Journal of Surgical Oncology 51:236–242 (1992)

Chromosome Aberrations of Human Small Cell Lung Cancer Induced by a New ^{111}In-Bleomycin Complex

DE-YAN HOU, MD, YOSH MARUYAMA, MD, AND JOSEPH R. DRAGO, MD
From the Department of Radiation Medicine, University of Kentucky Medical Center, Lexington, Kentucky (D.-Y.H., Y.M.) and the Division of Urology, Department of Surgery, The Ohio State University, Columbus, Ohio (D.-Y.H., J.R.D.)

A new ^{111}Indium labeled bleomycin complex (^{111}In-BLMC) was prepared and found to be effective for tumor imaging and therapy both in mouse glioma and human small cell lung cancer (SCLC) cells. Chromosome aberrations were studied in human SCLC cells to explore its mechanisms of killing cancer cells. SCLC cells (N417) were exposed to ^{111}In-BLMC, BLM, or $^{111}InCl_3$ (for control) for 1 hour, treated with colcemid, and chromosomal changes were analyzed. A dramatic increase in chromatic gaps, breaks, chromosome breaks, double minutes, rings, triradii, quadriradii, and chromosome stickiness were observed in the cells treated by ^{111}In-BLMC compared to BLM or $^{111}InCl_3$. These results indicated that ^{111}In-BLMC has therapeutic potential for combination chemo-radiotherapy of cancer (e.g., by Auger electrons and local energy deposition).

KEY WORDS: **^{111}Indium, bleomycin, chromosomes, tumor**

INTRODUCTION

Combined radiation and chemotherapy could be delivered to nuclear targets of tumor cells if radioactive labeled drugs are concentrated in the nuclear regions of tumor cells. Labeled drugs with high nuclear and DNA localization and tumor specificity could deliver radiochemotherapy to tumor cells and therefore represents an effective method for targetted tumor therapy. The radiotherapeutic effect would be considerably enhanced when the drug itself causes DNA strand breaks and is also a radiosensitizer. Bleomycin (BLM) is a cytotoxic antibiotic which binds to cellular DNA causing strand scissions [1,2] and localizes in the nucleus [3]; it is also a radiosensitizer [4]. Therefore, BLM is a candidate for this type of therapy and for studies where combined effects are sought. In addition, a suitable radiolabel (e.g., Auger emitter which deposits its energy locally) may cause more severe damage to the tumor cell and could synergically increase its potential benefit.

We prepared a new ^{111}Indium (Auger emitter) labeled bleomycin complex (^{111}In-BLMC) which has a high affinity for several tumors [5]. ^{111}In-BLMC was found to be effective for tumor imaging of mouse glioma, hepatoma, and rat mammary adenocarcinoma [6]. It also depressed tumor growth for a transplanted glioma [7]. These indicated a potential cytotoxic effect of the radiolabeled drug as well as the ability to localize in tumor.

Human small cell lung cancer (SCLC) is sensitive to chemotherapy, radiotherapy, and combined chemo-radiotherapy [8]. Experiments with ^{111}In-BLMC showed that it was more cytotoxic than BLM by a factor of 1.6–5.3 times for killing five human SCLC cell lines [9]. ^{111}In-BLMC localized mainly in the nucleus of the SCLC cells [10]. In order to explore possible mechanisms of tumor cell killing, chromosome aberrations induced by ^{111}In-BLMC in comparison with BLM and $^{111}InCl_3$ were analyzed. Our findings with cells treated by short exposure (60 minutes) to ^{111}In-BLMC indicated that an agent with high cell nucleus localization, when radiolabeled (e.g., by an Auger emitter), offered therapeutic potential

Accepted for publication August 13, 1992.
Address reprint requests to De-Yan Hou, MD, Division of Urology, Department of Surgery, University Hospital Clinic, Room 4980, The Ohio State University, 456 W. 10th Ave., Columbus, OH, 43210.

in cancer therapy by virtue of an ability to target radiation and cytogenetic effects in DNA and chromosomes.

MATERIALS AND METHODS

Preparation of ^{111}In-BLMC

One hundred to 500 μl of $^{111}InCl_3$, received in 0.45%–0.9% NaCl solution adjusted to pH 1–3 with HCl (Amersham Corporation, Medi-Physics, Arlington Heights, IL), was mixed with 1.0 mg solid BLM (Blenoxane, Bristol Laboratories, Syracuse, NY) or with BLM dissolved in 0.9% NaCl (N.S.). The final pH was 2.5 (2.0–3.0) by pH paper (pHydrion paper, Micro Essential Laboratory, Brooklyn, NY) [7] or a pH meter and then was quality controlled by thin-layer chromatography (TLC) and 5% gel electrophoresis [5,7]. The radiochemical purity of ^{111}In-BLMC was 99%. The Rf of ^{111}In-BLMC was 0.65 by TLC [7,10].

