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ON THE EFFECTS OF ARA-A AND ARA-C ON X-RAY INDUCED DNA LESIONS IN NORMAL HUMAN AND A-T CELLS: SIMILARITIES AND DIFFERENCES.

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ABSTRACT

A better understanding of the mechanism of chromosomal aberration formation could be obtained by using DNA repair inhibitors. Immortalized normal human (MRC5SVI) and ataxia telangiectasia (AT5BIVA) fibroblastic cell lines were treated with adenosine arabinoside (ara-A) and cytosine arabinoside (ara-C), both potent inhibitors of DNA dsb repair, alone or in combination with x-rays at G_2 or S-phase of the cell cycle. The length of G_2-phase for both cell lines was determined by autoradiographic labeling to be about 4.5-5 h. A similar result was obtained by scoring of chromosomally damaged cells following treatment with ara-A or ara-C for various time intervals before fixation. The results obtained in this study show that in spite of many similarities between the action of ara-A and ara-C, e.g., inhibition of DNA synthesis clastogenic effects at G_2 and S-phase and also lack of synergism as a possible consequence of these similarities, ara-A was found to have a different effect on rejoining of x-ray induced DNA lesions than that of ara-C. Ara-A caused inhibition of chromatid deletion rejoining, interpreted as inhibition of rejoining of DNA dsb at all sampling times before fixation, whereas ara-C showed a synergistic effect on radiation-induced DNA lesions, resulting in an increased frequency of chromatid deletions. Thus there appears that these inhibitors have different modes of action on x-ray induced DNA lesions, which may suggest a peculiar and important difference in the nature of these two nucleosides.

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INTRODUCTION

DNA is now thought to be the primary target for the induction of chromosomal aberrations (CA). Most known mutagens (DNA damaging agents) including x-rays are capable of inducing CA. Ionizing radiation induces a variety of damage in DNA including directly induced single and double strand breaks, various types of base damage as well as DNA-DNA and DNA-protein crosslinks. All primary lesions induced in DNA

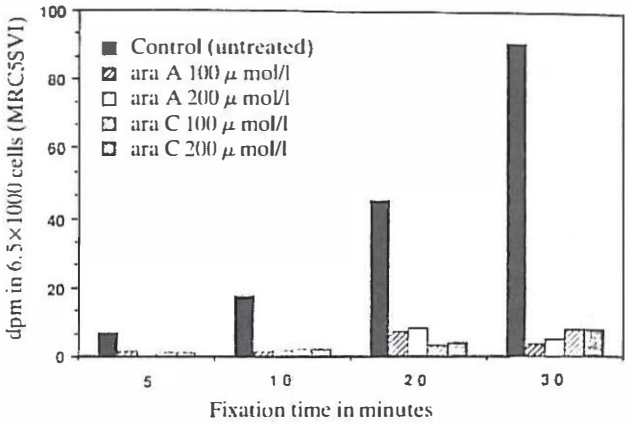

Fig.1. Incorporation of ^{3}H- TdR in normal cells at various periods time in the presence or absence of ara A and ara C. Cells were treated with inhibitors 0.5 h before being exposed to ^{3}H- TdR.

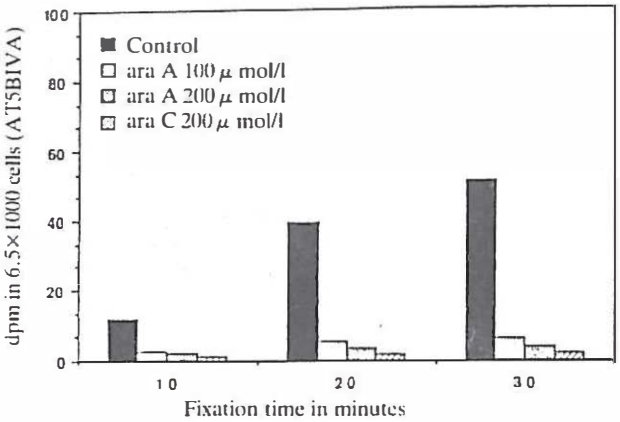

Fig.2. Incorporation of ^{3}H- TdR in A-T cells at various time intervals in the presence or absence of ara A and ara C. Cells were treated with ara A or ara C 0.5h before being exposed to ^{3}H- TdR.

