

HUMAN INTERFERON AND CELL GROWTH INHIBITION. V. EFFECT OF OUABAIN ON
INTERFERON ACTIVITIES

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SUMMARY: The effect of ouabain on the antiviral and anticellular activities of interferon and its components was tested in RS_b cells. These cells were found to be inhibited in growth by both components. Ouabain, known to block the antiviral effect of interferon without affecting the growth inhibitory effect, depressed both effects of the antiviral component. The effect of the growth inhibitory component was unaltered by the drug. The results support earlier suggestions that two different mechanisms may be involved in the growth regulation by interferon.

INTRODUCTION: Ouabain is a cardiac glycoside known to inhibit $\text{Na}^+ - \text{K}^+$ -dependent ATPase and to reduce synthesis of protein and DNA by cells and consequently their rate of growth (1-4). It has also been shown, that ouabain inhibits the antiviral activity of interferons (5-7), while the anticellular activity of human and mouse interferons seems to be unaffected. Kuwata and his colleagues have shown that the transformed human cell lines RS_a, RS_b and IF^r as well as the AKR mouse cell line, K_{3b} were additively suppressed in growth when treated with interferon and ouabain in combination (7-9). Under the same conditions the antiviral activity of interferon was blocked. This difference in effect of ouabain on interferon activities supports our notion that the two activities of interferon can be separated (10, 11). In a recent study (to be published) on the effect of the two components on growth of different human cell lines it was found, that most of the transformed cell lines studied were inhibited in growth by both components. As the growth rate of these cells is a result of the viral transformation it was suggested that the growth inhibition by the antiviral com-

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ponent may be a consequence of the antiviral activity and dependent on multiplication of the viral genome carried by the cells.

RSb cells are human fibroblasts double-transformed with Rous sarcoma virus and simian virus 40 (8). They are highly sensitive to the growth inhibitory effect of interferon. In a pilot study they were found to be reduced in growth by both components of interferon. Therefore, they seem to offer a model system for the study of growth inhibition by the two components. In the present study the effect of ouabain on the antiviral and growth inhibitory activities of interferon and its components is investigated in RSb cells. It is shown that both effects of the antiviral component are inhibited by ouabain, while the effect of the growth inhibitory component is unaffected. Thus, the growth inhibition of the two components as observed in RSb cells seems to be regulated by different mechanisms.

MATERIALS AND METHODS: Cells: RSb cells were originally isolated by T. Kuwata et al as a human embryonic fibroblast line successively transformed by Rous sarcoma virus and simian virus 40 (12). They were received in our laboratory from Dr. H. Schellekens, Rotterdam, The Netherlands. The cells were grown in Eagle's MEM (GIBCO) supplemented with 10 per cent inactivated calf serum and 0.044 per cent NaHCO_3 and maintained in the same medium with 2 per cent calf serum and 0.132 per cent NaHCO_3 .

Interferon: Partially purified human leukocyte interferon (P-IF) was a kind gift from Dr. K. Cantell, Helsinki, Finland. The preparation had a specific activity of 0.7×10^6 international units per mg of protein.

Preparation of interferon components: The procedure for preparation of antiviral and cell growth inhibitory components from human leukocyte interferon by sodium dodecyl sulphate has been described (11). The antiviral titer of the antiviral component was found to be 0.9×10^5 international units per ml in repeated tests. In dilution 1/25 this component did not reduce the growth of U-amnion cells. The anticellular component with an antiviral titer below detection limit reduced the cell number of U-amnion by 70-75 per cent in different tests.

Tests for antiviral activity: Antiviral activity was measured by an infectivity inhibition micromethod (13) using Vesicular stomatitis virus, 10 TCID₅₀ in 0.1 ml, as challenge. All titers given are expressed as international units per ml by correction to the international standard of human interferon, 69/19 B, if nothing else is indicated in the text.

Tests for effect on cell growth have been described in detail elsewhere (14). The results are expressed as per cent reduction in cell count as compared to the appropriate controls.

Ouabain was purchased from SIGMA. A 10^{-6} M stock solution was prepared in Eagle's MEM and kept at $+4^{\circ}\text{C}$. Based on a pilot study on the effect of the drug on growth of RSb cells a final dilution of $10^{-7.9}$ M was used throughout the study. In this dilution cell growth was inhibited by 20-40 per cent in different experiments.

Table 1. The effect of ouabain on antiviral activity of interferon and antiviral component.

	Interferon	Antiviral component
Untreated	6.45 ^{x+} -0.1	5.45 ⁺ -0.1
With $10^{-7.9}$ M ouabain	6.05	4.95

^x Log₁₀ antiviral units per ml.

RESULTS AND DISCUSSION: The effect of ouabain on the antiviral activity of unseparated interferon and the antiviral component was tested in RSB cells (Table 1). The dilution of the challenge virus was corrected to ensure that the same dose of virus was used in all titrations. As reported by others ouabain inhibited the antiviral activity. In different experiments the antiviral titer of both preparations was reduced 0.3-0.5 log₁₀ units by ouabain.

