A POSSIBLE CORRELATION BETWEEN THE GROWTH RATE AND THE EXTENT OF DNA DAMAGE INDUCED BY RADIODECAY IN MOUSE LYMPHOMA CELLS

P. L. Ribeiro, M. C. Martinez and R. S. Mitra

Program in Toxicology, Department of Environmental and Industrial Health,
School of Public Health, The University of Michigan,
Ann Arbor, Michigan 48109

The extent of DNA single strand breaks resulting from the beta radiodecay of incorporated [3H]thymidine in DNA of mouse lymphoma cells appears to be related to the degree of growth inhibition. The extents of damage to DNA and inhibition of growth seem to be functions of the concentration of radioactivity as well as the specific activity of the radiolabeled precursor in the medium. The differences in both concentration (μCi/ml) and in the specific activity of radiolabeled precursors, may help to explain the different responses encountered when using [3H] and [14C]thymidine. When cells exposed to [3H] TdR are transferred to fresh medium, the DNA damage can be repaired. The repair is followed by an increase in the cell number with the rate of growth being similar to that of unexposed cells. Cells exposed continuously to [3H] TdR in the medium can accommodate to the radioactive stressor by repairing the DNA damage and maintaining this repair capability throughout the exposure.   © 1985 Academic Press, Inc.

Radiolabeled precursors of DNA such as tritiated thymidine have been extensively used as valuable tools in cell biological research. However, in the majority of cases, the possible damage caused by radiodecay of such incorporated precursors per se has not been assessed. This possibility takes on more significance when one realizes that radiolabeled DNA is used in a number of different genotoxicity assays. In this case, one should establish if radiodecay of the incorporated radiolabeled precursors contributes to the genotoxicity especially when working in conjunction with other potential genotoxic compounds.

In 1958, Painter et al. (1) were the first to report growth inhibition of cultures attributed to the presence of high levels of tritiated thymidine in the medium. Since 1958, a number of papers report a range of deleterious effects resulting from the incorporation of ³H [TdR] including single strand breaks in DNA (2, 3), decreased survival of cells (4, 5), inactivation of transforming DNA

(6), perturbation of the cell cycle (7, 8) and aberrations of chromosomes (9,

10, 11).

In view of these studies, a direct causal relationship between the extent

of DNA damage and the effect on cell growth has largely been inferred and not

directly shown. In this paper, we have shown, using a eucaryotic cell line (12)

that the repair of the initial DNA damage could be closely followed by a return

to the normal growth pattern of these exposed cells.

METHODS

Culture Conditions

Mouse lymphoma cells type L5178Y/Tk+/- (obtained from Dr. David Clive,
Burroughs Wellcome Co., Research Triangle Park, N.C.) were grown in modified
Fischer's media according to the procedure of Turner et al. (12). The suspen-
sion cultures were grown in a Psychotherm shaker incubator (New Brunswick
Scientific Co., Inc., New Brunswick, NJ) at 37ºC at 120 rpm. Cells were counted
by Coulter counter and by trypan blue exclusion assay.

Exposure Conditions

For each experiment, cells obtained from a culture in log phase were inocu-
lated into separate glass screw cap erlenmeyer flasks to densities ranging from
7×10^4 to 1×10^5 cells/ml. After a 15 minute acclimatization, aliquots of
radiolabeled thymidine ([methyl-^{3}H]thymidine specific activity, 52 Ci/mmol; New
England Nuclear Corp.) or [methyl-^{14}C] thymidine (specific activity, 59
mCi/mmol; Amersham Corp.) were added to each suspension culture to achieve
concentrations ranging from 0.5 μCi - 2 μCi/ml for ^{3}H[TdR] and 0.5-1.0μ Ci/ml
for [^{14}C]TdR.

A volume of cell suspension containing approximately 2×10^6 cells was
taken from each flask at both the onset and end of the exposure period and
harvested by centrifuging at 5000 rpm for 10 min at 4ºC. The cells were then
washed twice with cold Ca^{2+}/Mg^{2+}-free PBS, pH 7.4 and the cell pellets were used
for DNA sedimentation studies.

