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COMMENTARY

ENDOTHELIAL INJURY CAUSED BY ANTINEOPLASTIC AGENTS

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Current antitumor agents damage both malignant and nonmalignant tissues; thus, a major pharmacologic goal remains the development of highly selective nontoxic therapies for the treatment of malignancies. Although many of our antineoplastic agents act by destroying rapidly proliferating cells, apparently with little regard for the malignant phenotype, some anticancer drugs injure tissues that lack a high mitotic index. With certain agents in this latter group, one of the first observable sites of toxicity is the endothelium [1–6]. The goal of this review is to examine the propositions that: (a) damage to the vascular endothelium by antineoplastic agents may be responsible, at least partially, for the unwanted toxicity manifested by nonmalignant tissues; and (b) endothelial cell damage by these agents may represent a novel target for the desired destruction of solid tumors.

Vascular endothelial biology

Almost all tissues depend upon blood, which flows through vessels lined with endothelium. The unique and strategic location of the vascular endothelium at the interface of blood and tissues imposes structural and functional demands. Thus, endothelial cells have evolved into highly specialized cells, which maintain a nonthrombogenic surface and regulate the movement of many circulating elements into and out of tissues. It is well recognized that the endothelium is both a physical and a metabolic barrier for many endogenous and exogenous substances [7, 8]. Important for the transendothelial transport properties of this cell type is the active vesicular transport system, which utilizes plasmalemmal structures termed caveolae. In addition, numerous receptors for endogenous circulating substances appear to exist on the luminal surface of the endothelium, some in the caveola, providing the essential means for attachment and subsequent movement across the endothelium into the surrounding tissue. Specific macromolecules (such as thrombomodulin and Factor VIII antigen) and enzymatic activities (such as angiotensin converting enzyme and 5′-nucleotidase) are also localized on the luminal surface of the plasma membrane of the endothelium. These surface endothelial components play an important homeostatic role and regulate interactions between blood-borne elements and the interstitium.

The endothelium exhibits regional morphological differences in intercellular junctions that form the basis of three distinct categories: (a) continuous endothelium, found in most arteries, veins and capillaries of the brain, lungs and skeletal muscle; (b) fenestrated endothelium, found in certain visceral capillaries, such as the adrenal gland; and (c) discontinuous endothelium, found in the sinusoids of the liver, spleen, and bone marrow [7, 8]. In addition, the organization of the gap junctions in the arterioles, arteries, capillaries, and veins of an organ appears to be different. Morphologic differences also exist in size and thickness (aortic cells thicker than those of the capillaries and veins), subcellular organelle content (Weibel–Palade bodies), and density of plasmalemmal vesicles (highest in capillaries). Accordingly, these distinct intra- (large versus small vessels) and inter-organ morphologic features may reflect altered functional characteristics. Consistent with this idea, differences in the biochemical composition of endothelial cells from various vascular beds have been reported. For example, the distribution of anionic and cationic binding sites on the plasmalemma of endothelial cells appears to be nonrandom and may vary in cells from different vascular beds [9]. Comparative studies with freshly isolated coronary microvessels and coronary arteries and veins indicate regional differences in prostaglandin synthetic capacity [10]. Results with short-term cultures that allow the generation of large numbers of cells to study biochemical and cellular physiological properties also suggest some differences. For example, Johnson [11] found that cultured endothelial cells from arteries had three to five times the angiotensin converting enzyme activity of cultured endothelial cells from veins. Significant differences are not always seen, however. In studies on phosphorylation systems, little difference has been noted in the protein kinase activities or endogenous substrate content of cultured bovine endothelial cells from the pulmonary artery compared to bovine aortic endothelial cells [12]. Since constitutive phenotypic expression may reflect the surface upon which this cell type resides, cells in culture may not display the characteristics seen *in vivo* in the absence of the normal basement membrane [13]. Thus, results from cultured cell models must be viewed with some caution until verified *in vivo*. An additional problem with these models has been the difficulty associated with obtaining and successfully culturing capillary endothelium from many vascular beds; this has limited comprehensive comparative studies of the capillary endothelium. This may reflect the lack of growth factors and the use of nonphysiologic plastic substrata or sera for growth. The development of more sophisticated culturing conditions could allow for the identification of biochemical and functional features unique to the endothelium of specific organs, which

may provide insight into interactions between toxins and the vascular bed.

