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Synergistic Antiproliferative Activity of Suramin and α2A-Interferon Against Human Colorectal Adenocarcinoma Cell Lines: *In vitro* Studies

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Suramin, a polysulphonated naphthylurea proven to be an effective anticancer agent against selected tumours, and α2A-interferon (α2A-IFN) were investigated for their combined effects on HCT-8, HCT-15, CL-D, SW-480 and SW-620 human colorectal adenocarcinoma cell lines. All lines were sensitive to clinically achievable concentrations of suramin in a dose-dependent manner, while α2A-IFN alone induced only a modest reduction of cell growth. Concomitant treatment with suramin and α2A-IFN resulted in a synergistic inhibition of cell viability in each cell line at all doses tested. However, when suramin and α2A-IFN were administered sequentially, inhibition of cell viability was clearly dependent on the timing of treatment schedule, with maximum effect obtained when α2A-IFN was administered prior to suramin. In contrast, pretreatment with suramin was markedly inferior to the former one. In conclusion, suramin and α2A-IFN exert a synergistic effect on human colorectal cell proliferation *in vitro* at clinically achievable concentrations. This observation may have clinical relevance although the mechanisms of interaction remain to be elucidated.

Key words: suramin, α-interferon, colorectal cancer, cell lines, *in vitro*
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INTRODUCTION

SURAMIN is a polysulphonated naphthylurea that has been used in the therapy of trypanosomiasis, onchocerciasis and acquired immunodeficency syndrome [1, 2]. More recently, new interest in suramin has been stimulated because of its capacity of binding and inactivating several growth factors, such as basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor α and β (TGFα and TGFβ), epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I) [3–6]. In fact, one of the most important developements in cancer research during the last years has been the discovery of the biochemical and genetic mechanisms of transformation, and within these, autocrine and paracrine growth stimulation through several growth factors, which are inactivated by suramin, has been well documented [7, 8]. In particular, in colorectal cancer, multi-autocrine loops involving EGF, TGFα, PDGF and TGFβ have been demonstrated [9, 10]. Therefore, we have evaluated the antiproliferative activity of suramin against a panel of human colorectal cell lines in an attempt to determine whether suramin merits clinical studies in colorectal cancer, a tumour which is very often resistant to currently available antitumour drugs. Furthermore, in an attempt to identify agents able to enhance the antitumour activity of suramin, which induces severe clinical toxicities at plasma concentrations over 300–350 μg/ml [11], we evaluated its combination with another antiproliferative agent, such as α-interferon (IFN). In fact, IFNs have important inhibitory effects on cell growth by interfering with mechanisms involved in cell proliferation, including gene expression, changes in the plasma membrane cytoskeletal complex and, in particular, downregulation of receptors for growth factors such as insulin, transferrin and EGF, that may affect suramin antiproliferative activity [12–16]. This study demonstrated that suramin efficiently inhibits cell proliferation of five human colorectal cell lines at clinically achievable concentrations, and that α2A-IFN administered before or together with suramin synergistically potentiates its antiproliferative activity.

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MATERIALS AND METHODS

Drugs and chemicals

Suramin was purchased from Bayer (Germany), as commercially available 1 g sterile vials, reconstituted in RPMI 1640 medium (Gibco), sterilised by ultrafiltration and stored in aliquots at −80°C until use. Recombinant α2A-IFN was purchased from Hoffmann-La Roche (Switzerland) as commerically available 3 million U vials, and appropriate dilutions were made in RPMI 1640 containing 10% heat inactivated fetal bovine serum (FBS) (Gibco) before each single experiment. The tetrazolium reagent 2,3-*bis*(2-methoxy-4-nitro-5-sulfophenil)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) was pur-

chased from Polyscience (Warrington, Pennsylvania, U.S.A.), and prepared at 1 mg/ml in prewarmed RPMI 1640 medium before use. Phenazine methosulphate (PMS) was purchased from Sigma (St. Louis, Missouri, U.S.A.), and prepared at 5 mM (1.53 mg/ml) in phosphate buffered saline (PBS) and stored at 4°C up to a maximum of 3 months.