Sedimentation of DNA through sucrose gradients

The cell pellet (approximate cell number 2×10^6) was resuspended in 2 ml
of 10 mM Tris-HCl/4 mM EDTA pH 8.0. For the analysis in alkaline sucrose
gradients, 0.5 ml sample of each suspension was lysed and layered on a 5-20%
sucrose gradient in 0.1 N NaOH-0.9 M NaCl according to the procedure of Mitra
and Bernstein (13). For analysis in neutral gradients, cells were lysed with
sodium lauryl sulfate and layered on a 5-20% sucrose gradient in 1 M NaCl-0.01 M
Tris-HCl, pH 7.5 (13).   The gradients were run in a Beckman L8-80 ultracentri-
fuge using a SW 41.1 rotor at 28000 rpm for two hours at 20ºC. After centrifu-
gation, the contents of each tube were fractioned by collecting drops through
the bottom of the tube.

Portions (0.1 ml) from each fraction were applied to paper strips (Whatman
3 MM), dried, and washed batchwise with three changes of cold 7% TCA and then
two changes of cold 95% ethanol. When dry, each paper strip was placed in a
vial with 5 ml of scintillation fluid (5.5 g Permablend I per liter of toluene)
and assayed for radioactivity in a Packard Tricarb Liquid Scintillation Spectro-

meter. Alkaline sucrose gradient fractions obtained from cells grown in the absence of any radiolabeled TdR were analyzed for DNA content by the fluoro-metric method of Zubroff and Sarma (14).

RESULTS AND DISCUSSION

In earlier, seemingly unrelated experiments (data not shown), we found that lymphoma cells after exposure to 1 μM Cd^{2+} in the medium, for 3 hr which in-hibited their growth, recovered when placed in fresh Cd^{2+}-free medium as seen by an increase in their rate of growth after an initial lag period. However, when this experiment was repeated with cells whose DNA was prelabeled overnight with [^{3}H]TdR (2 μCi/ml) prior to the cadmium exposure, this recovery was not noted. Upon closer inspection, we found that cell growth was severely inhibited during the prelabeling period by as much as 50%. On the other hand when [^{14}C] TdR (0.5 μCi/ml) was used as the label, cells grew at a rate similar to that of cells grown in the absence of radiolabeled precursor.

Preliminary experiments showed that the inhibition of growth was indeed a result of the presence of [^{3}H]TdR in the medium and was exclusive of any other components such as alcohol or unlabeled thymidine present in the [^{3}H] TdR stock solution. It was noted that increases in both the incidence of single strand breaks in DNA and the inhibition of growth seemed to correlate in a dose depend-ent manner with increasing concentrations of [^{3}H]TdR present in the medium (Fig. 1). Since these sedimentation profiles lacked an appropriate control, it could not be assumed that cells exposed to 0.5 μCi/ml [^{3}H]TdR had not experienced DNA damage. Although extensive single strand breakage was seen, no detectable double strand breakage in DNA occurred as assayed using neutral sucrose density gradients (data not shown).

The question still remained as to whether the much more pronounced inhibi-tion of growth resulting from [^{3}H]TdR as opposed to [^{14}C]TdR was a function of the concentration of the TdR in the medium, the specific activity of the radio-labeled compound or the path length of the decay particle. The tritium labeled compounds generally used have been usually on the order of 1000 times greater in specific activity than the [^{14}C] compounds. Therefore, in the case of [^{3}H]TdR,
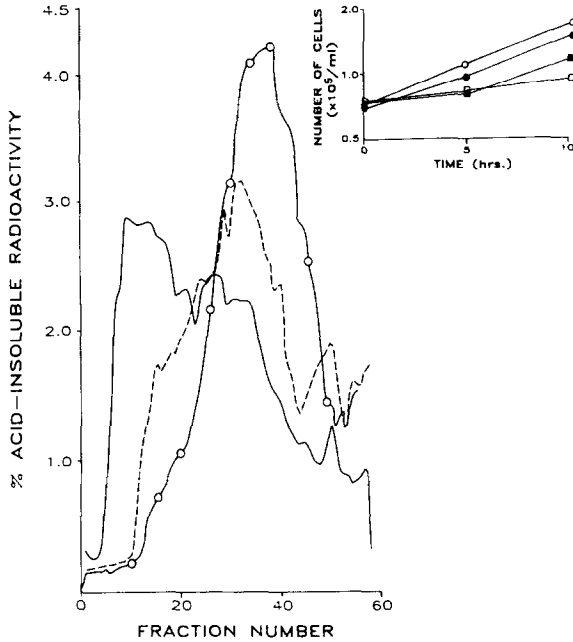
Figure 1. Effects of different concentrations of labeled thymidine on single
strand breaks in DNA. The cells were exposed to 0.5 µCi/ml (———),
1.0 µCi/ml (-----) and 2 µCi/ml (-O-O) of [3H] TdR for 4 hrs. The
cells were harvested, washed, lysed and were subjected to alkaline
sucrose density gradient centrifugation. The insert in figure 1 shows
the growth of cells when exposed to different concentrations of [3H]
TdR. At different intervals, the number of cells were determined by a
Coulter counter. Cells grown in media free of radiolabeled precursor
served as the control (-O-O); [3H] TdR was added to achieve the con-
centrations of 0.5 µCi/ml (-●-●-), 1.0 µCi/ml (-■-■-) and 2 µCi/ml
(-□-□-).