are thought to be subject to cellular repair processes; however unrepaired or misrepaired lesions may give rise to chromosomal aberrations. The question of which of these lesions is involved in aberration formation was the subject of controversy for many years and even now is not fully answered. However the weight of evidence shows that DNA dsb is the most probable lesion leading to CA after exposure to ionizing radiation.[1,2,3,4]

In the present study, DNA repair inhibitors ara-A and ara-C were used to study the effects of these drugs on the kinetics of x-ray induced CA. Ara-A and ara-C are synthetic nucleoside analogues[5] which are frequently used as antileukemic and cytotoxic agents. Both ara-A and ara-C are potent inhibitors of DNA polymerase α and β.[6]

It was also shown that clastogenicity is a feature of DNA synthesis inhibitors in S-phase cells.[1] Ara-A has also shown to enhance the chromosomal damage caused by x-rays,[7] an effect which has been related to its strong inhibition of repair of DNA double strand breaks.[8] A similar effect was shown for ara-C where a synergistic increase in chromosomal aberration in both G_0 and G_2 lymphocytes was observed.[9]

In the study described here various parameters were evaluated: 1) the effects of ara-A and ara-C on DNA synthesis was measured to compare the inhibitory effects of these drugs at similar concentrations on semi-conservative DNA synthesis judged by the reduction in incorporation of ^{3}H-TdRinto DNA,2) measurement of the clastogenicity of ara-A and ara-C, to confine the treatment time of cells in experiments with x-rays, thus also making it possible to estimate the duration of G_2- phase other than that made by autoradiography technique, and 3) the kinetics of x-ray induced chromatid aberrations in presence or absence of ara-A or ara-C.

Cell culture

Immortalized human fibroblastic lines: normal (MRC5SVI) and ataxia telangiectasia (AT 5 BIVA) were used. Cells were grown in Eagle's minimal essential medium supplemented with 15% foetal calf serum (MEMFCS). Expotentially growing cells were cultured in 75 cm^2 flasks after seeding at 4×10^5 cells in 10ml MEMFCS at about 44 hours prior to treatment.

DNA synthesis assay

For assay of DNA synthesis, suspensions of trypsinized AT 5 BIVA and MRC 5 SVI cells were prepared at about 6.5×10^5 cells per ml in 5 ml MEMFCS half an hour prior to labelling. Samples were treated with ara-A and ara-C at concentrations of 100 μ mol/L and 200 μ mol/L. Both control and treated samples were then labelled with 3.7 KBl/ml ^{3}H- TdR with a specific activity of 1.48 TBq (40 ci/mmol) and incubated in a water bath at 37°C and sampled at various time intervals. DNA was then prepared on filters. The fiberglass filters were placed in scintillation vials and 4.5 ml scintillation fluid was added. The samples were counted in a LKB 1214 Rack Beta liquid scintillation counter.

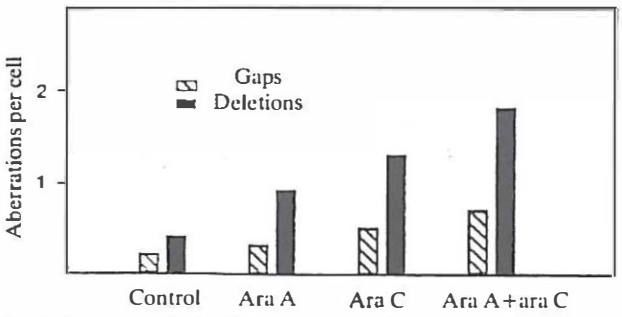

Fig.3. Frequencies of deletions and gaps induced by ara A (100 μ mol/l) or ara C (100 μ mol/l) alone or in combination in A-T cells exposed 4 h before fixation.

