Mutation Research, 232 (1990) 261–266
Elsevier

MUT 04904

Chromosome-type exchange aberrations are induced by inhibiting repair of UVC-induced DNA lesions in quiescent human lymphocytes

M. Holmberg * and E. Gumauskas

National Institute of Radiation Protection, S-10401 Stockholm (Sweden)

(Received 24 January 1990)
(Revision received 10 May 1990)
(Accepted 10 May 1990)

Keywords: Chromosome exchange; UVC; Arabinofuranosylcytosine

Summary

Human lymphocytes in the quiescent stage were UVC-irradiated and then incubated for 90 min in the presence of the DNA-repair inhibitor ara-C. The cells were then cultured and analyzed for chromosome aberrations. A single treatment with UVC or ara-C gives rise to a very low yield of dicentrics, whereas the combined treatment can induce a high frequency of these chromosome-type aberrations. The yield in the combined treatment is approximately proportional to the square of the UVC fluence in the range 1–3 J/m^2. In addition, the experiments demonstrate that synergistic effects arise when cells are treated with UVC + ara-C and then exposed to X-rays. The results can be explained on the assumption that the UVC + ara-C treatment induces DNA double-strand breaks which, to the first approximation, are randomly distributed over the chromosomes. These breaks are able to interact with each other as well as with X-ray-induced DNA double-strand breaks to form a chromosome-type exchange aberration.

When cells are exposed to ionizing radiation prior to DNA synthesis, the aberrations which arise at the subsequent mitosis are of the so-called chromosome type and comprise both chromosome fragments and exchange aberrations (dicentrics and reciprocal translocations). There is much evidence that these aberrations arise from the DNA double-strand breaks which are directly induced by ionizing radiation and there also is evidence that these aberrations are formed before the onset of the DNA synthesis phase (S-independence). In contrast, alkylating agents as well as UV radiation have an S-dependent behavior, i.e., when the cells are exposed to these agents in the interphase stage, DNA lesions are induced which might be transformed into chromatid-type aberrations during the S phase. In general the DNA lesion giving rise to these S-dependent aberrations affects only 1 DNA strand, even if a number of polyfunctional mutagenic agents inducing cross-links between the 2 DNA strands also give rise to chromatid-type aberrations in an S-dependent way. In UV-irradiated cells the DNA lesions giving rise to the chromatid-type aberrations might be cyclobutane pyrimidine dimers or (6-4) photoproducts.

There are, however, a few papers in which it is reported that chromosome-type aberrations might be induced by S-dependent agents under certain conditions. Thus, Preston and Gooch (1981) re-

Correspondence: Dr. E. Gumauskas, National Institute of Radiation Protection, Box 60 204, S-104 01 Stockholm (Sweden).

* Dr. M. Holmberg recently deceased.

Abbreviations: ara-C, 1-β-D-arabinofuranosylcytosine; PBS, phosphate-buffered saline; SSBs, single- and double-stranded DNA breaks and alkali-labile bonds.

ported that treatment of phytohemagglutinin (PHA)-stimulated human lymphocytes in the G_1 stage with methyl methanesulfonate (MMS) or 4-nitroquinoline-1-oxide (4NQO) followed by incubation with ara-C results in this type of aberrations. These observations were confirmed in similar experiments, where DNA lesions were induced in the G_0 stage, but the treatment with the DNA-repair inhibitor was carried out after PHA stimulation and lasted for 18 h (Kishi, 1987). The use of chemicals for the induction of DNA lesions might present problems if the chemical is not efficiently washed out of the cells before culturing and therefore it can be an advantage to induce the DNA lesions with UV radiation. There seems to be only one previously reported measurement of chromosome aberrations where cells have been treated with UV radiation and a DNA-repair inhibitor. Thus, Ejima and Sasaki (1986) report that UV when coupled to ara-C could cause dicentrics and rings in both normal and ataxia telangiectasia fibroblasts, even if the frequency was rather low ($< 10\%$), in density-inhibited fibroblasts from normal subjects.