Cell Preparation

The cell line of human SCLC cells (N417, NCI) was received from Drs. John D. Minna and Adi F. Gazdar (the NCI/Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, MD). Four × 10^6 cells were seeded into a 75 cm^2 flask containing 20 ml of medium supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% L-glutamine (200 mM, Gibco). Cells were incubated at 37°C in 5% CO_2. The cell line was maintained in exponential growth phase and passaged twice per week. The cell cycle time obtained by flow cytometry was 20 ± 3 hours for N417 cells, and the duration of its cell cycle phases was 4 hours for S phase; 4.4 hours for G-M, and 8.6 hours for G1 [11].

Drug Treatment and Preparation of Chromosomes

One–2 × 10^7 N417 cells in logarithmic growth phase were vigorously pipetted and then passed through sterile Nitex gause (NITEX Industrial, Elmford, NY), 30 μm diameter to obtain monodispersed cells. Cells were exposed to N.S., BLM (10 μg/ml), $^{111}InCl_3$ (50 μCi/ml, 1.85 MBq/ml), or ^{111}In-BLMC (20–50 μCi, 0.74–1.85 MBq/ml, carried by 10 μg BLM/ml) in a 37°C water bath for 60 minutes and washed with fresh medium three times. The cells were then treated with colcemid (Gibco, 10 mcg/ml, 40–50 μl for 1 × 10^6 – 10^7 cells in 10 ml) in a shaking water bath (37°C) for 30–40 minutes. Finally, the cells were treated with hypotonic solution (1% sodium citrate dihydrate: 0.075 M KCl, 1:4, v/v) for 30–60 minutes in 37°C incubator, and fixed in methanol: glacial acetic acid (3:1, v/v). Chromosome slides were prepared and stained with Giemsa solution. Chromosome spreads were analyzed under a Zeiss standard microscope and photographed [12–14].

Criteria of Chromatid and Chromosomal Aberrations

Chromatid and chromosome aberrations were identified on the basis of the international nomenclature for chromosome aberrations [15,16]. In our experiments, chromosomal aberrations were classified by the following criteria:

1. Chromatid gap: the length of chromatid deletion or gap is smaller or equal to one width of the chromatid;
2. Chromatid break: the length of the chromatid deletion is longer than the width of the chromatid; including chromatid fragment in this category, i.e., a chromatid deletion was displaced into fragments;
3. Chromosome break: the chromosome break occurred on both chromatids; separating to a) >10: the number of chromosome break is more than 10 in one cell; and b) <10: the number is less than 10 in one cell;
4. Double minute: two small chromatid fragments with length not greater than the width of a single chromatid;
5. Ring: chromosome was ring shaped;
6. Triradius: three chromosomes (or parts of chromosomes) stuck together;
7. Quadriradius: four chromosomes (or parts of chromosomes) stuck together;
8. Stickiness: more than six chromosomes stuck together;
9. No change: none of the above changes observed.

One hundred cells were enumerated in each group. The percentage of chromatid or chromosome aberrations in 100 cells was analyzed as: a) cell percentage (cell number of chromosomal changes in 100 cells); and b) frequency (total chromosomal changes in 100 cells/100, i.e., chromosome changes per cell).

RESULTS

Chromatid and Chromosome Aberrations

The cell percentages and frequencies of chromatid and chromosome aberrations of human SCLC cells treated with N.S., $^{111}InCl_3$, BLM, and ^{111}In-BLMC are shown in Tables I and II and in Figures 1 and 2. In the control (N.S.) group, there were very few chromosome aberrations. In the $^{111}InCl_3$ group, a higher percentage of double minutes was shown, and the chromosomal aberrations were higher than in the N.S. group ($P < 0.05$) in frequency (chromosome change per cell) (Table II). However, there were no significant differences between these two groups ($P < 0.25$) in cell percentage (change in cell number in 100 cells) (Table I). In the BLM group, the cell percentage and frequency of chromosome aberrations in

TABLE I. The Percentage of Chromosome Aberrations of N417 Cells by 0.9% NaCl, $^{111}InCl_3$, and ^{111}In-BLMC†