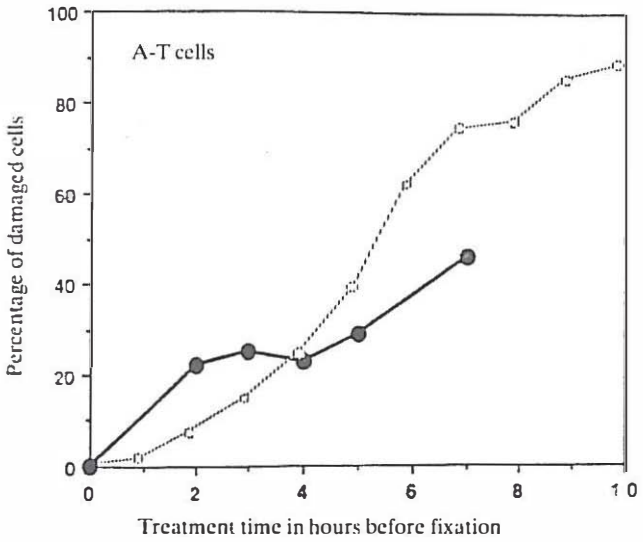

Fig.4. Percentage of damaged A-T cells following exposure to ara C (100 μ mol/l) for various time intervals before fixation. Values for each point are subtracted from the number of damaged cells in the controls. The dotted line shows the percentage of labelled mitoses as a function of time before fixation in A-T cells.

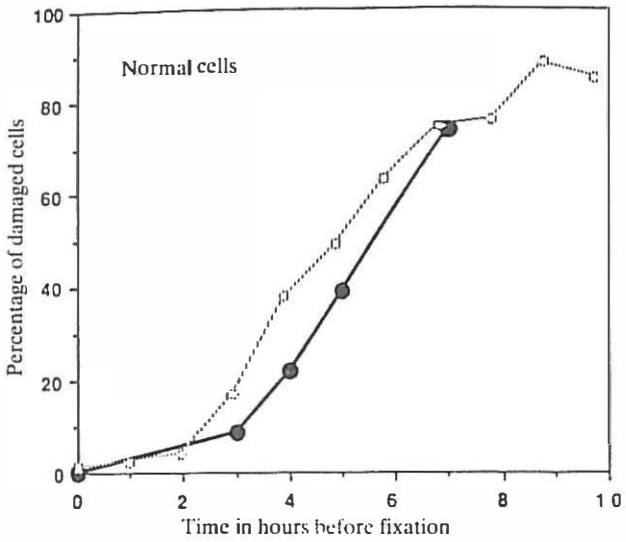

Fig.5. Frequency of damaged cells following exposure of normal cells to ara A (200 μ mol/l) for various time intervals before fixation. Values for each point are subtracted from the number of damaged cells in the controls. The dotted line shows the percentage of labelled mitoses as a function of time before fixation in normal cells.

X-irradiation

Cells were irradiated in medium as a monolayer with x-rays generated by a Siemens x-ray therapy unit operating at 250 kV and 14 mA filtered with a 0.5 mm Cu filter giving an absorbed dose- rate of 0.75 Gy/ minute. The x-ray dose was monitored by a Farmer-Baldwin dose- meter (ionization chamber).

Treatment with inhibitors of DNA synthesis

In experiments involving treatment with 9-ß-D arabino furanosyl adenine (ara-A) and 9-ß- D- arabinofuranosylcytosine (ara-C), ara-A and ara-C were added to the medium as a 10 mmol/L solution in Hank's balanced salt solution. These agents were adminstered routinely at four hours before mitotic collection. This was therefore at least 0.5 hour before x-irradiation in the case of G_2 experiments. Both drugs were left in the culture medium until fixation. X- irradiation was carried out in presence of inhibitors. Cells were then exposed to democolcine at a final concentration in the medium of 0.08 μ g/mL. Cells were then harvested by trypsinization and metaphase chromosomes prepared according to standard methods and stained in Giemsa (3%).

RESULTS

Inhibitory effects of ara-A and ara-C

Both ara-A and ara-C are known as inhibitors of DNA synthesis in mammalian cells. Experiments were designed to study the effects of ara-A and ara-C on DNA synthetic activities of S-phase normal and A-T cells. Figure 1 shows a sharp increase in ^{3}H- TdR incorporation by normal cells from 5-30 minutes in untreated samples, while the overall increase in incorporation in ara-A and ara-C treated samples was much lower. It can be seen that at 30 minutes post- labelling time, the overall incorporation of ^{3}H- TdR in ara-A and ara-C treated cells was only 10-15 percent compared to untreated controls. A similar result is shown in Figure 2 for A-T cells. These experiments show that both ara-A and ara-C reduce incorporation of ^{3}H- TdR to 85-90% in both cell lines. Thus both ara-A and ara-C were found to be strong inhibitors of DNA systhesis in the cell lines and ara-C was found to be more effective than ara-A at similar concentrations.

