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Effect of Fluosol-DA/O_2 on the antitumor activity and pulmonary toxicity of bleomycin*

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Summary. The effect of an oxygen-carrying perfluorochemical emulsion on bleomycin antitumor activity and pulmonary toxicity was examined. Fluosol-DA (0.3 ml/mouse, i. v.), combined with bleomycin (10 or 15 mg/kg, i. p.) and a 2 h exposure to 95% oxygen (BFO) increased by five- to six-fold the tumor growth delay of FSaIIC fibrosarcoma compared to bleomycin alone (B). Only a slight increase in tumor growth delay was noted with the incomplete combinations of bleomycin and O_2 (BO) and bleomycin and Fluosol-DA (BF). When bleomycin (10 mg/kg) was co-administered with 0.3 ml Fluosol-DA and 95% O_2, cell survival was reduced ten-fold compared to that seen with bleomycin alone. In contrast, the surviving fraction of cells obtained from FSaIIC tumors treated in vivo indicated that 0.3 ml Fluosol-DA per mouse or a 2 h exposure to 95% O_2 did not markedly alter the effects of bleomycin alone.

The pulmonary effects of the BFO combination were assessed during the course of the therapy by bronchoalveolar lavage (BAL) analysis and pulmonary hydroxyproline (OH-Pro) content. Mice treated with this combination had a 20-fold increase in total numbers of cells obtained in the BAL compared to control animals. An increased cellularity in the lungs was also seen morphologically. The composition of the cells in the lavage fluid was altered after BFO but not BO treatment and reflected a neutrophilic influx. Furthermore, total protein recovered in the BAL fluid was increased 5-fold in the BFO treatment group compared to that in the control mice. Pulmonary OH-Pro, an index of collagen and fibrosis, was unaffected acutely after three treatments of either BFO or BO compared to control mice. Thus, Fluosol-DA and O_2 can enhance the antitumor activity of bleomycin. The increased pulmonary cellularity suggests that this combination may also have adverse effects on lung tissue.

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Abbreviations: B, bleomycin; BF, bleomycin/Fluosol-DA; BO, bleomycin/95% O_2; BFO, bleomycin/Fluosol-DA/O_2; BAL, bronchoalveolar lavage; OH-Pro, hydroxyproline

Introduction

Bleomycin is an antitumor antibiotic used in the treatment of testicular carcinomas, squamous cell carcinomas of the head and neck, and lymphomas [2, 8, 27, 29, 36]. The antineoplastic properties of bleomycin appear to result from the fragmentation of DNA [22], although other sites of action may exist [1]. While the precise chemical state of bleomycin within cells is uncertain, in vitro data suggest that a DNA-bleomycin-ferrous ion-dioxygen complex is formed, which, via oxidation of the bound ferrous ion, reduces molecular oxygen to yield highly reactive species [12, 15, 30]. In the presence of appropriate reducing agents, a ferric-ferrous redox cycle can occur, generating as many as 5000 reactive species per minute [3]. These reactive free radicals then produce both single- and double-strand breaks in DNA. Differences in oxygen availability also appear to alter the type of DNA fragmentation products seen [5, 9, 10]. The importance of molecular oxygen in the cytotoxic action of bleomycin has been demonstrated directly; bleomycin was significantly more toxic to normally aerated malignant cells than to cells maintained in a hypoxic atmosphere [31].

Because of the role of molecular oxygen in the antitumor action of bleomycin, hypoxic cells in solid tumors can be assumed to be inherently resistant to this drug [31]. Oxygen-carrying perfluorochemical emulsions, such as Fluosol-DA, may offer a novel mechanism to enhance bleomycin action. Previous results from this laboratory and others [21, 32, 33] have demonstrated that Fluosol-DA can increase the antitumor action of ionizing radiation, which also requires oxygen. One objective of this study was to evaluate the effect of Fluosol-DA and oxygen on the antitumor actions of bleomycin.