Group	No change	Chromatid gap	Chromatid break	Chromosome break >10[a]	Chromosome break <10[a]	Double minute	Ring	Triradius	Quadri-radius	Stickiness	*P* value*
0.9% NaCl	85	0	2	0	0	13	1	0	0	0	
											<0.25
$^{111}InCl_3$ (50 μCi/ml)	54	4	10	1	2	24	12	5	0	4	
											<0.001
BLM (10 μg/ml)	21	39	29	7	10	20	23	8	1	3	
											<0.005
^{111}In-BLMC (20 μCi/10 μg BLM/ml)	8	33	36	8	29	33	47	31	11	14	
											>0.5
^{111}In-BLMC (50 μCi/10 μg BLM/ml)	1	53	49	11	54	46	55	46	16	18	
											<0.001**

† The cell number of chromatid and chromosome aberrations in 100 cells.
[a] The amount of chromosome break was >10 or <10 in one cell.
* *P* value for comparison of 0.9% NaCl with $^{111}InCl_3$, $^{111}InCl_3$ with BLM, BLM with ^{111}In-BLMC (20 μCi/10 μg BLM/ml), or ^{111}In-BLMC (20 μCi/10 μg BLM/ml) with ^{111}In-BLMC (50 μCi/10 μg BLM/ml) group, by χ^2 test (not including "no change" category).
** *P* value for comparison of ^{111}In-BLMC (50 μCi/10 μg BLM/ml) with BLM (10 μg/ml), or ^{111}In-BLMC (50 μCi/10 μg BLM/ml) with BLM (10 μg/ml) and $^{111}InCl_3$ (50 μCi/ml) groups, by χ^2 test (not including "no change" category).

TABLE II. The Frequency of Chromosome Aberrations of N417 Cells by 0.9% NaCl, $^{111}InCl_3$, and ^{111}In-BLMC†

Group	Chromatid gap	Chromatid break	Chromosome break (<10)[a]	Double minute	Ring	Triradius	Quadriradius	*P* value*
0.9% NaCl	0	2	0	18	1	0	0	
								<0.05
$^{111}InCl_3$ (50 μCi/ml)	4	12	2	32	18	7	0	
								<0.001
BLM (10 μg/ml)	111	44	15	28	31	8	1	
								<0.001
^{111}In-BLMC (20 μCi/10 μg BLM/ml)	97	68	62	62	81	41	13	
								>0.5
^{111}In-BLMC (50 μCi/10 μg BLM/ml)	192	112	119	91	125	68	24	
								<0.001**

† The total amount of chromatid and chromosome aberrations in 100 cells, frequency was this amount divided by 100.
[a] The total amount of chromosome break (<10 in one cell) in 100 cells.
* *P* value for comparison of 0.9% NaCl with $^{111}InCl_3$, $^{111}InCl_3$ with BLM, BLM with ^{111}In-BLMC (20 μCi/10 μg BLM/ml), or ^{111}In-BLMC (20 μCi/10 μg BLM/ml) with ^{111}In-BLMC (50 μCi/10 μg BLM/ml) group, by χ^2 test.
** *P* value for comparison of ^{111}In-BLMC (50 μCi/10 μg BLM/ml) with BLM (10 μg/ml), or ^{111}In-BLMC (50 μCi/10 μg BLM/ml) with BLM (10 μg/ml) and $^{111}InCl_3$ (50 μCi/ml) groups, by χ^2 test.

most categories was higher than that of the $^{111}InCl_3$ group (except for the double minutes). Differences were significantly different ($P < 0.001$) between these two groups (Tables I, II).

Interestingly, in the ^{111}In-BLMC group, we observed many more bizarre and frequent chromosome aberrations (chromatid gaps, chromatid breaks, double minutes, ring, triradius, quadriradius, and stickiness) in N417 cells than in the group treated by BLM alone (only chromatid gaps showed a lower percentage in the 20 μCi/ml ^{111}In-BLMC group) (Tables I, II). With increase in the radioactivity of ^{111}In-BLMC (from 20 μCi/10 μg BLM/ml to 50 μCi/10 μg BLM/ml) many more chromatid and chromosome aberrations were observed. However, there were no statistically significant differences between these two groups ($P > 0.5$). Both cell percentages and frequencies of chromosomal aberrations in the ^{111}In-BLMC (20 μCi or 50 μCi/10 μg BLM/ml) group were much higher than BLM alone ($P < 0.001$). For example, the frequencies of chromatid and chromosome aberrations per cell for ^{111}In-BLMC with 50 μCi/10 μg BLM/ml were much higher than those for the BLM (10 μg/ml) group by a factor of 1.7–24 (Table II). In addition, ^{111}In-BLMC groups (including 20 μCi and 50 μCi/10 μg