Synergism of ara-A and ara-C

Kihlman and Anderson (1985)[10] showed that if two inhibitors are combined and administered to cells, this might lead to the induction of chromosomal aberrations at higher frequencies than the sum of the aberrations induced by each inhibitor individually (at least by a factor of 1.5-2). Figure 3 shows the effects of inhibitors alone when they were applied individually or in combination. The frequency of aberrations induced by the combined treatment of ara-A and ara-C was high but not more or even equal to the sum of aberrations induced by ara-A and ara-C individually. This result therefore shows that the inhibitors did not act synergistically when applied to the cell culture together.

Clastogenic effects of ara-A and ara-C

On the basis of the assumption that ara-A and ara-C

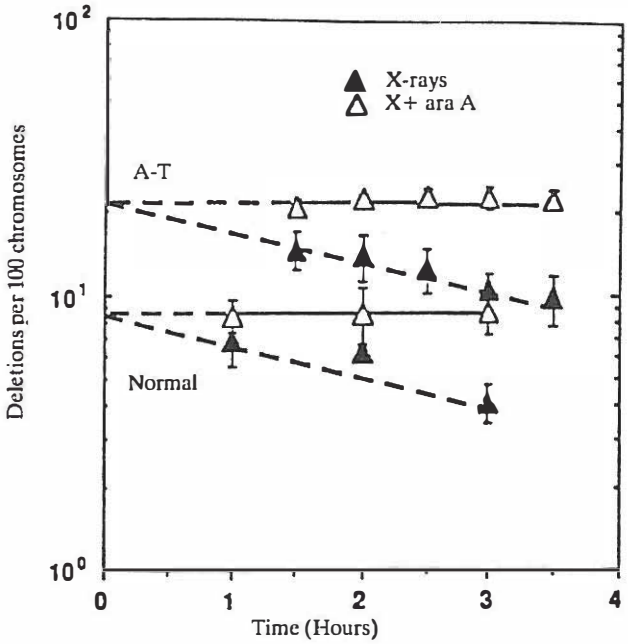

Fig.6. Frequencies of deletions in A-T and normal cells as a function of time between X-irradiation and fixation. Data for normal cells, originally for 2 Gy exposure has been recalculated for a dose of 1 Gy, on the assumption a linear induction over low dose range of X-rays. Error bars indicate standard deviation of mean values of three experiments. (Data is reploted from Mozdarani and Bryant, 1987).

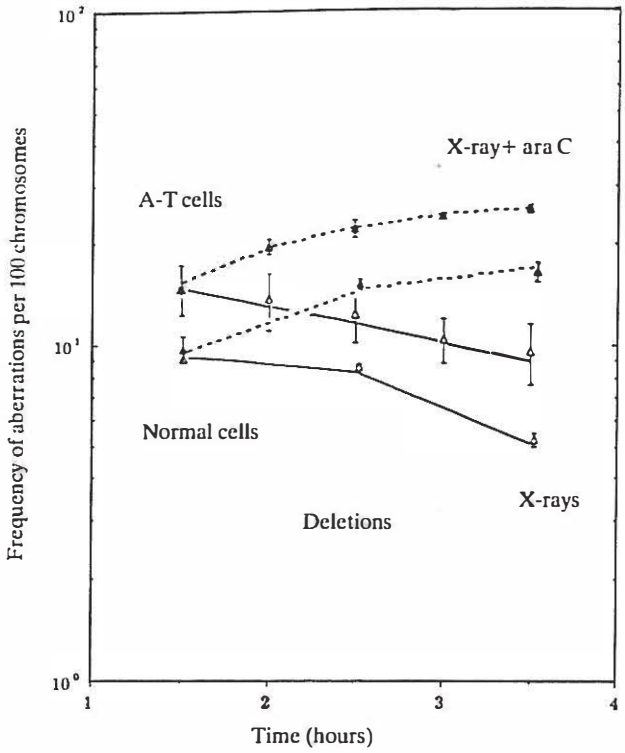

Fig.7. Frequency of chromatid deletions and gaps in normal and A-T cells as a function of timeafter a doseof 1Gy of X-rays in the presence or absence of ara C (100 μ mol/l). The time between irradiation and fixation includes a 1.5h treatment with coleemid. Error bars represent standard deviation of mean values of three experiments.