In contrast to most anticancer drugs, bleomycin causes little or no bone marrow damage. Its use has been limited by the occurrence of irreversible pulmonary fibrosis [17, 28, 38]. Although the precise mechanism of bleomycin-induced lung damage remains unknown, it is likely that the production of toxic oxygen radicals is involved [11]. A synergistic effect of oxygen supplementation and bleomycin on lung toxicity has been described [35]. Because perfluorochemicals are excreted via the lungs, they might influence this synergistic interaction. Thus, we have also evaluated the acute pulmonary toxicity of the bleomycin/Fluosol-DA/O_2 combination in mice.

Materials and methods

Drugs. Bleomycin (Blenoxane) was a gift from Bristol Laboratories (Syracuse, NY). Fluosol-DA, 20% (Green Cross Corporation, Japan) was provided by Alpha Therapeutic Corporation (Los Angeles, CA). The stem emulsion was stored at −20 °C and the complete emulsion was prepared immediately prior to use.

Tumor. FSaIIC fibrosarcoma [20, 32] was carried subcutaneously in the flanks of C3H/Be/FeJ male mice (Jackson Laboratories, Bar Harbor ME). For the experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted subcutaneously in the legs of C3H/Be/FeJ male mice 8–10 weeks of age. When the tumors were approximately 50 mm^3 in volume (about 1 week after tumor cell implantation), treatment was initiated.

Tumor growth delay experiments. The animals were given four injections of bleomycin of 10 or 15 mg/kg. The drug was administered intraperitoneally on days 6, 10, 13, and 16 after tumor implantation. Fluosol-DA (0.3 ml) was administered in the tail vein immediately following each drug dose and the animals were allowed to breathe air or were placed in a 95% oxygen/5% carbon dioxide (carbogen) atmosphere for 2 h then removed to air. The size of each tumor was measured using calipers thrice weekly until it reached a volume of 500 mm^3. Untreated FSaIIC tumors reached 500 mm^3 in about 12 days. Each treatment group had seven animals and the experiment was repeated three times; therefore, each point represents the treatment outcome for 21 animals.

Tumor cell survival experiments. FSaIIC tumor cells (2×10^6 cells), a subline of the FSaII fibrosarcoma that had been adapted for growth in culture [32], were implanted subcutaneously in the legs of C3H/Be/FeJ male mice 8 to 10 weeks of age. One week after tumor cell implantation, when the tumors were about 50 mm^3 in volume, the animals were given an intraperitoneal injection of bleomycin (10 mg/kg) immediately followed by intravenous administration of Fluosol-DA (0.3 ml) and 2 h carbogen breathing. Twenty-four hours after drug treatment, mice were killed and soaked in 70% ethanol. Their tumors were excised under sterile conditions in a laminar flow hood and were minced to a fine brei with small curved scissors. Four tumors (approximately 1 g total) were pooled to make each treatment group. Each brei was suspended in 20 ml Dulbecco's phosphate-buffered saline (Gibco, NY) containing deoxyribonuclease (93 μg/ml; Sigma) and trypsin (1.85 mg/ml; Gibco) in a 50-ml plastic centrifuge tube. The samples were incubated for 10 min at 37 °C, after which the cells were allowed to settle and the liquid was gently decanted and discarded. Tumor homogenates were resuspended in the enzyme-containing phosphate-buffered saline (PBS), mixed (Vortex mixer), incubated and rocked for 10 min, and mixed again. The deoxyribonuclease concentration was then increased to 2.5 mg/ml in each tube. After being thoroughly mixed, each sample was filtered through a 135-μm stainless steel mesh in a Nucleopore Swin-Lok holder into a 50-ml plastic centrifuge tube. The samples were centrifuged (E-model PR-5) at 500 *g* and 4 °C for 10 min, after which the supernatant was decanted and the pellets resuspended in Eagle's MEM containing 10% fetal bovine serum and antibiotics (Gibco, NY). The samples were centrifuged again, the supernatant fractions decanted, and the pellets resuspended in Eagle's MEM containing 10% fetal bovine serum and antibiotics. These suspensions were examined microscopically to ascertain that single cells were present. The number of viable cells was determined by trypan blue exclusion and the cells were plated for the colony-forming assay [32]. Cultures were incubated for 8 days in carbogen. Survival of a known number of tumor cells was determined with a colony-forming assay. Only colonies of >50 cells were counted. The control tumor cell plating efficiency was 5%–15%.