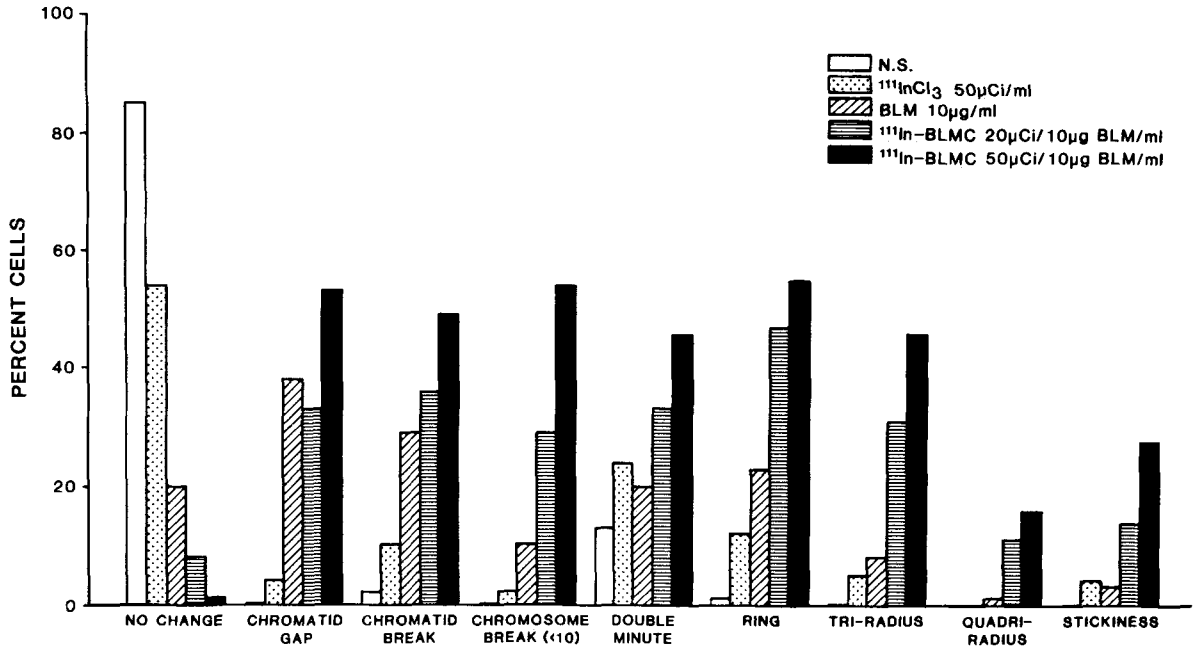

Fig. 1. The cell percentages of chromatid and chromosome aberrations for N417 cells. Cells were treated at 37°C for 60 minutes by N.S (0.9% NaCl); $^{111}InCl_3$ (50 μCi/ml); BLM (10μg/ml); ^{111}In-BLMC (20 μCi/10 μg/ml); or ^{111}In-BLMC (50 μCi/10 μg/ml). Same data are in Table I.

BLM/ml) showed more striking chromosomal aberrations than $^{111}InCl_3$ (50 μCi/ml) alone ($P < 0.005$, $P < 0.001$). The frequency of chromosomal aberrations was higher for ^{111}In-BLMC group (with 50 μCi/10 μg BLM/ml) than that of $^{111}InCl_3$ (10 μCi/ml) group by a factor of 2.8–48 (Table II).

Very interestingly, not only an enhancement effect but also a synergistic effect for chomosome aberrations was observed for the ^{111}In-BLMC groups compared with the BLM and $^{111}InCl_3$ groups (see Tables I, II). This was especially notable for the high radioactivity (50 μCi/10 μg BLM/ml) ^{111}In-BLMC group. The comparison of the chromosomal aberrations of the ^{111}In-BLMC group (50 μCi/10 μg BLM/ml) with the $^{111}InCl_3$ group (50 μCi/ml) and BLM group (10 μg/ml) showed that the chromosomal aberrations of the ^{111}In-BLMC group was significantly higher than those of the $^{111}InCl_3$ and BLM group by a factor of 1.1–18 or 1.5–24 in cell percentage or frequency analysis, respectively ($P < 0.001$).

