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Original Paper

Lack of Effect of Corticosteroids and Tamoxifen on Suramin Protein Binding and *In Vitro* Activity

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Stout and colleagues [*Proc Am Assoc Cancer Res* 1993, 34, p. 298] previously reported that both hydrocortisone and tamoxifen increased the free fraction of suramin in human plasma. We examined several corticosteroids as well as tamoxifen for their effects on suramin protein binding and also evaluated hydrocortisone for its ability to modulate suramin activity in PC-3 and MCF-7 cells. Greater than 99% of the suramin was protein bound in undiluted human plasma. However, the free fraction of suramin was increased with the reduced plasma protein levels and increased suramin concentrations. At concentrations ranging from 1 to 30 μM, neither tamoxifen, hydrocortisone, prednisone nor dexamethasone had any effect on the binding of suramin to human plasma, regardless of protein concentrations. Similar results were observed with fetal calf serum. Hydrocortisone also had no effect on suramin activity against PC-3 and MCF-7 cell *in vitro*. We conclude from these studies that neither corticosteroids nor tamoxifen affect suramin protein binding or its cytotoxic activity.

Key words: suramin, corticosteroids, hydrocortisone, prednisone, dexamethasone, tamoxifen
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INTRODUCTION

THERE ARE numerous reports describing drug interactions which have been attributed to plasma protein binding displacement (for reviews see [1, 2]). In most of these studies, the drug of interest is highly protein bound to plasma proteins such as albumin, and the 'modulating' drug can displace the primary agent from plasma proteins and thereby increase the free fraction of the drug in the blood [1]. Such drug–protein displacement reactions can lead to increased efficacy or increased toxicity. In patients treated with warfarin, increasing the level of free drug by agents such as phenylbutazone can lead to hypoprothrombinaemia and bleeding [3–5]. Corticosteroids can also have a dramatic effect on drug metabolism, pharmacokinetics and the binding of drugs to plasma proteins [6–11]. Stout and colleagues [12] previously examined this issue with suramin and found that hydrocortisone as well as tamoxifen increased the free fraction of suramin in human plasma. Modulation of suramin binding to plasma proteins could possibly affect suramin's antitumour activity. In addition, more potent corticosteroids might prove superior to hydrocortisone in modulating the protein binding of suramin. Current and planned clinical trials with suramin also included hydrocortisone as part of a hormone replacement therapy since suramin can cause adrenal insufficiency [13, 14]. We have, therefore, re-examined the effects of hydrocortisone, as well as prednisone, dexamethasone and tamoxifen, on suramin binding to plasma proteins. We have also determined the effect of hydrocortisone on suramin activity in PC-3 and MCF-7 cells in culture.

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MATERIALS AND METHODS

Chemicals

[^{3}H]Suramin, specific activity 50 Ci/mmol, was purchased from Moravek Biochemicals (Brea, California, U.S.A.). Suramin was obtained from the National Cancer Institute. Pooled human plasma was obtained from the blood bank. Dulbecco's modified Eagle's medium/F-12 (D-MEM/F-12) 1:1 mixture, Roswell Park Memorial Institute-1640 medium (RPMI-1640), fetal calf serum, and Hank's balanced salt solution (HBSS) were purchased from Gibco BRL (Gaithersburg, Maryland, U.S.A.). Centrifree micropartition tubes were purchased from Amicon (Beverly, Massachusetts, U.S.A.). Dialysis membrane tubing MWCO 12 000–14 000 S/P Brand Spectra/Por*2 was purchased from Baxter (McGaw Park, Illinois, U.S.A.) Hydrocortisone, prednisone, dexamethasone

and tamoxifen were purchased from Sigma (St Louis, Missouri, U.S.A.).

Cell lines

MCF-7, human breast cancer cells, obtained from the American Type Culture Collection (ATCC) (Rockville, Maryland, U.S.A.) were grown in growth medium consisting of D-MEM/F-12 supplemented with 10% fetal calf serum. PC-3, prostate cancer cells, obtained from the ATCC were grown in complete growth medium consisting of RPMI-1640 supplemented with 10% fetal calf serum.

Dialysis

Suramin binding to human plasma and serum proteins was examined by dialysis. Concentrations of suramin ranging from 7.14 to 714 μM containing 2 μCi/ml [^{3}H]suramin were incubated in the presence or absence of several concentrations of hydrocortisone for 3–4 h at 37°C in serum-free or 10% fetal calf serum diluted in D-MEM/F-12. Duplicate samples were dialysed overnight against three changes of D-MEM/F-12. Free and protein bound suramin were assayed by liquid scintillation determination.

