Chemotoxicity of Indium-111 Oxine in Mammalian Cells

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We have studied the uptake and toxicity of [^{111}In]oxine in Chinese hamster V79 lung fibroblasts. The incorporation of the radionuclide into these cells reached a plateau within 2 hr. Uptake was proportional to the extracellular radioactive concentration. Both radioactive and "decayed" [^{111}In]oxine exhibited similar toxicities, indicating that the observed toxicity was chemical in nature. These results are discussed in terms of the present status of this radiolabeling agent.

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Several recent communications have dealt with the "detrimental" effects of indium-111 (^{111}In) following intracellular localization. These effects have ranged from malignant transformation (*1*) to reduction of neutrophil chemotaxis (*2–6*), failure of lymphocytes to respond to phytohemagglutinin stimulation (*2,5*), decreased proliferative capacity of lymphocytes (*1*), induction of chromosomal aberrations (*1*), reduced phagocytosis of granulocytes (*4*), loss of ability of lymphocytes to recirculate (*6–9*), and decreased viability (*2,3,5,8,9*) and colony-forming capability of cultured mammalian cells (*6,10,11*). This has resulted in replies, counterreplies, and much confusion (*12–14*).

In the course of our work on the cytocidal effects of various Auger-electron emitters (*15–17*), we were interested in determining the radiotoxicity of ^{111}In in the form of [^{111}In]oxine. Unfortunately, "decayed" In-111 oxine exhibited chemical toxicity that overlapped the radiotoxicity of the ^{111}In. In view of the current interest in the matter, we now feel it appropriate to publish our observations, perhaps adding to the confusion.

MATERIALS AND METHODS

Cells

Chinese hamster V79 lung fibroblasts, with a doubling time of about 9 hr, were used in these experiments. These cells are maintained in culture as described previously (*15–17*).

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Radionuclide

The [^{111}In]oxine was dissolved in ethanol (20 mCi/ml) and diluted in minimum essential medium to yield a radioactive concentration of up to 50 μCi/ml. It was then passed through a 0.22-μm Millipore filter and used in the uptake and toxicity experiments.

Uptake studies

Following the incubation of V79 cells with varying radioactive concentrations of [^{111}In]oxine for up to 18 hr at 37°C, the cellular content of ^{111}In was determined by the microfuge method (*18*). Cells suspended in the radioagent are layered onto fetal bovine serum in microfuge tubes and spun at high speeds (12,800 *g*) for 1 min. With this method, the cells pass through the serum very quickly, leaving practically all the extracellular radioactivity in the upper layer of culture medium. The tubes are frozen rapidly in a dry ice/methanol mixture and the radioactive content of the cell pellets is counted in a gamma counter.

Survival assay

The toxicity of [^{111}In]oxine was determined by the colony-forming assay (*15*), in which cells exposed to an agent are serially diluted and seeded, in triplicate, into 25-cm^2 tissue-culture flasks. The number of cells is adjusted to yield 30–250 colonies 6–7 days later. The formed colonies are fixed, stained, and visually counted. The ratio of colony numbers in the experimental group to that in the control gives the survival ratio (S/S_0).

To study the chemotoxicity of the decayed [^{111}In]-oxine, the same incubation procedure was used as above except that the radionuclide sample was at least 4 wk old.

With the short half-life of this radionuclide (67 hr), essentially all the radioactivity has decayed.

RESULTS

Uptake studies

Figure 1 plots the uptake of [^{111}In]oxine by V79 cells following various incubation periods at 37°C. The incorporation of this radionuclide was most rapid within the first hour, and reached a plateau after 2 hr.

Uptake of [^{111}In]oxine was also measured as a function of the extracellular radioactive concentration following 2 or 18 hr of incubation at 37°C. Figure 2 is a semilogarithmic plot of the uptake data (correlation coefficient = 0.97). Since V79 cells have a diameter of 10.4 μm, with a corresponding volume of 570×10^{-12} cc/cell (*15*), it is possible to convert the intracellular content from pCi/cell to μCi/ml. The extent to which this radionuclide is concentrated by these cells can therefore be calculated. The data indicate that the intracellular-to-extracellular ratio for this radionuclide is >100:1.

Toxicity studies.