Pulmonary toxicity studies. To determine pulmonary toxicity during the course of therapy, we administered three intraperitoneal injections of bleomycin (15 mg/kg) on days 1, 4, and 8 to normal C3H/Be/FeJ male mice 8–10 weeks of age. Fluosol-DA (0.3 ml) was administered intravenously immediately after the bleomycin in the combined treatment group. Treatment groups were exposed for 2 h to either carbogen or air immediately after drug treatment, and then housed in air. Lungs were prepared for bronchoalveolar lavage (BAL), hydroxyproline (OH-Pro) assay and histology 4 days after the last drug injection.

Bronchoalveolar lavage and analysis of cells and fluid. The trachea was isolated and a tracheotomy performed. The abdomen was opened and the diaphragm cut. A 20-gauge plastic cannula was inserted into the trachea and tied in place. BAL was performed by a modification of our method for lavage of rat lungs [18, 19]. Saline was instilled in three 1-ml aliquots. Each aliquot was instilled and aspirated three times prior to collection by gentle syringe aspiration. Recovered aliquots were pooled, fluid recovery was measured with calibrated pipets, and cellular elements were counted by hemacytometer at high (400×) power. The fluid was then centrifuged (500 *g*, 4 °C, 10 min) and the supernatant removed from the cell pellets and stored at −20 °C for subsequent analysis. Cells were resuspended in Hanks balanced salt solution (Ca^{2+} and Mg^{2+}-free) and aliquots containing $50–100 \times 10^3$ cells were applied to glass slides by cytocentrifugation. Slides were stained with Wright's stain and 300 cell differential counts were performed. Total protein in supernatant fluid was assessed by an automated spectrophotometric method [13]. Data are expressed as total cells or protein recovered per lavage. This final value was calculated by multiplying the cellular or protein concentration by the recovered lavage volume.

Hydroxyproline assay. Since pulmonary OH-Pro is derived almost exclusively from collagen [4], whole pulmonary collagen content was estimated by measuring OH-Pro content. Following BAL, both lungs were removed, washed free of blood in PBS, and placed in 2 ml 6 *M* HCl. Trace amounts of [^{3}H] OH-Pro (0.2 μCi; New England Nuclear, Mass) were added to each sample as an internal standard for recovery. After sealing the tubes, the samples were heated for 24 h at 110 °C, resuspended in 20 ml g. d. H_2O and neutralized to pH 6–8 with NaOH. The sample was filtered through a Whatman paper filter and the eluant was assayed for OH-Pro using the spectrophotometric method of Woessner [39]. All values were corrected for recovery of radiolabeled OH-Pro, which was 60%–79%.

Histopathologic assessment of the lung. Animals were prepared as described for lavage except that the lungs were inflated with 4% buffered formalin at 20 cm H_2O distending pressure after tracheal cannulation. The left upper lobe was then embedded in paraffin and cut sections were stained with hematoxylin and eosin or Masson trichrome. Representative areas were photographed at 100× and 400× magnification [7, 24].

Data analysis. Data were analyzed by one-way analysis of variance (ANOVA). Differences between subgroup means were assessed by referring the calculated value of t to a t distribution corrected for multiple tests by the Bonferroni procedure [6]. Tumor growth delay data were analysed using unpaired *t*-tests and with the Dunn multiple-comparisons test after a very significant effect was found by ANOVA [32].