There seems to be no experiment in which both the induction of the DNA lesions and the inhibition of their repair were carried out in G_0 lymphocytes. Such experiments might be of particular interest because quiescent lymphocytes are commonly used to detect in vivo exposure to both chemicals and radiation and they might in some respects differ from stimulated lymphocytes with regard to the induction of chromosome aberrations (Heartline and Preston, 1985). Here we report that chromosome-type aberrations at high frequencies can be induced by UV radiation, if unstimulated human lymphocytes in the G_0 stage are treated with a DNA-repair inhibitor after the UV treatment. The yield of these aberrations was also measured as a function of UVC fluence in the UVC + ara-C-treated cells. It is further shown that synergistic effects arise when cells treated with UVC + ara-C are given a subsequent X-ray treatment.

Materials and methods

Buffy coat samples from healthy blood donors (Sabbatsberg Hospital, Stockholm) were sedimented above Ficoll-Paque (Pharmacia) and after centrifugation the lymphocyte layer was removed and washed twice in PBS. The cells were counted in a cell counter and thereafter suspended in 5 ml PBS in Petri dishes and irradiated with UVC (254 nm). After irradiation the contents of the Petri dishes were centrifuged in a refrigerated centrifuge and the cells were incubated in 10-ml tubes at 37 °C for 90 min in RPMI 1640 supplemented with 15% calf serum. Where used ara-C (Sigma) was added at a concentration of 5×10^{-5} M. After this incubation the cells in one experiment (subject I) were X-irradiated (250 kV, HVL, 2 mm Cu). The cells were then washed 3 times in PBS and 10^{-4} M deoxycytidine was added to the cultures. PHA was then added and the lymphocytes were cultured in the presence of bromodeoxyuridine (BrdU, 5 μg/ml) added to the culture medium (Medium 199, Flow Labs.) and harvested after 52–60 h. This rather long culture time was needed in order to get mitoses from the UVC + ara-C treatment. It can be remarked that it probably should have been an advantage to use RPMI 1640 for the chromosome cultures as well because it is a richer and faster medium than Medium 199. We preferred, however, to use Medium 199 because it was used in our previous experiments on chromosome aberrations (Holmberg and Gumauskas, 1986). Harlequin staining was performed according to Wolff and Perry (1974) and only first-division cells were analyzed. Further details of the experimental method on chromosome aberrations can be found elsewhere (Holmberg and Gumauskas, 1986).

For the experiment in which SSBs were measured $1–2 \times 10^6$ cells were gently added to alkali solution (0.15 M NaCl, 0.03 M NaOH at 20 °C). After 30 min the unwinding of DNA was terminated by adding neutralizing solution and the molecular weight of DNA was further reduced by sonication. Single- and double-stranded DNA was separated by hydroxylapatite chromatography and the DNA content in the various samples was measured fluorometrically after the DNA was stained with Hoechst 33258. Further details of the experimental method on DNA breaks can be found elsewhere (Holmberg et al., 1988; Holmberg, 1989).

Results

The present results show that the combined treatment with UVC plus ara-C induces high yields of chromosome-type aberrations as dicentric chromosomes (Table 1). In contrast, a single treatment of lymphocytes with UVC or ara-C induces a low yield (0–2%) of such aberrations. As can be seen from Table 1 there is a rather large variation in the frequency of induced dicentrics between the various blood donors. Blood lymphocytes from subjects I and III demonstrate a yield of about 0.8 dicentrics per cell for a treatment of UVC, 3 J/m^2 + ara-C, whereas for the same treatment the yield is only about 0.1 dicentrics per cell for subject IV.

