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INDUCTION OF MICRONUCLEI BY ^{125}IUDR AND ^{125}I-T_3 IN TWO MAMMALIAN CELL LINES

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ABSTRACT

The high effectiveness of ^{125}I Auger electrons in inducing damage to DNA has two practical aspects. The first aspect involves their use in cancer therapy as a modality with desirable radiobiological properties. The second introduces an undesirable, but highly specific, risk associated with the thyroid hormone (T_3) specific metabolic pathways and its target tissues and cells. In the target cells, the ^{125}I carrying thyroid hormone will be directed to the defined DNA sequences within the thyroid hormone regulated genes through the specific thyroid hormone nuclear receptors. Accordingly, ^{125}I will be localized there within the nanometer range of action and cause damage tantamount to high-LET-like effects. In this work an *in vitro* comparison of the damaging effectiveness of ^{125}I labeled to two different molecules is investigated: IUdR, the classical molecule used in experiments on the radiobiology of ^{125}I, and T_3.

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Two cell lines were employed that differ in responsiveness to T_3: GC, responsive and CHO, non-responsive. The kinetics of micronuclei formation served as a measure of the biological effectiveness of the radiochemical. It was found that ^{125}I-T_3 elicited significant damage to DNA at physiological concentrations in the hormone responsive cells and that its effectiveness for inducing micronuclei was 9 times lower than that of $^{125}IUdR$. The possibility of using Auger emitters bound to transcription regulating factors in fundamental research and therapy is discussed.

INTRODUCTION

The *in vivo* use of ^{125}I for diagnostic purposes (1) and attempts to utilize its severely damaging effect (observed *in vitro* and *in vivo*) in tumor therapy (2-4) implies the introduction of this radionuclide to organisms.

Iodine belongs to the class of biologically important trace elements. It is an indispensable constituent of thyroid hormone molecules. Insufficiency in this element leads to iodine deficiency diseases including cretinism, goiter, myxedema, Hashimoto's and Gull's diseases. To maintain a constant level of this element in organisms, vertebrates have adopted selective and highly effective mechanisms aimed at accumulating and preventing iodine excretion. Thus iodine nuclides, once introduced into the body of vertebrates by any means (food, air, medical treatment), become included into the iodine pools: the large thyroid compartment (8 - 10 mg for a 70 kg man), and a small extra-thyroid compartment (40 - 60 μg for a 70 kg man).

The nanometer range (5) of the large number of low-energy Auger electrons (6) emitted from decaying ^{125}I atoms poses a threat to DNA only when the decay occurs inside or in close proximity to the DNA molecule (7,8). When ^{125}I enters the thyroid compartment, which serves to accomplish the synthesis, storage, and secretion of the thyroid hormones - thyroxin and triiodothyronine - it is concentrated in the extracellular space of the follicular colloid. On the molecular scale this is far away from the cell nuclei of the thyroid gland. Thus, the risk to the thyroid gland can mainly be ascribed to the sparsely ionizing γ radiation from ^{125}I.

On the other hand, the risk from ^{125}I in the extra-thyroid compartment is inseparably connected with the regulating functions of thyroid hormones elicited in several genes in responsive tissues (9-11). These functions are

realized at the cellular level within the nanometer range of DNA-hormone interaction mediated by the thyroid hormone nuclear receptor (12-14). The interaction between these three components occurs in the following sequence: 1) activation of the receptor by its ligand, the thyroid hormone, 2) binding of the receptor-hormone complex to specific DNA base pairs, and finally 3) regulation of the transcription of responsive genes (14,15).

In order to probe the position of ^{125}I in the thyroid hormone receptor-thyroid hormone complex after binding to DNA, the damaging effect of ^{125}I measured by micronuclei formation in two cell lines (responsive and non-responsive to thyroid hormone) was determined. As a reference probe for DNA damage, the thymidine analog, ^{125}IUdR, was applied.

MATERIALS AND METHODS

Cells and their Growth Conditions

The number of thyroid hormone nuclear receptors is different in different tissues (16). Two cell lines were employed as the *in vitro* models: 1) Chinese hamster ovary (CHO) cells, originating from the reproductive organ known to have a very low number of thyroid hormone nuclear receptors, and 2) GC, a cell line originating from the anterior pituitary gland, the tissue richest in thyroid hormone nuclear receptors (16). The cells were grown as monolayers in plastic flasks or petri dishes (Nunc, Nunclon, Denmark) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The standard medium for CHO cells consisted of Ham's F-10 medium, 10% newborn calf serum, glutamine (0.02 M), penicillin (50 units/ml) and streptomycin (50 µg/ml). For GC cells Dulbecco's medium supplemented with 10% donor horse serum, 5% foetal bovine serum, and glutamine and antibiotics as above, was used. The doubling time (t_d) of the cells was 13 - 15 h and 28 - 30 h for CHO and GC cell lines, respectively.