the extent of incorporation of the hot precursor per unit length of DNA molecule

would be much higher than that of [14C]TdR. In an attempt to answer this

question, an experiment was designed to use the same concentration and specific

activity of both [14C] and [3H]TdR. An exposure of 4 hr to either radiolabeled

compound produced no differences in the alkaline sucrose density sedimentation

profiles (Fig. 2). These data suggest that the differences seen in the effects

imposed by [3H]TdR and [14C]TdR are at least partially attributable to the

different specific activities and the concentrations of the precursors in the

medium.

In an experiment designed to study the effects of prolonged exposure to

[3H]TdR, it was noted that DNA single strand breaks were evident as early as

four hours after the addition of [3H]TdR to the medium. A more important

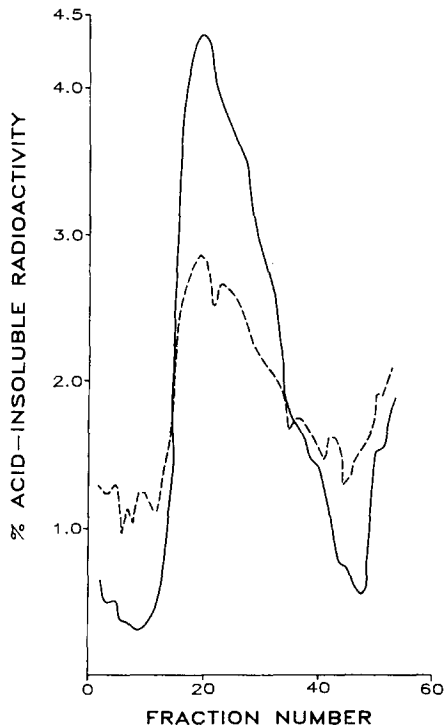observation was that the DNA of exposed cells which seemed to be comprised of

Figure 2. A comparison of the DNA sedimentation profiles in alkaline sucrose
gradients using the same specific activity for both [3H] and [14C]
TdR. A sufficient amount of non radioactive thymidine was added to
the [3H] TdR (———)to bring the specific activity down to the same
level of [14C] TdR (- - - -) which was 1 µCi/ 17.0 nmoles. Cells were
exposed to radioactivity for 4 hrs.

small fragments at four hours, did indeed begin to increase in size as the

exposure was continued in the presence of [3H]TdR (data not shown). This phe-

nomenon may be considered as a result of an accommodative response (15) by the

cells to the radioactive stressor. The apparent accommodative response by the

cells to such DNA damage may involve an inducible repair mechanism which was

maintained at a much higher level in the presence of [3H]TdR.

To prove that this increase in the molecular weight of DNA during the

continuous exposure was indeed the result of DNA repair, cells were grown in

medium containing [3H]TdR for 4 hrs after which an excess of unlabeled TdR was

added to the medium. Cells were collected for the analysis of the size of DNA

at various times following this addition. The results indicated (Fig. 3) that

the increase in the size of the DNA fragments in the exposed cells was indeed a

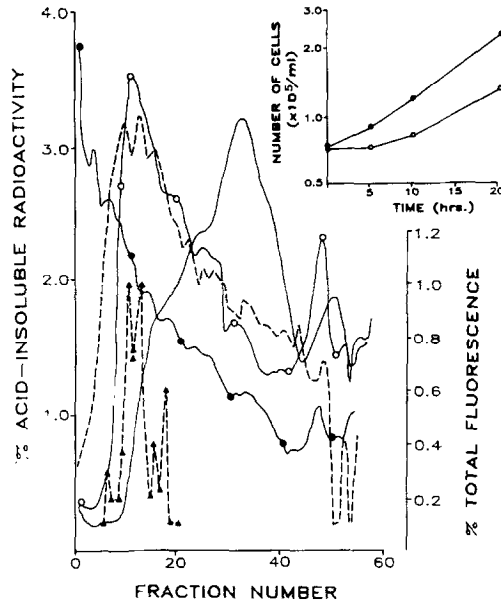result of repair. Along with this repair of DNA strand breaks, a concomitant

