Vol. 178, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1991 Pages 207-212

EFFECT OF SCHISANHENOL ON THE ANTITUMOR ACTIVITY OF ADRIAMYCIN

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Received May 13, 1991

SUMMARY: Schisanhenol (Sal) did not diminish the antitumor activity of adriamycin in mice bearing P388 ascites tumor. Sal did not antagonize the the suppressive effect of adriamycin on DNA synthesis and cell proliferation in an L1210 ascitic tumor cell culture. Furthermore, Sal at the concentration of 0.1, 0.25, or 1 mM accelerated adriamycin-dependent DNA damage in the presence of Fe^{3+} in vitro. It appears that Sal was able to protect against adriamycin induced heart mitochondrial toxicity, while it did not antagonize the antitumor activity of adriamycin.

The antitumor antibiotic adriamycin is one of the most important drugs in the field of cancer chemotherapy. It exhibits activity against a wide spectrum of human neoplasms and in particular, against solid tumors. Unfortunately, its clinical use has been compromised by an unusual and potentially lethal cardiotoxicity, which is related to free radicals. When free radical scavengers, such as tocopherol(1), are used to inhibit the formation of lipid peroxides, the toxicity of adriamycin is significantly reduced(2). As described in our previous work adriamycin induced cardiac mitochondrial toxicity was significantly reduced by schisanhenol (Sal). In this experiment, the effects of Sal on the antitumor activity of adriamycin were investigated *in vivo* and *in vitro* (Fig 1).

MATERIALS AND METHODS

Materials: Sal was supplied by Professor Yan-Yong Chen, Institute of Materia Medica, Chinese Academy of Medical Sciences. Male DBA mice weighing 18 to 20 grams were used. Deoxyribonuclic acid was obtained from Sigma. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma and dissolved in RPMI 1640 culture medium. The chemicals used were of analytical grade. The data presented are expressed as a mean ± SD and were statistically evaluated by Student's t-test.

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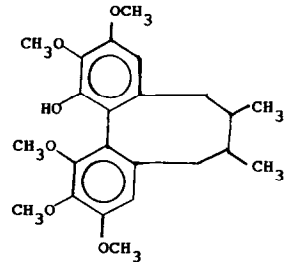

Schisanhenol

Fig. 1.

In vivo tests Eighty mice bearing P388 ascites tumor were divided into eight groups for two experiments. In each experiment, group A was used as the control. Group B was administered with Sal once daily. Group C received adriamycin alone. Group D received adriamycin and Sal as group B and C. In experiment 1, adriamycin (5 mg/kg, i.p.) was administered on days 1 and 2, and schisanhenol (100 mg/kg, p.o.) was given once daily from day 1 to day 4. In experiment 2, adriamycin (5 mg/kg, i.p.) was injected on day 1 and schisanhenol (150 mg/kg, p.o.) was given once daily from day 1 to day 7.

Incorporation of [^{3}H]TdR into cultured L1210 ascitic tumor cells L1210 ascitic tumor cells ($2x10^5$ cells/ml) were cultured in RPMI 1640 culture medium. Sal (1, 10 or 100 μM) and adriamycin (0.1, 1 or 10 μM) were incubated with tumor cells in a total volume of 1 ml at 37 °C, 95%O_2 + 5% CO_2 in a CO_2 incubator for 48 hrs. Tritiated thymidine, 20 μl (1 μCi/ml), was then added to the culture medium and incubation was continued under the same conditions for 4 hrs. Liquid scintillation counting was performed according to the method described in reference(3).

Cell growth test with MTT: L1210 ascitic tumor cells (2×10^5 cells/ml) were incubated with various concentrations of adriamycin and Sal in a total volume of 0.2 ml for 48 hrs. After the addition of MTT, the incubation was continued for 4 more hrs. Tumor cells were then harvested by centrifugation (1000 rpm, 5 min) and then washed free of phenol red and MTT with the culture medium . After lysis with isopropyl alcohol, the absorbance was detected at 520 nm.

Measurement of adriamycin-iron mediated degradation of deoxyribonuclic acid: DNA (0.5 ml, 1mg/ml in 0.15 M NaCl, buffered to pH 7.4 with sodium hydrogen carbonate) together with adriamycin 0.1 ml of a 1 mg/ml solution, 0.2 ml PBS (pH 7.4) and 0.1 ml Sal were mixed thoroughly. The reaction was started by the addition of ferrous ions, 0.1 ml of a 0.5 mM solution. The tubes were incubated at 37 °C for 20 min, followed by the addition of 1 ml 25% (v/v) HCl and 1 ml of 1% TBA reagent. The tubes were heated at 100 °C for 15 min to develop the MDA-TBA chromagen which was read at 532 nm (4,5,6).