Centrifree micropartition system

Suramin binding to human plasma proteins was carried out as described above but with several modifications. The concentrations of the corticosteroids (hydrocortisone, prednisone, dexamethasone) and tamoxifen ranged from 1 to 30 μM and the effect of ethanol and dimethyl sulphoxide (DMSO) on suramin binding were at a concentration of 10%. Duplicate samples were incubated at 37°C overnight and suramin binding to plasma protein determined using centrifree micropartition tubes according to the manufacturer's instructions. Radioactive suramin in the filtrate was determined by liquid scintillation counting.

Protein determination

Protein in diluted and undiluted plasma was determined following procedures described by Bradford [15] using bovine serum albumin as a standard.

Cellular effect

MCF-7 and PC-3 (5×10^4 cells/ml) were plated into 24-well plates and allowed to attach for 24 h. Cells were incubated for 6 days with several concentrations of suramin in the presence or absence of several concentrations of hydrocortisone. Cells were lysed and nuclei counted with a model Z_F Coulter Counter.

RESULTS

Greater than 99% of the suramin was protein bound in undiluted human plasma, even at suramin concentrations as high as 714 μM (Figure 1). However, the free fraction of suramin in plasma was increased if plasma was diluted (reduced plasma protein levels) or if the concentration of suramin was increased. The binding of suramin to either human plasma protein or 10% fetal calf serum gave similar results using the centrifree micropartition system and/or dialysis (Figure 1).

The effects of hydrocortisone, prednisone, dexamethasone and tamoxifen at concentrations from 1 to 30 μM on suramin binding to human plasma proteins were determined at two different dilutions of plasma using the centrifree micropartition tubes. Hydrocortisone had no effect on suramin binding to human plasma proteins, regardless of the concentration of hydrocortisone employed or the diultion of plasma tested (Figure 2a,b). In spite of the greater potency of prednisone and dexamethasone as corticosteroids [16], neither of these agents altered suramin binding to plasma proteins (Figures 3 and 4). In addition, tamoxifen, which altered suramin protein binding in the study of Stout and coworkers [12], also had no effect on suramin binding to plasma at concentrations as high as 30 μM (Figure 5). However, at higher concentrations than those employed in our studies, the solvents used to dissolve the corticosteroids and tamoxifen did have some effect on suramin binding to plasma proteins. In the present study, the final concentration of either ethanol or DMSO used for the corticosteroids or tamoxifen experiments never exceeded 0.5% and at this concentration, no effect of ethanol or DMSO was observed on suramin binding (data not shown). However, at concentrations of 10% ethanol or DMSO, an effect was observed on suramin binding to proteins (Figure 6). Both solvents increased the free fraction of suramin at both dilutions of human plasma.

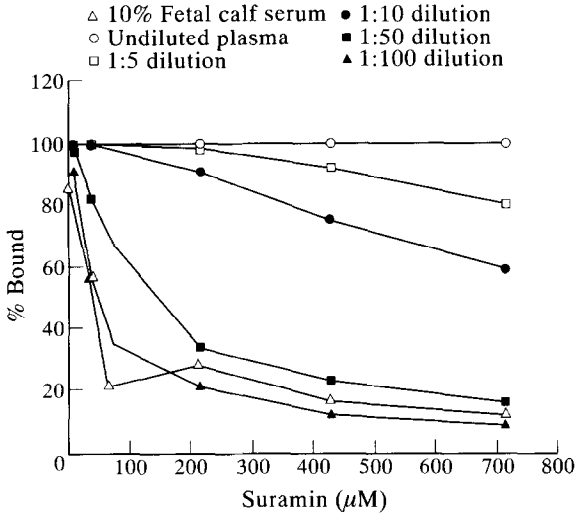

Figure 1. Suramin binding to human plasma proteins and 10% fetal calf serum tubes.

The effect of several concentrations of hydrocortisone on suramin activity against MCF-7 and PC-3 cells was also examined (Table 1). Concentrations of hydrocortisone as high as 30 μM had no significant effect of suramin activity in either cell line.

