. (A) Control cells grown for 17 days without drug. (B) HT29-D4 cells cultured for 15 days in the presence of a low concentration (10 μg/ml) of suramin. (C–H) HT29-D4 cells treated for 15 days with derivatives NF103 (C), NF058 (D), NF037 (F), NF201 (G), NF036 (H) or suramin (E). All drugs were used at a concentration of 100 μg/ml. Magnification × 300.

of 100 μg/ml) on the morphology of HT29-D4 cells are also given in Figure 2. We have noted three phenotypes according to the derivative used. When the cells were incubated in the presence of NF037 (Figure 2F) and NF201 (Figure 2G), they formed a monolayer resembling the one obtained upon suramin treatment. With NF103 (Figure 2C) and NF058 (Figure 2D) the cultures appeared less organized and heterogeneous. In contrast treatment of HT29-D4 cells with derivative NF036 led to a better state of organization of the monolayer (Figure 2H). In this latter case, the average size of the cells was smaller as compared to suramin-treated cells.

The effect of suramin and its derivatives on the morphology of HT29-D4 cells was further characterized at the ultrastructural level. As previously described[11] suramin induced a very elaborated state of enterocyte-like differentiation of HT29-D4 cell clone. After 15 days of culture in the presence of the drug the cells formed a regular monolayer of columnar cells exhibiting the morphological features of intestinal absorptive cells (Figure 3A). The apical plasma membrane formed a brush border with densely packed microvilli exhibiting their characteristic cytoskeletal core. Adjacent cells were joined together by tight junctions and numerous desmosomes. The nucleus