As with normal tissue, the endothelium plays a central role in controlling the growth of solid tumors. Detailed comparative studies of the differences between endothelium from normal and malignant tissues have not been conducted, although tumor blood vessels are generally more disorganized, tortuous, and dilated than blood vessels in normal tissues [14]. The endothelium from transplantable murine tumors does exhibit a much greater rate of proliferation. Hobson and Denekamp [15], for example, observed a doubling time in the normal tissue endothelium *in vivo* that was 20–2000 times longer than that seen in the endothelium of murine tumors (2.4 to 13 days). This agrees favorably with data derived from other laboratories [14, 16]. This rapid mitotic activity of endothelium in tumors is believed to be due to angiogenesis factors elaborated by tumor cells that stimulate the capillary endothelial cells to divide and to express proteolytic enzymes, such as plasminogen activator, which enables them to migrate through the basement membrane toward the tumor [14–16]. Endothelial cells proliferate in sufficient numbers to generate functional capillaries capable of delivering the requisite nutrients and oxygen for further tumor growth [14–16]. It is possible that these rapidly proliferating cells may have different transporters and biochemical properties than those in normal tissues.

Toxicity of anticancer agents to endothelium of normal tissues

The morphologic and metabolic properties of endothelial cells and their plasma membrane are major factors in maintaining a proper functional relationship between the blood and either normal or malignant tissues. In addition, the endothelium may control, through a poorly detailed mechanism, interstitial (e.g. fibroblast) cell migration and proliferation [17]. As mentioned above, sufficient heterogeneity exists in the properties of endothelial cells to allow speculation that some selective interaction could occur between pharmacologic agents and specific vascular beds. Obviously, if the agent were toxic, disruption of the endothelium and its attendant properties could have significant adverse effects on normal or tumor tissues. The herbicide paraquat, hyperoxia, and endotoxin all initially damage the endothelium and can cause significant organ toxicity [18–22]. Many antitumor agents are administered intravenously and are highly reactive. Consequently, the drugs presumably are presented to the endothelium in relatively high concentrations, at least for brief periods, and it is reasonable to consider them as potential toxins to the endothelium.

The best studied aspects of endothelial injury are the morphological changes, especially those in the pulmonary vasculature, Therefore, we will focus most of our attention on injury to that organ. The pulmonary endothelium appears to have a limited morphological repertoire of responses to toxins. Thus, with endotoxin [22], hyperoxia [21], antineoplastic agents [1–6, 23–25], or ionizing irradiation [17], the morphologic manifestations of the endothelial damage in the lungs are similar and do not offer a reliable method for distinguishing the etiological agent. These ultrastructural alterations due to acute injury appear in the following chronological order: (a) formation of blebs, which are cytoplasmic extrusions located on the luminal surface of the endothelium, (b) autolytic vacuole formation, (c) intracytoplasmic edema, and (d) detachment of degenerated endothelium [7]. Complementing these morphologic aberrations, biochemical and functional changes after toxin exposure also have been observed, as outlined below.

Morphological and biochemical investigations in both man and animals indicate that pulmonary endothelial damage occurs soon after treatment with some antitumor agents, including bleomycin, cyclophosphamide, nitrosoureas and ionizing irradiation.

