Conf-820594--2

Amer. Swedish Workshop

Plenum Press

Intrinsic factors that can affect sensitivity to chromosome-aberration induction

CONF-820594--2

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DE83 012099

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Research sponsored by the Office of Health and Environmental Research,
U. S. Department of Energy, under contract W-7405-eng-26 with the
Union Carbide Corporation.

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1. Introduction

The question to be addressed in this paper, namely are there individuals who are hypersensitive, or who are more likely to be hypersensitive, to the induction of chromosome aberrations by radiation and chemicals, is, at this time, difficult to answer. Furthermore, if such individuals can be identified there are social and legal implications concerning what action, if any, should be taken as far as their occupational or medical status is concerned. This will not be discussed here, as it is felt that such a topic is outside the domain of the author, a cytogeneticist. Whilst the emphasis will be on factors that could contribute to an increased sensitivity, it is also appropriate to consider if there are also factors that could contribute to a decreased sensitivity to chromosome aberration induction. Some of the discussion regarding DNA repair will be abbreviated, because much more detail can be found in the paper given by Dr. James Cleaver at this same Workshop

2. Identifiable syndromes conferring increased sensitivity to chromosome aberration induction or cell killing

There are a large number of published studies of the increased sensitivity to chromosome aberration induction, cell killing, or mutation induction of cells from persons having recognisable syndromes, that are associated with DNA repair or replication defects. These syndromes include xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, Fanconi's anemia, Cockayne's syndrome and Rothmund-Thompson syndrome. These probably represent the extremes

of increased sensitivity, and as such have been of value in providing information on DNA repair mechanisms, and the relationship between DNA repair or replication defects and other biological end-points. However, when considering individuals that might have a higher than normal sensitivity to chromosome aberration induction from occupational exposures to chemical or physical agents, they are unlikely to be a contributing factor. All these syndromes are clearly recognisable, generally at an early age, with early death being fairly common. Thus, the presence of such individuals in an occupational group is either unlikely or clearly identifiable.

3. Increases in sensitivity of persons heterozygous for DNA repair or replication defects

The frequency of persons in the population with identifiable DNA repair or replication defects is very low. However, since the genes responsible for the defects are recessive, it can readily be seen that the frequency of persons who will be heterozygous for these genes can be quite high - of the order of 1% of the population (Swift et al., 1976). It will, therefore, be important to know if these heterozygotes have an increased sensitivity to chromosome aberration induction by chemical or physical agents when compared to the sensitivity of some control group. It is appreciated that any control group could contain either unidentified heterozygotes for the same repair defects, or individuals

with increased sensitivities for unsuspected reasons. However, if a control group is fairly large, this problem is to some extent alleviated.

For obvious reasons, the heterozygotes that have been studied to date are obligate ones, ie they are the parents of individuals who have identifiable syndromes that are considered to be due to DNA repair or replication defects. Furthermore, the amount of information is small. Paterson et al. (1979) reported an increased sensitivity to cell killing by γ -rays given under hypoxic conditions for three of five presumed ataxia telangiectasia (AT) heterozygotes. The sensitivity was intermediate between that for children with AT and normal individuals. For two of the presumed heterozygotes the sensitivity was the same as that for normal individuals. The three heterozygotes of intermediate sensitivity to cell killing also carried out DNA repair replication at a level intermediate between normal and AT individuals. However, it is noteworthy that there was no difference in sensitivity between the AT heterozygotes and normal individuals when irradiations were given in oxic conditions. These results indicate some increase in sensitivity to cell killing by γ -rays under hypoxic conditions for some AT heterozygotes. Similar results have also been reported by Chen et al. (1978) and Arlett and Lehmann (1978).

It has also been reported that the spontaneous chromosome aberration frequency in AT heterozygotes is not significantly higher than for normal individuals, whereas it is often somewhat elevated in persons having AT. In addition, the increase in aberration frequency following radiation exposure is similar to that in normal individuals, whereas AT

individuals are markedly more sensitive to x-ray-induced chromosome aberration induction (Paterson and Smith, 1978; Harnden, 1978).