TABLE 1

NUMBER OF DICENTRICS OBSERVED AFTER SINGLE AND COMBINED TREATMENT WITH UVC AND ara-C

Subject	Treatment	Cells analyzed	Dicentrics	Dicentrics/ cell
I	–	200	1	0.005
	UVC, 3 J/m^2 + ara-C	100	74	0.74 ± 0.09
II	–	200	0	0
	UVC, 3 J/m^2 + ara-C	100	32	0.32 ± 0.06
III	–	100	0	0
	ara-C	76	0	0
	UVC, 2 J/m^2	100	1	0.01
	UVC, 2 J/m^2 + ara-C	100	18	0.18 ± 0.05
	UVC, 3 J/m^2 + ara-C	94	75	0.80 ± 0.09
IV	–	100	0	0
	ara-C	200	0	0
	UVC, 2 J/m^2	100	2	0.02
	UVC, 2 J/m^2 + ara-C	200	12	0.06 ± 0.02
	UVC, 3 J/m^2 + ara-C	145	18	0.12 ± 0.03
V	–	100	0	0
	ara-C	88	0	0
	UVC, 2 J/m^2	100	0	0
	UVC, 3 J/m^2	100	1	0.01
	UVC, 1 J/m^2 + ara-C	100	2	0.02
	UVC, 2 J/m^2 + ara-C	100	9	0.09 ± 0.03
	UVC, 3 J/m^2 + ara-C	98	25	0.26 ± 0.05

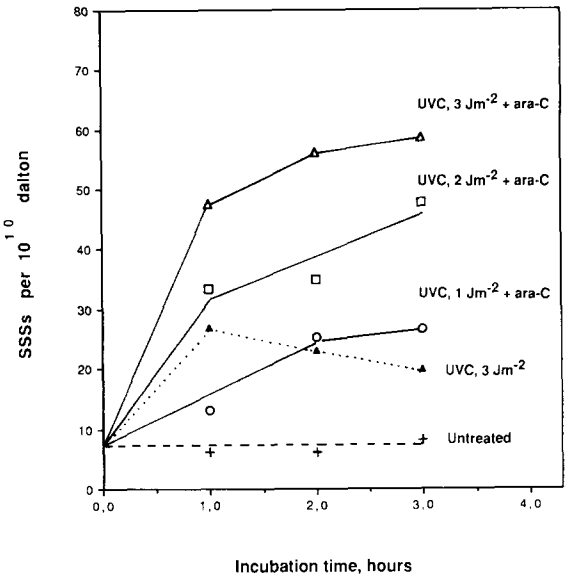

Fig. 1. SSBs as a function of incubation time after UVC irradiation at different fluences in the presence and absence of ara-C in the culture medium.

Fig. 1 shows the results of an experiment in which the yield of SSBs was measured as a function of UVC fluence in the UVC + ara-C-treated lymphocytes. As can be seen the yield of SSBs increases approximately linearly with UVC fluence in the range of 0–3 J/m^2 for incubation times in the range of 1–3 h in the presence of ara-C. A treatment of UVC, 1 J/m^2 + ara-C induces about the same yield of SSBs as a single UVC fluence of 3 J/m^2. A single ara-C treatment does not induce any measurable yield of SSBs (results not shown).

The effect of UVC fluence on the yield of aberrations in the UVC + ara-C-treated cells was examined in 3 experiments. As can be seen from the results given in Table 1 for subjects III, IV and V the yield of dicentric chromosomes after combined treatment with UVC and ara-C increases with increase in UVC fluence. The experimental points for subject V are plotted in Fig. 2 together with a curve proportional to the square of the UVC fluence and it can be seen that such a curve fits the experimental points. It can be mentioned that similar results on the dose response were obtained using aphidicolin as the DNA-repair inhibitor (data not shown). The results of an experi-

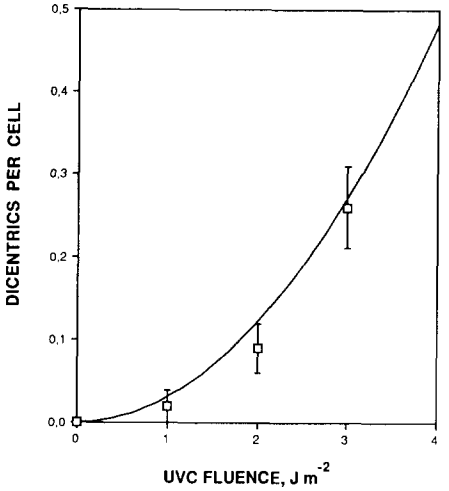

Fig. 2. The frequency of dicentric chromosomes per cell as a function of UVC fluence for blood lymphocytes treated with UVC + ara-C. The solid line is a dose-squared curve fitted to the experimental point at 3 J/m^2. Data from Table 1 (subject V).

ment in which UVC + ara-C-treated lymphocytes were X-irradiated are given in Table 2. As can be seen X-ray doses of 0.5 and 1.0 Gy induce 0.08 and 0.15 dicentrics per cell, respectively, in the absence of UVC + ara-C treatment. However, if the lymphocytes were treated with UVC + ara-C and then X-irradiated with 0.5 or 1.0 Gy the frequency increases from 0.74 dicentrics per cell to 1.07 and 1.69 dicentrics per cell, respectively.

