Vol. 106, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1982 Pages 546-552

IMPAIRED REPAIR CAPACITY OF DNA STRAND BREAKS INDUCED BY ^{125}I-TRIIODOTHYRONINE IN CHINESE HAMSTER CELLS

Synnöve Sundell-Bergman[1] and Karl J. Johanson[2]

[1)]The Gustaf Werner Institute, Box 531 S-751 22, UPPSALA, Sweden

[2)]The National Defence Research Institute, S-901 82, UMEÅ, Sweden

Received March 22,1982

^{125}I-labelled triiodothyronine which binds to specific nuclear receptors induce DNA strand breaks in Chinese hamster cells. A large fraction of these breaks is left unrepaired and seems to be double strand breaks. The efficiency of inducing such breaks is as high as after incorporation into DNA of [^{125}I-]iododeoxyuridine which is known to be very radiotoxic.

INTRODUCTION

Triiodothyronine binds to a specific nuclear receptor - a nonhistonprotein - which is bound to DNA (1,2,3). This complex seems to be involved in the regulation of transcription of RNA (4). The number of receptors per cell varies from 17 in the testis to about 5.800 in the anterior pituitary (5). In young age the number of nuclear T3 receptors per cell seems to be still higher (6).

It has been shown that ^{125}I incorporated into DNA as iododeoxyuridine is 3-20 times more toxic to the cells than ^{3}H incorporated into DNA as measured by cell survival (7,8), production of chromosome aberration (9,10) and induction of DNA strand breaks (11,12). The high radiotoxicity of ^{125}I is expected to depend on its decay process by electron capture which leads to a burst of low-energy electrons with very short ranges (13).

The aim of the present work has been to study the induction of DNA strand breaks in Chinese hamster cells after [^{125}I] T3 label-

Abbreviations: T3, triiodothyronine; PBS, phosphate buffer saline; SDS, sodiumdodecyl sulphate

0006-291X/82/100546-07$01.00/0

MATERIALS AND METHODS

Yeast enolase was isolated using a modification of the method of Westhead and McLain (12). The enzyme was deionized, concentrated and assayed for residual "conformational" metal ion as described (1,13). Protein concentrations were calculated assuming the molecular weight is 93,300 and the extinction coefficient at 280 nm is 0.895 cm^2/mg (1).

Solutions were prepared from deionized water (Continental Deionized Water Service) and "Ultrapure" potassium chloride (Alfa Chemical Co.) or twice-recrystallized tris or AR Reagent Grade chemicals, where these were available. Plasticware was used whereever possible. 2-phosphoglycerate and phosphoenolpyruvate were obtained as the sodium salts from Calbiochem. The chromophoric substrate analogues, 3-aminoenolpyruvate-2-phosphate (AEP) and D-tartronate semialdehyde-2-phosphate (TSP) were synthesized as described by Hartman and Wold (14) and Spring and Wold (15).

Absorbance readings were made using a Bausch and Lomb Spectronic 200 spectrophotometer equipped with a digital readout. Measurements of pH were made using a Corning Model 10 pH meter.

RESULTS

The inhibition of enolase by fluoride in the absence of P_i increases with time. The time-courses of control and fluoride-inhibited substrate dehydrations are shown in Figure 1. The lower half of the Figure shows the percent inhibition as a function of time. These values were obtained from relative slopes of the curves in the upper half of the Figure, taken at equivalent midpoint values of OD_{230}. This must be done because the rate of the control enolase reaction, expressed as ΔOD_{230}/minute, decreases with time as the solution approaches equilibrium. The inhibition by 10-20 mM fluoride increases rapidly, then remains essentially constant. The final level of inhibition and the rate of attainment of the final level are highly dependent on fluoride concentration. There is also some dependence on Mg^{2+} concentration. The reverse reaction (not shown) is affected more slowly, with 10 mM fluoride producing half of its maximum inhibition (80-90%) in 10 minutes. Control experiments have shown that incubation of the enzyme with any one or two of Mg^{2+}, fluoride or substrate is not sufficient for inhibition; all three must be present.

Use of extrapolated very early (0-10 seconds) reaction rates indicate that fluoride is initially a weak (K_I about 15 mM) inhibitor (16), competitive with substrate or product (not shown). If velocities of the forward and reverse reactions are taken at times beyond one minute of reaction, the inhibition appears to be more noncompetitive (16), as Wang and Himoe found (11).