Labeling with ^{125}IUdR or ^{125}I-T_3

When experiments with ^{125}I-labeled triiodothyronine (L- 3,5,3'[^{125}I]-T_3, of specific activity of 81.4 TBq/mM, Dupont De Nemours GMBH NEN Products, Germany) were set up, the cells were seeded into T_3-depleted media. Depletion of T_3 from the standard sera was performed by repeated incubation of the commercially purchased standard sera with resin (AG 1-X 8, Bio-Rad

Laboratories, Richmond, California, USA; 5 mg resin/100 ml of serum during 5 and 18 h) (17). After a lag period and about one t_d in the T_3-depleted medium, the cells were labeled with ^{125}I-T_3 at an activity concentration of 74 kBq/ml medium, which was 2 to 3 times higher than the molar T_3 concentration of the euthyroid serum. The cells were incubated in ^{125}I-T_3 medium either 1) For one t_d (pulse labeling mode) and thereafter the cells continued to grow in their proper standard culture medium, or 2) The cells were cultured in the radioactive medium up to 6 t_d (continuous mode of labeling). For the dose-response curve (only GC cells), the cells were cultured with ^{125}I-T_3 activity concentrations ranging from 0 up to 74 kBq/ml during 4 t_d.

Labeling with $^{125}IUdR$ (^{125}I-5-iodo-2'-deoxyuridine with specific activity of 74 TBq/mM, Amersham, England) was performed by substitution of the proper standard medium with $^{125}IUdR$ containing medium at various ^{125}I-activity concentrations during one t_d incorporation period. Afterwards the cells were cultured in the proper standard medium up to 5 t_d.

The experiments were performed in replicate sets of petri dishes for various ^{125}I-T_3 concentrations and incubation times. The initial number of cells was constant and was 3×10^4 (CHO) and 5×10^4 (GC) per dish. The control cells were cultured in their proper standard medium except for the ^{125}I-T_3 dose-response study where the control cells were cultured in T_3-depleted medium supplemented with 1 nM of cold T_3.

^{125}I Activity Measurements

As 95% of $^{125}IUdR$ is found to be incorporated into nuclear DNA of labeled cells (18), the measurement of ^{125}I activity was performed on whole cells. The T_3 concentration in nuclei depends on the nuclear receptor density which varies considerably from tissue to tissue (16). However, T_3 can be bound specifically or non-specifically to cytoplasmic proteins (19,20). Therefore we measured ^{125}I-T_3 activity in both intact cells as well as in nuclei. Preparation of the nuclei is described elsewhere (21).

After harvesting by trypsinization (0.25% trypsin in EDTA) the cells were suspended in culture medium and counted with a Coulter electronic particle counter. One ml of the cell suspension was taken for both $^{125}IUdR$ and ^{125}I-T_3 activity measurements. The ^{125}I activity measurement in the suspension of cell nuclei (in 1 ml of buffer B (21)) using a Packard NaI Auto-

Gamma counter with a 15-80 keV window. To obtain the kinetics of the labeled compounds, the activity determination was done after every doubling time over 5-6 t_d.

Micronuclei Assay

Micronuclei (MN) are observed in interphase cells and are formed from chromosome and/or chromatid acentric fragments arising in the preceding mitoses (22). The acentric fragments reflect damage of chromosomes caused by clastogenic agents, in particular by ionizing radiation. The formation of micronuclei depends on cell division and thus the scoring of MN should be restricted only to the proliferating cells in order to get reliable results. Practically, this restriction was obtained by using cytochalasin B (CB) which arrests cytokinesis of the post-mitotic cells (23). Cells which divided once in the presence of CB appear as binuclear (BN), *i.e.*, as containing two nuclei.

CB (3 μg/ml) (23) was added to the culture medium at the beginning of n_{th} t_d and the cells were harvested at the end of the n_{th} t_d, n = 1,2,...6 (this time interval will subsequently be denoted as t_{dn}). In this way we could follow the kinetics of appearance of micronucleated binuclear cells throughout the investigated time period. After incubation with CB for one t_d the cells were trypsinized, ^{125}I activity measured, and the cells were prepared for scoring micronucleated binuclear cells. The trypsinized, suspended cells were centrifuged for 2 min at 1500 rpm, the cell pellet was gently resuspended in about 1 ml of 1% sodium citrate, and the cells were immediately centrifuged (1-2 min, 1500 rpm). The pellet of hypotonically treated cells was resuspended in about 1 ml of fixative (ethanol:acetic acid - 3:1). An improvement in preservation of the cytoplasm around the nuclei was obtained when the fixative was added directly to the suspension of cells in the 1% sodium citrate before centrifugation, and then centrifuged and resuspended again in fixative. The fixed cells were dropped on a clean glass slide, air-dried and stained in 5% Giemsa solution in Sorensen's buffer for 20 min (GC cells) or 30 min (CHO cells). Slides were scored under a light microscope (Zeiss-Axioplan) at 1000 X magnification. An average of 500 binuclear cells were scored per slide. It should be noted that the term micronucleation has been used to denote the frequency of binuclear cells with micronuclei regardless of the number of micronuclei per cell and has constantly been applied throughout the paper.

