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The Influence of AZQ on the DNA Distribution of Human Cerebral Tumours in Short-term Culture

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Abstract—*The influence of AZQ, a chemotherapeutic agent, on the DNA-distribution of human cerebral tumours in short-term culture was studied by means of a MPV II cytofluorometer. The cell cultures were exposed to increasing concentrations of AZQ during* 5 days. *The results showed that AZQ has no cell phase-specific activity. The influence on the DNA-distribution varied considerably in the examined tumours. It is hypothesised that the effect of AZQ might depend upon the initial DNA-distribution of human cerebral tumours, hyperdiploid tumours being more vulnerable.*

INTRODUCTION

2,5-DIAZIRIDINYL-3,6-BIS-CARBOETHOXYAMINO-1,4-BENZOQUINONE (AZQ), an alkalyting agent, has recently been introduced as a chemotherapeutic agent in the treatment of central nervous system tumours. Its effectiveness had been established in animal tumour models as well as its ability to cross the blood-brain barrier [1, 2].

The purpose of our study was the evaluation of the effect of AZQ on the distribution of nuclear DNA in human cerebral tumour cells in short-term culture. DNA distribution studies have been used previously to determine the effect on the cell cycle of human tumours. These substances include arabinose C [3], 1-β-D-arabinofuranosylcytozine, daunorubicin, 6-thioguanine [4], vindesine, vinblastine and vincristine [5], methotrexate and citrovorum factor [6] and interferon [7].

These studies make it possible to determine whether a product has cell phase-specific activity or whether it acts by eliminating a specific stemline in a cell population.

The advantages of using cell cultures in studying cell sensitivity have been reviewed by Dendy [8] and the importance of cell kinetics in human brain tumour in order to establish an effective chemotherapeutic protocol has been reviewed by Hoshino and Wilson [9].

The aim of this study is to determine whether the sensibility for AZQ of a tumour can be determined by means of DNA distribution studies.

MATERIALS AND METHODS

Tissue culture

Biopsy material of ten human intracranial tumours was used. The material included two benign tumours (a hypophyseal adenoma and an oligodendroglioma) and eight malignant tumours (four glioblastoma multiforme, one anaplastic astrocytoma, one anaplastic oligodendroglioma and two meduloblastomas).

Each biopsy was rinsed in Hanks' basic salt solution and chopped into small cubes of 1 mm^3 which were left overnight in a collagenase solution. The resulting cells were suspended in F10 Ham nutrient medium containing 20% foetal calf serum and transferred to five Leighton tubes (2 ml per tube; minimum 10^6 cells/ml). Forty-eight hours later the medium was replaced by an AZQ-containing medium, each tube with a different concentration of AZQ (one control tube, 0.2, 2, 20 and 200 μg/ml).* The cultures were left for 5 days at 37°C and fixed in AFA.

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*The AZQ was obtained from the EORTC.

DNA measurement

Fluorescence intensity of pararosanilin-feulgen-stained sections was measured with the MPV II cytofluorometer (Leitz, Wetzlar, S.D.R.). In order to minimise photophading during measuring the nucleus was centred and focused in the measuring diaphragm in orange light using phase-contrast (filter in use OG2/4 mm). In this way the excitation by light from a mercury high-pressure lamp (HBO 100 W) was reduced to 1 sec with electronically guided shutters. SP 560, LP 530 and BG 36 filters were used in combination with a dichrome mirror with 50% reflection at 560 nm for excitation. As secondary filters LP 580 and RG 2 (4 mm) were used. During the 1-sec measuring time a sample of 200 readings was taken from the photomultiplier by the AD converter connected to the PDP 11/03 microcomputer (digital) and the mean reading stored on a floppy disk. The fluorescence intensity of the leucocytes was used as the diploid reference value. Standardisation of the fluorometer was done with uranyl glass and values were expressed as arbitrary units, as a relative DNA amount.

A second program was used to analyse the measured data. This program ranked the data in ascending order after conversion to the diploid reference and was plotted as a histogram. In each tumour 100 cells were measured.

RESULTS

The results are summarised in Table 1. The control cultures of the malignant tumours showed in one case (case 1) a diploid DNA distribution and in one case a tetraploid (case 2). One tumour was hypodiploid (case 5), one tumor hyperdiploid (case 6) and two were aneuploid (cases 3 and 4). The two medulloblastomas had a hypodiploid DNA distribution.

The hypophyseal tumour was diploid, while the oligodendroglioma showed a double stemline, 2c and 4c.

When submitted to an increasing concentration of AZQ no major changes in DNA content of distribution was observed in cases 1, 4, 5, 7 and 10. In cases 2, 3 and 6 a definite diminution of the mean DNA content of the cell population occurred (Fig. 1), while in tumour 8 a diploid stemline emerged from a hypodiploid population.

In cases 1, 3, 4, 7 and 10 at a concentration of 200 μg/ml AZQ no remaining cells were found.

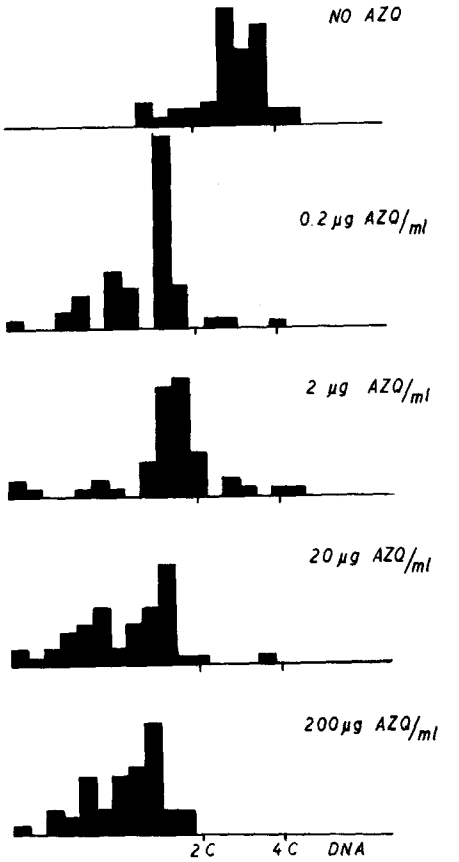

Fig. 1. DNA distribution of an anaplastic oligodendroglioma (case 6) exposed to increasing concentrations of AZQ. In this aneuploid tumour a marked change in DNA content does occur at low concentrations of the drug.