Following a 2-hr or an 18-hr incubation of V79 cells with various radioactive concentrations of [^{111}In]oxine, cell survival was determined and plotted as a function of extracellular radioactive concentration (μCi/ml). The data from both incubation periods fell on the same survival curve (Fig. 3), which was characterized by a wide shoulder, an exponential portion, and a D_{37} of 10.5 μCi/ml, equivalent to 0.26 pCi/cell. Furthermore, the incubation of these cells with the "decayed" [^{111}In]oxine produced a curve that was superimposable on the radioactive [^{111}In]oxine curve, indicating that the observed toxicity was not due to the radioactive decay of ^{111}In.

DISCUSSION

Indium-111 is a commercially available radionuclide that is suitable for external detection, as it decays to stable cadmium with the emission of two gamma photons of 173 keV (89%) and 247 keV (94%). This radionuclide has a half-life of 67 hr, which is long enough for studies of cell migration while avoiding excessive long-term residual radiation.

The development of the lipophilic [^{111}In]oxine complex as a radiolabel for isolated polymorphonuclear leukocytes, lymphocytes, and platelets has provided a useful technique with many potential applications in clinical and biological investigations. This complex has been used extensively to locate abscesses (*19,20*), to study myocardial infarction (*21*), and for in vitro studies (*5,22*).

The validity of all these studies has depended heavily on the presumption that unless the ^{111}In complex and/or the labeling procedure are toxic to the radiolabeled cells,

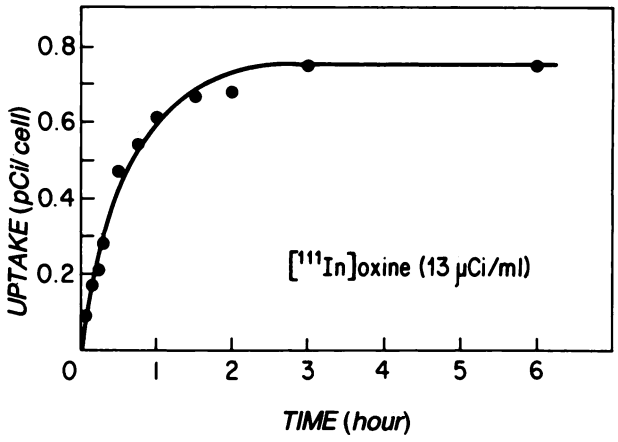

FIGURE 1
Uptake (pCi/cell) of [^{111}In]oxine by V79 cells as function of incubation time (hr). Each point represents mean of three samples

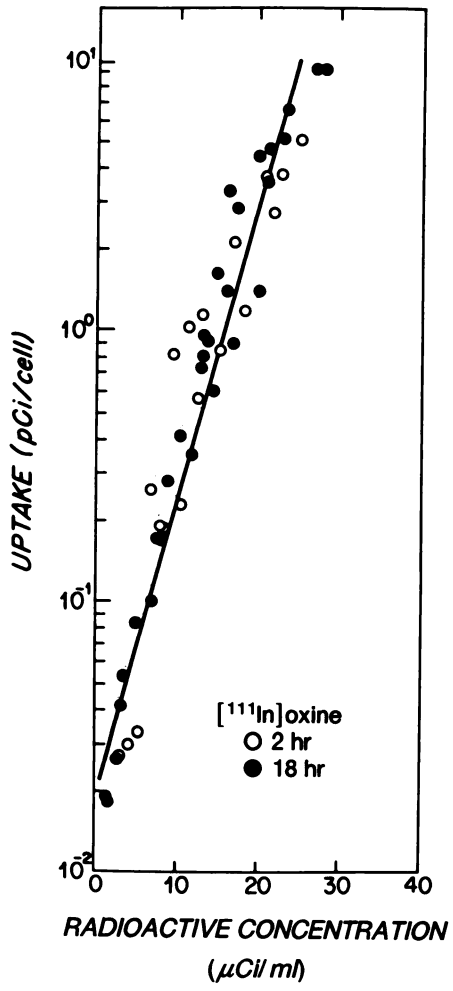

FIGURE 2
Uptake (pCi/cell) of [^{111}In]oxine by V79 cells following 2-hr (O) or 18-hr (●) incubation (37°C) in various radioactive concentrations (μCi/ml) of radionuclide. Each point represents mean of three samples