DISCUSSION

The displacement of protein-bound drugs from plasma proteins by other interacting agents can potentially increase the free active form of the drug and thus potentially increase both its pharmacological action and/or its toxicity. However, the role that plasma protein binding interactions plays clinically is of considerable debate [1, 2]. There are a number of reports that have suggested that corticosteroids can modulate drug binding to plasma proteins or alter drug metabolism and the pharmacokinetics of certain drugs [6–11]. For example, patients treated with a combination of prednisolone and isoniazid had a decrease in the plasma isoniazid concentrations leading to a lower exposure of isoniazid [6]. Patients treated with corticosteroids in combination with salicylate had

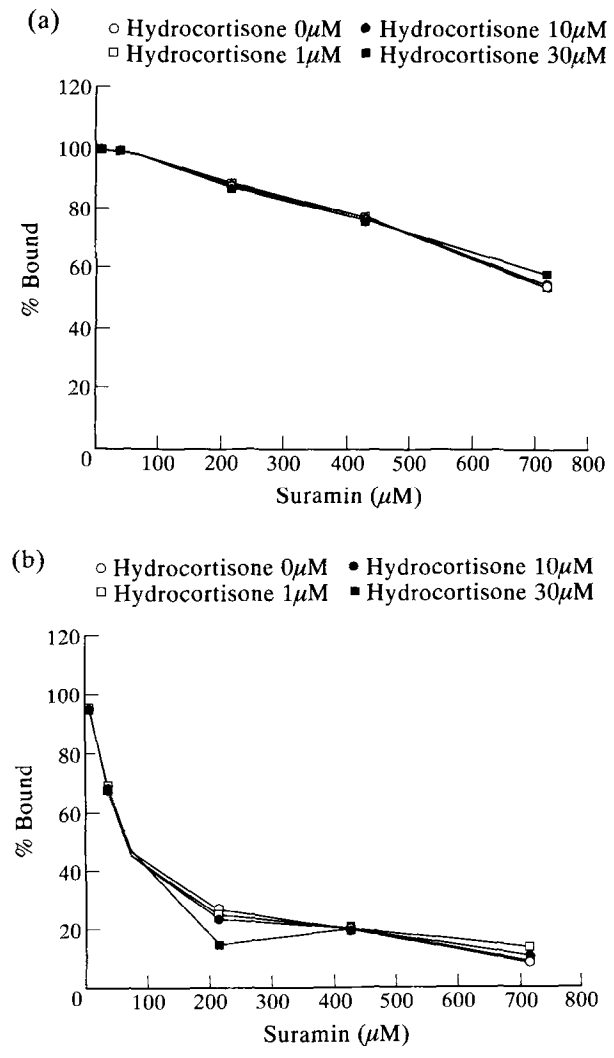

Figure 2. Effect of hydrocortisone on suramin binding to human plasma protein. (a) 8 mg/ml. (b) 0.8 mg/ml.

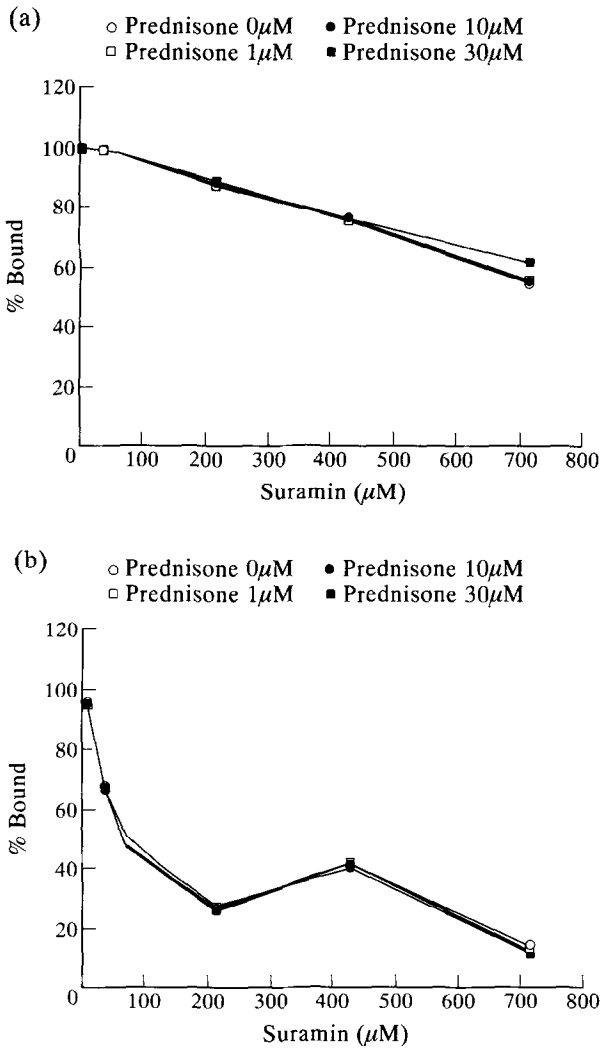

Figure 3. Effect of prednisone on suramin binding to human plasma protein. (a) 8 mg/ml. (b) 0.8 mg/ml.