RESULTS

Cellular Uptake of the ^{125}I-labeled Molecules

The ^{125}IUdR and ^{125}I-T_3 cellular uptake is presented in Fig. 1 (A and B). M_1, the exponent of the power fit $y = M_0 x^{M_1}$), where y is the activity per cell and x is the concentration of activity in the medium, was 0.95 for CHO cells and 0.97 for GC cells indicating a linear uptake of ^{125}IUdR in both cell lines. For ^{125}I-T_3, the exponent was 0.76, which indicates a saturation of the uptake of this compound in GC cells. The CHO cells showed a very low uptake of ^{125}I-T_3 at 74 kBq/ml (2 to 3 times higher than the physiological T_3 concentration) and therefore the concentration dependence was not investigated.

Cellular Retention of the Radiochemicals

The ^{125}IUdR activity concentration decreased with every doubling time in both cell lines (Fig. 2A and 2B). In contrast, the level of ^{125}I-T_3 in GC cells in the continuous mode of labeling decreased during the first three t_d and then a steady state was established. The diminishing of ^{125}I could be ascribed to the down-regulation of the T_3 receptors by an excess of its ligand (24,25). In CHO cells, the ^{125}I-T_3 activity concentration was, on average, one order of magnitude lower than in GC cells, but increased with incubation time.

Micronuclei

Comparison of ^{125}I-T_3 and ^{125}IUdR using the same experimental conditions was not possible. After labeling for one doubling time there was a rapid decrease of ^{125}I-T_3 when the radioactive medium was removed, whereas ^{125}IUdR decreased more slowly with cell divisions (Fig. 2A and 2B). Therefore, to construct the dose-response curve for ^{125}I-T_3, we chose the continuous labeling mode which was more comparable with the dilution of ^{125}IUdR due to the down-regulation of receptors (and thus ^{125}I activity per nucleus) at the first three t_d. The micronuclei were scored at t_{d4} when a steady state of ^{125}I-T_3 in the nuclei was obtained. For ^{125}IUdR, the dose-response curve was constructed as an average of micronucleated cells observed at four consecutive doubling times after labeling.