The effect of ouabain on the growth inhibitory activity of interferon and its components was then investigated (Figure 1). RSB cells, 10^5 per tube, 5 tubes in parallel, were seeded with increasing concentrations of interferon with or without ouabain. Controls were similarly seeded with and without the drug. The cells were counted after 3 days of incubation in 5 per cent CO₂-atmosphere. The results are shown in Figure 1a. The additive suppression of growth by combined treatment with interferon and ouabain reported (8) is confirmed in the present study. However, when the effect of the antiviral component was studied in the same way (Figure 1b) a reduction in the growth inhibitory effect of this component was observed in the presence of ouabain. This reduction is well correlated with the observed reduction in antiviral activity of the component by ouabain. For the anti-cellular component (Figure 2) no effect of ouabain on the growth inhibitory activity could be observed in agreement with suggestions by Kuwata et al (8).

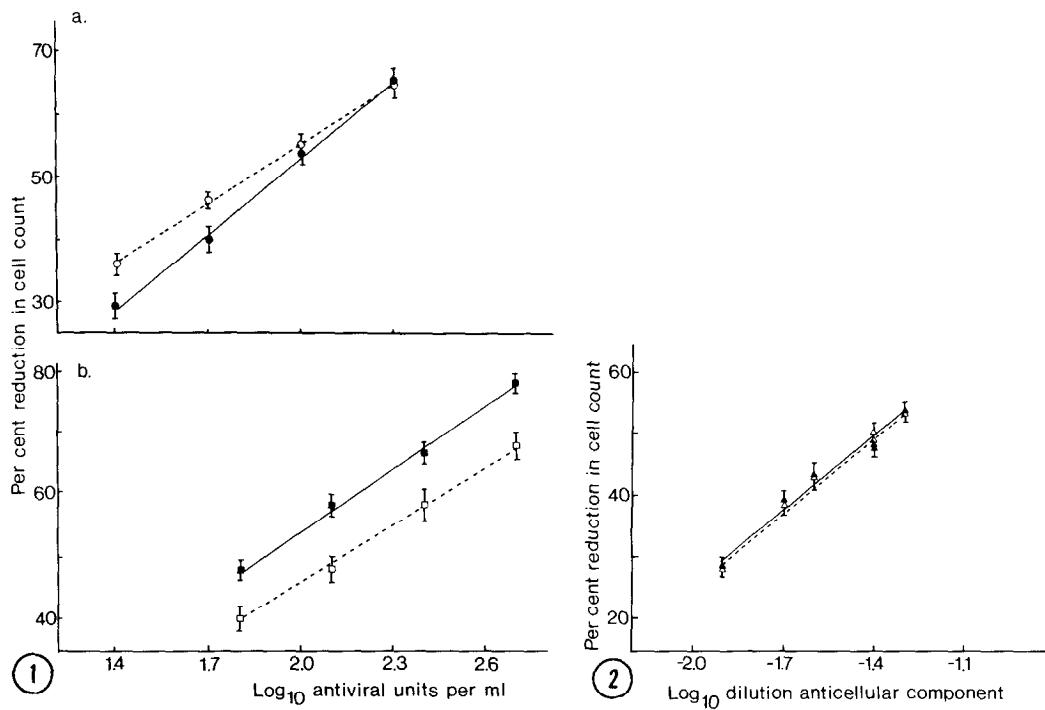


Figure 1a: Effect of ouabain on growth inhibition by interferon. \bullet — \bullet interferon alone in per cent of untreated control. \circ — \circ interferon + ouabain $10^{-7.9}M$ in per cent of ouabain-treated control.

Figure 1b: Effect of ouabain on growth inhibition by the antiviral component. \blacksquare — \blacksquare antiviral component alone in per cent of untreated control. \square — \square antiviral component + ouabain $10^{-7.9}M$ in per cent of ouabain-treated control.

Figure 2: Effect of ouabain on growth inhibition by the anticellular component. \blacktriangle — \blacktriangle anticellular component alone in per cent of untreated control. \triangle — \triangle anticellular component + ouabain $10^{-7.9}M$ in per cent of ouabain-treated control.

When the effects of the antiviral component was tested with increasing concentrations of ouabain (not shown) the anticellular effect of 100 antiviral units was completely blocked by $10^{-7.3}M$ ouabain. In agreement with this the same concentration of ouabain reduced the antiviral titer by $1.75 \log_{10}$ units or about 98 per cent. However, increasing cytotoxicity of ouabain made the readings less reliable.

The present results support the hypothesis that more than one mechanism may be involved in growth regulation by interferon in different cell lines. The observed growth inhibition by the antiviral component was found to be inhibited by ouabain to the same degree as the antiviral activity in-

dicating a close relationship between the mechanism of the two activities of the component. The effect of the growth inhibitory component was not affected by the drug. This finding suggests a completely different mechanism of action for growth inhibition by the antacellular component. Therefore, the physical separation of activities of interferon (10, 11) is confirmed in the present study and the results may explain why others were unable to demonstrate a separation of activities even after drastic denaturation of interferon (15-17). Further studies using other inhibitors of cellular functions and different cell lines are in progress supporting the hypothesis that different mechanisms of action are involved in growth regulation by interferon depending on the nature of the cell lines employed.

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