Biophysical Aspects of Auger Processes

Kurt G. Hofer

From the Institute of Molecular Biophysics, Florida State University, Tallahassee, USA

Correspondence to: Kurt G. Hofer, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, USA

Acta Oncologica Vol. 39, No. 6, pp. 651–657, 2000

In this review I begin with a brief history of Auger processes, and then present a more detailed review of recent literature reports on physical, molecular, and cellular aspects of Auger emitters, and potential therapeutic applications.

Received 10 August 1999
Accepted 29 January 2000

HISTORY

The 'Auger effect' is named in honor of the French physicist Pierre Auger, who discovered this process in 1925 (1). Auger was investigating the pattern of electron tracks produced by low-energy x-rays in a cloud chamber and noted that in many cases multiple electron tracks emanated from point sources in the chamber. He concluded that this phenomenon was caused by electron transitions that are initiated when low-energy x-ray photons impinge on target atoms and eject inner shell electrons. The resulting vacancy cascade leads to the emission of characteristic x-rays and low-energy Auger electrons by Auger, Coster-Kronig, and super Coster-Kronig transitions. It was later discovered that the Auger effect also occurs in radionuclides that decay by electron capture or internal conversion (2).

The biological significance of Auger electrons was not appreciated for many years, because the energy carried by Auger electrons is usually negligible compared to the total energy released from the decaying radionuclide. In 1964, Carlson & White (3) showed that the decay of the Auger emitter ^{125}I in methyl or ethyl iodide leads to molecular fragmentation. A few years later, Hofer et al. (4, 5) and Feinendegen et al. (6, 7) reported that ^{125}I incorporated into DNA in the form of ^{125}I-iododeoxyuridine (^{125}IUdR) was strikingly toxic to mammalian cells. Cell lethality from ^{125}I decays in DNA resembled the pattern of cell death observed after exposure to α particles or other high LET radiations. These findings were soon confirmed by other investigators (8–11). Nevertheless, research on the biological effects of Auger emitters attracted little attention until Feinendegen organized a conference on 'Radionuclides in Biology and Medicine', held in Jülich in 1975. This meeting brought together physicists, chemists, and biologists working on Auger emitters, and recruited scores of new scientists to the field.

By the early 1980s certain firm conclusions about the Auger effect had emerged. We were aware from the start that this type of decay entails copious emission of low-energy electrons. This, in turn, results in a highly charged daughter atom and a high density of electron irradiation in the immediate vicinity of the decay site. Microdosimetry calculations from several laboratories confirmed this conclusion (12, 13) and provided an explanation for the observation that the biological action of Auger emitters depended critically on the cellular location of the radionuclides. Auger emitters located outside the cell nucleus were found to be relatively non-toxic to cells (14–16), but intranuclear decays at the DNA caused pronounced high-LET-type cellular damage ranging from DNA strand breaks (10, 11, 17), mutations (18, 19), chromosome aberrations (20, 21), malignant transformations (19), division delay (22, 23), to cell death (4–7).

So the early work with Auger emitters clearly demonstrated that radiation death in mammalian cells results from damage to the cell nucleus. By implication, these findings refuted the enzyme release hypothesis and other hypotheses that explained radiation death in terms of cytoplasmic or membrane effects rather than nuclear damage (14, 15). The results also had important ramifications for nuclear medicine. It was obvious that for diagnostic applications, where it is desired to keep cellular damage as low as possible, Auger emitters should remain outside the cell, or at least outside the cell nucleus. Conversely, therapeutic applications (e.g., radionuclide therapy of cancers) require radiochemicals that target Auger emitters to the DNA within the cell nucleus.

 ISSN 0284-186X

After this brief excursion into history I will now turn to my main assignment, a review of recent literature reports dealing with physical, molecular, and cellular aspects of Auger processes and the implications of these studies for potential clinical application.

MOLECULAR EFFECTS OF AUGER EMITTERS

Molecular damage induced by Auger emitters has fascinated investigators ever since Krisch et al. (10, 11) and Painter et al. (17) first demonstrated that ^{125}I decays in DNA induce double-strand breaks (DSB) with nearly 100% efficiency. Martin and Haseltine (24) extended this work to oligodeoxynucleotides and showed that ^{125}I decays are highly efficient in producing DNA strand breaks within 10 bases of the decay site in both the labeled and unlabeled DNA strands. However, the actual mechanism of DNA damage by Auger emitters is not yet fully understood.

In recent studies, Adelstein & Kassis (25), Kassis et al. (26), and Sahu et al. (27) demonstrated that the magnitude of DNA damage depends critically on the position of Auger emitters relative to DNA. These investigators examined single-strand breaks (SSB) and double-strand breaks (DSB) induced in plasmid DNA by ^{125}I decays close to DNA or at a distance from DNA. Plasmid DNA was exposed to ^{125}I-labeled compounds such as ^{125}I-iodo-Hoechst 33342 which bind to the minor groove of DNA, or to ^{125}I-iodoantipyrene, which remains free in solution. The results indicated that ^{125}I decays in solution and in the minor groove of DNA are equally effective in producing SSB, but for DSB groove-bound ^{125}I is ~ 7 times more efficient than ^{125}I in solution. Kassis et al. (28) also evaluated the contribution of direct and indirect effects to ^{125}I-induced DNA breaks by comparing DNA break yields from ^{125}I decays accumulated in the presence and absence of the radical scavenger dimethyl sulfoxide (DMSO). DMSO proved very effective in protecting DNA from ^{125}I decays in solution (dose-modifying factor ~ 60), but less effective for decays occurring in the minor groove (dose-modifying factor ~ 4). The authors attribute this difference to the inability of DMSO to protect DNA from local DSB produced by groove-bound ^{125}I and conclude that local DNA lesions formed by decays directly at the DNA differ in nature from lesions caused by decays in solution.