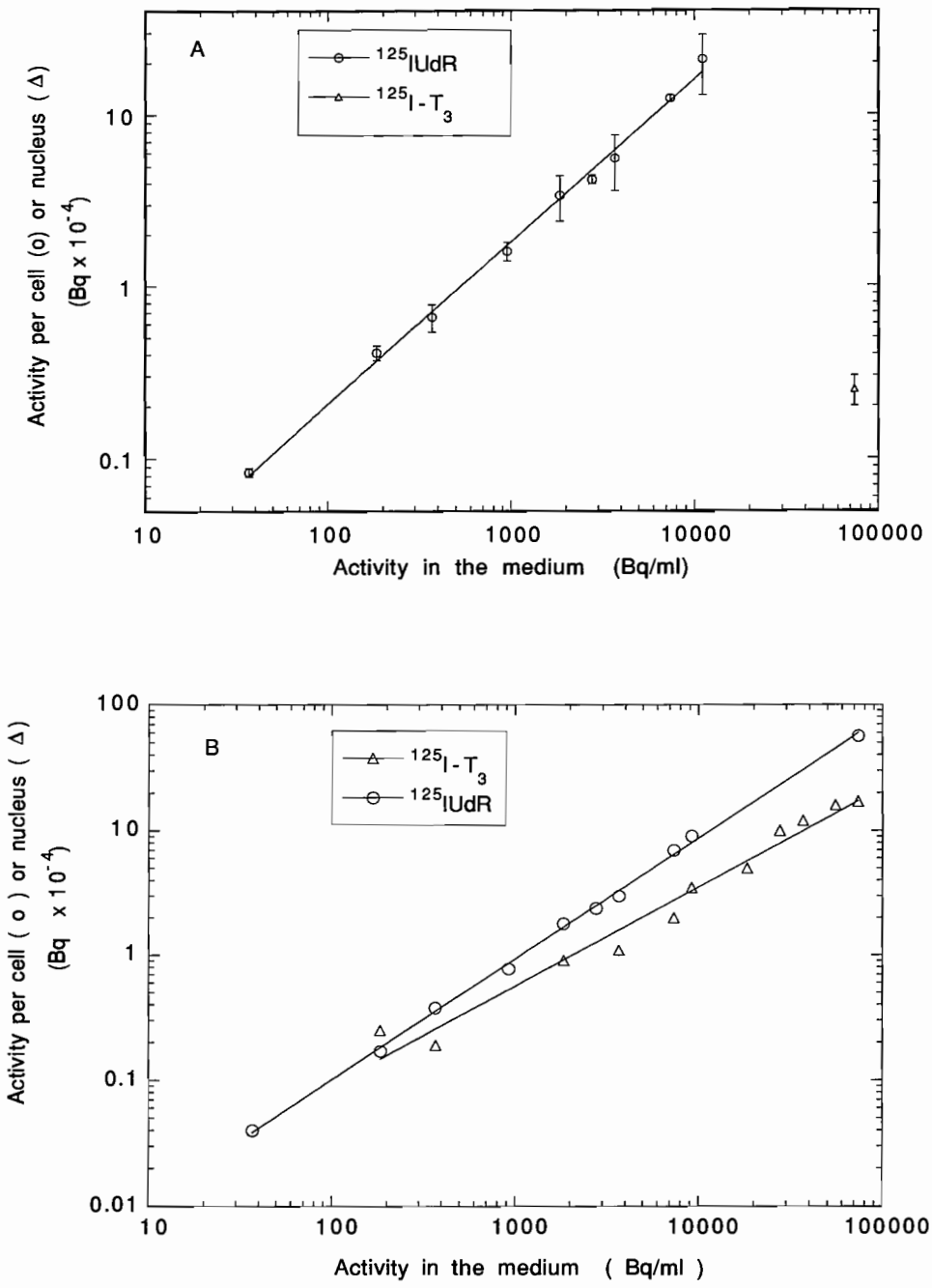

FIG. 1. Cellular uptake of ^{125}IUdR (open circles) and ^{125}I-T$_3$ (open triangles) in CHO cells (A) and GC cells (B).