Results

In tumor growth delay experiments with four doses of bleomycin given at 4-day intervals, there was approximately five-fold enhancement of bleomycin antitumor activity in combination with Fluosol-DA and carbogen breathing (BFO; Table 1). There was no significant difference between the tumor growth delay achieved with either 10 mg/kg or 15 mg/kg bleomycin under any of the experimental treatment conditions; however, treatment with the higher dose of drug produced greater weight loss in the animals which was not influenced by the presence of Fluosol-DA. Bleomycin and carbogen breathing (BO) produced a significant increase in tumor growth delay compared to bleomycin alone (B; $p < 0.05$). BFO led to a markedly improved treatment outcome compared with B ($p < 0.0001$). BFO also produced a significant increase in tumor growth delay compared to BO and bleomycin plus Fluosol-DA (BF; $p < 0.0001$ and $p < 0.0003$ respectively).

Using the cell survival assay with the FSaIIC fibrosarcoma, there was approximately a ten-fold increase in tumor cell kill in animals treated with BFO compared with B (Table 2). Fluosol-DA alone had no effect on the survival of the tumor cells. With a single dose of B, BO, or BF there was no significant cell killing; however, BFO produced approximately one log of cell kill.

Hydroxyproline content, histology, and cellular and protein composition of the lungs of treated and normal mice were examined to determine the acute pulmonary toxicity of the combination BFO. Lavage fluid recovery was 2.15 ml/control mouse and was similar in all groups examined in Fig.1 ($p > 0.1$ for all comparisons, unpaired *t*-test). Animals treated with BO had increases in the mean values for all the observed cell populations and in total BAL protein; however, these values were not statistically significantly different from control ($p > 0.05$ for all comparisons). By contrast, animals receiving the combination BFO had significant increases in all cell populations and in total recoverable lavage protein. For example, the triple combination resulted in a five-fold increase in total pulmonary lavage protein compared to control mice and a 2.5-fold increase compared to the BO group. The total cell number in the lavage fluid of mice treated with BFO was 20- and five-fold greater than that seen in control and BO mice respectively ($p < 0.05$ for BFO compared to BO). Among the morphologically distinguishable cells in lavage fluid, a marked elevation was observed in alveolar macrophages (20-fold), lymphocytes (45-fold) and neutrophilic granulocyte numbers (560-fold) compared to control mice. Significant increases in these cell types were also seen with BFO compared with the BO group.

As shown in Table 3, 4 days after the last drug injection there was no difference in OH-Pro content between lungs of animals receiving the combination BFO and lungs of control or BO animals.

Histological assessment of animal lungs revealed findings similar to those illustrated in Fig. 2. An apparent increase in cellularity was noted in sections obtained from animals treated with BFO. The most prominent cells were large alveolar macrophages. Alveolar walls appeared thickened, and sections from one of three examined animals treated with the combination showed evidence of alveolar wall fibrosis on Masson staining.

Table 1. Enhancement in FSaIIC fibrosarcoma tumor growth delay

Treatment group	Tumor growth delay (days)[a]	
	Bleomycin dose[b] 10 mg/kg	15 mg/kg
B	2.3 ± 0.7	3.7 ± 1.0
BO[c]	4.7 ± 1.0	5.6 ± 1.5
BF	5.5 ± 2.3	5.9 ± 1.6
BFO	14.6 ± 2.9	16.9 ± 2.9

B, bleomycin; BO, bleomycin + carbogen; BF, bleomycin + Fluosol-DA; BFO, bleomycin + Fluosol-DA + carbogen

[a] Based on days for the tumors to reach 500 mm^3 in volume compared to untreated controls. The experiment was repeated three times with groups of seven animals in each condition and untreated controls. Means ± SEM

[b] Bleomycin was given on days 6, 10, 13, and 16. Fluosol-DA was given immediately after bleomycin

[c] Carbogen breathing was maintained for 2 h

Table 2. Cell survival from FSaIIC fibrosarcoma tumors

Treatment group	Surviving fraction
Control	1.00 ± 0.01
F[a]	1.00 ± 0.01
B[b]	0.90 ± 0.01
BO[c]	0.95 ± 0.01
BF	0.84 ± 0.01
BFO[c]	0.096 ± 0.02