The results obtained with plasmids stand in contrast to findings by Walicka et al. (29), who reported that in mammalian cells DMSO can reduce the number of ^{125}I-induced DSB. Walicka and co-workers suggest that these differences can be explained by the fact that, unlike naked DNA in plasmids, DNA in mammalian cells exists in the form of tightly packaged nucleoprotein. Such compaction should enable clusters of OH radicals from ^{125}I decays to attack both a local DNA site at the decay, and sites that in terms of linear distance are hundreds or thousands of base pairs away from the site of decay. This implies that a single ^{125}I decay in nuclear DNA can produce more than one DSB and that DMSO scavenges a fraction of the OH radicals responsible for the distant DSB, but does not offer significant protection against local DSB in either plasmid or mammalian DNA.

A different approach to the investigation of site-specific action of ^{125}I was taken by Panyutin & Neumann (30, 31), who examined DNA damage induced by triplex-forming oligonucleotides. The authors examined the spatial distribution of DNA breaks induced by ^{125}I decays in the C5 position of a cytosine residue on an oligonucleotide that formed a triple helix with a DNA double helix. More than 90% of all breaks in the target duplex were located within 10 bp around the decay site. However, the frequency of the breaks in the two strands of the double helix differed by a factor of 2. The asymmetry in break yield corresponded closely to the geometry of the triple helix, that is, the larger number of breaks was seen in that strand of the double helix that was located closer to the labeled oligonucleotide. Addition of DMSO did not significantly change the yield or distribution of breaks, suggesting that these local breaks are produced primarily by direct radiation action. These studies and similar work by Karmychev et al. (32) show that ^{125}I can induce SSB and DSB even under conditions where the radionuclide is located on a separate DNA strand attached to a double helix in the form of a triple helix.

Another interesting example of DNA damage by Auger emitters is the work of DeSombre et al. (33), Schwartz et al. (34) and Yasui et al. (35), who studied SSB and DSB induced in mammalian DNA by Auger emitters covalently bound to estrogen. Yasui et al. (35) exposed estrogen receptor-positive CHO cells to ^{125}I in the form of ^{125}IUdR or ^{125}I-labeled estrogen and found that ^{125}I-estrogen was 4–8 times more efficient in inducing DNA DSB than ^{125}I administered as ^{125}IUdR. More recent data by the same authors suggest a differential closer to 10 (personal communication). Yasui proposes that this surprising effect is caused by non-random placement of ^{125}I estrogen at the base of DNA loop domains, where individual ^{125}I decays may cause multiple DSB.

These data pose a challenge to theorists. In recent years, several investigators have developed sophisticated models for simulating DNA damage by Auger emitters. The first model, developed by Charlton & Humm (36), simplified the molecular structure of DNA to geometric volumes representing the bases and the sugar-phosphate moieties of the two strands. More detailed models developed by Pomplun (37), Terrissol (38) and Nikjoo et al. (39) account for each individual atom in DNA, including water molecules associated with DNA. To approximate the complex organization of DNA in mammalian cells, Terrissol (40) and Pomplun et al. (41, 42) developed a model for simulating DNA damage in nucleosomes. In this model the nu-

cleosome was represented as a DNA double helix with 146 basepairs and 9056 atoms surrounding the histones.

Regardless of the model used, computer simulations generally indicate that ^{125}I decays in DNA cause about 0.6–1.0 DSB/decay. This is in good agreement with experimental results obtained for naked DNA (e.g., plasmids), but does not account for reports of multiple DSB per decay for mammalian cells. Walicka et al. (29) found that ^{125}I in mammalian cell DNA causes more than one DSB/decay. If we interpret 'more than one' as indicating 2 DSB/decay and consider Yasui's finding that ^{125}I-estrogen is 10 times more effective than ^{125}IUdR in producing DSB (35), then combining these data implies that under appropriate conditions ^{125}I can produce as many as 20 DSB/decays in mammalian DNA. If correct, this would suggest that existing physical models do not adequately represent the situation in mammalian systems. Alternatively, it is possible that no conceivable physical arrangement can account for these findings and that multiple DSB in mammalian DNA arise as a result of biological damage magnification.