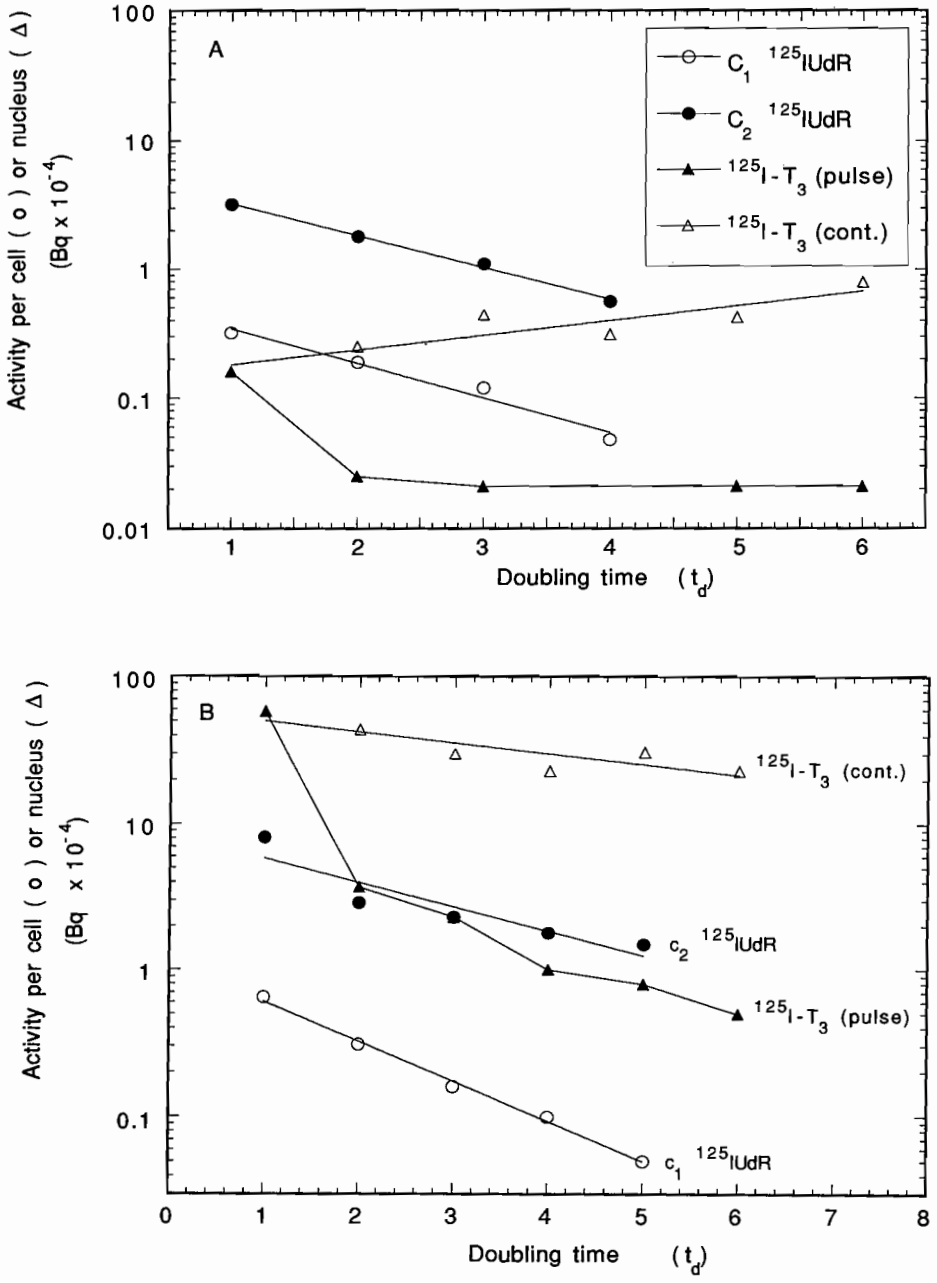

FIG. 2. Kinetics of ^{125}IUdR (open circles, solid circles) incorporated during one t_d, and of ^{125}I-T_3 permanently present in the medium (open triangles) or for one t_d (solid triangles): A) - CHO cells, B) - GC cells; initial ^{125}IUdR is taken at two different concentrations.

In GC cells the micronucleation was linear along the whole range of ^{125}I activity in the nucleus. The micronucleation induced by $^{125}IUdR$ increased linearly with ^{125}I activity up to 0.3 - 0.4 mBq/cell and then reached a plateau. The ratio of the slopes was 9 (Fig. 3B).

The uptake of ^{125}I-T_3 in the nuclei of CHO cells was very low at the high external activity concentrations used in the experiment (about 40 μBq/nucleus at 74 kBq/ml medium). The micronucleation induced by this ^{125}I activity did not significantly differ from the control (3 ± 1 vs 3.1 ± 0.8) and therefore the dose-response curve for lower activity concentrations (comparable with the physiological T_3 concentration) was not performed. The micronucleation induced by $^{125}IUdR$ was linear up to about 0.8 mBq/cell, where a deviation from linearity was seen (Fig. 3A).

DISCUSSION

The results obtained in this study show that the ^{125}I-labeled thyroid hormone induces a significant number of micronucleated cells in hormone-responsive cells but with lower efficiency than the reference compound, $^{125}IUdR$. In the non-responsive cells, ^{125}I-T_3 at physiological T_3 concentrations did not significantly increase micronucleation. The reasons for the observed differences in efficacy of inducing micronuclei may be of geometrical, biochemical or dosimetric nature.

The proximity of ^{125}I to the DNA molecule is considered to be crucial for the magnitude (7) and severity (6) of damage imparted. $^{125}IUdR$ is unspecifically incorporated into dividing cells. When incorporated into the DNA molecule, $^{125}IUdR$ is retained in it until the end of G_2 phase. During division the ^{125}I-activity per cell is halved (7). However, the distance of ^{125}I to the DNA constituents is close and remains unchanged. Hence, the activity measurements carried out with whole cells actually reflects the ^{125}I-activity in DNA. Thus, for $^{125}IUdR$, the position of ^{125}I in the DNA molecule is constant and independent of the particular phase of the cell cycle.

The intracellular concentration of ^{125}I-T_3 is governed by the abundance of the thyroid hormone nuclear (TR) receptors in a given cell type (16), and the regulation of the number of receptors by the ligand itself within this cell type (24,25). The limited number of nuclear receptors, therefore, gives a cell-