Tumors were excised 24 h after treatment. Values are means of five separated experiments ± SEM. For abbreviations, see Table 1

[a] Fluosol-DA was given as an intravenous injection of 0.3 ml

[b] Bleomycin was given as an intraperitoneal injection of 10 mg/kg

[c] Carbogen breathing was maintained for 2 h

Discussion

Oxygen has been implicated as directly involved in the mechanism of cytotoxicity of several antineoplastic agents, including bleomycin, adriamycin streptonigrin, and the hematoporphyrins. Previously it was demonstrated that removing oxygen from cells or DNA being treated with bleomycin significantly reduced or completely inhibited

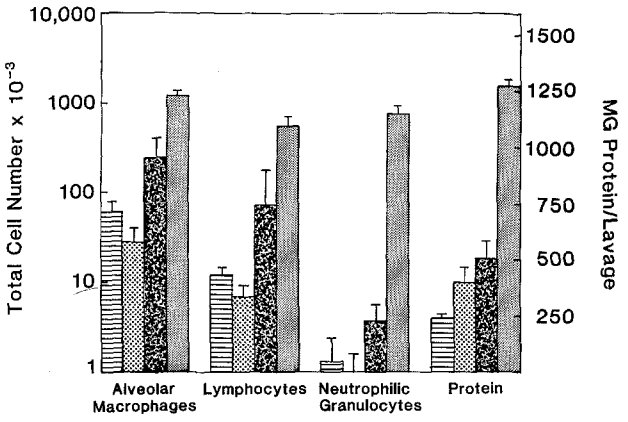

Fig. 1. Bronchoalveolar lavage assessment of whole lungs after bleomycin (15 mg/kg) and 95% O_2 (2 h) on days 1, 4, and 8 with and without Fluosol-DA (n = 5–7). ⊟, controls; ▨, FO; ▩, BO; ▩, BFO. Means ± SEM

damage by the drug [23]. In this study, we have demonstrated that intravenous Fluosol-DA and carbogen breathing for 2 h following intraperitoneal administration of bleomycin significantly improved tumor growth delay and enhanced the tumor cell kill produced by this drug. It is interesting that tumor growth delay was independent of bleomycin dose in the range tested. The addition of carbogen or Fluosol-DA increased the growth delay but neither intervention alone produced a significant effect. These findings suggest that intratumor oxygen may limit the therapeutic action of bleomycin. We propose that Fluosol-DA with carbogen breathing allows increased oxygen delivery to previously hypoxic areas and thus enhances the antitumor activity of bleomycin. We cannot rule out a pharmacokinetic component of drug enhancement. Bleomycin solvation on the perfluorochemical particles might result in an increase in the drug's exposure-time index.

Table 3. Hydroxyproline content of whole lungs

Treatment group	Hydroxyproline (μg)
Control	378 ± 28
BO	356 ± 47
BFO	382 ± 35

Hydroxyproline was measured in both lungs [16]. Bleomycin (15 mg/kg) was administered on days 1, 4, and 8. Groups receiving Fluosol-DA were given it immediately after bleomycin. Carbogen breathing was maintained for 2 h. For abbreviations see Table 1

It has been shown that complete transfusion of animals with perfluorochemical emulsions produces no acute pulmonary toxicity [25, 26]. Bleomycin, however, produces dose-limiting pulmonary toxicity, which may be exacerbated by elevated ambient oxygen tension [35]. The oxygen-carrying perfluorochemicals are excreted by expira-