Ivanova et al. (1980) analysed chromosome aberrations in lymphocytes of AT heterozygotes and normal individuals following treatment with thiophosphamide. The results indicated that there was no overall difference in aberration frequency between the two groups. The authors suggested that there was a significant increase in the frequency of aberrant cells in the AT heterozygotes (not manifest as an increase in aberration frequency or a per cell basis), but it would appear that further analysis is needed before this can be considered conclusive.

Auerbach et al. (1980) measured the frequency of chromosome aberrations in the lymphocytes from individuals with Fanconi anemia (FA), heterozygotes for FA, and normal individuals following in vitro treatment with diepoxybutane. The spontaneous frequency of chromosome aberrations in FA heterozygotes and normal individuals was identical, and considerably lower than that for FA individuals. The frequencies of aberrations induced by diepoxybutane were significantly elevated in some of the eleven FA heterozygotes compared to those in normal individuals, but the mean frequency was some 50 times lower than that for FA homozygotes. This was a fairly small study, and only sensitivity to diepoxybutane was measured. Thus more data are needed on FA heterozygotes, and heterozygotes for other disorders in order for any general conclusion to be drawn.

It seems to be clear that even in those few cases where an increased sensitivity of a heterozygous condition is indicated, it is not consistent, ie only some heterozygotes for a specific condition

appear to have an increased sensitivity. Clearly more work in this area is needed. It is also particularly important to note that the studies to date on specific heterozygotes are for obligate heterozygotes, and where the homozygous condition is known to be especially sensitive. The question of particular importance is whether or not it is possible to identify persons who might be only very slightly more sensitive to chromosome aberration or mutation induction by specific agents, and for whom there is no available genetic information that would indicate such an increased sensitivity. If such individuals are identifiable, then with a sufficient quantity of information it might be possible to determine what intrinsic factors could be responsible for an increased sensitivity to aberration or mutation induction. In parallel with such studies it would also be essential to determine what extrinsic factors could also influence the sensitivity of individuals to chemical or physical agents.

4. The mechanism of induction of chromosome aberrations and differences in sensitivity

It is very difficult to determine the best, or even a suitable approach for identifying persons who might show an increased sensitivity to the induction of chromosome aberrations by chemical or physical agents, and subsequently to determine if there are intrinsic factors that can influence this sensitivity. The approach we have taken as a starting point is to try and better understand the mechanism of induction of chromosome aberrations, and then begin

to determine what steps in the induction process are subject to alteration, or variation and if such alterations or variations could result in increases or decreases in sensitivity to aberration induction.

Our initial results on the use of DNA repair inhibitors for studying the mechanism of aberration induction by radiation and chemicals have been published (Preston, 1980; Preston, 1981; Preston & Gooch, 1981; Preston, 1982a; Bender and Preston, 1982; Preston, 1982b) and will not be presented in detail here. The conclusions from these studies are, however, important for the subsequent discussion.

It was shown that cytosine arabinoside (ara-C) can inhibit excision repair following UV, x-ray, or chemical treatment. This inhibition probably occurs at the polymerase step, and results in single strand gaps in the DNA (Hiss and Preston, 1977 ; Dunn and Regan, 1979). The inhibition can be reversed by the addition of deoxycytidine. If the single strand gaps accumulated by ara-C incubation, following x-irradiation for example, can interact to form aberrations, on reversing the inhibition with deoxycytidine, then the aberration frequency would be expected to be greatly enhanced for cells x-irradiated and incubated with ara-C, compared to that in cells x-irradiated but with no post-treatment with ara-C. This was the rationale behind the experiments. If the time required to repair all the induced DNA damage that can result in aberrations is long, of the order of an hour or more, then the aberration frequency should increase with increasing duration of post-treatment ara-C incubations.

All the chromosome aberration studies reported here utilised human lymphocytes, largely because they represent a rather synchronous population before and after mitogenic stimulation, at least for the first in vitro cell cycle, that was all that was necessary for these experiments. The first in vitro cell cycle is also long (about 44-48 h.) and so it is possible to carry out post-treatment incubations in ara-C for several hours without the complication of cells progressing into subsequent stages of the cell cycle (particularly G_1 to S).