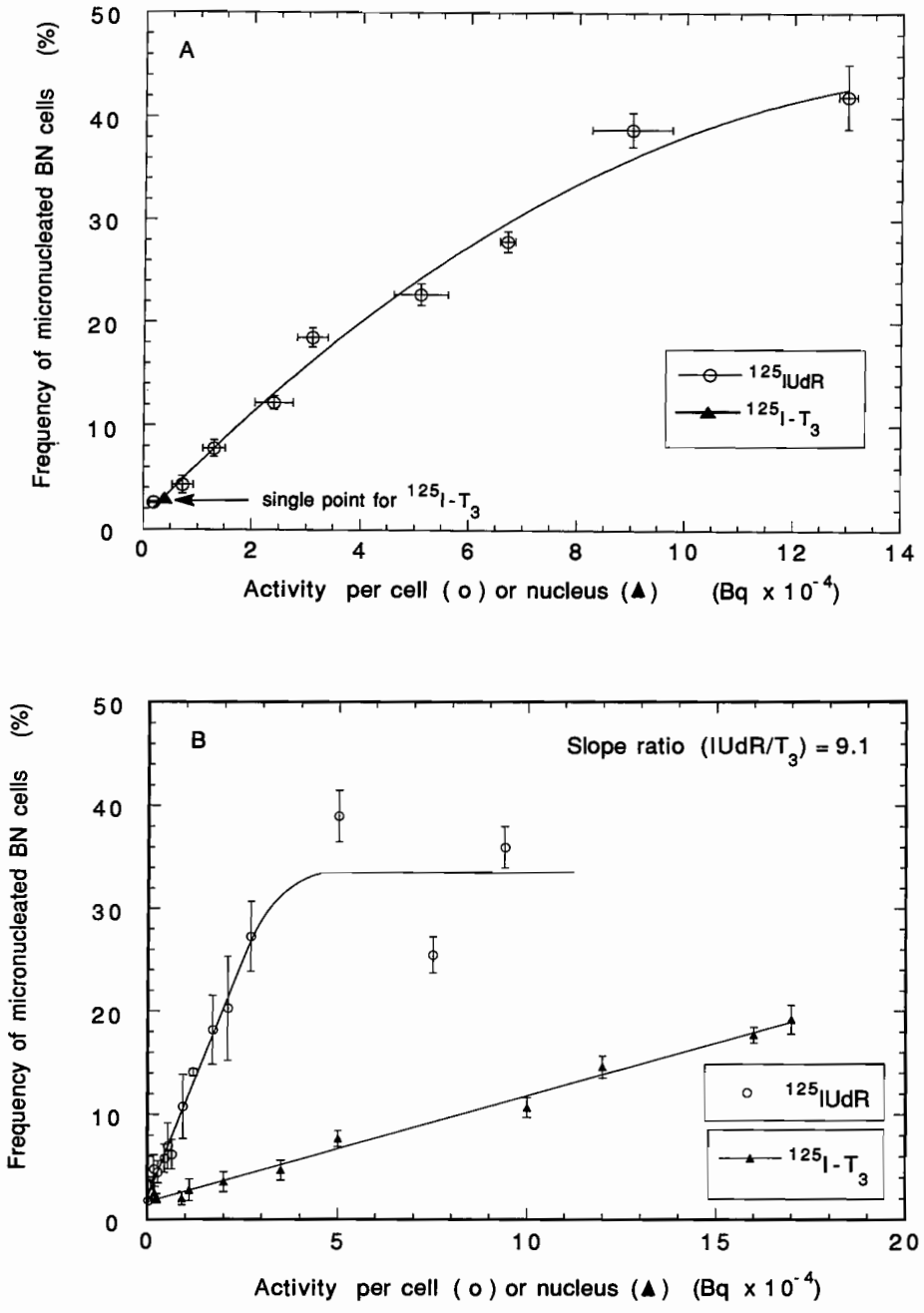

FIG. 3. Micronucleation of CHO cells (A) and GC cells (B) after one t_d labeling with ^{125}IUdR (open circles) or after continuous incubation with ^{125}I-T$_3$ (solid triangles) for 4 t_d.

specific upper limit for the dose delivered to the nucleus. TR belongs to ligand-dependent transcription regulating proteins of specific genes. This protein consists of three distinct structural and functional domains: ligand-, DNA- and histone- binding domains (26). When the ligand-receptor complex is formed the binding of the complex to DNA is enabled. The receptor binds to DNA in the form of a dimer. Each DNA-binding domain of the receptor creates two finger-like structures coordinated by zinc atoms (14,32). These zinc fingers recognize and interact with specific DNA sequences through the major groove at a distance of about 200 base pairs in the 5' flanking DNA from the transcription starting point of the regulated genes (12,15).

The nuclear receptors regulate the duration of association of ^{125}I-T_3 with DNA. The mean half-life of the receptors is approximately 4 h (13), and thus the mean time of association of ^{125}I-T_3 to the receptor is shorter than the cell cycle (30 h). Moreover, the action of the receptor on regulated genes is exerted during a limited time of the cell cycle, for instance during the G_1 phase (10). In the S phase the receptors probably dissociate from the DNA and move to a nuclear subcompartment distant from DNA (*i.e.*, from the nuclear matrix) (27). Thus, the measurement of ^{125}I activity in the nucleus is not tantamount to the ^{125}I activity measurement in DNA.

This study indicates that ^{125}I-T_3, which is not incorporated into the DNA, interacts with DNA indirectly through its nuclear receptor and induces concentration dependent damage at physiological concentrations of the hormone (below 0.5 nM of T_3). This implies that the mediator itself, the thyroid hormone nuclear receptor, can be used as a targeting molecule for ^{125}I to investigate distance-related damage to the DNA helix and/or can be applied for therapeutic purposes owing to the receptor's tissue and gene specificity.

The thyroid hormone nuclear receptor and functionally related proteins from the large steroid- and thyroid-hormone receptor superfamily show structural similarities with other transcription regulating proteins (28,29). The structural similarities can be seen in their three-domain construction, zinc-finger feature in the DNA-binding domain, and the dimerization of the COOH terminal domain. ^{125}I labeling of the zinc finger amino acids involved in interaction with DNA should damage the specific nucleotide sequences (6,30). The expected specificity and efficacy of damage caused by this, and other Auger emitters, might be useful for therapeutic purposes. The use of ^{125}I in therapy was suggested by Martin *et al.* (31) and

applied by Adelstein *et al.* (2). However, the compounds proposed by Martin *et al.* (31) as well as IUdR used by Adelstein *et al.* (2) did not have cell and/or tissue specific DNA affinity.