Figure 3 shows representative chromatid and chromosome aberrations in N417 cells treated by N.S., $^{111}InCl_3$, BLM, and ^{111}In-BLMC. Many more bizarre chromosome aberrations were found in the ^{111}In-BLMC group.

DISCUSSION

X-irradiation induces chromosome aberrations in mammalian cells [16,17]. In addition, the order of the relative biological effectiveness (RBE) of different radiation emissions follows alpha rays > beta rays > γ rays (~ = X-ray). It has been reported by comparison of the chromosome aberrations with X-ray (220 Kv) and α-particles (5.3 Mev), that the RBE of α-particles was 15–25 for the induction of sister chromatid exchanges in mouse cells [18]. Therefore, α-emitters are a good candidate for radionuclide therapy of cancer, based on the high RBE for producing chromosome damage.

Currently, Auger emitters have been proposed for cancer therapy [19–23]. Auger electrons have very low energies and can deposit their energy locally (e.g., in 10Å) and may induce severe cell damage. Recent studies revealed that the DNA binding compound ^{125}IdU (iododeoxyuridine) was about 1.6 times more effective in killing V79 cells than 5.3 Mev α-particles from intracellularly localized ^{210}Po-citrate [21]. The experiments in vivo also indicated similar results, i.e., the Auger emitter ^{125}I bound to DNA was equally effective as the α-emitter ^{201}Po [22]. Therefore, Auger emitters are good candidates for consideration for cancer therapy. There are many radionuclides emitting Auger electrons, e.g., ^{51}Cr, ^{99}Tc, ^{59}Fe, ^{67}Ga, ^{111}In, ^{125}I, etc. ^{111}In is one that has more electrons and higher potential lethality for cells [23].

DNA breaks and chromosome aberrations by BLM have also been reported frequently [1,2,24]. BLM has high nuclear binding specificity and localizes to the nucleus of certain tumor cells. It is of interest to study what happens by labeling ^{111}In (an Auger emitter) to BLM for its ability to produce chromosome aberrations.

In these studies, the chromosome aberrations by $^{111}InCl_3$ were much lower than those of the ^{111}In-BLMC group. Our previous data [9] also indicated that there were no significant difference in cell survival between the

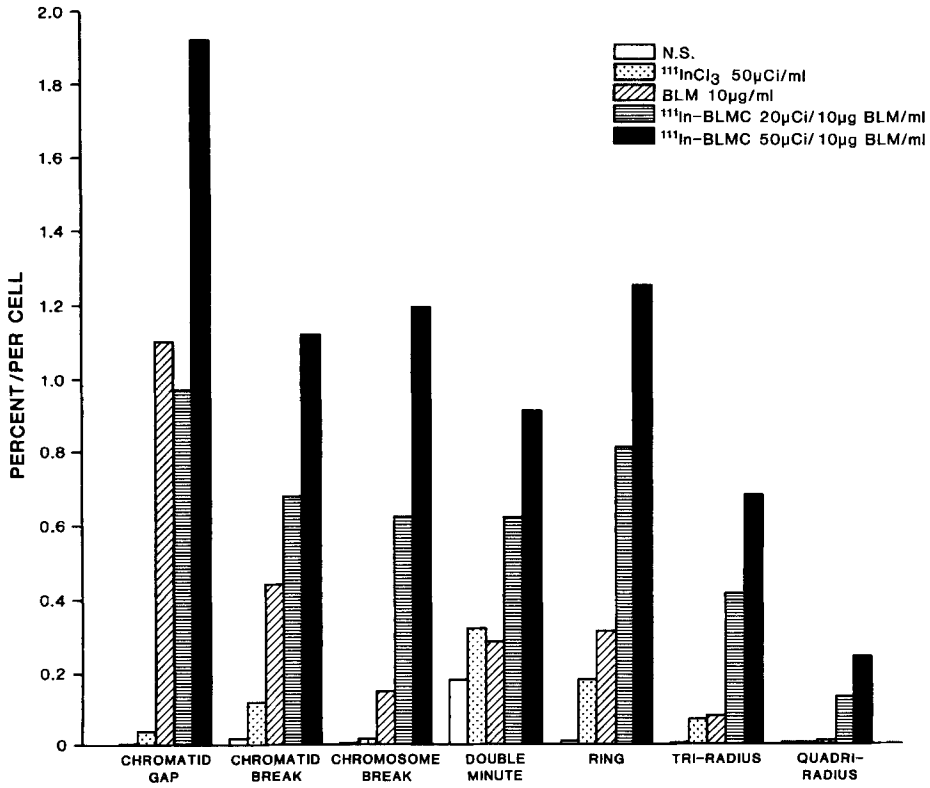

Fig. 2. The frequencies (percentage per cell) of chromatid and chromosome aberrations for N417 cells. Cells were treated at 37°C for 60 minutes by N.S. (0.9% NaCl); $^{111}InCl_3$ (50 μCi/ml); BLM (10 μg/ml); ^{111}In-BLMC (20 μCi/10 μg/ml), or ^{111}In-BLMC (50 μCi/10 μg BLM/ml). Same data are in Table II.