208

Figure 3. Repair of single strand breaks in DNA in cells exposed to labeled
thymidine. Cells were exposed to 1.0 µCi of [3H] TdR per ml for 4 hrs
(——). After that period, a 500 fold excess of nonradioactive thymi-
dine was added to the medium and aliquots at 5 hrs of post addition (-
0-0-) and 20 hrs of post addition (-●-●-) were withdrawn for the
analysis of size of DNA in alkaline sucrose gradients. Cells labeled
with [14C] TdR (0.5 µCi/ml for 20 hrs) were used as the control (----
). Cells grown in media free of radiolabeled precursor also serving
as control (-▲-▲-) were identically processed. DNA content in each
fraction was estimated fluorometrically. The insert in the figure
shows the recovery of cell growth during the repair of DNA in cells
exposed to [3H] TdR (-O-O-). Cells exposed to [14C] TdR were used as
the control (-●-●-). The number of cells were determined by trypan
blue exclusion assay using a hemocytometer.

return of the rate of growth to that of unexposed cells was observed. Within

the first 5 hrs after the addition of nonradioactive thymidine, the DNA repair

was essentially complete. Longer incubation resulted in a much larger product

which sedimented to the bottom of the gradient. On the other hand, no signifi-

cant differences in either the rates of growth or DNA sedimentation profiles

were exhibited (Figure 3) when cells grown in medium containing [14C]TdR (0.5

µCi/ml) were compared to cells grown in the absence of radiolabeled precursors.

This suggests that at least at the concentration employed, 14C did not cause

inhibition of growth or damage to the DNA.

In summary, the inhibition of growth exhibited by cells exposed to [3H]TdR

can be attributed to the DNA damage produced by the beta radiodecay of the

incorporated radiolabeled precursor. This damage seems to be related to both

the concentration of radioactivity and the specific activity of the precursors used. The extent of such damage can be minimized if both of these parameters are kept to a minimum. During the recovery of cells from the radiotoxic effects, the repair of the DNA damage seems to be followed closely by a return of the rates of growth to those of unexposed cells. This accommodative mechanism must be considered when one uses radiolabeled cells to assess DNA damage or the repair of such damage by other genotoxic agents.

REFERENCES

1. Painter, R. B., Drew, R. M., Hughes, W. L., (1958) Science, 127, 1244-1245.
2. Cleaver, J. E., Thomas, G. H., (1972) Science, 177, 996-998.
3. Burki, H. J., Bonker, S., Ritter, M., Cleaver, J. E., (1975) Radiation Res., 62, 299-312.
4. Marin, G., Bender, M. A., (1963) Intern. J. Rad. Biol., 7(3), 221-233.
5. Burki, H. J. and Okada, S., (1970) Radiation Res., 41, 409-424.
6. Rosenthal, P. N. and Fox, M. S., (1970) J. Mol. Biol., 54, 441-464.
7. Ehmann, U. K., Williams, J. R., Nagle, W. A., Brown, J. A., Belli, J. A. and Lett, J. T., (1975) Nature, 258, 633-636.
8. Pollack, A., Bagwell, C. B., Irvin III, G. L., (1979) Science, 203, 1025-1027.
9. Klesecz, R. R., Hsu, T. X., (1964) Proc. Natl. Acad. Sci., U.S.A., 52, 811-814.
10. Geard, C. R., (1975) Radiation Res., 61, 118-127.
11. Olivieri, O., Bodycote, J., Wolff, S., (1984) Science, 223, 594-597.
12. Turner, N. T., Batson, A. G., Clive, D., (1984) Handbook of Mutagenicity Procedures, Chapter 11, Second Edition Revised, Elsevier Sci. Pub., B. U.
13. Mitra, R. S. and Bernstein, I. A., (1978) J. Bacteriol., 133, 75-88.
14. Zubroff, J. and Sarma, D. S. R., (1976) Anal. Biochem., 70, 387-396.
15. Mitra, R. S., Gray, R. H., Chin, B. and Bernstein, I. A., (1975) J. Bacteriol., 121, 1180-1188.