The carboxy-terminal end of the zinc finger transcription regulation proteins/nuclear receptors participate in dimer formation. It was shown (20) for C/EBP (CAT/Enhancer-Binding Protein) that two homo- and hetero-molecules are pieced together by a characteristic arrangement of leucine residues in the COOH-domain. A mode for the association of hormone receptor molecules has not yet been reported, but it has been suggested that binding of the hormone to this domain seems to be necessary for the receptor dimerization (14,32). The superfamily of the leucine-zipper transcription factors can offer a new tool for experimental elucidation of the distance-dependence of damage caused by ^{125}I and other Auger emitters. Labeling of the seven leucine residues of the transcription factors with ^{125}I, which probably are at different distances from the DNA molecule, should create a molecular scale of distances of ^{125}I from the DNA, which could be used to investigate the damage-distance relationship.

CONCLUSIONS

The ^{125}I labeled thyroid hormone places ^{125}I nanometers from the DNA where a high-LET-like effect is exerted and the dose-related damage is linear. This effect is specific for cells with large numbers of nuclear receptors for the thyroid hormone. The relative ^{125}I-T_3 toxicity is about one order of magnitude lower than ^{125}IUdR. Because the effect of ^{125}I bound to the thyroid hormone depends on the thyroid hormone nuclear receptor, we suggest an application of structurally related molecules, *i.e.* zinc finger proteins, labeled with Auger emitters for tumor therapy and fundamental research of Auger-electron emitting radionuclides.

REFERENCES

1. E.E. CAMERGO, G.S. JOHNSTONE, S.M. LARSON, and H.N. WAGNER, In *Textbook of Nuclear Medicine: Clinical Applications*, (A.F.G. ROCHA and J.C. HARBERT, Eds.) Philadelphia: Lea & Febiger, 440-455, 1979.
2. S.J. ADELSTEIN, A.I. KASSIS, J. BARANOWSKA-KORTYLEWICZ, A.D. VAN DEN ABBEELE, G. MARIANI, and S. ITO, Potential for tumor therapy with iodine-125 labeled immunoglobulins. *Nucl. Med. Biol.* **18**, 43-44 (1991).

3. K.D. BAGSHAWE, S.K. SHARMA, P.J. SOUTHALL, J.A. BODEN, G.M. BOXER, T.A. PATRIDGE, P. ANTONIW, and R.B. PEDLEY, Selective uptake of toxic nucleoside (^{125}IUdR) by resistant cancer. *Brit. J. Radiol.* **64**, 37-44 (1991).
4. D.V. WOO, D. LI, J.A. MATTIS, and Z. STEPLEWSKI, Selective chromosomal damage and cytotoxicity of ^{125}I-labeled monoclonal antibody 17-1a in human cancer cells. *Cancer Res.* **49**, 2952-2958 (1989).
5. A.I. KASSIS, F. FAYAD, B.M. KINSEY, K.S.R. SASTRY, R.A. TAUBE, and S.J. ADELSTEIN, Radiotoxicity of ^{125}I in mammalian cells. *Radiat. Res.* **111**, 305-318 (1987).
6. D.E. CHARLTON and J. BOOZ, A Monte Carlo treatment of the decay of ^{125}I. *Radiat. Res.* **87**, 10-23 (1981).
7. A.I. KASSIS, K.S.R. SASTRY, and S.J. ADELSTEIN, Kinetics of uptake, retention and radiotoxicity of ^{125}IUdR in mammalian cells: implications of localized energy deposition by Auger processes. *Radiat. Res.* **109**, 78-89 (1987).
8. D.E. CHARLTON, The range of high LET effects from ^{125}I decays. *Radiat. Res.* **107**, 163-171 (1986).
9. C.R. DEFESI and M.I. SURKS, 3,5,3'-triiodothyronine effects on the growth rate and cell cycle of cultured GC cells. *Endocrinology* **108**, 259-267 (1981).
10. C.R. DEFESI, E.C. FELS, and M.I. SURKS, Triiodothyronine stimulates growth of cultured GC cells by action in the G_1 period. *Endocrinology* **114**, 293-295 (1984).
11. M.H. KUMARA-SIRI and M.I. SURKS, Regulation of growth hormone mRNA synthesis by 3,5,3'-triiodo-L-thyronine in cultured growth hormone-producing rat pituitary tumor cells (GC cells). *J. Biol. Chem.* **260**, 14529-14537 (1985).
12. J.W. APRILETTI, J.D. BAXTER, and T.N. LAVIN, Large scale purification of the nuclear thyroid hormone receptor from rat liver and sequence-specific binding of the receptor to DNA. *J. Biol. Chem.* **263**, 9409-9417 (1988).
13. J. CASANOVA, Z.D. HOROVITZ, R.P. COPP, W.R. MCINTYRE, A.PASCUAL, and H.H. SAMUELS, Photoaffinity labeling of thyroid hormone nuclear receptors. *J. Biol. Chem.* **259**, 12084-12091 (1984).
14. R.M. EVANS, The steroid and thyroid hormone receptor superfamily. *Science* **240**, 889-895 (1988).
15. R.P. LARSEN, J.W. HARNEY, and D.D. MOORE, Sequences required for cell-type specific thyroid hormone regulation of rat growth hormone promoter activity. *J. Biol. Chem.* **261**, 14373-14376 (1986).
16. J.H. OPPENHEIMER, H.L. SCHWARTZ, and M.I. SURKS, Tissue differences in the concentration of triiodothyronine nuclear binding sites in the rat: Liver, kidney, pituitary, heart, brain, spleen and testis. *Endocrinology* **95**, 897-903 (1974).
17. H.H. SAMUELS, F. STANLEY, and J. CASANOVA, Depletion of L-3,5,3'-triiodothyronine and L-thyroxine in euthyroid calf serum for use in cell culture studies of the action of thyroid hormone. *Endocrinology* **105**, 80-85 (1979).
18. E.W. BRADLEY, P.C. CHAN, and S.J. ADELSTEIN, The radiotoxicity of iodine-125 in mammalian cells. *Radiat. Res.* **64**, 555-563 (1975).
19. K. ICHIKAWA, K. HASHIZUME, M. KOBAYASHI, and T. YAMADA, Evidence for induction by thyroid hormone of cytosolic proteins which control mitochondrial protein synthesis. *Endocrinology* **117**, 1749-1758 (1985).