Unstimulated (G_0) lymphocytes were irradiated with 200 rad of x-rays and incubated with ara-C for 0, 1, 2, or 3 hours. The ara-C inhibitory action was reversed by the addition of deoxycytidine, and the cultures were incubated for 48 h., following mitogenic stimulation with phytohemagglutinin (pha). The frequency of chromosome-type aberrations was considerably enhanced when cells were incubated with ara-C after x-irradiation, and the increase was approximately linear with increasing ara-C incubation times (Preston, 1980). These results show that ara-C inhibits the repair of DNA damage that is converted into aberrations, and that the damage resulting in aberrations takes 3 h. or more to be completely repaired. Since ara-C inhibits excision repair, and not the repair of directly induced strand breaks (Hiss and Preston, 1977), and because the aberration frequency increases with ara-C incubation times up to 3 h., it was concluded that x-ray-induced base damage was the DNA lesion that was converted into aberrations during the repair process. In order to obtain an aberration a single strand induced event has to be converted into a double strand gap during repair and this can be accomplished either by a single strand nuclease (Natarajan and Obe, 1978)

or by having coincident base damages on the two strands of the DNA double helix (Ahnstrom and Bryant, 1982).

These results led us to propose a general hypothesis for the induction of chromosome aberrations (Preston, 1980). An aberration can be formed by the interaction (or misrepair) of two repairing lesions. The repair of these two lesions must be coincident in time and they must be close together spatially (within the rejoining distance) for an aberration to be formed. The probability of obtaining an aberration will be dependent upon the likelihood of having coincident lesions. This in turn will be dependent upon the amount of the specific DNA damage that can be converted into aberrations, and upon the rate of repair of this damage -- the more rapid the repair the greater the probability of coincident repairing lesions.

It can be seen that this hypothesis will explain the induction of aberrations by chemical agents, and also the aberration spectrum and cell cycle variations observed with most chemical agents. If cells are treated in G_1 with the majority of chemical agents, the aberrations observed at the first post-treatment metaphase are of the chromatid-type, and are formed during or after DNA synthesis.

If the repair of chemically-induced damage that can be converted into aberrations is slower than that for X-ray-induced damage, then the probability of obtaining coincident lesions will be lower. Thus, the probability of producing aberrations in G_1 cells is low. As a consequence of the replication of damaged DNA, the probability of two lesions being coincident during or after DNA synthesis is much higher than in G_1 cells, and aberrations can be formed -- these will be of the chromatid-type.

Pha-stimulated lymphocytes were treated with 4-nitroquinoline-N-oxide (4NQO) and methyl methanesulfonate (MMS) in G_1 (9 h. after stimulation), and no aberrations were observed. If cells were treated with 4NQO or MMS in G_1 and then incubated with ara-C for 6 h. aberrations were observed. These aberrations were unequivocally of the chromosome type, and had to have been formed during G_1 , ie prior to DNA synthesis (Preston and Gooch, 1981). Thus it can be concluded that ara-C inhibits the repair of 4NQO or MMS-induced damage that can be converted into aberrations, accumulating single-strand gaps that can interact to form aberrations on reversing the ara-C inhibition with deoxycytidine. The probability of obtaining coincidentally repairing regions is greatly increased by essentially accumulating 6 h. of repairing regions by ara-C inhibition. If this probability is increased, then aberrations can be produced in G_1 following chemical treatment as evidenced by the observation of chromosome-type aberrations. There is supportive evidence for this hypothesis, since it has been shown that the repair of 4NQO- and MMS-induced DNA damage is inhibitable by ara-C, that this repair is of the "long-patch" type, and that the duration of repair of this chemically induced damage is considerably longer than that for X-rays (Waters et al., 1982; Snyder and Regan, 1982).

If chromosome aberrations are indeed produced by the interaction of two coincidentally repairing regions, then it would be predicted that if the rate of repair of the damage leading to aberrations is more rapid in a particular cell type then the aberration frequency would be higher in that cell type at any dose level. As a possible example of such a situation we utilised lymphocytes of Down individuals. It was

reported by Sasaki and Tonomura (1969) and Evans and Adams (1970) that the frequencies of x-ray-induced chromosome-type aberrations in G_0 lymphocytes of Down individuals were about 1.6 times as high as the frequencies in normal lymphocytes. Since the amount of x-ray-induced DNA damage is expected to be the same because the DNA content of the Down cells is only about 1% higher than in normal cells, it seemed plausible that some differences in rate of repair of the damage leading to aberrations could account for this increased sensitivity of Down cells.

