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Three alkaloids as selective destroyers of the proliferative capacity of cancer cells

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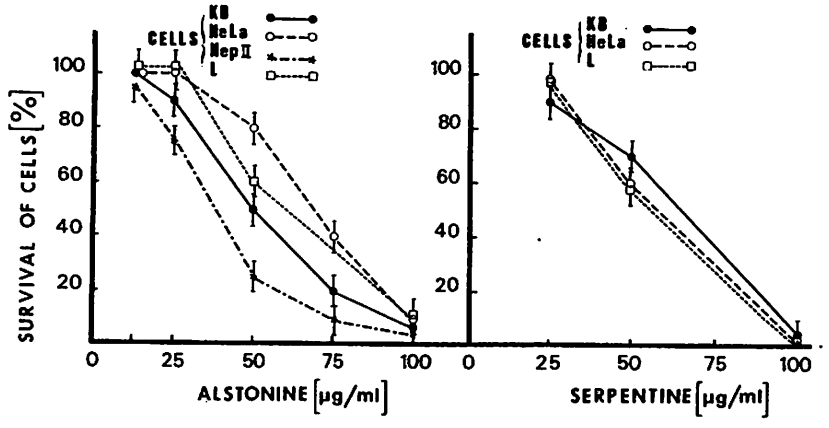
Most of the anti-cancer drugs at present used in cancer chemotherapy exhibit tissue toxicity and cause severe damage to haematopoietic cells (1, 2). In addition, they are mutagenic and/or carcinogenic in animals (3) and in plants (4). Using the Oncotest (5, 6), we have selected three alkaloids, alstonine, serpentine and sempervirine that possess the capacity to distinguish *in vitro* between DNAs isolated from cancerous and healthy mammalian (6) and plant (7) tissues. They bind to the initiation sites of destabilized cancer DNAs (DNAs which have more unpaired chain areas than normal cell DNAs (8)), thus preventing cancer DNA synthesis, without affecting that of DNAs from healthy tissues (6). Here we demonstrate that each of the three alkaloids, which remain inactive against normal eukaryotic cells, selectively and completely destroys the proliferative potential of various established cancer cell lines maintained in an *in vitro* culture.

Materials and methods: Established cancer cell lines, KB, HeLa, Hep II, L (clone from BHK cells) and normal eukaryotic cells, Vero (monkey kidney), RC (rabbit cornea) and BHK (baby hamster kidney) were maintained in monolayer confluent cells on Eagle's medium supplemented with 10% calf serum (37°C). Each cell type was free of mycoplasma. The stock culture was duplicated once per week after addition of trypsin + 0.05% EDTA in order to obtain dispersed cells. Aureomycin (100 μg/ml) was used as an antibacterial agent. Streptomycin and pencillin were avoided since at the required concentration (100 μg and 100 U/ml respectively) they make normal cells to some extent sensitive to the alkaloids used here. Each cell type ($10-20 \times 10^4$ cells/ml) was cultured in multiple samples. The surface area of each flask was 25 cm².

Alstonine and serpentine were isolated and purified in our laboratory from Rauwolfia plants (6). Their purity was characterized by thin layer chromatography and UV absorption spectra. Other alkaloids (sempervirine, ajmalicine, ajmaline and reserpine) were purchased from Roth-Sochiel, Lauterbourg, France. Each compound was dissolved in sterile distilled water (2 mg/ml) and after the pH had been adjusted to 7.6 the solution was filtered under sterile conditions. The cytotoxic effect of the alkaloids was determined, after addition of increasing concentrations of each alkaloid to the culture medium either at the same time as cell inoculum or three days post-inoculation. Using "Thoma cell" the destructor effect was determined by counting viable cells every other day for 14 consecutive days. Staining of dead cells with erytrosine B was also performed. In some instances the viability of cells was controlled by injecting the alkaloid-treated cells into nude mice.



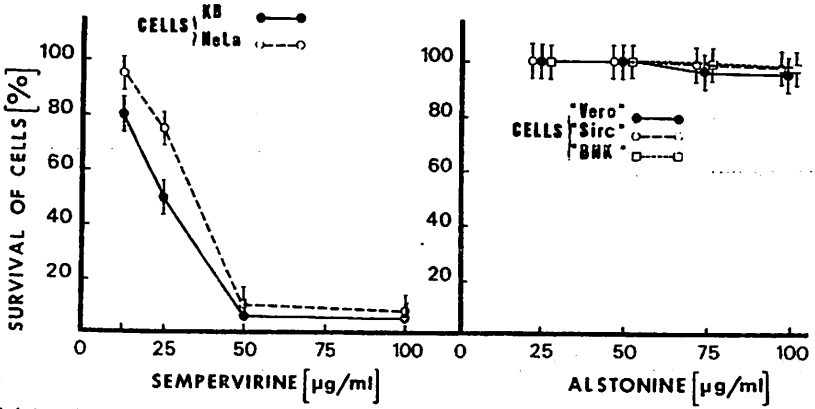


Figure 1 (above): Percent survival of various cell lines treated with alstonine or serpentine. Figure 2 (below): Percent survival of various cell lines treated with sempervirine or alstonine.