N.S. and $^{111}InCl_3$ groups. $^{111}InCl_3$ exposure also did not induce severe damage to SCLC cells. In addition, in these studies, BLM (10 μg/ml) alone induced more chromosome aberrations than those of the $^{111}InCl_3$ group. However, these aberrations of the BLM group were much lower than those of the radiolabeled ^{111}In-BLMC (20–50 μCi carried by the same amount of BLM, 10 μg/ml) group. Furthermore, these studies indicated that there was a dramatic synergistic effect of chromosome aberrations in the cells treated with ^{111}In-BLMC (especially with the higher radioactivity, 50 μCi/10 μg BLM/ml) in comparison with the BLM and $^{111}InCl_3$ groups. This means that the remarkable increase in chromatid and chromosome aberrations of SCLC cells by the ^{111}In-BLMC group was caused by the ^{111}In labeled BLM compound, i.e., by the combination chemo-radiotherapy of the cancer cells.

The mechanisms postulated for these results include the following:

1. ^{111}In is an Auger emitter and it (carried by BLM) localized mainly in the nuclei and DNA of the SCLC cells.

For intracellular ^{111}In exposure, as little as 4.4 mBq/cell (~4.4×10^3 Bq/ml of culture) was able to affect cell division, however, for extracellular ^{111}In, it had little effect even when the amount of radioactivity was increased to 1.15 MBq/ml (i.e., 2.6×10^2 times higher than that of intracellular concentration) [25]. The lethality of ^{111}In (Auger emitters) was mainly related to its nuclei and DNA-bound fraction [20,22,23]. ^{111}In-BLMC localizes to the cell nuclei and nuclear membrane (78.3%) as shown by autoradiography [10], and was bound to DNA (60% of nuclei fraction, unpublished data). Therefore, the Auger electrons from ^{111}In carried by BLM to specific binding sites in the DNA probably resulted in the greater yield of chromosome abnormalities.

2. BLM alone has activity as an alkylating agent and binds to DNA and causes DNA strand breaks [2,26].
3. BLM can enhance the cytotoxicity of radiation from ^{111}In for tumor cells if both are present in the nuclear target area at the same time. BLM and X-ray have been shown to interact synergically in producing increased cytotoxic effects [4,26]. BLM could therefore act as a radiosensitizer for ^{111}In radiations.

Our finding showed that ^{111}In-BLMC induced more chromosome aberrations than were caused by BLM alone for human SCLC cells. These studies indicate a potential