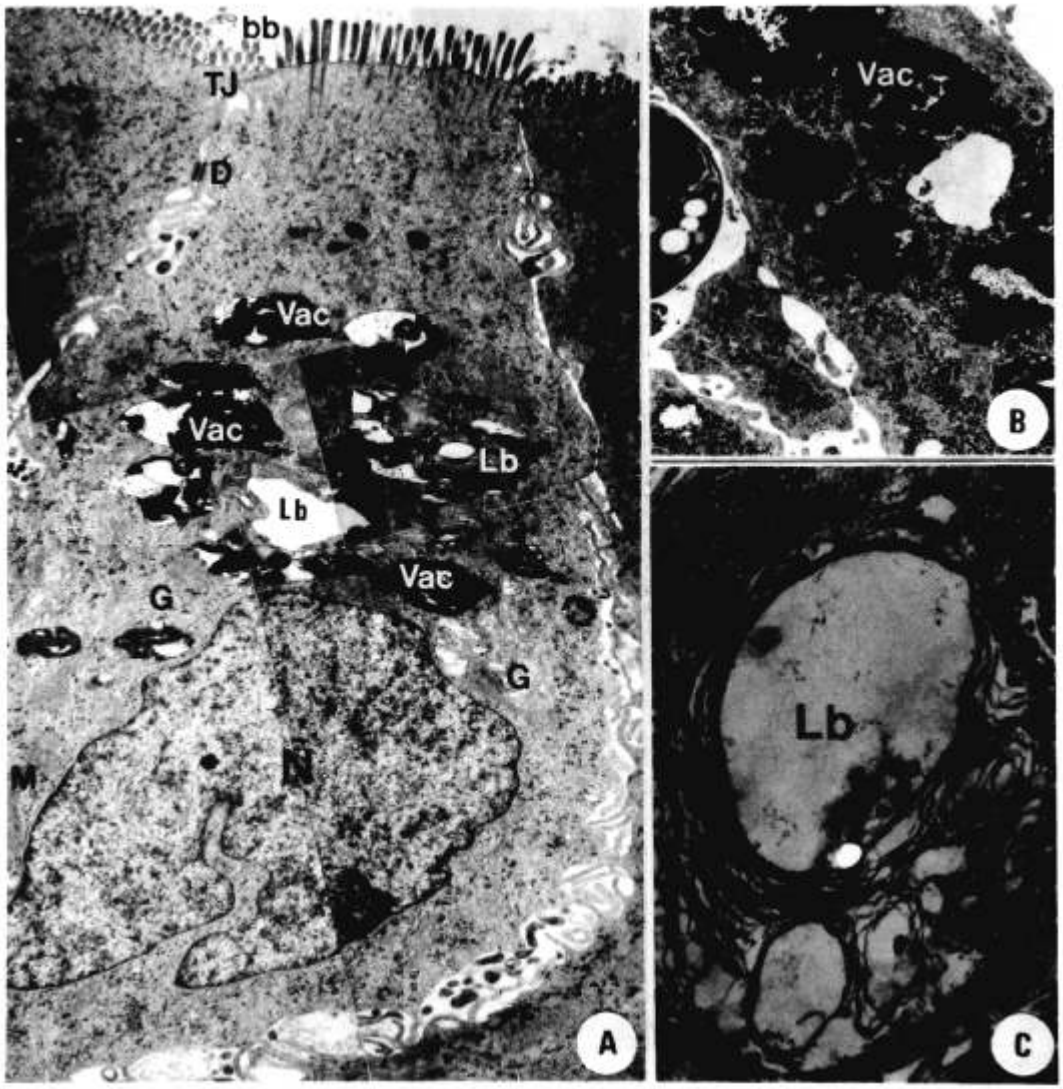

Figure 3. Suramin-induced enterocyte-like differentiation of HT29-D4 cells and lysosomal disorder. BB, brush border; D, desmosome; G, Golgi apparatus; Lb, lamellar body; M, mitochondria; N, nucleus; TJ, tight junction; Vac, autophagic vacuoles.
Note the presence of numerous autophagic vacuoles (B) and lamellar bodies (C). Magnification: (A) ×8300; (B) ×10000; (C) ×16000.

was always rejected at the basal side of the cells. The apical part of the cytoplasm contained a functional Golgi apparatus and a largely perturbated lysosomal system (Figure 3A). This impairment consists of an accumulation of autophagic vacuoles (Figure 3B) and occurrence of typical lamellar inclusion bodies (Figure 3C). Since the lysosomal system was not altered in HT29-D4 cells cultured in standard medium,[11] it can be assumed that the perturbation is caused by suramin. The treatment of HT29-D4 cells by derivative NF103 led to a poorly organized multilayer. In this case, only the upper cell layer exhibited some differentiation characteristics, namely the presence of tight junctions (not shown) and a brush border with rare microvilli (Figure 4A). Moreover the cells showed numerous autophagic vacuoles located in all parts of the cytoplasm. Chronic treatment with NF058 led to a better if not perfect state of differentiation. The multilayer showed two types of organization (Figure 4B). The upper cell layer exhibited a well-defined brush border and tight junctions. The lower layers formed numerous cysts with a well-organized brush border facing the intercellular lumina. The lysosomal system was enriched in autophagic vacuoles and lamellated bodies. The differentiation induced by derivative NF037 was very similar to that induced by suramin. The cells formed a regular monolayer and the lysosomal system contained typical autophagic vacuoles and lamellar bodies (Figure 4C–E). When HT29-D4 cells were cultured in the presence of NF201, they also differentiated but in this case they never displayed lamellar inclusion bodies (Figure 5A). However the lysosomal system was far to be normal as strengthened by the presence of large autophagic vacuoles in the apical part of the cytoplasm (Figure 5B,C). Finally the most elaborate state of differentiation was achieved by using NF036 derivative. The columnar cells obtained in this case were exceptionally tall (Figure 5D). The microvilli of the brush border showed a cytoskeletal axis extending very deep in the apical cytoplasm (Figure 5E). Most importantly, the noteworthy feature of these fully differentiated cells is that their lysosomal system is not impaired.