Cell lines

The five human colorectal adenocarcinoma cell lines used in this study (HCT-8, HCT-15, CL-D, SW-480, SW-620) have been characterised previously [9, 17–20] and were kindly provided by Dr Ming Chu (Providence, Rhode Island, U.S.A.). Exponentially growing cells were grown in RPMI 1640 medium

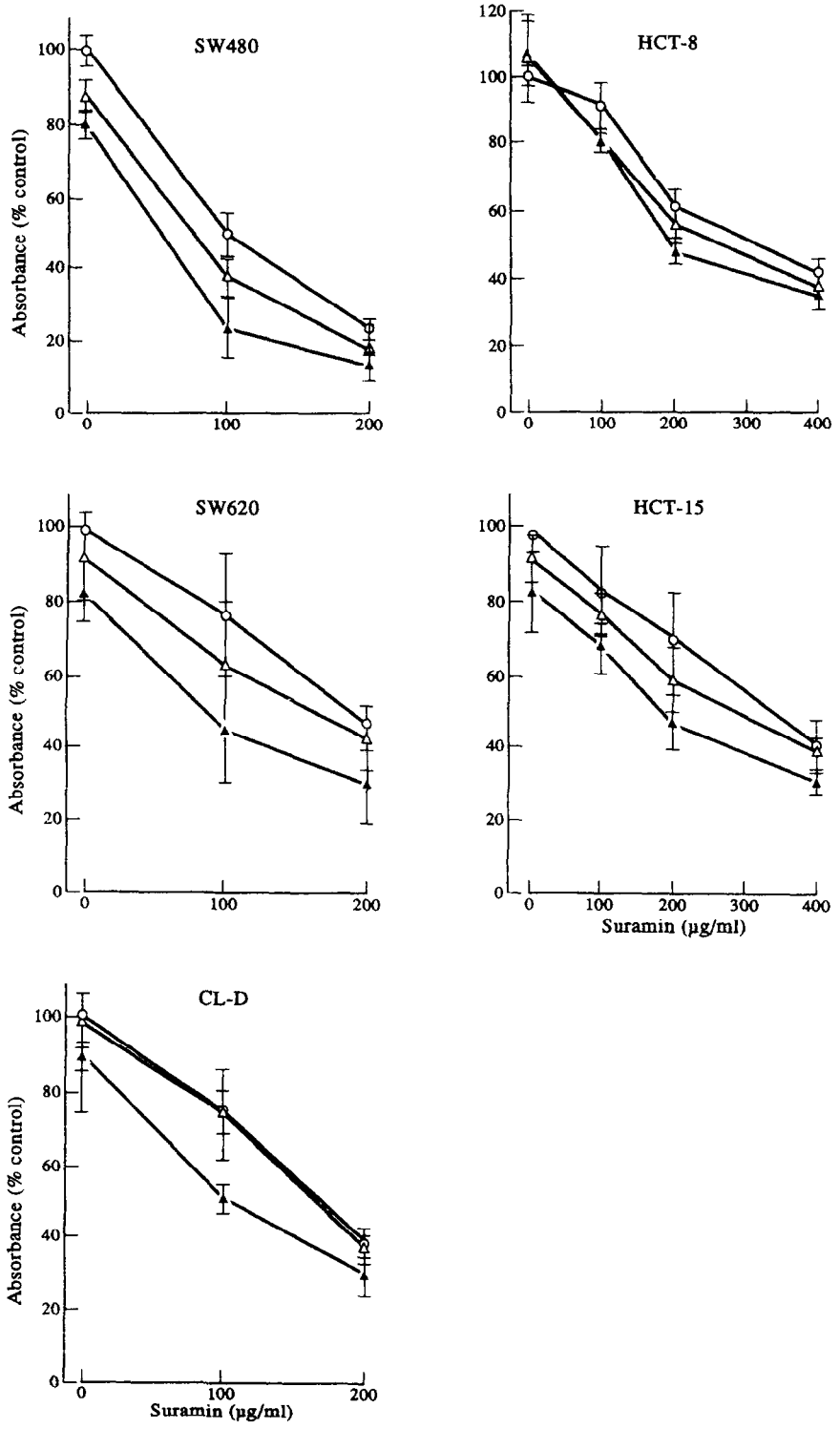

Figure 1. Effect of suramin alone (-○-) and in combination with α2A-IFN 100 (-△-) and 1000 (-▲-) U/ml on human colorectal adenocarcinoma cell lines. Points are the mean of duplicate experiments (HCT-8), or triplicate experiments (HCT-15, CL-D, SW480 and SW620), each performed in triplicate, with S.D. shown by vertical bars; if not represented, bars are within the plotting symbol.