CELLULAR EFFECTS OF AUGER EMITTERS

It is well established that Auger emitters incorporated into DNA are extremely toxic to mammalian cells, but identical decay events in the cytoplasm, at the plasma membrane, or outside of the cells are relatively non-toxic. For example, Warters et al. (15) subjected CHO cells to ^{125}I decays in the DNA versus the plasma membrane by labeling the cells with either ^{125}IUdR or ^{125}I-labeled concanavalin A. For DNA-bound ^{125}I, 60 decays/cell were sufficient to cause 50% cell death, but when ^{125}I was attached to the plasma membrane, $\sim$20000 decays/cell were required to produce the same effect. Other investigators obtained similar results and confirmed that Auger emitters located at extranuclear sites act as low-LET radiation sources with an RBE of $\sim$1 (14–16, 43–45).

Based on these findings, it was initially assumed that Auger emitters decaying inside the cell nucleus are always highly cytotoxic because such decays damage the DNA, the presumed primary radiosensitive target within the cell. However, Yasui et al. (46) showed that toxic effects from DNA-bound ^{125}I are non-uniform and depend on the region of the DNA in which the radionuclide is incorporated. Kassis et al. (47) confirmed that the magnitude of the damage induced by intranuclear Auger emitters is not always directly correlated with intranuclear energy deposition. For example, ^{125}I attached to DNA via a DNA intercalator yields an RBE of $\sim$4–5 as compared to an RBE of $\sim$7–9 for covalently bound ^{125}I (47–49).

These differences pale by comparison with toxicity data obtained by Sedelnikova et al. (50), who examined the cytotoxicity of ^{125}I-labeled oligodeoxyribonucleotides in HT-1080 fibrosarcoma cells. The labeled oligodeoxyribonucleotides were introduced into cells by liposomes and the ^{125}I activity accumulated within the cell nucleus. However, there was no significant triplex formation with cellular DNA because the base sequence chosen was such that it could form a triplex with only one single target DNA sequence in the genome. Therefore, the radionuclide was localized within the cell nucleus, but was not in direct contact with nuclear DNA. When the authors evaluated the radiotoxic effects, they found that ^{125}I administered in this fashion was almost three orders of magnitude less toxic than ^{125}I incorporated into DNA via ^{125}IUdR. In other words, the toxicity differential was almost as large as that observed with DNA-bound versus membrane-bound ^{125}I. These results demonstrate that nuclear localization of ^{125}I by itself does not ensure high radiotoxicity. The findings also improve the prospects for selective gene therapy where specific gene sequences in the genome must be inactivated without causing excessive radiation damage to cells.

The notion that intranuclear decay of Auger emitters invariably produces high-LET-type radiation effects is also challenged by reports which show that the toxic effects of ^{125}I can be mitigated by chemical radioprotectors. For example, Rao et al. (51), Narra et al. (52, 53) and Harapanhalli et al. (54) found that treatment with radioprotectors (MEA, AET, vitamin C, vitamin A) offers remarkable protection against ^{125}I damage in mouse spermatogonia. More recently, Walicka et al. (55) and Bishayee et al. (56) demonstrated equally pronounced radioprotective effects on mammalian cells in tissue culture using DMSO as a radical scavenger. In contrast, no protection was noted in cells exposed to α particles from ^{210}Po (56). These findings indicate that damage from DNA-associated Auger emitters is caused mainly by indirect radiation action. However, this does not automatically prove that ^{125}I is not a high-LET radiation source. As pointed out by Chapman (57), indirect effects play a role in cell inactivation for all types of ionizing radiations, and sufficiently high concentrations of DMSO can modify radiation damage even in cells exposed to high-LET radiations such as charged particle beams.

Part of the difference between ^{125}I and α particles may be due to biological factors that modify the action of point sources, but do not affect the action of linear radiation sources. One such phenomenon is the 'chase effect', described by Hofer et al. (58–60). When CHO cells are pulse-labeled with ^{125}IUdR and then frozen at different times after labeling to accumulate ^{125}I decays, the biological effects show a marked shift in the pattern of cell death. Cells harvested within 1 h after labeling yield a typical low-LET radiation-survival curve (pronounced shoulder, D_0 135 decays/cell) but identically labeled cells harvested 5 h after labeling show a response characteristic of high-LET radiation (no shoulder, D_0 42 decays/cell). A similar shift in ^{125}I action can be observed for micronucleus induction

(60), but not for the induction or rejoining of DNA DSB (61). To account for this discrepancy, the authors postulate that DNA DSB may not be the sole cause of cell killing, and that damage to higher-order structures in the cell genome may contribute to radiation-induced cell death.

This interpretation in strengthened by data from Schneiderman et al. (personal communication), who performed chase experiments on repair-deficient CHO irs-20 cells that are highly sensitive to external and internal irradiation. However, a very unusual response was observed when CHO K1 cells and CHO irs-20 cells were pulse-labeled with ^{125}IUdR and frozen 60 min later for decay accumulation. At low dose levels (up to 120 decays/cell) both cell lines exhibited identical patterns of cell killing. At higher doses the survival of CHO K1 cells showed a continued exponential decline, but irs-20 cells exhibited a plateau at the 30% survival level. No additional cell death was observed at doses as high as 1280 decays/cell. Examination of cell samples by autoradiography and by the comet assay revealed that the cells were uniformly labeled and that the DNA was heavily damaged in all cells. It must therefore be concluded that, under appropriate experimental conditions, even massive DNA damage from DNA-bound ^{125}I fails to induce cell death. To explain these unusual results, the authors postulate the existence of a second critical target in the genome. According to this hypothesis, the enormous ^{125}I-resistance of irs-20 cells can be explained by assuming that in these cells newly replicated DNA segments are not yet attached to the second target 60 min after labeling. If the second target (assumed to be the cell structure that is responsible for radiation repair) is not damaged, the cells would retain the ability to repair DNA damage induced by ^{125}I decays. After longer chase periods, the newly labeled DNA segments become associated with the second target and ^{125}I decays induce high-LET effects in all cells, because at that point the decays can damage both the DNA and the second target.