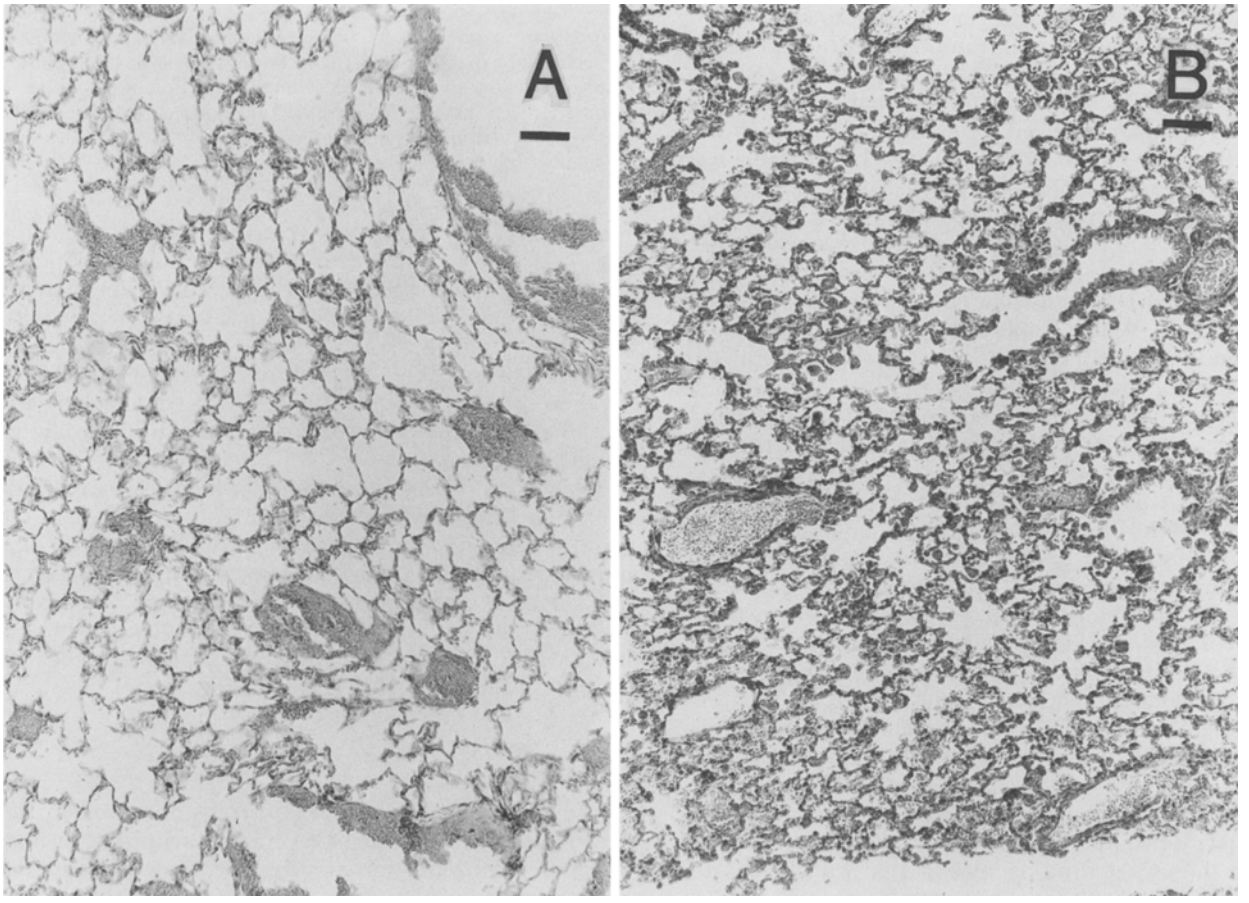

Fig. 2 A, B. Lung sections from mice. Inflated lungs from control **(A)** and BFO-treated **(B)** mice were fixed and portions of the left lobe were embedded in paraffin, cut, and stained with hematoxylin and eosin. *Bar* = 20 μm

tion and may deliver increased amounts of oxygen to bleomycin residing in the pulmonary parenchyma. Thus, we examined the lungs of treated animals for potentiation of or protection from bleomycin-induced pulmonary toxicity. During BFO treatment we observed no increase in collagen content as measured by OH-Pro shortly after treatment, indicating that there is probably no major change in the kinetics of OH-Pro deposition in the lungs. BAL of BFO-treated animals indicated a marked increase in cellularity and protein recovery. The increase noted in the numbers of polymorphonuclear leukocytes and alveolar macrophages in lavage specimens of BFO-treated animals suggest the recruitment of both monocytes and polymorphonuclear leukocytes to the alveolar structures. Both cell types can secrete mediators which injure cells [37]. The increase in lavage protein suggests a toxic effect of treatment on the alveolar wall, leading to increased protein flux. Athough we did not reproducibly see pulmonary fibrosis in histologic specimens, the alterations in lavage cells and protein suggest significant lung injury. The consequences of these acute changes cannot be predicted from our results, and these pulmonary derangements may be transient or may portend irreversible lung damage.