Discussion

After the discovery by our group that suramin was a potent inducer of cell differentiation, we investigated in this report the action of suramin-related drugs, all tested at the same concentration (100 μg/ml), on the differentiation of the cell clone HT29-D4. The compounds tested, namely NF058, NF037, NF103 and NF201, have six sulfonic acid groups like suramin, while compound NF036 has only three acidic groups and possesses a 7-chloro-4-quinolinyl residue.[15] Suramin is known to induce a lysosomal storage disorder which results in inadequate digestion of different macromolecules which entered the vacuolar apparatus of cells. Most of these observations have been done in animals (essentially rats and mice) perfused with suramin.[16–18] Because the lysosomal storage disorder was observed constantly in different tissues originating from suramin-treated animals, it can be speculated that this impairment of lysosomal function is responsible, at least in part, for the cytotoxicity observed in humans. Yet little is known about the effect of suramin on the lysosomal system of cells in culture.

We have taken advantage of the impressive response of the human colonic adenocarcinoma cell line HT29-D4 to suramin to investigate carefully the effect of the five suramin-related compounds on: (i) the induction of cellular differentiation; (ii) the perturbation of the lysosomal system by the observation of abnormal inclusions such as lamellated inclusion bodies and hypertrophied autophagic vacuoles. By comparison to the differentiation-effect of suramin, the different compounds can be classified as follows: NF103 and NF058 generated cell multilayers with a profound disorder in the lysosomal system and by consequence these suramin-derived drugs were ineffectual to promote cell differentiation except that intercellular cysts are often visible with the NF058 compound.

NF037, NF201 and NF036 are in contrast effective in promoting the organization of cells in monolayers. NF037, the unmethylated suramin analog, was found as effective as suramin with the same extent of lysosomal storage perturbation. NF201 behaved like NF037 but we have never observed lamellated bodies as abnormal inclusions. NF036, in our hands, was the suramin derivative that generated the most spectacular effect on cell differentiation. HT29-D4 cells became particularly well organized on the plastic culture dishes. The cells reached a height never previously obtained with suramin. This better state of organization was never associated with an impairment of lysosomal storage because NF036-treated cells were free of abnormal inclusions constantly found in suramin-treated cells. It is of interest to note that NF036 was found to be 10 times less