Results and discussion: The proliferative capacity of various cancer cells cultured *in vitro* was destroyed by alstonine, serpentine and sempervirine. The number of killed cancer cells depended on the concentrations used as illustrated by

dose-response curves (Figures 1 and 2). None of the cancer cells were viable 48 h after the addition of each alkaloid (100 μg/ml). They became round and a large number of vacuoles were often seen in the cytoplasm on the periphery of these cells. The nucleus became picnotic and alkaloid-treated cells did not stay attached to the surface of the flask. Lysis of many cells occurred. These results were observed whether alkaloids were added at the beginning of cell incubation or three days later. In the case of malignant melanoma cells maintained in *in vitro* culture supplemented with 200 μg of serpentine/ml, only 80% of cells were killed within 48 h of incubation (preliminary data not shown here). Normal primary cells or established cell lines from different origins and cultured under the same experimental conditions (Figure 2) survived in the presence of each alkaloid for at least 7 days, or more depending on the origin of the cell line. In those instances where alkaloid-treated cancer and normal cells (10^6 cells) were injected into nude mice, tumours did not appear and such mice survived in excellent condition. These results demonstrated that all alkaloid-treated cancer cells were destroyed and that alkaloid-treated and undestroyed normal cells did not aquire the potential for tumor induction. Only

Tumour induction in nude mice injected with cancer cells, either untreated or alkaloid treated

Cell type	Treatment	State of cells	Number of mice injected	Tumor development
KB	none	normal	4	4/4
KB	100 μg alkaloid	lysis (80%)	4	0/4
Hep II	none	normal	6	6/6
Hep II	200 μg alkaloid	lysis (70%)	6	0/6

untreated cancer cells led to the appearance of tumors and death of mice (Table 1). The proliferative capacity of cancer cells and of normal cells was unaffected by ajmalicine, ajmaline and reserpine, alkaloids which are chemically related in varying degrees to alstonine or serpentine (Figure 3). In contrast to alstonine, serpentine and sempervirine, these alkaloids did not inhibit cancer cell DNA synthesis *in vitro*

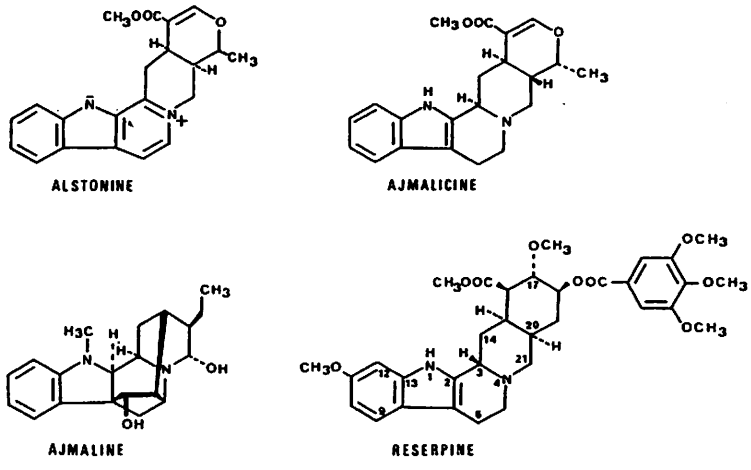


Figure 3: Chemical structure of alstonine, ajmalicine, ajmaline and reserpine.

Used at appropriate concentrations, alstonine, serpentine and sempervirine selectively and completely destroy the proliferative capacity of many different cancer cells, maintained *in vitro*. They do not appreciably affect the proliferation of normal cells. These data support our previous observations; all three alkaloids selectively inhibit cancer cell DNA synthesis *in vitro* whilst that of DNA from normal cells is not seriously affected. The fact that the nucleus of alkaloid-treated cancer cells became picnotic indicates that alkaloids reach the nucleus of these cells where they bind to destabilized DNA forming an alkaloid-DNA complex (6, 8). The presence of alkaloids bound to DNA can be ascertained by the fluorescence which is one of the characteristics of these alkaloids. At the concentrations used, the alkaloids appear to ignore the healthy cells and their stabilized DNA (6), unlike most of the cytotoxic-antitumour agents which interact strongly with double or single stranded DNA (8, 9). From data described elsewhere (6) and those presented here, one may expect that alstonine, serpentine and sempervirine will be of help for the treatment of certain cancers in mammals and humans.

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