RESULTS

Effects of Sal on the antitumor activity of adriamycin in vivo The P388 ascites tumor was used in this study because it is the murine tumor most sensitive to adriamycin(7). As shown in Fig 2, Sal alone showed no antitumor activity in mice bearing P388

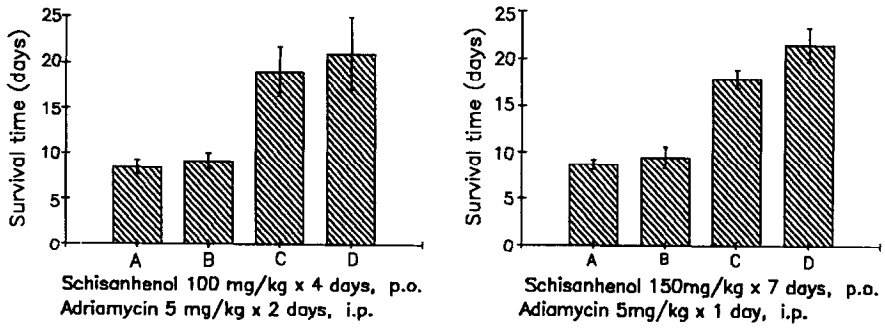

Fig. 2. Effect of Sal on the antitumor activity of adriamycin in vivo. A: Control, B: Sal, C: Adriamycin, D: Sal + Adriamycin.

tumor. Adriamycin exhibited significant anti-tumor activity as the survival time of the mice were prolonged. When adriamycin was administered in combination with Sal, its antitumor potency was not antagonized.

Effects of Sal on the antitumor activity of adriamycin in vitro The results in Fig 3 indicated that Sal at the concentration of 0.1 mM has antitumor activity on L1210 ascitic tumor cells assayed by the MTT method. The various concentrations (1, 10, 100 μM) of Sal did not significantly affect the antitumor activity of adriamycin (Fig 3A). The antitumor activity of adriamycin in various concentrations was not affected by 100 μmol/L Sal (Fig 3B).

Effect of Sal on adriamycin-induced inhibition of [^{3}H]TdR incorporation into L1210 tumor cells As shown in Fig 4, the depression of [^{3}H]thymidine incorporation into L1210 ascitic tumor cells by adriamycin was not affected by Sal. Sal alone at

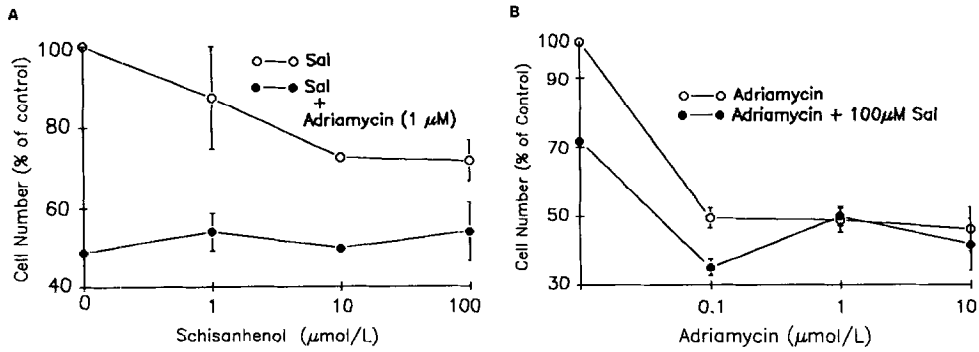

Fig. 3. Effects of Sal on the antitumor activity of adriamycin on the L1210 ascite tumor cell culture. After a 48 hr incubation with Sal, the tumor cells were mixed with MTT and incubation was continued for a further 4 hrs. After the tumor cells were lysed with isopropyl alcohol, the absorbance was detected at 520 nm.