act as S-phase specific clastogens, the length of G_2-phase was determined by treatment of cells for various times before fixation with ara-A or ara-C other than autoradiography. 100 μ mol/L ara-C was applied to five cultures from two up to seven hours prior to harvesting. Figure 4 shows the results for A-T cells. The results of score of chromosomal lesions were expressed as the percentage of cells containing lesions. Ara-C was found to have a slight clastogenic effect on G_2 A-T cells during the first 3h following treatment since the number of damaged cells were greater than the number of labelled mitoses obtained for A-T cells (Figure 4, dotted line).

The result of scores of chromosomal lesions induced by 200 μ mol/L ara-A in normal cells were also expressed as the percentage of cells containing aberrations. When comparing the frequencies of damaged cells with the values for labelled mitoses in normal cells, it seems that ara-A only affected cells in DNA synthesis (Figure 5).

Effects of ara-A and ara-C on the kinetics of x-ray induced chromatid breaks

The result of experiments with normal and A-T cells are summarized in Figures 6 and 7. In cells exposed to x-rays alone, the frequency of deletions decreased with increasing post-irradiation incubation time, indicating the rejoining of breaks. This frequency decreased by a factor of approximately two for both cell lines over a 2h interval. In cells exposed to x-rays in the presence of 200 μ mol/L ara-A (Figure 6) the frequency of deletions remained constant at a value higher than that of x-rays alone for the various intervals between irradiation and fixation. In contrast cells x-irradiated and held in the presence of ara-C (100 μ mol/L), the number of deletions increased with time almost doubling with the two hour interval before fixation (Figure 7).

DISCUSSION

Inhibitory action of ara-C and ara-A on DNA synthesis is shown in Figures 1 and 2 for both cell lines as proposed by Moore and Hodgson (1983)[12]. These drugs act as clastogenic agents when applied to G_2 cells. This proposal appears to be supported by the data presented in Figures 4 and 5 when ara-A and ara-C were administered to the cells individually for a period of time before fixation. These figures show that both ara-A and ara-C increased the background level of aberrations during 4h treatment. The large increase in aberrations in ara-C treated A-T cells might either be a consequence of A-T hypersensitivity to chemical

mutagens or greater effectiveness of ara-C than ara-A. Kihlman and Anderson (1985)[10] did not find synergism with ara-C and caffeine even with x-ray. They therefore suggested that lack of synergism between two inhibitors might be expected when two inhibitors compete for the same site on an enzyme; one inhibitor might reduce the uptake of the other. This proposal seems true for the lack of synergism between ara-A and ara-C (Figure 3).

For x-irradiated A-T and normal cells with ara-A (Figure 6) the frequency of deletions was found to be constant for all post-irradiation intervals which was interpreted as the result of inhibition of dsb repair by ara-A.[13] The results for treatment of cells with ara-C after x-irradiation is shown in Figure 7. The results presented here for deletions are similar to those experiments on G_2 human lymphocytes.[9] Preston (1980)[9,14] interpreted these data as indicating that chromatid deletions are induced as a result of base damage which was being incised during dsb inhibition leading to an increasing number of dsb and consequent chromatid breaks. It therefore appears that ara-C has a different mode of action from that of ara-A on x-ray induced chromatid deletions. In view of results with ara-A the notion proposed by Preston (1980)[9] that ara-C is simply inhibiting repair of dsb and thus allowing incision of base damage can not be accepted. The synergistic action of ara-C on x-ray induced breaks might be explained by the clastogenic property of this drug reported by Panthelias and Wolff (1985)[15]. However, this hypothesis would not be supported by the fact that ara-C alone in our experiments did not of itself lead to significant levels of aberrations during last 4h of the G_2 phase (Figure 4).

Similarities between the action of these two drugs when applied to G_2 or S-phase cells, lack of synergism as a possible consequence of these similarities and yet on the other hand, different action of ara-A and ara-C on x-ray induced DNA lesions in G_2- cells, suggest a peculiar and important difference in the nature of these two nucleosides. Thus ara-A and ara-C appear to have a different mode of action on x-ray induced DNA lesions. The mechanism by which ara-C enhances the frequency of x-ray induced chromosomal aberrations in G_2 cells is not understood.

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