reduced serum salicylate levels which decreased the salicylate effect [9]. Previous studies and planned clinical trials with suramin also include hydrocortisone for hormone replacement therapy [17–20] since suramin has been reported to cause adrenal failure [13,14]. Thus, the report by Stout and colleagues [12] that hydrocortisone, as well as tamoxifen, modulated suramin binding to human plasma proteins was of potential interest because of the possibility that a hydrocortisone-induced increase in free suramin might increase the antitumour activity of the suramin in clinical trials. We have, therefore, re-investigated the effect of hydrocortisone and tamoxifen as well as two more potent corticosteroids, prednisone and dexamethasone, on suramin binding to plasma proteins. Using concentrations similar to those employed by Stout and coworkers [12], we examined the binding of suramin to human plasma and fetal calf serum proteins by dialysis and by the centrifree micropartition system.

First, in agreement with previous studies [21, 22], we found that greater than 99% of suramin was bound to plasma proteins even if the suramin concentration was greater than 700 μM (Figure 1). However, dilutions of human plasma in HBSS resulted in increased suramin in the free fraction with increasing suramin concentrations. Hydrocortisone had no effect on suramin protein binding, regardless of the concentration of hydrocortisone or the dilution of plasma used. The more potent corticosteroids [16], prednisone and dexamethasone, also had no effect on suramin binding to plasma protein. In addition, hydrocortisone had no effect on suramin binding to fetal calf serum proteins (the serum used to grow MCF-7 and PC-3 cells) and also had no effect on suramin activity against either MCF-7 or PC-3 cells over 6 days of drug treatment. If hydrocortisone altered the binding of suramin to serum proteins, it is likely that the additional free drug would have resulted in increased activity against these two cell lines. It should also be noted that the lack of effect of the corticosteroids or tamoxifen on suramin binding would probably not change in whole blood. Dewey and Wormall [23] previously demonstrated that suramin is almost completely confined to plasma and does not appear to bind to red blood cells.

In the present study, the solvents, DMSO and ethanol, used to dissolve the corticosteroids or tamoxifen did not exceed 0.5% and had no effect on suramin's binding. However, there is an effect of both solvents when their concentrations are increased to 10%. In fact, both solvents increased the free fraction of suramin to approximately the level reported by Stout and colleagues [12]. It is possible, therefore, that the

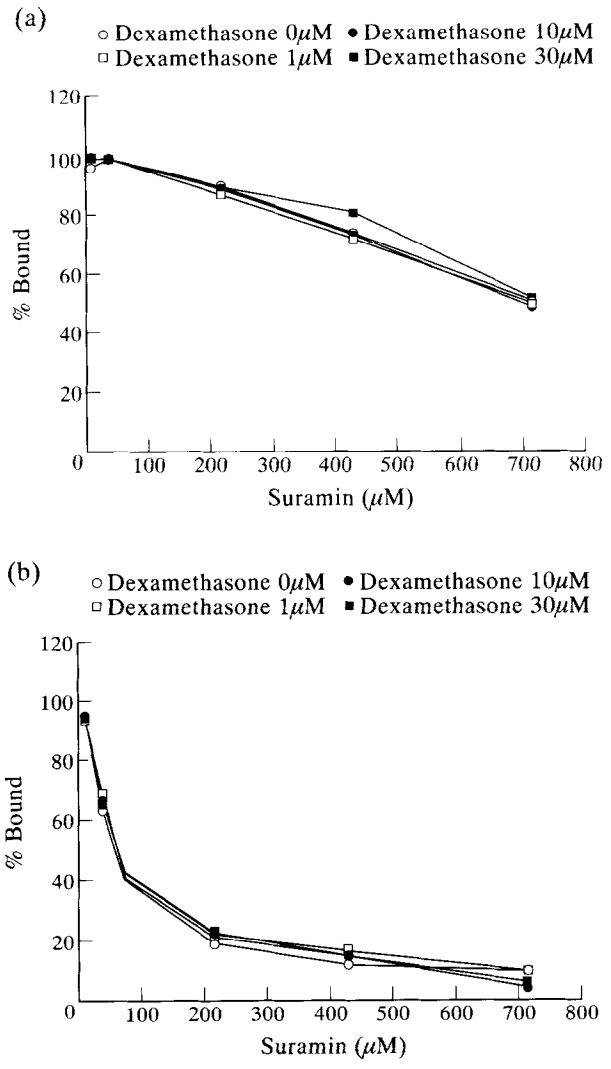

Figure 4. Effect of dexamethasone on suramin binding to human plasma protein. (a) 8 mg/ml. (b) 0.8 mg/ml.