(a) *Bleomycin.* Best studied has been bleomycin, which produces a dose-limiting pulmonary toxicity that often is fatal [24, 25]. Adamson and Bowden [2] first reported in mice that the initial site of pulmonary toxicity after bleomycin treatment is to the capillary endothelium. Within 2 weeks after repeated bleomycin injections, bleb formation is seen on endothelium of the pulmonary arteries and veins followed by intracytoplasmic edema and capillary involvement by 4 weeks. Subsequent studies by a number other investigators [1–5, 26] confirmed these morphologic changes. Aso *et al.* [1], in fact, found that pulmonary capillary endothelial damage occurs as soon as 3 days after bleomycin injection. Biochemical abnormalities, which may reflect damage associated with the endothelium, also appear to be a prominent feature of bleomycin treatment. For example, bleomycin appears to decrease the single-pass transpulmonary removal of norepinephrine, 5-hydroxytryptamine, and prostaglandin E_2 (PGE_2) [3–5, 27]. These processes are believed to be mediated by the endothelium. Some circulating substances, such as serum PGE_2, thromboxane B_2, 6-keto-PGF_1 and angiotensin converting enzyme, are also believed to reflect endothelial integrity and are altered by bleomycin [3–5, 28]. Many of these changes may precede pulmonary fibrosis and may be reversible [3]. Evidence of endothelial damage by intravenous or intraarterial injection of bleomycin has also been reported as an untoward effect in humans [26, 29–31]. For example, Burkhardt *et al.* [26] found detachment of the arteriole endothelium from the underlying tissue in addition to endothelial edema, intracytoplasmic vacuole formation, and luminal projections. Pohler *et al.* [30] identified changes in human serum angiotensin converting enzyme activity after bleomycin therapy. Hilgard and Hossfeld [31] observed a transient decrease in the peripheral platelet counts in patients treated with bleomycin and postulated that platelets were sequestered in lung capillaries due to bleomycin-induced early endothelial damage. Thus, it seems likely that bleomycin produces endothelial damage in both animals and humans.

The molecular mechanism by which bleomycin produces endothelial injury is unclear. Bleomycin, when complexed with iron(II) and fueled with reducing equivalents, can catalytically generate radical species perhaps at the rate of 5000 moles per min per mole of bleomycin, which could react with cellular

nucleophiles, most notably DNA and membranes [32]. Binding to plasma membrane of malignant cells and alterations in plasma membrane enzyme function have been observed [33–35]. Whether similar events occur with endothelial cells remains to be established, although exposure of cultured bovine endothelial cells to bleomycin can induce cellular retractions and alters platelet and tumor cell binding [35]. It seems possible that damage to the pulmonary endothelium could result in more drug entering the interstitial space and interacting with fibroblasts or pneumocytes [25]. Alternatively, loss of endothelium could reduce the degradation of substances, such as bleomycin or PGE_2, or block the release of factors, which affect collagen production and the interstitial extracellular matrix [27]. This may explain the observed enhanced pulmonary toxicity reported with bleomycin and either X-irradiation or oxygen [24].

The relative sensitivity of the pulmonary endothelium to bleomycin may relate to the levels of bleomycin hydrolase, which cleaves ammonium from the carboxamide moiety on the β-alanine region of bleomycin to yield the inactive desamidobleomycin product [36, 37]. The cytosolic enzyme, which has not been fully characterized, is heterogenously expressed in pulmonary cells. In rabbits but not bovine pulmonary artery endothelial cells high levels are seen compared to that found in epithelial cells [37]. The levels of bleomycin hydrolase in pulmonary cells from other species have not been determined, nor is much known about the intracellular regulation of the enzyme activity. Antibodies to this enzyme should permit cellular mapping in lung tissue. It is possible that reported murine strain differences [38] in sensitivity to bleomycin may reflect altered content or distribution of this protective enzyme.

(b) *Cyclophosphamide.* All of the alkylating agents in clinical use, with the exception of thiotepa and nitrogen mustard, have been implicated as pulmonary toxins [39]. Most notably the alkylating agent cyclophosphamide can produce both acute respiratory complications and chronic pneumontitis with interstitial fibrosis, leading to fatal respiratory failure [39–40]. As with bleomycin, one of the earliest observable pulmonary effects of cyclophosphamide treatment appears to be endothelial damage. Gould and Miller [6] found by transmission electron microscopy endothelial bleb formation, degeneration, and focal sloughing 24 hr after a single intraperitoneal injection of 200 mg/kg cyclophosphamide into rats. Cellular injury was not limited, however, to the pulmonary endothelium because there also was focal sloughing of pneumocytes, basal lamina denudation, and septal edema. At the level of light microscopy, the earliest detectable changes were seen at 48 hr after cyclophosphamide administration and comprised irregularly distributed foci of perivascular edema, septal widening, capillary congestion, and minute hemorrhages.