AUGER EMITTERS IN CANCER THERAPY

As pointed out by Feinendegen (62), 'molecular surgery' by Auger emitters may one day provide the means of realizing Paul Ehrlich's dream of a 'magic bullet' in cancer therapy. Conventional radiation therapy invariably involves massive exposure of normal tissues. In contrast, with Auger emitters, overlap irradiation is minor, even from one cell compartment to the next (15), and overlap exposure from labeled cancer cell to adjoining normal cells is virtually nil. Moreover, DNA-bound Auger emitters exhibit a high RBE and a correspondingly low oxygen enhancement ratio (63). Thus, Auger emitters could be extremely effective in cancer therapy if suitable carrier agents are developed that selectively, or at least preferentially, target radionuclides to the genome of cancer cells.

With current targeting agents, the most serious obstacles are tumor heterogeneity and incorporation of radionuclides into normal body tissues. As pointed out by Strand et al. (64) and Humm et al. (65), evaluation of radionuclide carriers for cancer therapy requires data on both subcellular radionuclide distribution and on the heterogeneity in cellular activity within tumors. This was confirmed experimentally by Humm et al. (66), who injected radiolabeled antibodies into tumor-bearing mice and then studied the microdistribution of radioactivity in the tumor, spleen, liver, and kidneys. The results showed that inhomogeneity in dose distribution is a serious problem for low-energy electron emitters. This has important implications for the choice of radionuclides for therapy. Dosimetry calculations by Howell et al. (67) show that Auger emitters should be highly effective in treating micrometastases, but large tumors would respond better to high-energy β emitters.

Despite these obstacles, many researchers are investigating a wide variety of potential carrier agents for Auger emitters. These include the thymidine analogue iododeoxyuridine, radiolabeled hormones or anti-hormone drugs such as estrogen or tamoxifen, tumor-specific antibodies, epidermal growth factors, bleomycin and other anti-cancer drugs, and oligonucleotides targeted against specific nucleotide sequences in the genome of cancer cells (for a review see Ref. 68).

Unfortunately, many of these agents have proven problematical. For example, tumor-specific antibodies would seem to be ideal carrier agents for radionuclide therapy, but so far most trials with radiolabeled antibodies have yielded disappointing results. Problems include the failure to find cell surface groups (epitopes) that are truly unique to cancers, heterogeneity in epitome expression within cancers, limited penetration of antibodies into solid tumors, and low levels of antibody attachment to tumor cells (69). Nevertheless, promising results were reported by Behr et al. (70), who evaluated the therapeutic efficacy of ^{125}I and ^{131}I attached to internalizing antibodies in a human colorectal cancer model and concluded that ^{125}I linked to internalizing antibodies provides therapeutic benefits that are clearly superior to those obtained with ^{131}I.

Another interesting approach to radionuclide therapy of cancers is the use of estrogen labeled with Auger emitters. Studies by McLaughlin et al. (71) and DeSombre et al. (72) had shown that such agents can kill estrogen receptor-positive cells while sparing ER-negative cells. A potential problem with this system is that ER-positive tumor cells have only about 10000–30000 receptor sites per cell, and under in vivo conditions it is nearly impossible to saturate all these sites. Moreover, the residence time of iodoestrogen in cells is relatively short. To overcome these obstacles, DeSombre et al. (72) proposed to use ^{123}I-labeled estrogens with high specific activity. Recently, Kearney et al. (73) tested this approach by subjecting spheroids of ER-rich

MCF-7 breast cancer cells to ^{123}I-labeled estrogen. The ^{123}I treatment resulted in a significant reduction in cell growth, with nearly half the treated spheroids showing pronounced necrosis within 18 days. Survival studies on cell suspensions prepared after treatment showed cell killing with a D_0 of about 700 decays/cell. The authors believe that this approach has merit for future clinical application, especially in ovarian cancers where steroid receptor-rich micrometastases can be directly targeted.

Nevertheless, for most investigators the carrier compound of choice remains IUdR. Like other targeting agents, IUdR is subject to serious limitations. Rapid dehalogenation makes it difficult to achieve therapeutic radionuclide levels in cancers (74). In addition, the presence of non-proliferating cells in solid tumors necessitates repeated injections or continuous infusions of IUdR to destroy the dividing cells and recruit non-dividing cells into active proliferation. Another complication arises from the fact that IUdR is incorporated into normal and neoplastic cells. As a result, prolonged treatment with high doses of IUdR could cause severe toxic side effects to host organs that undergo continuous cell renewal (e.g., intestine, bone marrow). To overcome this obstacle, investigators are developing strategies that introduce IUdR into tumors, either by direct infusion or by injection into an arterial blood supply that precedes the tumor. This approach is most successful with ovarian cancers that can be treated by intraperitoneal injection, bladder cancers that respond to intravesical administration, hepatic cancers that can be labeled by intra-arterial infusion, and cancers in the central nervous system, breast, and prostate that are accessible by direct infusion into the tumor.