In conclusion, the antitumor activity of bleomycin was potentiated by Fluosol-DA and carbogen breathing; however, caution must be expressed regarding possible enhancement of pulmonary toxicity.

References

1. Berry DE, Kilkuskie RE, Hecht SM (1985) DNA damage induced by bleomycin in the presence of bibucaine is not predictive of cell growth inhibition. Biochemistry 24: 3214–3219
2. Carter SK, Crooke ST, Umezawa H (eds) (1978) Bleomycin: current status and new developments. Academic, New York
3. Caspary WJ, Niziak C, Lango DA, Friedman R, Bachur NR (1979) Bleomycin A_2: a ferrous oxide. Mol Pharmacol 16: 256–260
4. Crystal RG (1974) Lung collagen, definition, diversity and development. Fed Proc 33: 2246–2255
5. Cunningham ML, Ringrose PS, Lokesh BR (1984) Inhibition of the genotoxicity of bleomycin by superoxide dismutase. Mutat Res 135: 199–202
6. Duncan RC, Knapp RG, Miller MC (1983) Introductory biostatistics for the sciences. Wiley, New York
7. Dungworth DL, Schwartz LW, Tyler WS (1976) Morphological methods for evaluation of pulmonary toxicity in animals. Ann Rev Pharmacol 16: 381–399
8. Ervin TJ, Weichselbaum R, Miller D, Meshad M, Posner M, Fabian R (1981) Treatment of advanced squamous cell carcinoma of the head and neck with cisplatin, bleomycin and methotrexate (PBM). Cancer Treat Rep 65: 787–791
9. Kross J, Henner WD, Haseltine WA, Rodriguez L, Levin MD, Hecht SM (1982) Structural basis for the deoxyribonucleic acid affinity of bleomycin. Biochemistry 21: 3711–3721
10. Kross J, Henner WD, Hecht SM, Haseltine WA (1982) Specificity of deoxyribonuclease acid cleavage by bleomycin, phleomycin and tallysomycin. Biochemistry 21: 4310–4317
11. Lazo JS, Humphreys CJ (1983) Lack of metabolism as the biochemical basis of bleomycin-induced pulmonary toxicity. Proc Natl Acad Sci USA 80: 3064–3068
12. Lown JW, Kim S-K (1977) The mechanism of the bleomycin-induced cleavage of DNA. Biochem Biophys Res Commun 77: 1150–1157
13. Merrill WW, O'Hearn E, Rankin JR, Naegel GP, Matthey RA, Reynolds R (1982) Kinetic analysis of respiratory tract proteins recovered during a sequential lavage protocol. Am Rev Resp Dis 126: 610–617
14. Moulder JE, Rockwell S (1984) Hypoxic fractions of solid tumors: experimental techniques, methods of analysis, and a survey of existing data. Int J Radiat Oncol Biol Phys 10: 695–712
15. Oberley LW, Buettner GR (1979) The production of hydroxy radical by bleomycin and iron (II). FEBS Lett 97: 47–49
16. Orr FW, Adamson IYR, Young L (1986) Promotion of pulmonary metastasis in mice by bleomycin-induced endothelial injury. Cancer Res 46: 891
17. Parvinen LM, Kilkku P, Makinen E, Luikko P, Gronroos M (1983) Factors affecting the pulmonary toxicity of bleomycin. Acta Radiol 22: 417–421
18. Rankin JR, Merrill WW, Hitchcock M, Askenase PW (1982) IgE dependent release of leukotriene C4 from alveolar macrophages. Nature 297: 329–331
19. Rankin JR, Hitchcock M, Merrill WW, Huang SS, Brashler FR (1984) IgE immune complexes induce immediate and prolonged release of leukotriene C4 (LTC4) from rat alveolar macrophages. J Immunol 132: 1993–1999