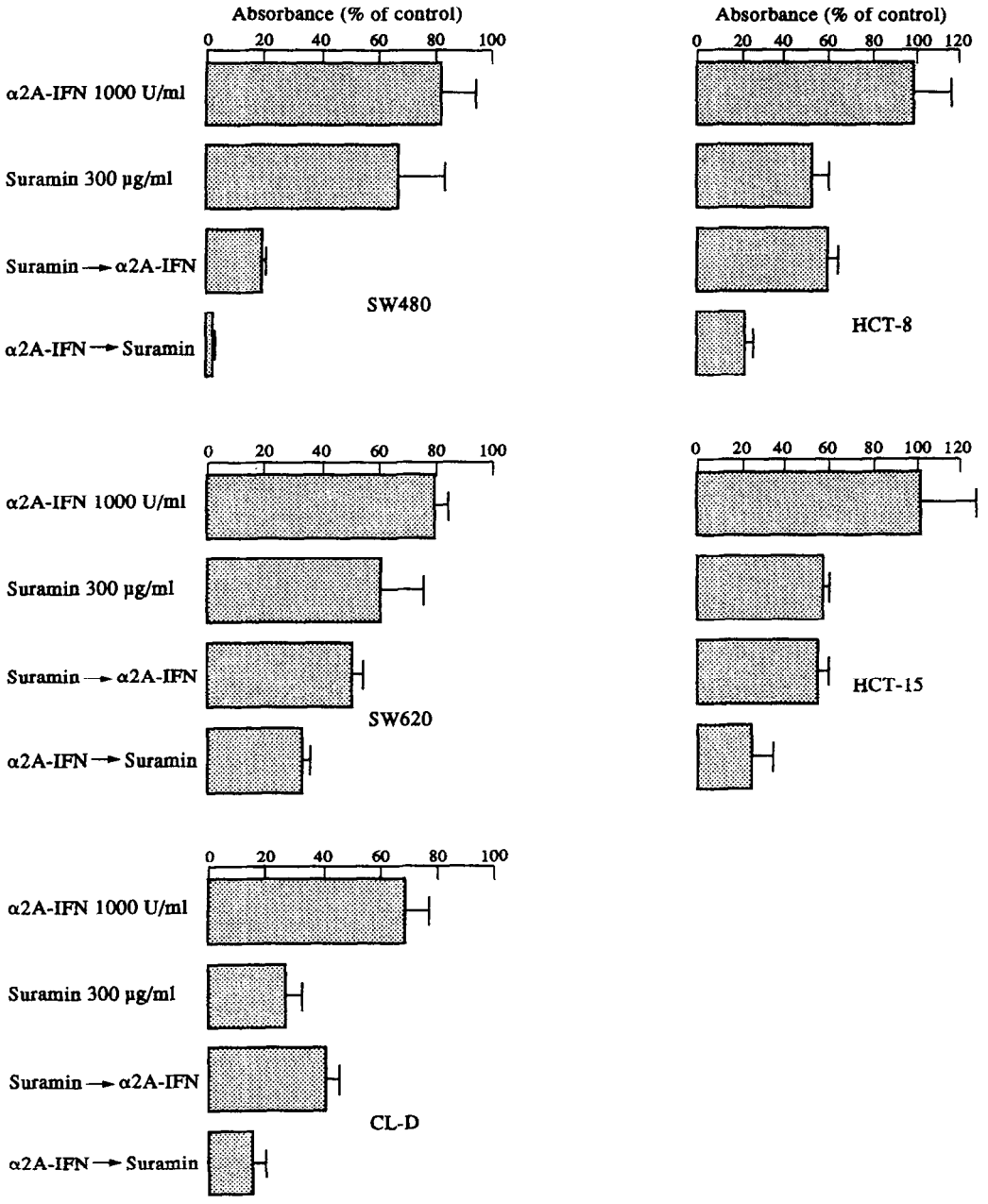

Figure 2. Effect of treatment with suramin or α2A-IFN alone or in sequential combination on human colorectal carcinoma cell lines; arrow (→) indicates the timing of treatments. Bars are the mean value of three experiments each performed in triplicate with S.D. shown by vertical bars.

supplemented with 10% FBS, and maintained in a humidified incubator at 37°C in an atmosphere of 5% CO_2.

Drug sensitivity testing

The XTT assay was used to determine drug sensitivity, and was performed as previously reported [21]. In brief, single cell suspensions were prepared by trypsinisation and cells counted by a haematocytometer. Between 1000–2000 cells were suspended in 100 μl of RPMI 1640 + 10% FBS, seeded into each well of a 96-well microtitre plate (Gibco), and incubated at 37°C with 5% CO_2. After 16–24 h 100 μl of RPMI 1640 containing 10% FBS and drugs at appropriate concentrations were added. Cells were incubated for an additional 5 days in the experiments of concomitant treatment with suramin and α-IFN, or for 3+3 days in the sequential experiments, where after 3 days, media containing 10% FBS and drugs was substituted with new media RPMI 1640 + 10% FBS + drugs at appropriate concentrations. Subsequently, 50 μl of RPMI 1640 media containing 50 μg of XTT and 0.38 μg of PMS were added, cells incubated for additional 3–4 h and then absorbance measured at 450 nm with a microplate autoreader (Bio-Teck Instruments). Cell growth inhibition was expressed as the percentage of control (no drugs) absorbance.

Statistical analysis