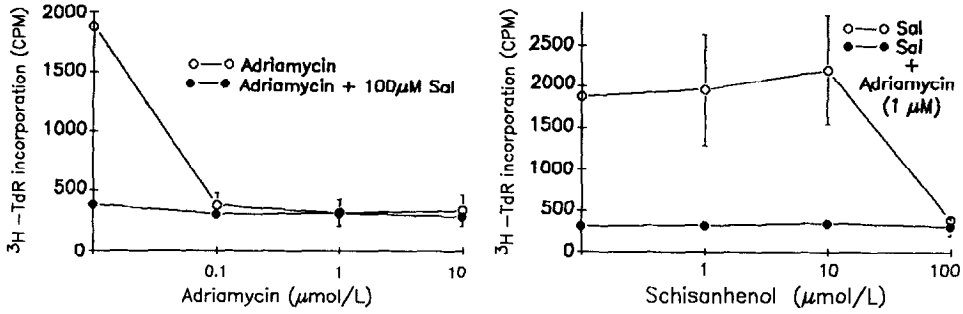

Fig. 4. Effect of Sal on the suppression of [^{3}H]TdR incorporation into cultured L1210 ascite tumor cells by adriamycin. After a 48 hr incubation with Sal, tumor cells were mixed with tritiated thymidine and incubation was continued for 4 hrs more. After lysis with Triton X-100, the radioactivity was counted in Beckmann liquid scintillation counter.

the concentration of 0.1 mM significantly inhibited [^{3}H]TdR incorporation, in accordance with the results of cell growth tested by MTT.

Effect of Sal on adriamycin-iron mediated degradation of deoxyribonuclic acid Figure 5 shows that addition of Sal led to more DNA degradation induced by adriamycin and Fe^{2+} at pH 7.4. The potentiation of adriamycin-induced DNA degradation was increased by Sal until the optimum Sal concentration was reached (250 μM) and then became smaller with a further increase in Sal (Fig 5). Sal alone did not induce DNA degradation.

DISCUSSION

It has been assumed that the antitumor action of adriamycin can be dissociated from its cardiotoxicity(1). In order to

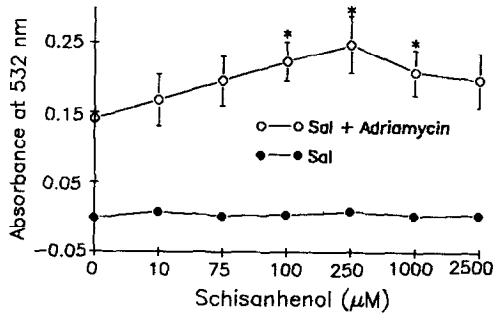

Fig. 5. Effect of Sal on adriamycin-Fe^{2+}-mediated DNA degradation. After addition of ferrous sulfate (50 μM), the mixture containing DNA (0.5 mg/ml) and adriamycin (0.1 mg/ml) in the presence or absence of Sal was incubated at 37 °C for 20 min. The reaction was stopped by adding 1 ml 25%(v/v) HCl. After addition of 1 ml 1% TBA reagent, the tubes were heated at 100 °C for 15 min to develop the MDA-TBA chromagen, which was read at 532 nm.

minimize adriamycin cardiotoxicity, different approaches with the aim of increasing the adriamycin therapeutic index have been proposed. The most frequently tested drugs are free radical scavengers(1,8). It was reported that combination use of tocopherol and adriamycin prevented adriamycin induced cardiomyopathy, while it did not diminish the therapeutic potency of adriamycin in mice bearing P388 ascites tumor, leading to a significant increase of the therapeutic index. As described in a companion paper (12), the mitochondrial toxicity of rat heart induced by adriamycin was significantly reduced by Sal. The results in the present study showed that the responsiveness of the murine ascitic tumor cells to adriamycin was not significantly affected by Sal both in vivo and in vitro.

The incorporation of [^{3}H]TdR into cancer cells as an indication of cell proliferation has been extensively studied. The responsiveness of experimental tumors to chemotherapeutics depends on this proliferative activity(9,10). The inhibition of DNA synthesis and therefore, of cell multiplication can be considered as a "common final pathway" of cytotoxic drugs, whatever their modes of action are(10). Adriamycin binding to mammalian DNA causes fragmentation of DNA and inhibition of DNA synthesis. This DNA binding has been proposed as a mechanism for the antitumor effect of adriamycin(11). In order to further study the interactions of Sal and adriamycin, the effect of Sal on the suppression of DNA synthesis in tumors by adriamycin was investigated. As shown in Fig 4, Sal did not significantly affect the ability of adriamycin to inhibit DNA synthesis in L1210 ascitic tumor cell culture.

The results on the TBA-MDA adduct formed from DNA after adriamycin-iron damage, indicated that Sal, an effective antioxidant in iron-catalyzed lipid peroxidation, showed reactive pro-oxidant activity in the adriamycin iron catalyzed damage to DNA.

These results indicated that Sal is able to protect against adriamycin induced heart mitochondrial damage, without affecting the antitumor activity of adriamycin. It is reasonable to speculate that Sal may have potential use as an adjuvant with adriamycin during cancer treatment.

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