Discussion

The present data show that chromosome-type aberrations such as dicentric chromosomes can be induced in human lymphocytes in the G_0 stage if the cells are treated with UV radiation and the DNA-repair inhibitor ara-C. Each of these 2 agents induces a very low yield of such aberrations. The observations reported here on the induction of dicentric chromosomes in unstimulated G_0 lymphocytes agree with those reported by Ejima and Sasaki (1986) on UV-irradiated human fibroblasts treated with ara-C, even if a rather low frequency (about 10%) was observed in cells from normal subjects in this latter experiment. The observations previously reported by Preston and Gooch (1981) and Kishi (1987) on stimulated G_1 lymphocytes treated with various chemicals in combination with either ara-C or aphidicolin also show that chromosome exchange aberrations can be induced by this treatment before the S-phase. It is commonly believed that this type of aberrations, when induced by ionizing radiation in cells before the onset of DNA synthesis, is caused by DNA double-strand breaks. Therefore it is of particular interest that Bradley and Taylor (1983) have reported that if UV-induced DNA lesions in human fibroblasts are accumulated by using ara-C or aphidicolin, this results in a significant yield of double-strand breaks as measured with the neutral filter elution method. Consequently, it is of interest to know whether the models, commonly used to explain the dose–response relationship for chromosome aberrations induced by ionizing radiation, also can explain the observed yield of dicentrics as a function of UVC fluence in the UVC + ara-C-treated cells as well as the observation of a synergistic effect when the UVC + ara-C-treated cells are given a subsequent X-ray exposure.

The yield of dicentrics as a function of UVC fluence in the UVC + ara-C-treated cells demonstrates a non-linear response in the UVC range of 1–3 J/m^2 (Fig. 2). In contrast, the measurements with the DNA-unwinding method show that the yield of DNA breaks (single-stranded and double-stranded DNA breaks plus alkali-labile lesions) increases approximately linearly with increase in UVC fluence (Fig. 1). This can indicate that the yield of double-stranded DNA breaks also demonstrates a linear increase as a function

TABLE 2

NUMBER OF DICENTRICS OBSERVED AFTER SINGLE AND COMBINED TREATMENT WITH X-RAYS AND UVC + ara-C

Subject	Treatment	Cells analyzed	Dicentrics	Dicentrics/cell
I	–	200	1	0.05
	0.5 Gy	100	8	0.08 ± 0.03
	1.0 Gy	100	15	0.15 ± 0.04
	UVC, 3 J/m^2 + ara-C	100	74	0.74 ± 0.09
	UVC, 3 J/m^2 + ara-C + 0.5 Gy	100	107	1.07 ± 0.11
	UVC, 3 J/m^2 + ara-C + 1.0 Gy	100	169	1.69 ± 0.13

of UVC fluence, because it has been suggested (see below) that a UVC + ara-C-induced DNA double-strand break originates from endonuclease cutting of the intact DNA strand opposite the gap which results after the incision of the UVC-induced DNA lesion. If so, the explanation for the non-linear dose response in the yield of dicentrics as a function of UVC fluence in the UVC + ara-C-treated cells can be similar to the explanation used to account for the non-linear dose response of the exchange aberrations induced by ionizing radiation. In this latter case, it is assumed that a dose-squared response results from interactions between DNA breaks randomly distributed in the cell nucleus (and where the DNA breaks are located in different ionization tracks), whereas interactions between 2 DNA breaks in a single ionization track should lead to a linear dose response. Thus, for the UVC + ara-C treatment it is assumed that the interactions take place between DNA lesions randomly distributed over the genome and consequently the yield of aberrations as a function of UVC fluence is expected to demonstrate a dose-squared response. This is in good agreement with the data (Fig. 2).