The percentage of growth was calculated from the formula: $100 \times (\text{absorbance}_{\text{treated cells}}/\text{absorbance}_{\text{control cells}})$. Interaction between α2A-IFN and suramin was analysed using a multiplicative model as described previously [22, 23]. Briefly, the multiplication of the surviving fraction (SF) due to either drug alone (SF_{suramin} or $SF_{\alpha 2A\text{-IFN}}$) is compared with the observed result. If $SF_{\text{suramin + }\alpha 2A\text{-IFN}} < (SF_{\text{suramin}} \times SF_{\alpha 2A\text{-IFN}})$ the interaction is

synergistic, if $SF_{suramin + \alpha 2A\text{-}IFN} = (SF_{suramin} \times SF_{\alpha 2A\text{-}IFN})$ the interaction is additive, and if $SF_{suramin + \alpha 2A\text{-}IFN} > (SF_{suramin} \times SF_{\alpha 2A\text{-}IFN})$ it is antagonistic.

RESULTS

Treatment with suramin produced a dose-dependent inhibition of cell viability in each cell line (Figure 1). α2A-IFN induced only a modest reduction of cell viability ranging from −2.2 to −12.2% at 100 U/ml and from −11 to −20% at 1000 U/ml in HCT-15, CL-D, SW480 and SW620 cell lines, while cell viability in HCT-8 cells was increased compared to controls. Concomitant treatment with suramin and α2A-IFN resulted in a synergistic inhibition of cell viability in each cell line at all doses tested (Figure 1). However, when suramin and α2a-IFN were administered sequentially, inhibition of cell viability was clearly dependent on the timing of treatment schedule (Figure 2); if α2A-IFN was administered prior to suramin, the inhibition of cell proliferation by combined treatment was highly synergistic, while if suramin was added first and then α2A-IFN, the resulting effect was antagonistic in the case of HCT-8, CL-D and SW620 cell lines, borderline synergistic in the HCT-15 cell line, and synergistic in SW480 cells.