Kassis et al. (75) demonstrated that ^{125}IUdR infused intracerebrally is effective in prolonging the survival and producing some cures in rats bearing brain tumors. Van den Abbeele et al. (76) subjected rats with bladder cancer to intravesical infusion of ^{125}IUdR or ^{131}IUdR and found that the radionuclides were incorporated into the hyperplastic and carcinomatous urothelium, but not in the normal urothelium. IUdR penetrated deeply into the bladder wall, but other normal body tissues such as intestine or bone marrow remained free of radioactivity. Sahu et al. (77) achieved equally promising effects with intrathecal injection of ^{125}IUdR in rats with leptomeningeal tumor metastases. Mariani et al. (78) and Kassis et al. (79) extended this approach to humans and demonstrated efficient radionuclide uptake in patients with brain cancer and colorectal cancer.

A very different application of Auger emitters in cancer therapy was proposed by Schneiderman et al. (80), who used ^{125}IUdR for selective purging of cancer cells from bone marrow. This rationale is that cancer therapy often involves exposure of patients to supralethal chemotherapy. In such cases, bone marrow is harvested from the patient, stored in liquid nitrogen for the duration of the treatment, and then transplanted back into the patient to rescue the hematopoietic system. However, stem cell harvests from cancer patients are often contaminated with metastatic cancer, so the use of autologous bone marrow transplants may lead to relapse of the cancer by reinfusing tumor cells. To eradicate the tumor cells, stem cell harvests are purged with cytotoxic drugs, but these drugs also destroy up to 99% of stem cell population. Schneiderman's cell cycle-based purging technique employs ^{125}IUdR to eliminate tumor cells from bone marrow harvests, with little or no damage to normal stem cells. This technique was tested on bone marrow harvested from mice bearing RAW117 lymphoma cells. When untreated bone marrow from such mice was injected into new recipient mice, 90% of the recipient mice died, but when the bone marrow was treated in vitro with ^{125}IUdR, only 10% of the mice died. Equally important, the ^{125}I treatment regimen used to destroy the contaminating tumor cells did not have any detectable effect on the viability of normal stem cells. If similar selectivity can be demonstrated in purging of cancers from human bone marrow, this technique may have considerable promise for future clinical application.

ACKNOWLEDGEMENTS

This work was supported by Grant No. DHP-179 and Grant No. RPG-96-112-03-LBC from the American Cancer Society.

REFERENCES

1. Auger P. Sur les rayons β secondaires produit dans un gaz des rayons X. Comp Rend 1925; 180: 65–8.
2. Sastry KSR. Biological effects of the Auger emitter ^{125}I: a review: report No. 1 of AAPM Nuclear Medicine Task Group No. 6. Med Phys 1992; 19: 1361–70.
3. Carlson TA, White RM. Formation of fragment ions from $HC_3^{125}Te$ and $C_2H_5^{125}Te$ following the nuclear decays of $CH_3^{125}I$ and $C_2H_5^{125}I$. J Chem Phys 1964; 38: 2930–4.
4. Hofer KG, Prensky W, Hughes WL. Death and metastatic distribution of tumor cells in mice monitored with ^{125}I-iododeoxyuridine. J Nat Cancer Inst 1969; 43: 763–73.
5. Hofer KG, Hughes WL. Radiotoxicity of intranuclear tritium, iodine-125, and iodine-131. Radiat Res 1971; 47: 94–109.
6. Feinendegen LE, Ertl HH, Bond VP. Biological toxicity associated with the Auger effect. In: Ebert H, ed. Proceedings of the Symposium on Biological Aspects of Radiation Quality. Vienna: IAEA, 1971: 419–30.
7. Ertl HH, Feinendegen LE, Heiniger HJ. Iodine-125, a tracer in cell biology: physical properties and biological aspects. Phys Med Biol 1970; 15: 447–56.
8. Burki HJ, Roots R, Feinendegen LE, Bond VP. Inactivation of mammalian cells after disintegration of ^{3}H or ^{125}I in cell DNA at − 196 degrees C. Int J Radiat Biol 1973; 4: 363–75.
9. Bradley EW, Chan PC, Adelstein SJ. The radiotoxicity of mammalian cells. I. Effects on the survival curve of radioiodine incorporated into DNA. Radiat Res 1975; 64: 555–63.
10. Krisch RE, Ley RD. Induction of lethality and DNA breakage by the decay of iodine-125 in bacteriophage T_4. Int J Radiat Biol 1974; 25: 21–30.