Unstimulated lymphocytes from Down individuals were x-irradiated and incubated with ara-C for 0, 1/2, 1, or 2 h. It was found that the increase in frequency of chromosome-type aberrations as a function of ara-C incubation time was more rapid in Down cells than normal cells (Preston, 1981). Thus, for example, the frequency of dicentrics in Down cells for 1 h. post-treatment in ara-C is about the same as the frequency after 2 h. ara-C incubation for normal cells. Thus, the repair of x-ray-induced DNA damage, that can be inhibited by ara-C, and that can be converted into chromosome aberrations, appears to be more rapid in Down G_0 lymphocytes than normal lymphocytes. This more rapid repair will increase the probability of having coincidentally repairing regions that are able to interact to form aberrations. A possible explanation for differences in the rate of repair of x-ray-induced DNA damage, that is converted into aberrations, in Down and normal lymphocytes has been offered by Leonard and Merz (1981). They have shown that the G_0 Down lymphocytes behave as though partially stimulated, ie the time of entry into the S-phase, following mitogenic stimulation, and the first

cell cycle in Down cells are shorter than in normal cells. It is also important to note that Down cells are equally sensitive to aberration induction when irradiated in G_0 or G_1 , following stimulation, whereas normal lymphocytes are more sensitive when irradiated in G_1 compared to irradiation in G_0 , ie a G_1 -irradiated normal lymphocyte has the same sensitivity to aberration induction as Down lymphocytes irradiated in G_1 or G_0 (Lambert et al., 1976; Leonard and Merz, 1981).

These results indicate that aberration frequency can be influenced by the rate of repair of the specific DNA damage that is converted into chromosome aberrations -- the more rapid the rate of repair, the higher the probability of having coincidentally repairing regions, and the higher the probability of producing an aberration. It is possible, therefore, to argue that differences in sensitivity to chromosome aberration induction by chemical and physical agents can be reflective of differences in the rate of repair of the specific DNA damage that results in aberrations.

The preceding discussion relates rather specifically to how differences in the rate of repair of DNA damage induced in G_1 cells could influence the frequency of chromosome-type aberrations. However, it can also be argued that an increase in this rate of repair could decrease the frequency of aberrations, specifically chromatid-type aberrations formed during or after DNA replication. If, for example, the DNA damage induced in cells prior to replication is repaired more rapidly than normal (or average), less of this damage will be present at the time of replication, and thus the probability of producing a chromatid-type aberration will be lower.

The converse would be expected for a slower rate of repair than normal. Therefore, for a more rapid repair of damage than normal, the probability of producing chromosome-type aberrations is increased, but the probability of producing chromatid-type aberrations will be decreased, and vice versa for a slower than normal rate of repair. The transmission and possible consequences of the different aberration types may be quite different, and so increases or decreases of different types could be very important.

As discussed in Sections 2 and 3, increased sensitivity to aberration induction can be the result of the presence of defective DNA repair or replication processes. It was considered that such defects represent the extreme of sensitivity. The influences of perhaps small changes in the rate of repair of DNA damage in apparently normal individuals would account for much smaller differences in sensitivity. Furthermore, it is the consideration of small differences in sensitivity that is probably the most important for estimating potential consequences of occupational or environmental exposures, because there is unlikely to be a phenotypic characteristic that will identify such slightly sensitive individuals. Clearly the hypotheses presented needs further study, particularly with regard to attempting to relate differences in rate of repair for specific DNA damage with sensitivity to aberration induction for a range of chemical and physical agents. It is perhaps important to add here that differences in rate of repair could also account, in part, for differences in sensitivity between the same cell type in different species, or between different cell types in the same species. Such considerations could well be valuable when attempting to assess

potential health effects of exposure to chemical or physical agents, where extrapolation generally have to be made from data obtained with laboratory animals to man, or from somatic to germ cells.

It is appreciated that the preceding discussion deals with only one possible intrinsic factor that could influence sensitivity. However, it is proposed simply as a first step towards trying to understand what is clearly a most complex problem. It is hoped that by determining the mechanism of induction of chromosome aberrations, that it will be possible to learn where to start looking for factors that could influence this process.

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