Unlike most antitumor agents, cyclophosphamide must be activated by the monooxygenase system, which leads to the formation of 4-hydroxycyclophosphamide and aldophosphamide [41]. 4-Hydroxycyclophosphamide is quite reactive and unstable in aqueous solutions. The pulmonary injury associated with cyclophosphamide may be due to the direct toxic effect of 4-hydroxycyclophosphamide or products of aldophosphamide produced from cyclophosphamide *in situ* [41]. The relative sensitivity of the pulmonary vasculature may be due to a slow rate of deactivation of the biologically active metabolites [41]. Since cyclophosphamide can disrupt carbohydrate, lipid, energy, amino acid, protein, and nucleic acid metabolism [41], it is possible that actions other than DNA alkylation may have greater importance in damaging the endothelium and normal pulmonary tissue.

(c) *Nitrosoureas.* The toxic lung damage associated with the nitrosoureas, such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), is a fibrosing alveolitis and interstitial pneumonitis, which can produce pulmonary fibrosis, restrictive pulmonary failure, and death [23, 24, 39]. Although acute pulmonary toxicity has been reported in both monkeys and dogs [42], in contrast to studies with bleomycin and cyclophosphamide, attempts appear not to have been made to characterize histologically both the temporal sequence of events and the cell types damaged. Smith and Boyd [23] have noted changes in pulmonary and serum angiotensin converting enzyme activity in animals after BCNU treatment and concluded that this reflected initial endothelial injury. Nicolson and Custead [35] reported that BCNU causes cultured endothelial cells to retract. Most nitrosoureas maintain both alkylating and carbamoylating actions towards nucleophilic sites. Although the molecular target and mechanism of nitrosourea-induced endothelial and pulmonary toxicity have not been established, Weiss *et al.* [39] speculated, based upon a review of clinical case reports, that DNA damage due to alkylation was responsible for lung injury. The availability of nitrosoureas with different alkylating and carbamoylating components should provide powerful tools to determine in experimental models which reactive moiety is important in endothelial damage and pulmonary fibrosis.

(d) *Ionizing irradiation.* The endothelium also appears to be the initial pulmonary site of damage after ionizing irradiation, such as that used in cancer radiotherapy. For example, between 1 and 2 weeks after 650 rads, endothelial injury accompanied by interstitial edema was seen morphologically in mouse lungs [2, 43]. As with anticancer chemotherapeutic agents, a period of endothelial proliferation followed. Thus, acute endothelial injury may be repaired while, after larger doses of irradiation (1000 rads), regeneration is affected and fibrosis proceeds. Kwock *et al.* [44] found that after irradiation amino acid transport is readily impaired in cultured endothelium but not in fibroblasts. Thus, the lung toxicity associated with ionizing irradiation may reflect early toxicity to endothelium, although the cause of cellular specificity for this damage is unclear.

Toxicity to the endothelium of solid tumors

As previously mentioned, solid tumors require an endothelial-laden vasculature. The endothelial cells in solid tumors have a much higher mitotic rate than that found in normal tissue [14, 15, 45, 46]. During certain phases of tumor growth, the mitotic rate of the endothelium actually approaches and may equal that of the malignant cells, perhaps due to the