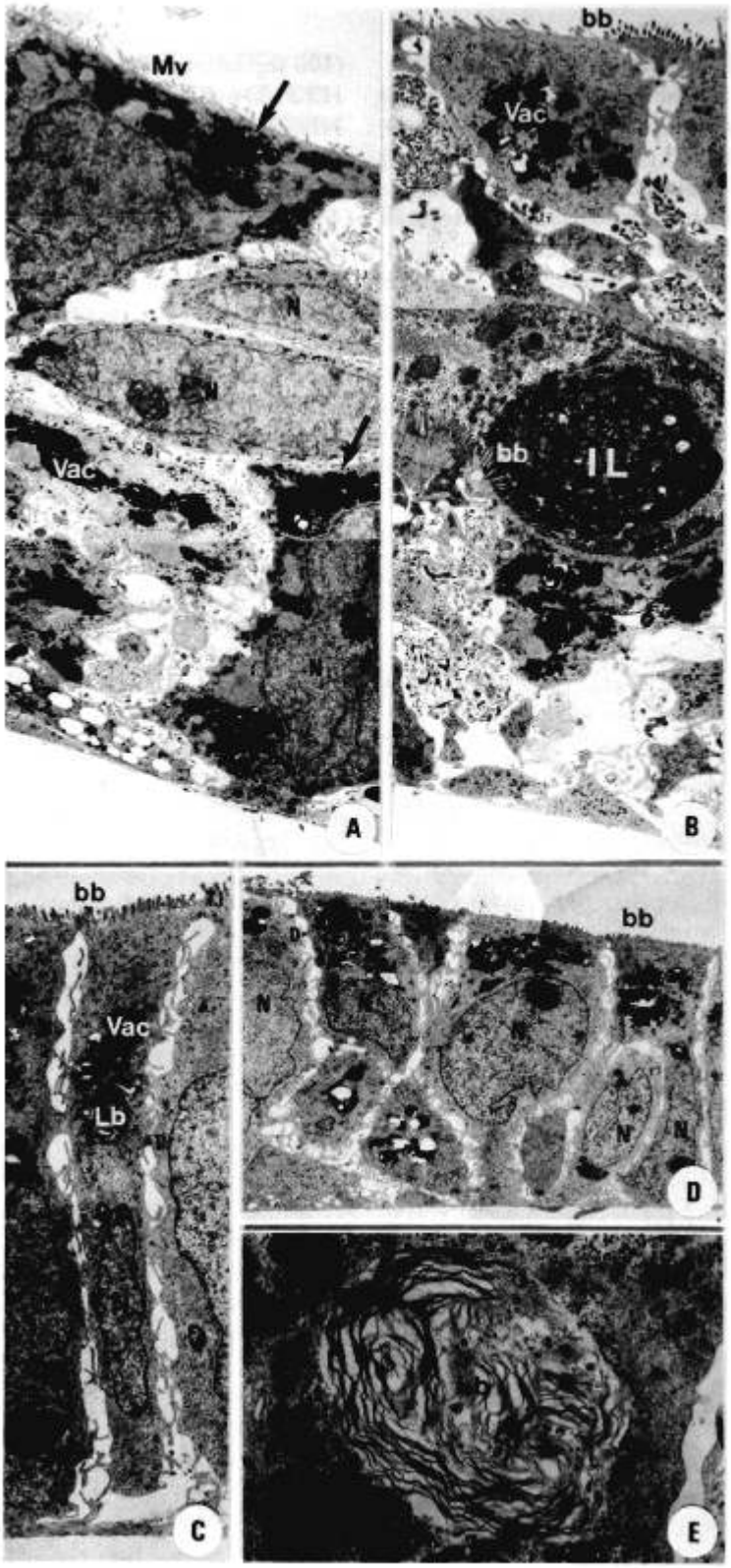

Figure 4. Morphology of HT29-D4 cells treated with derivatives NF103 (A), NF–058 (B) or NF037 (CE).
↙, glycogen; BB, brush border; D, desmosome; IL, intercellular lumina; Lb, lamellar body; Mv, microvilli; N, nucleus; TJ, tight junction; Vac, autophagic vacuoles. Magnification: (A–C) ×5000; (D) ×2000; (E) ×16000.