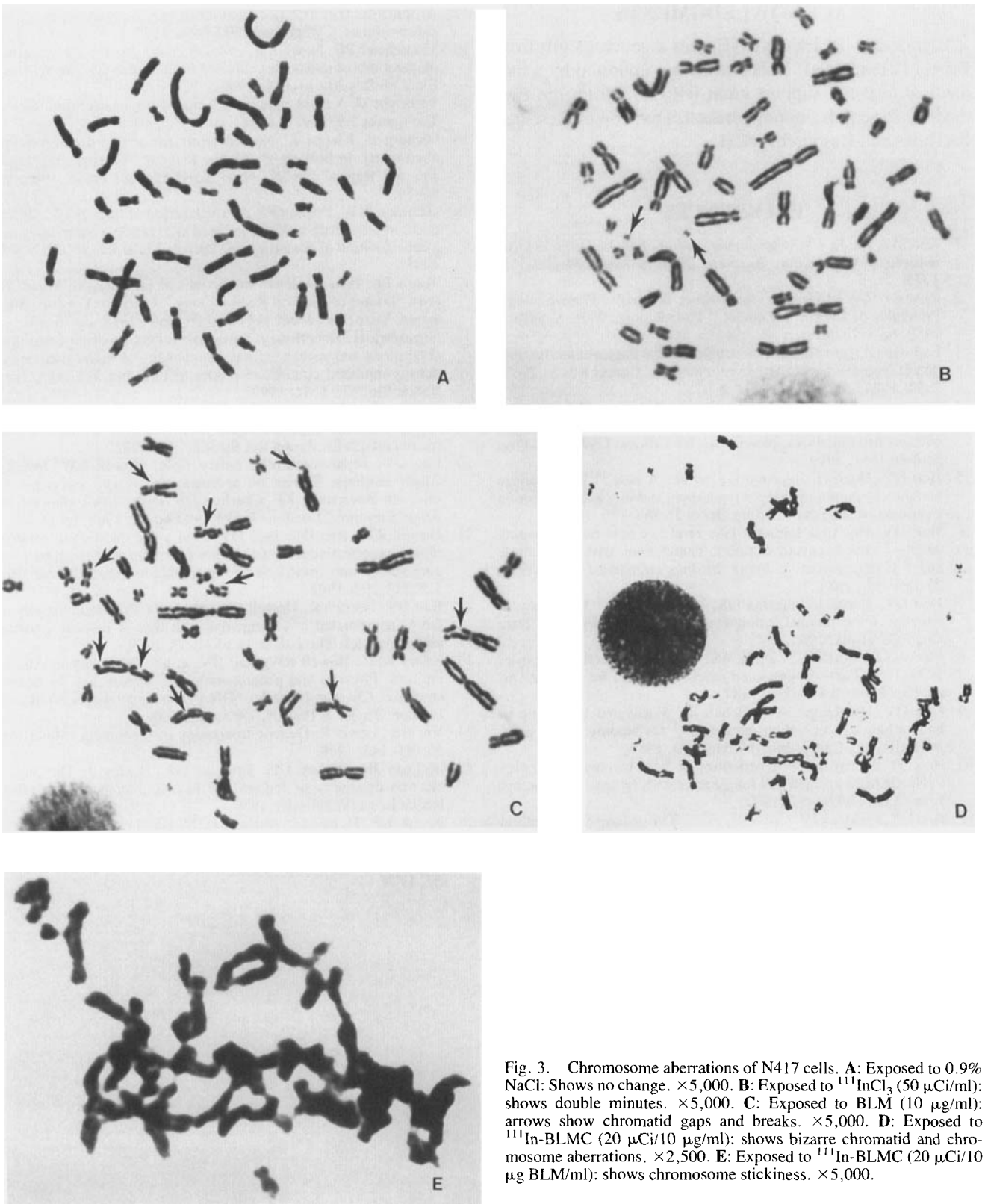

Fig. 3. Chromosome aberrations of N417 cells. **A**: Exposed to 0.9% NaCl: Shows no change. ×5,000. **B**: Exposed to $^{111}InCl_3$ (50 μCi/ml): shows double minutes. ×5,000. **C**: Exposed to BLM (10 μg/ml): arrows show chromatid gaps and breaks. ×5,000. **D**: Exposed to ^{111}In-BLMC (20 μCi/10 μg/ml): shows bizarre chromatid and chromosome aberrations. ×2,500. **E**: Exposed to ^{111}In-BLMC (20 μCi/10 μg BLM/ml): shows chromosome stickiness. ×5,000.

role for radiolabeled chemotherapeutic agents with high DNA, nuclear, and tumor specificity. When agents of high tumor specificity are radiolabeled with appropriate radioisotopes, they may prove useful for targetted tumor therapy by synergically increasing cytotoxicity of both agents.

ACKNOWLEDGMENTS

Bleomycin (BLENOXANE) was a generous gift from Bristol Laboratories. This work was supported by a biomedical research support grant RR05374 from the Biomedical Research Support Branch, Division of Research Facilities and Resources, NIH.