11. Krisch RE, Krasin F, Sauri CJ. DNA breakage, repair, and lethality of ^{125}I decay in rec^{+} and recA strains of Escherichia coli. Int J Radiat Biol 1976; 29: 37–50.
12. Hofer KG, Keough G, Smith JM. Biological toxicity of Auger emitters: molecular fragmentation vs. electron irradiation. Curr Top Radiat Res Q 1977; 12: 335–54.
13. Charlton DE, Booz J. A Monte Carlo treatment of the decay of ^{125}I. Radiat Res 1981; 87: 10–23.
14. Hofer KG, Harris CR, Smith JM. Radiotoxicity of intracellular ^{67}Ga, ^{125}I, and ^{3}H: nuclear versus cytoplasmic radiation effects in murine L1210 leukemia. Int J Radiat Biol 1975; 28: 225–41.
15. Warters RL, Hofer KG, Harris CR, Smith JM. Radionuclide toxicity in cultured mammalian cells: elucidation of the primary target of radiation damage. Curr Top Radiat Res Q 1977; 12: 389–407.
16. Kassis AI, Adelstein SJ, Haydock C, Sastry KSR. Radiotoxicity of Se-75 and S-35: theory and application to a cellular model. Radiat Res 1980; 84: 407–25.
17. Painter RB, Young BR, Burki HJ. Non-repairable strand breaks induced by ^{125}I incorporated into mammalian DNA. Proc Nat Acad Sci USA 1974; 71: 4836–8.
18. Miyazaki N, Fujiwara Y. Mutagenic and lethal effect of [5-^{125}I]iodo-2′-deoxyuridine incorporated into DNA of mammalian cells, and their RBEs. Radiat Res 1981; 88: 456–65.
19. Little JB, LeMotte PK, Liber HL. Quantitative studies of cytotoxicity, mutagenesis and oncologic transformation by radioisotopes incorporated into DNA. In: Harris CC, Auhup HN, eds. Human Carcinogenesis. New York: Academic Press, 1983: 545–59.
20. Chan PC, Lisco E, Lisco H, Adelstein SJ. The radiotoxicity of iodine-125 in mammalian cells. II. A comparative study on cell survival and cytogenetic responses to ^{125}IUdR, ^{131}IUdR and ^{3}H-TdR. Radiat Res 1976; 67: 322–43.
21. Sundell-Bergman S, Bergman R, Johanson KJ. Chromosome damage induced by decay of ^{3}H and ^{125}I incorporated into DNA of Chinese hamster cells. Mutat Res 1985; 149: 257–63.
22. Warters RL, Hofer KG. Radionuclide toxicity in cultured mammalian cells: elucidation of the primary site for radiation-induced mitotic delay. Radiat Res 1977; 69: 348–58.
23. Schneiderman MH, Hofer KG. The target for radiation-induced division delay. Radiat Res 1980; 84: 462–76.
24. Martin RF, Haseltine WA. Range of radiochemical damage to DNA with decay of iodine-125. Science 1981; 213: 896–8.
25. Adelstein SJ, Kassis AI. Strand breaks in plasmid DNA following positional changes of Auger-emitting radionuclides. Acta Oncol 1996; 35: 797–801.
26. Kassis AI, Harapanhalli RS, Adelstein SJ. Comparison of strand breaks in plasmid DNA after positional changes of Auger electron-emitting iodine-125. Radiat Res 1999; 151: 167–76.
27. Sahu SK, Kortylewicz ZP, Baranowska-Kortylewicz J, Taube RA, Adelstein SJ, Kassis AI. Strand breaks after the decay of iodine-125 in proximity to plasmid pBR322 DNA. Radiat Res 1997; 147: 401–8.
28. Kassis AI, Harapanhalli RS, Adelstein SJ. Strand breaks in plasmid DNA following positional changes of Auger-electron-emitting iodine-125: direct versus indirect effects. Radiat Res 1999; 151: 530–8.
29. Walicka MA, Adelstein SJ, Kassis AI. Indirect mechanisms contribute to biological effects produced by decay of DNA-incorporated iodine-125 in mammalian cells in vitro: double-strand breaks. Radiat Res 1998; 149: 134–41.
30. Panyutin IG, Neumann RD. Sequence-specific DNA breaks produced by triplex-directed decay of iodine-125. Acta Oncol 1996; 35: 817–23.