response to tumor angiogenesis factors [47]. The relationship between vascular development and tumor demands for nutrients and oxygen is likely to be fragile. Thus, an untested but reasonable hypothesis is that some of the specificity of our current antitumor agents to solid tumors may reflect their ability to damage the capillary endothelium of the tumors with subsequent loss of nutrients and necrosis [45]. Irradiation and cytokines, such as tumor necrosis factor [48], may also act in part by destruction of the solid tumor endothelium. An equally provocative question is whether agents can be developed that selectively recognize the presumed phenotypically unique properties of the tumor endothelium. For example, toxins, such as ricin or abrin, linked to antibodies directed against the antigens on the plasma membrane of the tumor endothelium could be ideal since their large mass would restrict their initial interactions to vascular targets. Perhaps the high proliferation rate of endothelial cells in tumors permits the expression of specific antigens that could be exploited [14, 15, 45, 46], although accelerated proliferation of endothelial cells in normal organs during healing or after drug- or radiotherapy-induced injury might limit this approach. It is unfortunate that such agents would evade detection in our current cultured tumor cell models or peritoneally-maintained mouse ascites tumor screens for clinically useful antineoplastic agents. Efforts should be directed toward examining these questions.

Summary

The vascular lining of the blood vessels to normal organs and malignant tissues would be expected to reflect the functional demands placed upon it. These functional requirements may be accomplished by specific biochemical macromolecules, some of which are localized on the plasma membrane of the endothelium. Considerable evidence exists that toxicity to at least some normal organs caused by antineoplastic agents is heralded by endothelial damage. This endothelial damage may reflect a specific drug–endothelium interaction, the mechanism and basis of which are not yet understood. The possibility also exists that destruction of solid tumors by currently employed antitumor agents is mediated in part by local loss of essential vasculature. Selective destruction of the tumor endothelium could be a rational and novel target to which drugs could be designed.