20. Rice L, Urano M, Suit HD (1980) The radiosensitivity of a murine fibrosarcoma as measured by three cell-survival assays. Br J Cancer 41 [Suppl 4]: 240–245
21. Rochwell S (1985) Use of a perfluorochemical emulsion to improve oxygenation in a solid tumor. Int J Radiat Oncol Biol Phys 11: 97–103
22. Sausville EA, Peisach J, Horwitz SB (1978) Effect of chelating agents and metal ions on the degradation of DNA by bleomycin. Biochemistry 17: 2740–2746
23. Sausville EA, Stein RW, Peisach J, Horwitz SB (1978) Properties and products of the degradation of DNA by bleomycin and iron (II). Biochemistry 17: 2746–2754
24. Schachter EN, Buck MG, Merrill WW, Askenase PS, Witek TJ Jr (1986) Skin testing with an aqueous extract of cotton bract. J Allergy Clin Immunol (in press)
25. Schneeberger EE (1982) Circulating proteins and marcomolecular transport across continuous nonfenestrated endothelium. Ann NY Acad Sci 1982: 25–37
26. Schneeberger EE, Neary BA (1982) The bloodless rat: a new model for macromolecular transport studies across lung endothelium. Am J Physiol 242 (Heart Circ Physiol 11): H890–H899
27. Shah PM, Shukla SN, Patel KM, Baboo HA, Patel DD (1981) Effect of bleomycin-radiotherapy combination in management of head and neck squamous cell carcinoma. Cancer 48: 1106–1109
28. Sikic BI, Young DM, Mimnaugh EG, Gram TE (1978) Quantification of bleomycin pulmonary toxicity in mice by changes in lung hydroxyproline content and morphometric histopathology. Cancer Res 38: 787–792
29. Spaulding MB, Kahn A, De Los Santos B, Klotch D, Lore JM (1982) Adjuvant chemotherapy in head and neck cancer; an update. Am J Surg 144: 432–436
30. Sugiura Y (1979) Production of free radicals from phenol and tocopherol by bleomycin-iron (II) complex. Biochem Biophys Res Commun 87: 649–653
31. Teicher BA, Lazo JS, Sartorelli AC (1981) Classification of antineoplastic agents by their selective toxicities toward oxygenated and hypoxic tumor cells. Cancer Res 41: 73–81
32. Teicher BA, Rose CM (1984) Perfluorochemical emulsions can increase tumor radiosensitivity. Science 223: 934–936
33. Teicher BA, Rose CM (1984) Oxygen-carrying perfluorochemical emulsion as an adjuvant to radiation therapy in mice. Cancer Res 44: 4285–4288
34. Thrall RS, McCormick JR, Jack RM, McReynolds RA, Ward PA (1979) Bleomycin-induced pulmonary fibrosis in the rat. Am J Pathol 95: 117–130
35. Tryka AF, Skornik WA, Godleski JJ, Brain JD (1982) Potentiation of bleomycin-induced lung injury by exposure to 70%

oxygen: morphologic assessment. Ann Rev Resp Dis 126: 1074–1079
36. Twentyman PR (1984) Bleomycin-mode of action with particular reference to the cell cycle. Pharm Therapeut 23: 417–441
37. Weiss SJ, Regiani S (1984) Neutrophils degrade subendothelial matrices in the presence of alpha-1-proteinase inhibitor. J Clin Invest 73: 1297–1303
38. Willson JKV (1978) Pulmonary toxicity of antineoplastic drugs. Cancer Treat Rep 62: 2003–2009
39. Woessner JF (1961) The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch Biochem Biophys 93: 440–447

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