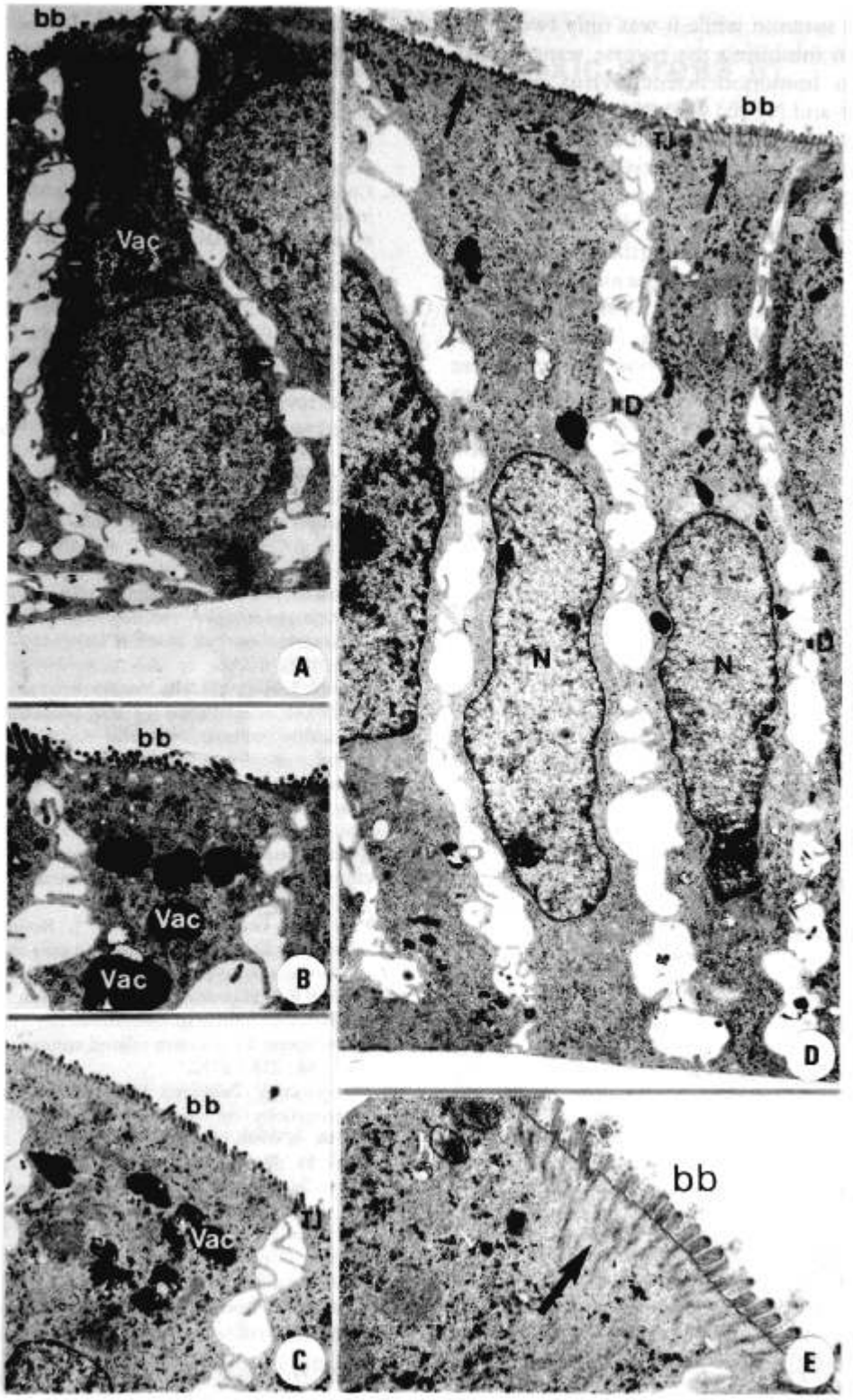

Figure 5. Morphology of HT29-D4 cells treated with derivatives NF201 (A–C), or NF036 (D–E).
↗, filamentous axis of the microvilli; BB, brush border; D, desmosome; N, nucleus; TJ, tight junction; Vac, autophagic vacuoles. Magnification: (A) ×5000; (B, C) ×10000; (D) ×66000; (E) ×16000.

cytotoxic than suramin while it was only two times less effective in inhibiting the reverse transcriptase of the human immunodeficiency virus HIV-1.[15] NF201, NF058 and NF103 were also found 10 times less cytotoxic than suramin while they were as effective as or even more effective (NF201) than suramin in inhibiting the reverse transcriptase.[15] Therefore we cannot draw a clear relationship between the cytotoxic effect described by Jentsch *et al.*[15] as the drug concentration at which lymphocyte proliferation was impaired in the absence of HIV-I, and the action of the drug either on cell differentiation or on the inhibition of the reverse transcriptase of HIV-I. Moreover, no relationship can be drawn between the molecular size and the number of acidic groups and the differentiation effect of these compounds. We found that the smallest molecular size and the lowest number of acidic groups was associated with the most effective derivative on cell differentiation, namely NF036.

Conclusion

In conclusion we have described the effect on cell differentiation of five suramin-related drugs. We have shown that one of them, NF036, was a more potent inducer of cell differentiation than suramin, without an associated morphological disturbance of the lysosomal system. It would be of great interest to investigate at the clinical level this derivative in tumor-bearing patients or in patients with AIDS.

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(Received 23 August 1990; accepted 30 August 1990)