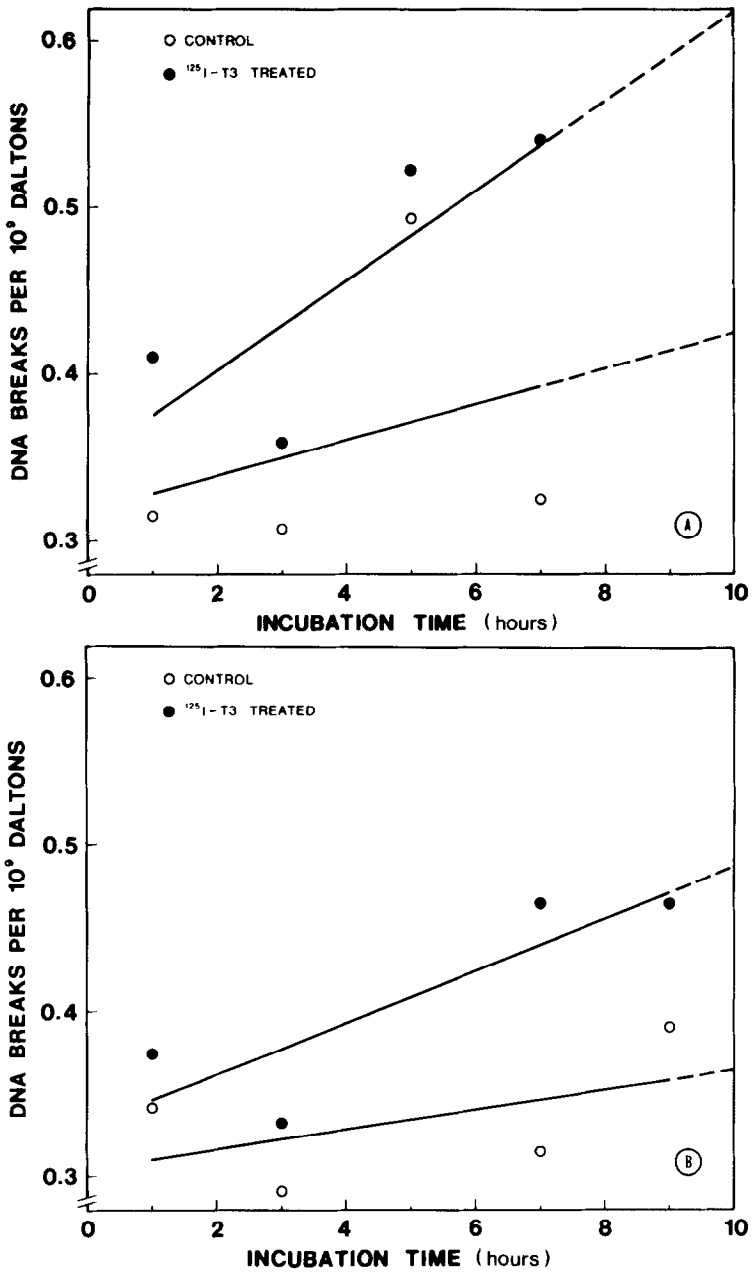

Figure 1. Induction of DNA strand breaks in CHO cells (A) and Cl:1 cells (B). [^{125}I]T3 was added to the cells at zero time. The dpm per cell was 0.4 for CHO cells and 0.7 for Cl:1 cells.

a certain incubation time is the most relevant value when comparing the treatments. A t-test using paired groups on the N_{10} values show a significant increase in the number of DNA strand breaks in [^{125}I]T3 treated cells as compared with non-treated cells ($p < 0.01$). We have also studied the effects in V79, HeLa

Table 1. Induction of DNA strand breaks in [^{125}I] T3 treated cells. The procedure is the same as that used in figure 1.

Cell line	dpm per cell	k for curves in fig. 1		number of DNA strand breaks per 10^9 Daltons left unrepaired after 10 hours incubation in ^{125}I-T3		125-I-T3 induced breaks	
						per 10^9 daltons	cell^{-1}.dpm^{-1}
		control	^{125}I-T3	control	^{125}I-T3		
CHO	0.09	0.0116	0.0220	0.403	0.469	0.066	3667
	0.23	-0.0117	0.0166	0.257	0.431	0.174	3783
	0.4	0.0107	0.0279	0.424	0.625	0.201	2513
	1.4	-0.0124	0.0342	0.458	0.815	0.357	1275
C1:1	0.13	0.0247	0.013	0.427	0.382	-0.045	-1731
	0.35	0.0188	0.0335	0.365	0.498	0.133	1900
	0.7	0.0063	0.0157	0.366	0.488	0.122	871
	0.9	-0.020	-0.044	0.324	0.332	0.008	44
						Mean	1540

Table 2. Comparison of the efficiency of induction of unrepaired DNA strand breaks in [^{125}I]iododeoxyuridine and [^{125}I]T3 labelled cells. Data from table 1 and (11).