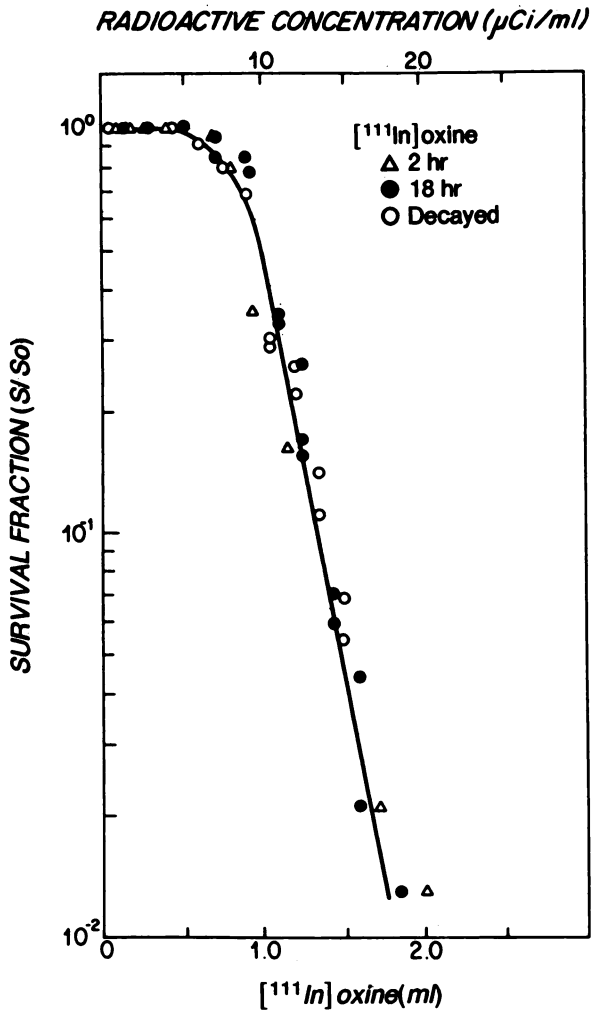

FIGURE 3
Survival of V79 cells following incubation in various concentrations of [^{111}In]oxine (Δ: 2 hr; ●: 18 hr) and its "decayed" residue (O). Each point represents mean of three samples

the normal metabolic functioning of these cells, as defined by the uses to which they are put, will not be compromised. The successful and extensive use of this radionuclide in nuclear medicine attests to the validity of this presumption. However, several studies have been concerned with the biological effects of the complex on the carrier cells, and in particular on the possible malignant transformation of these cells (*1,13,14*). Experiments have examined such parameters as the recirculation of the tagged cells (*6–9*), certain in vitro responses of lymphocytes to mutagenic agents (*1,2,5*), and the toxicity, presumably due to radioactivity, as measured by dye-exclusion assays (*2,3,5,8,9*) or by the colony-forming capacity of these cells (*6,10*). However, no overall picture has emerged, even though a certain degree of concern over the fate, health, and welfare of the labeled cells is becoming evident.

The results presented in this communication indicate, beyond any doubt, the presence of a toxic species, presumably within the oxine-metal complex, that is cytotoxic to cultured V79 lung fibroblasts. Our results are at odds with those of ten Berge et al. (*1*) who reported that "decayed" [^{111}In]oxine (no decay time given) did not affect the proliferative capacity of human peripheral lymphocytes and did not cause chromosomal aberrations, whereas both end points showed effects with the radioactive complex. However, these authors exposed their cells to both the radioactive and the cold complex for 30 min only. Perhaps some effects would have been observed if the cells had been exposed for the longer time periods used in our study. While the conditions of the radiolabeling and the cells used in these experiments are different from those used in human studies, it must be kept in mind that, in general, mammalian cells respond similarly to various agents. Furthermore, even though our period of incubation with the ^{111}In complex was rather lengthy (2 or 18 hr), the radioactive concentration (μCi/ml)—and consequently the molar concentration of the chemotoxic species—was much lower than that experienced by white cells in human studies.

In its decay, ^{111}In emits many Auger electrons with very short ranges. These electrons are biologically detrimental only if localized close to the radiosensitive target of the cell, i.e., within the cell nucleus (*15–17*). The fact that we were unable to demonstrate any radiotoxicity of this agent in our studies may be due either to overshadowing by the chemotoxic process or to the exclusion of the radionuclide from the nucleus. Further studies are needed to clarify this point.

In conclusion, our results emphasize the chemotoxicity of the [^{111}In]oxine complex in a cultured mammalian cell line. The nature of this toxicity is unknown; its identification and elimination are most pertinent to the valid use of radiolabeled cells in nuclear medicine.

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