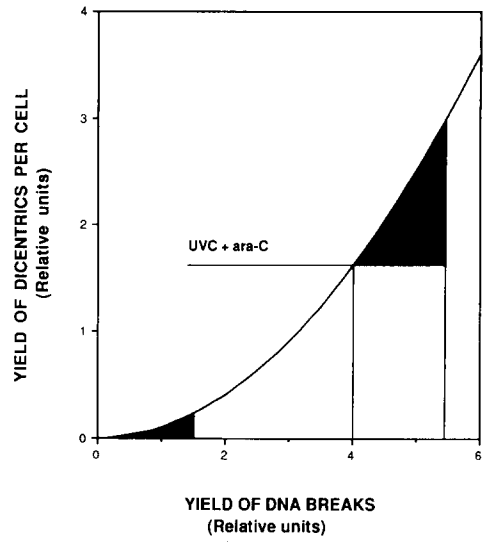

Fig. 3. The yield of dicentrics as a function of the number of DNA breaks on the assumption that the yield is proportional to the square of the number of breaks. Relative scales are used and it is apparent that for an X-ray dose which induces a constant number of DNA breaks, the effect on the yield of dicentrics after X-irradiation is much larger if the cells contain UVC + ara-C-induced DNA breaks than when the cells do not contain any DNA breaks before the X-ray treatment.

The experiments in which UVC + ara-C-treated cells are given a subsequent X-ray exposure show that the chromosome aberration yield is higher in comparison with the sum of the UVC + ara-C-induced yield and the X-ray-induced yield. This is expected because previous experiments have shown that pretreatment of lymphocytes with UVC radiation or posttreatment with ara-C gives a 2-fold increase in X-ray-induced dicentric yield (Holmberg and Gumauskas, 1986). The explanation was that these treatments delay the repair of short-lived X-ray-induced DNA breaks. Therefore, combined treatment of the cells with UVC and ara-C followed by X-irradiation is expected to give a 2-fold increase of the X-ray-induced yield if UVC and ara-C act on the same X-ray-induced DNA lesions, whereas a 4-fold increase is expected if different sites are affected. As can be seen from Table 2 a 4-fold increase agrees with the data for an X-ray dose of 0.5 Gy whereas the observed yield is higher than the expected yield for an X-ray dose of 1.0 Gy. Even if these data are not conclusive our other data (results not shown) indicate that the observed dicentric yield in cells treated with UVC + ara-C and given a subsequent X-ray exposure is higher than the yield calculated on the assumption that UVC + ara-C gives a 4-fold increase of the X-ray-induced yield.

An excess yield of aberrations in cells treated with UVC + ara-C and given a subsequent X-ray exposure can be explained on the assumption that the DNA breaks induced by X-rays can interact with the DNA breaks that result from the accumulation of the DNA breaks generated in the repair of UVC-induced DNA lesions. The number of excess exchange aberrations should then be proportional to the product of the DNA breaks induced by UVC + ara-C and X-rays separately. This is exemplified in Fig. 3 where, on an arbitrarily chosen scale, a dose-squared yield of dicentrics is plotted as a function of number of DNA breaks. It can be seen that an excess yield of dicentrics is expected when UVC + ara-C-treated cells are X-irradiated because the effect on the yield of dicentrics after a constant dose of X-rays is much larger in the cells which contain DNA breaks before the X-ray treatment (on the assumption that these breaks can interact with the X-ray-

induced breaks). The present results on UVC + ara-C-treated lymphocytes given a subsequent X-ray dose of 1 Gy indicate that the DNA breaks induced by UVC + ara-C can interact with the X-ray-induced DNA breaks and form exchange aberrations.