Table 1. Results of the study (AZQ concentration expressed as mg/ml)

Case	Histology	DNA stemline after AZQ administration 0	0.2	2	20	200	Overall change
1	glioblastoma	2c	2c	hypodipl.	2c	-	no change
2	glioblastoma	4c	4c	hyperdipl.	hyperdipl.	-	diminution
3	glioblastoma	aneupl.	aneupl.	2c	aneupl.	-	diminution
4	glioblastoma	aneupl.	aneupl.	aneupl.	aneupl.	-	no change
5	anapl. astrocytoma	hypodipl.	hypodipl.	hypodipl.	hypodipl.	hypodipl.	no change
6	anapl. oligodendrogl.	hyperdipl.	hypodipl.	hypodipl.	hypodipl.	hypodipl.	diminution
7	medulloblastoma	hypodipl.	hypodipl.	hypodipl.	hypodipl.	-	no change
8	medulloblastoma	hypodipl.	hypodipl.	hypodipl.	2c	2c	increase of 2c
9	hypof. adenoma	2c	2c	2c	2c	2c	no change
10	oligodendroglioma	2c,4c	2c,4c	2c,4c	hypodipl.	-	diminution

2c = diploid; 4c = tetraploid.

A definite accumulation of cells in one DNA class was not observed in any case.

We did not observe cellular nor nuclear changes in our cultures except in case 10, where cell lysis occurred at a concentration of 20 μg/ml AZQ.

DISCUSSION

Our results show that AZQ does not seem to have any cell phase-specific activity. No accumulation of cells occurred in the G_{0-1}, S or G_2 phases of the cell cycle. This accords with the presumed alkylating effect of the agent.

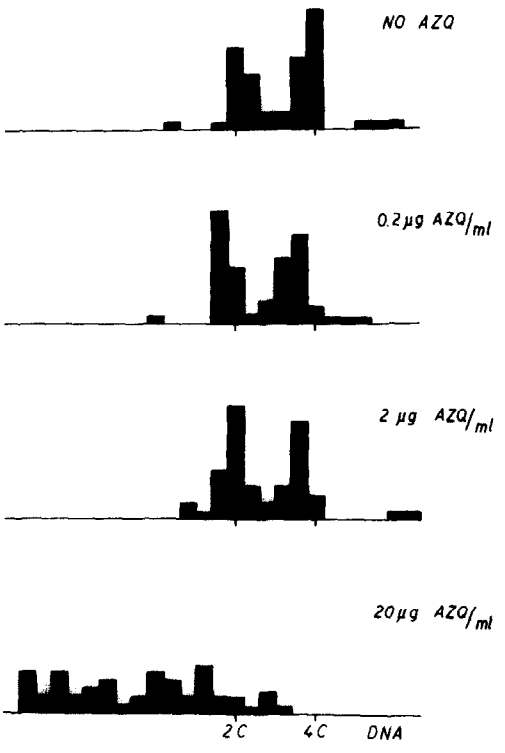

Fig. 2. DNA distribution of a low-grade oligodendroglioma (case 10) exposed to increasing concentrations of AZQ. In these tumours with a 2c, 4c DNA distribution no change occurs at low concentration of the drug. At higher concentrations cell-lysis is noted, with destructuration of the DNA distribution curve.

However, in some tumours a clear change in DNA distribution does occur. In tumours 2, 3 and 6 there was a reduction of the mean DNA content of the cell population since we observe more cells with a lower DNA content. Whether this is due to specific sensitivity of a stemline or to preferential activity of AZQ against an abnormal DNA structure is uncertain. It is, however, remarkable that in tumours 1, 9 and 10 with a diploid stemline no changes in the DNA distribution occurred at therapeutic concentrations of the drug: this applied to both benign and malignant tumours. This might be explained by the greater sensitivity of hyperdiploid cells, whereas diploid cells are vulnerable only at higher concentrations of AZQ; tumour 10 provides such an example (Fig. 2). This might indicate a higher sensitivity of hyperdiploid subpopulations in the tumour or could point to a higher sensitivity of cells in the S phase. The differentiation of these two possibilities cannot be made because the methods presently used do not allow differentiation between cells that are synthesising DNA and those that are not.

At concentrations well above the therapeutic levels used in patients a definite cell-toxic effect occurs, killing all cells in most cultures (tumours 1, 2, 3, 4, 7 and 10). In one aneuploid tumour (case 4) and in two hypodiploid tumours (cases 5 and 7) no changes in DNA distribution were observed.

Our findings suggest that the effect of AZQ depends upon the initial stemline of the tumours. Therefore, in view of the great heterogenity of DNA distribution and cell kinetics of cerebral tumours [10-12], biological effects such as those which were shown could be used to explain the outcome of current chemotherapeutic trials and also in designing clinical trials to test drugs with potentially more tumour-specific activity. Cytofluorometric analysis of the DNA content of cerebral tumours is a useful tool in testing sensitivity and resistance of the cell lines of these lesions.

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