Changes in the Mitotic Cycle Induced by α-Solanine

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Summary. Low concentrations of α-solanine stimulated the growth of cultured human fibroblasts, while higher concentrations (> 30 μg ml⁻¹) had a markedly inhibitory effect. Autoradiographic studies indicated that the stimulation of cell growth was due to a shortening of the G₁ phase. Feulgen microdensitometry of cells treated with high doses of α-solanine revealed an abnormal accumulation of cells in G₂. The response of cultured human fibroblasts to low doses of α-solanine is comparable to that of sex hormones on target tissues. It is concluded that by virtue of either its stimulatory or its inhibitory effect on cell growth, α-solanine could act as a human teratogen.


Introduction

Subsequent to the suggestion that blighted potatoes may contain a teratogen (Renwick, 1972), it has been shown that α-solanine may cause skeletal abnormalities in rats (Swinyard, 1973). α-solanine, a naturally occurring steroid glycoalkaloid in potatoes, is toxic toward fungi (Allen, 1968), nematodes (Allen and Feldmesser, 1971) and man (Wilson, 1959). It causes hyperglycemia in rats (Satoh, 1967), inhibits human plasma cholinesterase in vitro (Orgell et al., 1958), and apart from mimicking the effects of cardiac glycosides on the heart (Nishie et al., 1971), little information concerning its pharmacological or biochemical effects is available. We have examined the effects of α-solanine on human fibroblasts in tissue culture.

Materials and Methods

Cells. All fibroblasts used for these studies were normal human diploid cells derived from skin biopsies, none having undergone more than 20 culture passages.

Scoring for Mitoses. Cells were plated at 10⁶ ml⁻¹ in Eagle's minimum essential medium (MEM) supplemented with 10% foetal calf serum, Hepses Buffer (10 mM), glutamine (0.02 mM), gentamycin (48 μg ml⁻¹) and penicillin (100 International Units ml⁻¹) in 3 cm. Nuclcon petri dishes containing glass coverslips. α-solanine was introduced at the different concentrations
in the above medium 21 hrs after plating, being previously dissolved in dimethylformamide (DMF) to give a final concentration of 6.6 μl of DMF/ml of medium for all α-solanine concentrations. Controls (as in all experiments described) received similar amounts of the solvent DMF. Cells were harvested by removing a coverslip, rinsed in saline, fixed in 95% methanol and stained by the Feulgen procedure. Mitoses were scored using an oil immersion objective.

**Densitometry.** DNA values of Feulgen stained interphase nuclei, as scored for mitoses in Table 1, were measured with a Barr and Stroud integrating microdensitometer. The readings were converted into logarithms to the base 2 and delineated into the 3 major DNA classes, 2C, 4C and intermediate, as described previously (Mittwoch and Wilkie, 1971). Nuclei with the 2C amount of DNA were classed as G1, those with twice this amount as G2, while nuclei with intermediate values were regarded to be in the S phase of DNA synthesis. A small proportion of cells had DNA values greater than 4C and these must have been polyploid.

**Growth Curves.** Growth curves of fibroblasts were obtained essentially as described previously (Raff and Houck, 1969). Normal human diploid fibroblasts were seeded into Leighton tubes at a concentration of 4 × 10^4 cells per 1.5 ml of medium, (the medium being identical to that described for scoring mitoses, except that 20% human serum was used). The α-solanine was added in DMF as described previously. Cells were incubated at 37°C and counts were made every day using a Whipple eyepiece and an inverted microscope.

**Autoradiography.** Cells were seeded on coverslips at 10^5 cells ml^-1 in Eagle’s MEM as described before, supplemented with 20% human serum. After 24 hrs the cells were pulse labelled with tritiated thymidine (1 μCi ml^-1, sp.ac. 15 mCi/mM) for 30 min, rinsed 3 times with Hank’s Balanced Salt Solution and fresh media introduced containing α-solanine dissolved in DMF as described previously. Cells were incubated at 37°C and cover slips were periodically harvested, rinsed in saline, fixed in 95% methanol and stained by the Feulgen reaction. Kodak AR10 stripping film was applied, given an exposure time of 12 days and developed with Kodak D19b Developer. Slides were randomised, coded and scored for labelled metaphase cells. The fraction of labelled metaphases was established from 25 randomly detected metaphases.

### Results

The effects of various concentrations of α-solanine on the growth of human fibroblasts is recorded in Table 1, where it can be clearly seen that the response is not a simple one. Concentrations up to 33.3 μg ml^-1 show a marked increase in mitotic indices compared to controls, whereas 66.6 μg ml^-1 lead to a marked reduction in the number of cells reaching mitosis. This differential response elicited by α-solanine in both stimulating and inhibiting fibroblasts, as reported here, has been observed by us in repeated experiments. From Table 1 it is also seen that no α-solanine concentration has any apparent effect on the flow of cells through the mitotic phases, although the small numbers involved make it difficult to be certain about this. However, measurement of DNA content of cells by microspectrophotometry, given in Table 2, shows that the high α-solanine concentration (66.6 μg ml^-1) causes an abnormal accumulation of cells in the G2 (4C DNA value) phase of the mitotic cycle as illustrated by a higher G2 Index (0.34) compared to the control (0.24). No inhibitory effects on other parts of the cycle at this concentration were detectable in the present data.

Confirmation of the stimulatory properties of α-solanine at low concentrations has been obtained by comparing growth rates of fibroblasts in the presence and absence of 5 μg ml^-1 of α-solanine. Growth was followed for at least 2 cell generations and was exponential up to the end of the 3 day period investigated, as is seen from the log curves in Fig. 1. The α-solanine treated cells showed significantly