Treatment	Cell line	dpm per cell	Number of ^{125}I-induced DNA strand breaks per cell left unrepaired		
			after labelling period	dpm^{-1}. cell^{-1}	dpm^{-1}. cell^{-1}. hour^{-1}
^{125}I-T3	CHO and C1:1	See table 1	See table 1	1540	154
^{125}IUdR	C1:1	0.7	1050	1500	
		0.2	850	4250	
		0.1	450	4500	
		1.0	1200	1200	
			Mean	2863	136

and GH1 cells and found similar results (to be published elsewhere).

The dose rate was very low - 0.4 dpm per cell corresponds to about 0.3 rad per minute to the cell nucleus, assuming all ^{125}I decays occuring in the cell nucleus. Single strand breaks should thus be rejoined very efficiently so it seems that there are double strand breaks or more serious DNA damages which are left unrepaired.

T3 is bound to a specific nuclear receptor (1,2,3). The ^{125}I decays may thus be located very close to DNA. Decay of ^{125}I appears to result in multiple breaks on each strand. For maximum efficiency the ^{125}I decay should be located no more than 15 to 20 Å from double helix (16). Our results indicate that the ^{125}I-decay were within 60 nm from DNA where ^{125}I decays are giving a high LET-type radiation (13). They are probably even closer to DNA since we observed such a high damaging efficiency.

In a previous work we have studied the induction of unrepairable DNA strand breaks after ^{125}I labelling of DNA using iododeoxyuridine (11). Surprisingly for us, [^{125}I]T3 seems to be as efficient as ^{125}I incorporated into DNA in inducing such breaks (table 2). T3 is involved in the regulation of RNA synthesis (4) which may change the DNA superstructure giving a more vulnerable DNA. The yield of single strand breaks after irradiation seems to be much larger in active chromatine than in inactive (17).

What are the implications of this findings? Future work will show if a reevaluation of the radiation protection standards for iodine isotopes is necessary. The critical organ may be some other than the thyroid gland. Some cells of the anterior pituitary have a high number of receptors (5000 to 6000 per cell). The number seems to be still higher during growth. Based on the knowledge we have today, we found it possible that the anterior pituitary may be the critical organ in children resulting in a disturbed hormon production. Another implication can be the use of [^{125}I] T3 in therapy of tumours with a high number of nuclear receptors for T3.

Acknowledgement

We thank Mrs U. Johanson for excellent technical assistance. The work has been financially supported by Studsvik Energiteknik AB, Sweden.

References:

1. Oppenheimer, J.H., Koerner, D., Schwartz, H.L., and Surks, M.I. (1972) J. Clin. Endocrinol. Metab. 35, 330-333.

2. Samuels, H.H., and Tsai, T.S. (1974) J. Clin. Invest. 53, 656-659.

3. Surks, M.I., Koerner, D., Dillman, W., and Oppenheimer, J.H. (1973) J. Biol. Chem. 248, 7066-7072.

4. Dillman, W.H., Mindecki, J., Koerner, D., Schwartz, H.L., and Oppenheimer, J.H. (1978) Endocrinology 102. 568-575.

5. Oppenheimer, J.H. (1979) Science 203, 971-979.

6. Schwartz, H.L., and Oppenheimer, J.H. (1978) Endocrinology 103, 943-948.

7. Hofer, K.G., and Hughes, W.L. (1971) Radiat. Res. 47, 94-109.

8. Burki, H.J., Roots, R., Feinendegen, L.E., and Bond, V.P. (1973) Int. J. Radiat. Biol. 24, 363-375

9. Chan, P.C., Lisco, E., Lisco, H., and Adelstein, S.J. (1976) Radiat. Res. 67, 332-343

10. Burki, H.J., Koch, C., and Wolff, S. (1977) Curr. Top. Radiat. Res. 12, 408-425

11. Sundell-Bergman, S., and Johanson, K.J. (1980) Radiat Environ. Biophys. 18, 239-248.

12, Painter, R.B., Young, B.R., and Burki, H.J. (1974) Proc. Nat. Acad. Sci. USA 71, 4836-4838.

13. Charlton, D.E., Booz, J., Fidorra, J., Smit, Th., and Feinendegen, L.E. (1978) Proc. of the Sixth Symposium on Microdosimetry. Vol. 1. pp 91-110, Brussels Euratom 6064, DE-EN-FR.

14. Rydberg, B. (1975) Radiat. Res. 61, 274-287.

15. Ertl, H.J. (1970) Jühlich Manuscript No Jül. -668-ME (KFA)

16. Martin, R.F., and Haselfine, W.A. (1981) Science 213, 896-898.

17. Chiu, S.M., and Oleinick, N.L. (1982) Int. J. Radiat. Biol. 41, 71-77.