REFERENCES

1. Kuo MT, Haidle CW: Characterization of chain breakage in DNA induced by bleomycin. Biochem Biophys Acta 335:109–113, 1973.
2. Chabner BA: Bleomycin. In Chabner BA (ed): "Pharmacologic Principles of Cancer Treatment." Philadelphia: W.B. Saunders, 1982, pp 377–386.
3. Fujimoto J: Autoradiographic studies on the intracellular distribution of bleomycin-^{14}C in mouse tumor cells. Cancer Res 34:2969–2974, 1974.
4. Twentyman PR, Kallman RF, Brown JM: The effects of time between X-irradiation and chemotherapy on the growth of three solid mouse tumors vs. bleomycin. Int J Radiat Oncol Biol Phys 5:1499–1501, 1979.
5. Hou DY, Hoch H, Johnston GS, et al.: A new ^{111}In-bleomycin complex for tumor imaging: Preparation, stability and distribution in glioma-bearing mice. J Surg Oncol 25:168–175, 1984.
6. Hou DY, Hoch H, Johnston GS, et al.: A new tumor imaging agent—^{111}In-bleomycin complex. Comparison with ^{67}Ga-citrate and ^{57}Co-bleomycin in tumor bearing animals. J Surg Oncol 27:189–195, 1984.
7. Hou DY, Hoch H, Johnston GS, et al.: A new ^{111}In-bleomycin complex for combined radiotherapy and chemotherapy. J Surg Oncol 29:91–98, 1985.
8. Morstyn G, Idhe DC, Lichter AS, et al.: Small cell lung cancer 1973–1983: Early progress and recent obstacles. Int J Radiat Oncol Biol Phys 10:515–539, 1984.
9. Hou DY, Hamburger AW, Beach JL, Maruyama Y: Killing of human lung cancer cells using a new [^{111}In]bleomycin complex, [^{111}In/BLMC. Cancer Invest 7:543–550, 1989.
10. Hou DY, Maruyama Y: Distribution of ^{111}In-bleomycin complex (^{111}In-BLMC) in small cell lung cancer cells by autoradiography. J Surg Oncol 49:93–97, 1992.
11. Hou DY, Ordonez JV, Cross RJ, et al.: Killing lung cancer cells at cell-cycle phase by a new Indium-III- bleomycin complex. J Surg Oncol 40:73–78, 1989.
12. Arrighi FE, Hsu TC: Localization of heterochromatin in human chromosomes. Cytogenetics 10:81–86, 1971.
13. Moorehead PS, Nowell PC, Mellman WJ, et al.: Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 20:613–616, 1960.
14. Seabright M: A rapid banding technique for human chromosomes. The Lancet 2:971–972, 1971.
15. Seeberg E, Kleppe K: Nomenclature for acquired chromosome aberrations. In Seeberg E, Kleppe K (eds): "Chromosome Damage and Repair". ISCN. New York: Plenum Press, 1985, pp 66–76.
16. Heartlein MW, Preston RJ: An explanation of interspecific differences in sensitivity to X-ray induced chromosome aberrations and a consideration of dose-response curves. Mutat Res 150:299–305, 1985.
17. Travis EL: Basic biologic interactions of radiation. In Travis EL (ed): "Primer of Medical Radiobiology." Chicago, London, Boca Raton: Year Book Medical Publishers, Inc., 1989, pp 33–45.
18. Nagasawa H, Robertson J, Little JB: Induction of chromosomal aberrations and sister chromatid exchanges by alpha particles in density-inhibited cultures of mouse 1011/2 and 313 cells. Int J Radiat Biol 57:35–44, 1990.
19. Kassis AI, Adelstein SJ, Haydock C, Sastry KSR: Lethality of Auger electrons from the decay of Bromine-77 in the DNA of mammalian cells. Radiat Res 90:362–373, 1982.
20. Rao DV, Mylavarapu VB, Sastry KSR, Howell RW: Internal Auger emitters: Effects on spermatogenesis and oogenesis in mice. In Baverstock KF, Charlton DE (eds): "DNA Damage by Auger Emitters." London: Taylor and Francis, 1988, pp 15–25.
21. Howell RW, Rao DV, Hou DY, et al.: The question of relative biological effectiveness and quality factor for Auger emitters incorporated into proliferating mammalian cells. Radiat Res 128:282–292, 1991.
22. Rao DV, Narra VR, Howell RW, et al.: In-Vivo radiotoxicity of DNA-incorporated ^{125}I compared with that of densely ionising alpha-particles. The Lancet 16:652–653, 1989.
23. Sastry KSR, Howell RW, Rao DV, et al.: Dosimetry of Auger-emitters: Physical and phenomenological approaches. In Baverstock KF, Charlton DE (eds): "DNA Damage by Auger Emitters." London: Taylor & Francis, 1988, pp 27–38.
24. Vig BK, Lewis R: Genetic toxicology of bleomycin. Mutat Res 55:121–145, 1978.
25. McLean JR, Blakey DH, Douglas GR, Bayley J: The Auger electron dosimetry of Indium-111 in mammalian cells in vitro. Radiat Res 119:205–218, 1989.
26. Povirk LF, Hogan M, Dattagupta N: Binding of bleomycin to DNA, intercalation of the bithiazle rings. Biochemistry 18:96–101, 1979.