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CYTOTOXIC ACTIVITY OF AZIRIDINYL PUTRESCINE ENHANCED BY POLYAMINE DEPLETION WITH ALPHA-DIFLUOROMETHYLORNITHINE

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The human and rat prostate glands contain higher concentrations of the polyamines putrescine, spermidine, and spermine, and polyamine synthetic enzymes than most other tissues (1). In spite of this, i.v. administration of [^{14}C]-putrescine results in a greater uptake of putrescine by the prostate than by most other tissues (2). Thus, it appears that cytotoxic analogs of putrescine may prove to be useful as chemotherapeutic agents against prostatic cancer. We synthesized the monoaziridinyl analog of putrescine (AZP) and examined it for cytotoxic activity against a prostate-derived tumor line.

MATERIALS AND METHODS

AZP was synthesized previously as a precursor in the syntheses of antiradiation compounds (3). We used this method for the synthesis of AZP. The human prostatic carcinoma cell line PC-3 was obtained from the American Type Culture Collection, Rockville, MD. For the clonogenic assays, 10^3 PC-3 cells were plated in 3 ml of RPMI 1640 medium with 10% fetal calf serum in 60 mm^2 petri dishes. These were incubated with or without 100 μM alpha-difluoromethylornithine (DFMO) for 48 hr. The medium was then aspirated and replaced with medium containing 100 μM aminoguanidine with or without 50 μM AZP, 1 mM putrescine (PUT), or both AZP and PUT. After 1 hr the medium was aspirated and replaced with drug-free medium, and the incubation was continued for 5 more days. The medium was then aspirated, the cells were rinsed with Hanks' balanced salt solution, fixed with methanol, and stained with Harris' hematoxylin, and colonies were enumerated at a magnification of 10x. [^{14}C]- putrescine uptake assays were performed on PC-3 cells in a manner similar to that described previously (4).

RESULTS AND DISCUSSION

In vitro DFMO pretreatment of PC-3 cells significantly enhanced their subsequent uptake of [^{14}C] - putrescine from 15 to 73 pmoles/20 min/10^4 cells. AZP appeared to block the uptake of putrescine of DFMO pre-treated cells with 10 μM AZP reducing the uptake of 10 μM [^{14}C] - putrescine by 85%. AZP at 50 μM was toxic and inhibited the growth of the PC-3 cells by 60% (Table 1). DFMO had little effect on clonogenicity but, when AZP was combined with DFMO pretreatment, enhanced suppression of the growth of these cells was observed. AZP appears to compete with putrescine for the polyamine transport system. Since AZP appears to use the polyamine uptake mechanism for entrance into the cell, putrescine should reverse the effects of AZP.

Putrescine did reverse the effect of DFMO + AZP and restored the number of clonogenic survivors to near the control level.

Table 1. Effect of treatment on PC-3 cells

Treatment	Clonogenic survivors	
1. Control	584 ± 70*	(100%)
2. DFMO	549 ± 57	(94%)
3. AZP	210 ± 30	(36%)
4. DFMO + AZP	5 ± 2	(1%)
5. DFMO + AZP + PUT	505 ± 76	(86%)

* Represents mean number of colonies ± one s. d. of triplicate assays. Numbers in parentheses are percent of control. Statistical differences ($p<.01$) were observed for: 1 vs 3, 1 vs 4, 2 vs 3, 2 vs 4, 3 vs 4, 3 vs 5, and 4 vs 5.

Although not included in the table, putrescine by itself had no effect on the number of clonogenic survivors; the addition of putrescine was also able to reverse the cytotoxic effect of AZP that had not been preincubated with DFMO. Aminoguanidine was included to inhibit serum diamine oxidase activity. It had no effect on PC-3 cell clonogenicity or [^{14}C]- putrescine uptake.

The chemotherapeutic agent methylglyoxal bis-guanylhydrazone (MGBG) uses the polyamine transport system for its cellular uptake and its cytotoxic activity can be enhanced by DFMO pretreatment (5,6). The search continues for other cytotoxic agents which use the polyamine transport system for their uptake (7). AZP demonstrated cytotoxic activity, and that cytotoxic activity was enhanced by DFMO and decreased by simultaneous administration of putrescine. Thus, AZP might be a useful cytotoxic drug especially for tumors that can be manipulated into increasing their uptake of putrescine-like compounds (4).

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