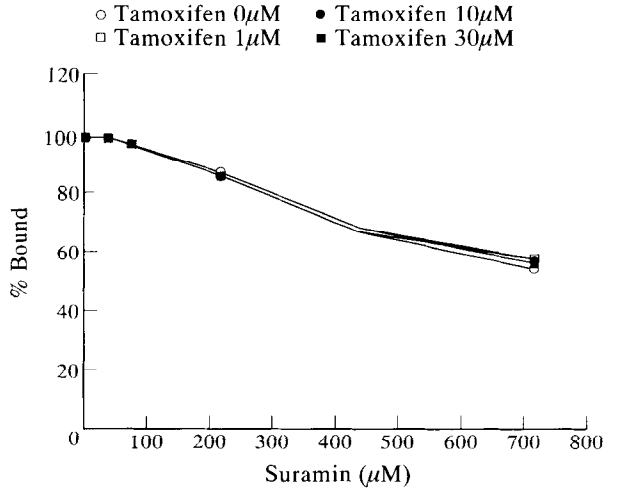

Figure 5. Effect of tamoxifen on suramin binding to human plasma protein (8 mg/ml). Plasma was diluted to 1:10 in HBSS and plasma proteins determined. Suramin binding to plasma proteins was determined as described in Figure 1.

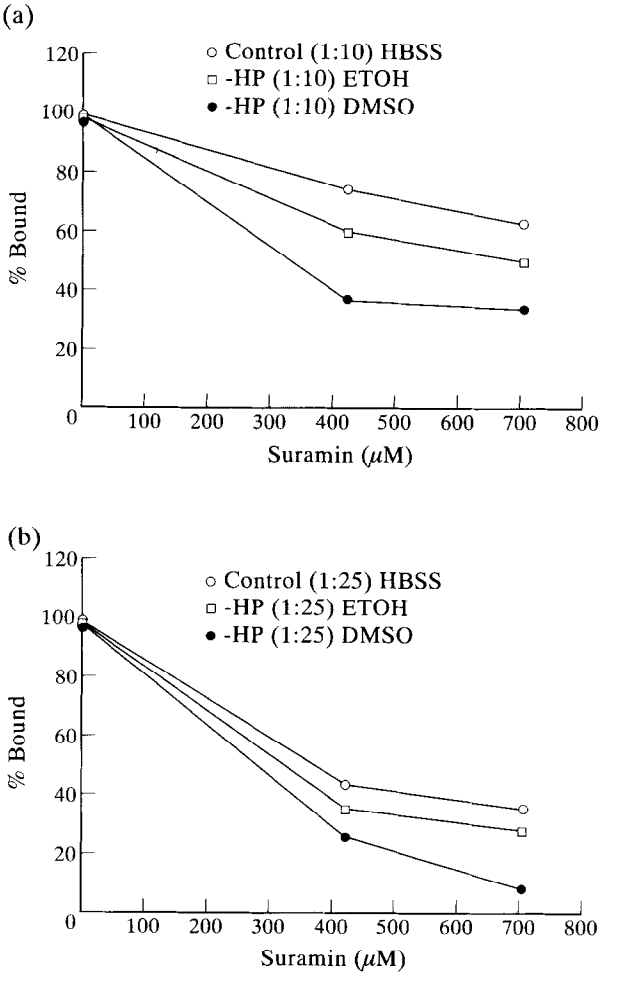

Figure 6. Effect of 10% ethanol or DMSO on suramin binding to human plasma protein (HP). (a) 8 mg/ml. (b) 3 mg/ml.

Table 1. *Effect of hydrocortisone on suramin activity in MCF-7 and PC-3 cells*

Hydrocortisone (μM)	ID_{50}(μM)* MCF-7 cells	ID_{50}(μM)* PC-3 cells
0	163.6	92.0
0.1	174.2	95.3
1	172.6	96.4
10	171.4	99.4
30	166.4	108.7

*MCF-7 and PC-3 cells were treated for 6 days in several concentrations of suramin ± the indicated concentrations of hydrocortisone. ID_{50}, the concentration that inhibits 50% growth of cells.

effect observed by Stout and colleagues was more related to the solvent effect on suramin protein binding rather that the effect of hydrocortisone or tamoxifen.

In conclusion, neither corticosteroids nor tamoxifen affected the binding of suramin to proteins in pooled plasma. The rationale of including hydrocortisone or other more potent corticosteroids in suramin clinical trials (other than as hormone replacement therapy) in order to modulate suramin plasma binding and potentate drug activity does not appear to have merit.

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