31. Panyutin IG, Neumann RD. Radioprobing of DNA: distribution of DNA breaks produced by decay of ^{125}I incorporated into a triplex-forming oligonucleotide correlates with geometry of the triplex. Nucl Acid Res 1997; 25: 883–7.
32. Karamychev VN, Panyutin IG, Reed MW, Neumann RD. Effect of radionuclide linker structure on DNA cleavage by ^{125}I-labeled oligonucleotides. Antisense Nucleic Acid Drug Dev 1997; 7: 549–57.
33. DeSombre ER, Hughes A, Landel CC, Greene G, Hanson R, Schwartz JL. Cellular and subcellular studies of the radiation effects of Auger electron-emitting estrogens. Acta Oncol 1996; 35: 833–40.
34. Schwatz JL, Mustafi R, Hughes A, DeSombre ER. DNA and chromosome breaks induced by iodine-123-labeled estrogen in Chinese hamster ovary cells. Radiat Res 1996; 146: 151–8.
35. Yasui LS, DeSombre ER. DNA damage induction by ^{125}I-estrogen. Acta Oncol 1996; 35: 841–7.
36. Charlton DE, Humm JL. A method of calculating initial DNA strand breakage following the decay of incorporated ^{125}I. Int J Radiat Biol 1988; 53: 353–65.
37. Pomplun E. A new DNA target model for track structure calculations and its first application to I-125 Auger electrons. Int J Radiat Biol 1991; 59: 625–42.
38. Terrissol M. Modelling of radiation-induced DNA damage: The early physical and chemical events. Int J Radiat Biol 1994; 66: 447–51.
39. Nikjoo H, O'Neill P, Terrissol M, Goodhead DT. Modelling of radiation-induced DNA damage: the early physical and chemical events. Int J Radiat Biol 1994; 66: 453–7.
40. Terrissol M. Modelling of radiation damage by ^{125}I on a nucleosome. Int J Radiat Biol 1994; 66: 447–51.
41. Pomplun E, Terrissol M. Low-energy electrons inside active DNA models: a tool to elucidate the radiation action mechanisms. Radiat Environ Biophys 1994; 33: 279–92.
42. Pomplun E, Terrissol M, Demonchy M. Modelling of initial events and chemical behaviour of species induced in DNA units by Auger electrons from ^{125}I, ^{123}I and carbon. Acta Oncol 1996; 35: 857–62.
43. Jonkhoff AR, vDieren EB, Huijgens PC, et al. Biological effectiveness of 67-gallium in HL60 cells compared with external low dose rate gamma irradiation: effects on proliferation, G2 arrest, and clonogenic capacity. Int J Radiat Oncol Biol Phys 1994; 30: 117–24.
44. Narra VR, Howell RW, Harapanhalli RS, Sastry KSR, Rao DV. Radiotoxicity of some iodine-123, iodine-125, and iodine-131-labeled compounds in mouse testes: implications for radiopharmaceutical design. J Nucl Med 1992; 33: 2196–201.
45. Yasui LS, Hofer KG. Role of mitochondrial DNA in cell death induced by ^{125}I decay. Int J Radiat Biol 1986; 49: 601–10.
46. Yasui LS, Hofer KG, Warters RL. Inhomogeneity of the nucleus to ^{125}IUdR cytotoxicity. Radiat Res 1985; 102: 1–18.
47. Kassis AI, Fayad F, Kinsey BM, Sastry KSR, Adelstein SJ. Radiotoxicity of an I-125-labeled DNA intercalator in mammalian cells. Radiat Res 1989; 118: 283–94.
48. McLean RN, Wilkinson D. The radiation dose to cells in vitro from intracellular indium-111. Biochem Cell Biol 1989; 67: 661–5.
49. Rao DV, Sastry KSR, Grimmond HE, et al. Cytotoxicity of some indium radiopharmaceuticals in mouse testes. J Nucl Med 1988; 29: 375–84.
50. Sedelnikova OA, Panyutin IG, Thierry AR, Neumann RD. Radiotoxicity of iodine-125-labeled oligodeoxyribonucleotides in mammalian cells. J Nucl Med 1998; 39: 1412–8.