DISCUSSION

Despite recent advances in chemotherapy, the treatment of metastatic colorectal cancer remains unsatisfactory, making it a logical target for new treatment approaches [24]. During the last years, there have been important advances in knowledge on the mechanisms involved in transformation and in tumour cell progression and proliferation. In particular, autocrine and paracrine growth stimulation through several growth factors has been well documented in many human tumours including colorectal cancer [7–10]. Therefore, suramin, which binds and inactivates several of these growth factors [3–6], might represent a new agent to be evaluated for the therapy of colorectal cancer. Indeed, our study demonstrates that suramin has significant antiproliferative activity against five human colorectal cell lines at clinically achievable concentrations. Other authors recently reported a similar activity of suramin *in vitro* against human colorectal tumour cells [25–28]; therefore, further clinical studies with suramin in colorectal cancer patients should be encouraged.

α-IFN is a cytokine which acts as a negative growth regulator and affects the expression of many genes and proteins involved in the control of cell proliferation [12–16]. In the present *in vitro* model, α2A-IFN possessed only minimal antiproliferative activity, but synergistically enhanced suramin-induced inhibition of cell growth. The synergism was assessed by a multiplicative model which is a simple, although sometimes weak method of defining synergism; nevertheless, the application of different and more complex methods would not have modified the conclusions from the data.

Because of these findings and considering the important suramin-related clinical toxicities, which are plasma concentration dependent [11, 29], this observation might have clinical relevance, especially if the combination will not result in synergic toxicities in humans. However, our preliminary results in *in vivo* preclinical models in mice and rats have shown that α2A-IFN does not affect suramin plasma kinetics and toxicities (data not shown).

The observation that α-IFN enhances suramin antiproliferative activity has already been reported in human melanoma cell lines [30]. The mechanisms of interaction between α-IFN and suramin are not known and have not been elucidated in this study. Nevertheless, the observation that a synergistic inhibition of cell viability in all the five cell lines studied occurred only when they were exposed to α-IFN before or together with suramin, indicates that α2A-IFN may induce cellular changes that can make tumour cells more sensitive to suramin and not *vice versa*. The nature of these changes has not yet been investigated, although other authors described effects of α-IFN on the expression of growth-related proteins, like EGF receptor, which may enhance the antiproliferative activity of suramin [14–16, 31]. However, it cannot be excluded that the nature of the interaction is not at the level of growth control mechanisms, because suramin may have other important antiproliferative effects, like inhibition of DNA polymerase and topoisomerase II [32, 33].

In conclusion, this study demonstrates that suramin has significant antiproliferative activity against five human colorectal cell lines, and that this effect can be synergistically enhanced by α2A-IFN. This observation may have clinical relevance, although the mechanisms of interaction between suramin and α2a-IFN remain to be eludicated.

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Modulation of Cisplatin Cytotoxicity by Human Recombinant Interferon-γ in Human Ovarian Cancer Cell Lines

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Cytotoxic interactions between recombinant human interferon-γ (IFNγ) and cisplatin have been studied in six ovarian cell lines (IGROV1, NIHOVCAR3, SKOV3, OVCCR1, 2008 and its cisplatin resident variant 2008/C13*). Studies were performed using a cell survival assay. Results were assessed using median effect analysis. Synergy between these two drugs was observed in cell lines sensitive to IFNγ, whatever their relative sensitivity or resistance to cisplatin, suggesting that IFNγ enhances the cytotoxic activity of cisplatin. This interaction is not due to an increase in platinum accumulation in cells. This combination of drugs should be evaluated against human ovarian cancer xenografts in nude mice before its use in clinical practice.

Key words: interferon-γ, cisplatin, interactions, median effect analysis, human ovarian cancer cell lines, combination index, synergism

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INTRODUCTION

THE INCORPORATION of biological agents, often termed biological response modifiers (BRM) into combination regimens with standard chemotherapeutic agents offers an important challenge to medical oncologists, since the assumptions for their use are likely to differ from those for chemotherapeutic agents. The interferons (IFN) are a family of naturally occurring glycoproteins which share antiviral, immunomodulatory and antiproliferative effects. Interferon-γ (IFNγ) has a somewhat more potent antitumour activity than the other classes of interferons