20. N.B. PLIAM and I.D. GOLDFINE, High affinity thyroid hormone binding sites on purified rat liver plasma membranes. *Biochem. Biophys. Res. Commun.* **79**, 166-172 (1977).
21. S. SUNDELL-BERGMAN, K.J. JOHANSON, and G. LUDWIKOW, Radiotoxic effect of ^{125}I-labeled thyroid hormones with affinity to cellular chromatin. In *DNA damage by Auger Emitters*, (K.F. BAVERSTOCK and D.E. CHARLTON, Eds.) London: Taylor & Francis, pp. 147-157, 1988.
22. P.I. COUNTRYMAN and J.A. HEDDLE, The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutation Res.* **41**, 321-332 (1976).
23. M. FENECH and A.A. MORLEY, Measurement of micronuclei in lymphocytes. *Mutation Res.* **147**, 29-36 (1985).
24. H.H. SAMUELS, F. STANLEY, and L.E. SHAPIRO, Dose-dependent depletion of nuclear receptors by L-triiodothyronine: Evidence for a role in induction of growth hormone synthesis in cultured GH1 cells. *Proc. Nat. Acad. Sci. USA* **73**, 3877-3881 (1976).
25. B.M. RAAKA and H.H. SAMUELS, Regulation of thyroid hormone nuclear receptors levels in GH1 cells by 3,5,3'-triiodo-L-thyronine. *J. Biol. Chem.* **256**, 6883-6889 (1981).
26. K. ICHIKAWA and L.J. DEGROOT, Separation of DNA binding domain from hormone and core histone binding domains by trypsin digestion of rat liver nuclear thyroid hormone receptor. *J. Biol. Chem.* **261**, 16540-16546 (1986).
27. M.H. KUMARASIRI, L.E. SHAPIRO, and M.I. SURKS, Cell cycle dependence of thyroid hormone nuclear receptors in cultured GC cells: Relationship to nuclear matrix. *Endocrinology* **122**, 1897-1904 (1988).
28. N.P. PAVLETICH and C.O. PABO, Zinc finger-DNA recognition: Crystal structure of a Zif268-DNA complex at 2.1 Å. *Science* **252**, 809-817 (1991).
29. S.L. MCKNIGHT, Molecular zippers in gene regulation. *Sci. Am.*, **264**, 32-39 (1991).
30. V. MURRAY and R.F. MARTIN, Sequence specificity of ^{125}I-labeled Hoechst 33258 in intact human cells. *J. Mol. Biol.* **201**, 437-442 (1988).
31. R.F. MARTIN, T.R. BRADLEY, and G.S. HODGSON, Cytotoxicity of ^{125}I-labeled DNA-binding compound that induces double-stranded DNA breaks. *Cancer Res.* **39**, 3244-3247 (1979).
32. M. BEATO, Gene regulation by steroid hormones. *Cell* **56**, 335-344 (1989).