51. Rao DV, Narra VR, Howell RW, Lanka VK, Sastry KSR. Induction of spermhead abnormalities by incorporated radionuclides: dependence on subcellular distribution, type of radiation, dose rate and presence of radioprotectors. Radiat Res 1991; 125: 89–97.
52. Narra VR, Harapanhalli RS, Howell RW, Sastry KSR, Rao DV. Vitamins as radioprotectors in vivo: I. Protection by vitamin C against internal radionuclides in mouse testes: implications to the mechanism of the Auger effect. Radiat Res 1994; 137: 394–9.
53. Narra VR, Harapanhalli RS, Goddu SM, Howell RW, Rao DV. Radioprotection against biological effects of internal radionuclides in vivo by S-(2-aminoethyl)isothioronium bromide hydrobromide (AET). J Nucl Med 1995; 36: 259–66.
54. Harapanhalli RS, Narra VR, Yaghmai V, et al. Vitamins as radioprotectors in vivo. II. Protection by vitamin A and soybean oil against radiation damage caused by internal radionuclides. Radiat Res 1994; 139: 115–22.
55. Walicka MA, Adelstein SJ, Kassis AI. Indirect mechanisms contribute to biological effects produced by decay of DNA-incorporated iodine-125 in mammalian cells in vitro: clonogenic survival. Radiat Res 1998; 149: 142–6.
56. Bishayee A, Rao DV, Bouchet LG, Bolch WE, Howell RW. Protection by DMSO against cell death caused by intracellularly localized iodine-125, iodine-131, and polonium-210. Radiat Res 2000; 153 (in press).
57. Chapman JD. Biophysical models of mammalian cell inactivation by radiation. In: Meyn RE, Withers HR, eds. Radiation biology in cancer research. NY: Raven Press, 1980: 21–32.
58. Hofer KG, VanLoon N, Schneiderman MH, Charlton DE. The paradoxical nature of DNA damage and cell death induced by ^{125}I decay. Radiat Res 1992; 130: 121–4.
59. Hofer KG, VanLoon N, Schneiderman MH, Charlton DE. High LET and low LET cytocidal effects of DNA-associated iodine-125. In: Howell RW, Narra VR, Sastry KSR, Rao DV, eds. Biophysical aspects of Auger processes. Woodburry, NY: American Institute of Physics, Inc, 1992: 227–48.
60. Hofer KG, Bao SP. Low-LET and high-LET radiation action of ^{125}I decays in DNA: effect of cysteamine on micronucleus formation and cell death. Radiat Res 1995; 141: 183–92.
61. Hofer KG, Lin X, Bao SP. DNA damage, micronucleus formation, and cell death from ^{125}I decays in DNA. Acta Oncol 1996; 35: 825–32.
62. Feinendegen LE. Biological damage from the Auger effect, possible benefits. Radiat Environ Biophys 1975; 12: 85–99.
63. Koch CJ, Burki HJ. The oxygen-enhancement ratio for reproductive death induced by 3H or ^{125}I damage in mammalian cells. Int J Radiat Biol 1975; 5: 417–25.
64. Strand SE, Jönsson BA, Ljungberg M, Tennvall J. Radioimmunotherapy dosimetry—a review. Acta Oncol 1993; 32: 807–17.
65. Humm JL, Howell RW, Rao DV. Dosimetry of Auger-electron emitting radionuclides: report No. 3 of the AAPM Nuclear Medicine Task Group No. 6. Med Phys 1994; 21: 1901–15.
66. Humm JL, Macklis RM, Lu XQ, et al. The spatial accuracy of cellular dose estimates obtained from 3D reconstructed tissue autoradiographs. Phys Med Biol 1995; 40: 163–80.
67. Howell RW, Rao DV, Sastry KSR. Macroscopic dosimetry for radioimmunotherapy: nonuniform activity distributions in solid tumors. Med Phys 1989; 16: 66–74.
68. Hofer KG. Biophysical aspects of Auger processes: a review. Acta Oncol 1996; 35: 789–96.
69. Wheldon TE. Targeting radiation to tumours. Int J Radiat Biol 1994; 65: 109–15.
70. Behr TM, Sgouros G, Vougiokas V, et al. Therapeutic efficacy and dose-limiting toxicity of Auger-electron vs. beta emitters in radioimmunotherapy with internalizing antibodies: evaluation of ^{125}I vs. ^{131}I-labeled CO17-1A in a human colorectal cancer model. Int J Cancer 1998; 76: 738–48.
71. McLaughlin WH, Milius RA, Pillai KMR, Edasery JP, Blumenthal RD, Bloomer WD. Cytotoxicity of receptor-mediated 16α [^{125}I]iodo-estradiol in cultured MCF-7 human breast cancer cells. J Nat Cancer Inst 1989; 81: 437–40.
72. DeSombre ER, Shafi B, Hanson RN, Kiuvanen PC, Hughes A. Estrogen receptor-directed radiotoxicity with Auger electrons: specificity and mean lethal dose. Cancer Res 1992; 52: 5752–8.
73. Kearney T, Hughes A, Hanson RN, DeSombre ER. Radiotoxicity of Auger electron-emitting estrogens in MCF-7 spheroids: a potential treatment for estrogen receptor-positive tumors. Radiat Res 1999; 151: 570–9.
74. Klecker RW Jr, Jenkins JF, Kinsella TJ, Fine RL, Strong JM, Collins JM. Clinical pharmacology of 5-iodo-2′-deoxyuridine and 5-iodouracil and endogenous pyrimidine modulation. Clin Pharmacol Ther 1985; 38: 45–51.
75. Kassis AI, Wen PY, Van den Abbeele AD, et al. 5-[^{125}I]iodo-2′-deoxyuridine in the radiotherapy of brain tumors in rats. J Nucl Med 1998; 39: 1148–54.
76. Van den Abbeele AD, Tutrone RF, Berman RM, et al. Tumor-targeting potential of radioiodinated iodo-deoxyuridine in bladder cancer. J Nucl Med 1996; 37: 315–20.
77. Sahu SK, Wen PYC, Foulon CF, et al. Intrathecal 5-[^{125}I]iodo-2′-deoxyuridine in a rat model of leptomeningeal metastases. J Nucl Med 1997; 38: 386–90.
78. Mariani G, Sacco SD, Volterrani D, et al. Tumor targeting by intra-arterial infusion of 5-[^{123}I]iodo-2′-deoxyuridine in patients with liver metastases from colorectal cancer. J Nucl Med 1996; 37: 22S–5S.
79. Kassis AI, Tumeh SS, Wen PYC, et al. Intratumoral administration of 5-[^{123}I]iodo-2′-deoxyuridine in a patient with brain tumor. J Nucl Med 1996; 37: 19S–22S.
80. Schneiderman MH, Jackson JD, Joshi SS, Clark-Pierce L, Murphy BJ, Schneiderman GS. Cell cycle-based purging of lymphoma cells from bone marrow harvests using radioiodinated 5-iodo-2′-deoxyuridine. Radiat Res 1998; 149: 147–54.