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REFERENCES

1. Y. Aso, K. Yoneda and Y. Kikkawa, *Lab. Invest,* **35**, 558 (1976).
2. I. Y. R. Adamson and D. H. Bowden, *Am. J. Path.* **77**, 185 (1974).
3. J. S. Lazo, J. D. Catravas, K. J. Dobuler and C. N. Gillis, *Toxic. appl. Pharma.* **69**, 276 (1983).
4. J. S. Lazo, J. D. Catravas and C. N. Gillis, *Biochem. Pharmac.* **30**, 2577 (1981).
5. J. D. Catravas, J. S. Lazo and C. N. Gillis, *J. Pharmac. exp. Ther.* **217**, 524 (1981).
6. V. E. Gould and J. Miller, *Am. J. Path.* **81**, 513 (1975).
7. N. Freudenberg, K. H. Riese and M. A. Freudenberg, *Veroff. Path.* **120**, 1 (1983).
8. B. Thorgeirsson and A. L. Robertson, *Am. J. Path.* **93**, 803 (1978).
9. M. Simionescu, N. Simionescu, F. Santoro and G. E. Palade, *J. Cell Biol.* **100**, 1396 (1985).
10. M. E. Gerritsen and M. P. Printz, *Circulation Res.* **49**, 1159 (1981).
11. A. R. Johnson, *J. clin. Invest.* **65**, 841 (1980).
12. K. Mackie, Y. Lai, A. C. Nairn, P. Greengard, B. R. Pitt and J. S. Lazo, *Fedn Proc.* **44**, 2904 (1985).
13. J. A. Madri, *Progress in Hemostasis and Thrombosis* (Ed. T. H. Spaef), Vol. 6, p. 1, Grune & Stratton, Florida (1982).
14. H. I. Peterson, *Tumor Blood Circulation: Angiogenesis, Vascular Morphology and Blood Flow of Experimental and Human Tumors* (Ed. H. I. Peterson), p. 1, CRC Press, Florida (1979).
15. B. Hobson and J. Denekamp, *Br. J. Cancer* **49**, 405 (1984).
16. I. F. Tannock, *Cancer Res.* **30**, 2470 (1970).
17. I. Y. R. Adamson and D. H. Bowden, *Am. J. Path.* **112**, 224 (1983).
18. M. A. Hollinger, S. N. Giri, S. Patwell, J. E. Zuckerman, A. Gorin and G. Parsons, *Am. Rev. resp. Dis.* **121**, 373 (1980).
19. M. A. Hollinger, S. W. Patwell, J. E. Zuckerman, A. B. Gorin, G. Parsons and S. N. Giri, *Am. Rev. resp Dis.* **121**, 795 (1980).
20. R. Huxtable, D. Ciaramitaro and D. Eisenstein, *Molec. Pharmac.* **14**, 1189 (1978).
21. C. N. Gillis and J. D. Catravas, *Ann. N.Y. Acad. Sci.* **384**, 458 (1982).
22. M. A. Reidy and S. M. Schwartz, *Lab. Invest.* **48**, 25 (1983).
23. A. C. Smith and M. R. Boyd, *Biochem. Pharmac.* **32**, 3719 (1983).
24. F. M. Muggia, A. C. Louie and B. I. Sikic, *Cancer Treat. Rev.* **10**, 221 (1983).
25. J. S. Lazo and M. P. Hacker. *Fedn Proc.* **44**, 25 (1983).
26. A. Burkhardt, W. J. Holtje and J. O. Gebbers, *Virchows Arch. A. Path. Anat. Histol.* **372**, 227 (1976).
27. D. B. Chandler, R. M. Jackson, A. D. Briggs, W. C. Fuller and J. D. Fulmer, *Prostaglandins* **19**, 139 (1985).
28. D. B. Chandler and S. N. Giri, *Am. Rev. resp. Dis.* **128**, 71 (1983).
29. A. Burkhardt, J. O. Gebbers and W. J. Holtje, *Dt. Med. Wschr.* **102**, 281 (1977).
30. E. Pohler, R. Schmiedl and R. Thoma, *Verh. dt. Ges. inn. Med.* **87**, 1053 (1981).
31. P. Hilgard and D. K. Hossfeld, *Eur. J. Cancer* **14**, 1261 (1978).
32. W. J. Caspary, C. Niziak, D. A. Lango, R. Friedman and N. Bachur, *Molec. Pharmac.* **16**, 256 (1976).
33. J. Fujimoto, *Cancer Res.* **34**, 2969 (1974).
34. I. L. Sun and F. L. Crane, *Biochem. Pharmac.* **34**, 617 (1985).
35. G. L. Nicolson and S. E. Custead, *Cancer Res.* **45**, 331 (1985).
36. J. S. Lazo and C. J. Humphreys, *Proc. natn. Acad. Sci. U.S.A.* **80**, 3064 (1983).
37. J. S. Lazo, W. W. Merrill, E. T. Pham, T. J. Lynch, J. D. McCallister and D. H. Ingbar, *J. Pharmac. exp. Ther.* **231**, 583 (1984).
38. D. J. Schrier, R. G. Kunkel and S. H. Phan, *Am. Rev. resp. Dis.* **127**, 63 (1983).
39. R. B. Weiss, D. S. Poster and J. S. Penta, *Cancer Treat. Rev.* **8**, 111 (1981).

40. A. A. Topilow, S. P. Bothenberg and T. S. Cottrell, *Am. Rev. resp. Dis.* **108**, 114 (1973).
41. D. L. Hill, *A Review of Cyclophosphamide*. Charles C. Thomas, Springfield (1975).
42. S. K. Carter and J. W. Neuman, *Cancer Chemother. Rep.* **1**, 115 (1968).
43. W. F. Ward, N. H. Solliday, A. Molteni and C. D. Port, *Radiat. Res.* **96**, 294 (1983).
44. K. Kwock, W. H. J. Douglas, P. S. Lin, W. E. Baur and B. L. Fanburg, *Am. Rev. resp. Dis.* **125**, 95 (1982).
45. J. Denekamp, *Br. J. Cancer* **45**, 136 (1982).
46. J. Denekamp and B. Hobson, *Br. J. Cancer* **46**, 711 (1982).
47. B. R. Zetter, *Nature, Lond.* **285**, 41 (1980).
48. L. J. Old, *Science* **230**, 630 (1985).