Even if 2 DNA double-strand breaks are needed for the induction of a chromosome exchange aberration, neither the exact nature of these breaks nor the exact mechanisms for the formation of the exchange complex are known. The experiments with restriction enzymes have shown, however, that endonucleases that produce blunt-ended DNA double-strand breaks as well as the ones giving rise to cohesive ends are able to induce exchange aberrations of the chromosome type (Bryant, 1984; Natarajan and Obe, 1984). It has been assumed that X-rays induce both types of DNA double-strand breaks but it is not known whether the DNA-repair mechanisms differ for the 2 types of DNA breaks (Bryant, 1988). As previously mentioned, Bradley and Taylor (1983) reported that using ara-C or aphidicolin to accumulate UV-induced DNA breaks leads to the production of DNA double-strand breaks. The authors suggest several possible mechanisms that can lead to the induction of these DNA double-strand breaks. One suggested explanation is that 2 single-strand DNA breaks might occur close together on opposite DNA strands. Simple calculations on the UVC + ara-C-induced DNA breaks show, however, that for a random occurrence of the DNA breaks, the probability is very low that 2 single-strand DNA breaks on opposite strands will be as close together as 40–50 bp. Another explanation suggested by Bradley and Taylor is that a cellular endonuclease cuts the intact DNA strand opposite the gap which results after the incision of the UVC-induced DNA lesion and which is held open by the DNA-repair inhibitor. If so, one can expect that the resulting DNA double-strand break should have asymmetrical sticky ends with lost bases.

In conclusion, the present results show that dicentrics can be induced in quiescent human lymphocytes by agents other than ionizing radiation if the repair capacity of the cells is impaired. This might be of importance when chromosomal aberrations are used to detect in vivo exposure of quiescent lymphocytes to chemicals or radiation.

Acknowledgement

This work was supported by grants from the Swedish Natural Science Research Council.

References

Bradley, M.O., and V.I. Taylor (1983) Repair-induced DNA double strand breaks after ultraviolet-light and either aphidicolin or 1-β-D-arabinofuranosylcytosine/hydroxyurea, Carcinogenesis, 4, 1513–1517.

Bryant, P.E. (1984) Enzymatic restriction of mammalian cell DNA using *Pvu*II and *Bam*HI: evidence for the double strand break origin of chromosomal aberrations, Int. J. Radiat. Biol., 46, 57–65.

Bryant, P.E. (1988) Use of restriction endonucleases to study relationships between DNA double-strand breaks, chromosomal aberrations and other end-points in mammalian cells, Int. J. Radiat. Biol., 54, 869–890.

Ejima, Y., and M.S. Sasaki (1986) Enhanced expression of X-ray- and UV-induced chromosome aberrations by cytosine arabinoside in ataxia telangiectasia cells, Mutation Res., 159, 117–123.

Heartline, M.W., and R.J. Preston (1985) The effect of 3-aminobenzamide on the frequency of X-ray- or neutron-induced chromosome aberrations in cycling or non-cycling human lymphocytes, Mutation Res., 148, 91–97.

Holmberg, M. (1989) The effect of deoxynucleosides on repair of DNA breaks in UVC-irradiated human lymphocytes, Mutation Res., 218, 33–39.

Holmberg, M., and E. Gumauskas (1986) The role of short-lived DNA lesions in the production of chromosome-exchange aberrations, Mutation Res., 160, 221–229.

Holmberg, M., M. Lagerberg, B. Niejahr and L. Rödin (1988) Simultaneous detection of DNA strand breaks and unscheduled DNA synthesis in mutagen-treated human lymphocytes in the absence of hydroxyurea, Mutation Res., 202, 111–118.

Kishi, K. (1987) Effects of repair inhibition in the G_1 phase of clastogen-treated human lymphocytes on the frequencies of chromosome-type and chromatid-type aberrations and sister-chromatid exchanges, Mutation Res., 176, 105–116.

Natarajan, A.T., and G. Obe (1984) Molecular mechanisms involved in the production of chromosomal aberrations, III. Restriction endonucleases, Chromosoma, 90, 120–127.

Preston, R.J., and P.C. Gooch (1981) The induction of chromosome-type aberrations in G_1 by methyl methanesulfonate and 4-nitroquinoline-*N*-oxide, and the non-requirement of an S-phase for their production, Mutation Res., 83, 395–402.

Wolff, S., and P. Perry (1974) Differential staining of sister chromatids and the study of sister chromatid exchanges without autoradiography, Chromosoma, 48, 341–353.