Cancer Chemother Pharmacol (1991) 28: 308–310
034457049100107A

Cancer Chemotherapy and Pharmacology

Short communication

Experimental basis for increasing the therapeutic index of carboplatin in brain tumor therapy by pretreatment with WR compounds*

François Doz, Michael E. Berens, Donna R. Spencer, Dolores V. Dougherty, and Mark L. Rosenblum

Brain Tumor Research Center of the Department of Neurological Surgery, School of Medicine, University of California, San Francisco, CA 94143, USA

Received 31 August 1990/Accepted 8 February 1991

Summary. We tested an experimental strategy to decrease the dose-limiting hematotoxicity of carboplatin without compromising its activity against brain tumors. The effect of pretreatment with WR-1065, a chemomodifier that penetrates brain poorly, on carboplatin's cytotoxicity was evaluated in human hematopoietic granulocyte-monocyte progenitor cells and in three human glioblastoma cell lines. WR-1065 reduced bone marrow toxicity without decreasing carboplatin's activity against glioblastoma cells. These results suggest that the therapeutic index of carboplatin might be increased in the treatment of malignant brain tumors.

Introduction

Cisplatin and carboplatin have comparable activity against brain tumor cells in vitro [4]. Carboplatin penetrates brain better [10] and has lower neuro- and nephrotoxicities, but has a dose-limiting hematological toxicity. Decreasing bone-marrow toxicity without altering antitumor activity might increase the therapeutic index of carboplatin for the treatment of malignant brain tumors. To evaluate this possibility, we studied the effect of 2-(aminopropyl)-amino-ethanethiol (WR-1065) [11], the active metabolite of WR-2721, a chemomodifier that protects normal tissues from cisplatin's toxicity [6], on carboplatin's toxicity to normal human bone marrow cells and three human glioma cell lines. These in vitro carboplatin concentrations were chosen because they achieve a clinically relevant exposure [4].

* This study was supported by Grants CA-13525 and CA-31882 from the National Cancer Institute, and by a grant from the Preuss Foundation. F. Doz was supported by the Ligue Nationale Française Contre le Cancer. Some of the data have already been presented in a paper for which F. Doz was awarded the Schweisguth Prize of the International Society of Paediatric Oncology at its annual meeting in Rome, October, 1990

Offprint requests to: Mark L. Rosenblum, Department of Neurological Surgery, c/o The Editorial Office, 1360 Ninth Avenue, Suite 210, San Francisco, CA 94122

Materials and methods

Drugs. WR-1065 was provided by the Drug Synthesis and Chemistry Branch of the National Cancer Institute (Bethesda, Md.). Carboplatin was provided by Bristol-Myers (Syracuse, NY). Cells were cultured in complete medium consisting of Eagle's minimum essential medium (MEM) with 10% fetal calf serum (FCS) and gentamicin. In general, cells were exposed for 30 min to MEM or WR-1065 (2.6 mM) [11], rinsed twice, treated for 2 h with MEM or carboplatin (24-270 μM), rinsed twice, incubated at 37° C in 5% CO_2 and 95% humidified air, and assayed for growth changes.

Human bone marrow cells. Bone marrow samples were collected with preservative-free heparin (Sigma, St. Louis, Mo.) from 3 subjects without hematologic disease who gave informed consent to participate in the study. Mononuclear cells were collected by Ficoll-Hypaque centrifugation, suspended in complete medium, and incubated overnight. Nonadherent cells were collected, resuspended in MEM, treated as described above, and plated in MEM with 0.1% gentamicin, 20% FCS, 20% phytohemagglutinin A-stimulated, leukocyte-conditioned medium (Terry Fox Laboratories, Vancouver, BC) [5] and 0.3% agarose type VII (Sigma) on pregelled agarose layers in 4-well plates (Nunc, Roskilde, Denmark). Plates were incubated for 10–12 days; granulocyte-monocyte colonies (CFU-GM) containing at least 40 cells were counted with an inverted microscope. Experiments were done in quadruplicate.

Human glioblastoma cells. Cell lines U251-MG and U87-MG (Jan Ponten, Uppsala, Sweden) [1] and SF-126 [7] were studied. Exponentially growing cells (1000/well) suspended in complete medium were placed in flat-bottom, 96-well plates (Falcon, Lincoln Park, NJ), incubated for 24 h, treated with WR-1065 or MEM and then with carboplatin as described above, and refed with complete medium. After 6–9 days (equal to 6–7 cell doublings), 125 μg 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) in 25 μl of Hank's balanced salt solution was added to each well [2]. The resulting formazan crystals were dissolved in mineral oil. Absorbance was measured at 540 nm (Titertek Multiskan, Flow Lab., Inc., McLean, Va.). Previous experiments under similar conditions in our laboratory established a linear relationship between cell number and the absorbance obtained after subtraction of the background absorbance in wells containing medium alone. The fractional absorbance was calculated as the mean of the test sample divided by the mean of the untreated samples from six replicate wells. The

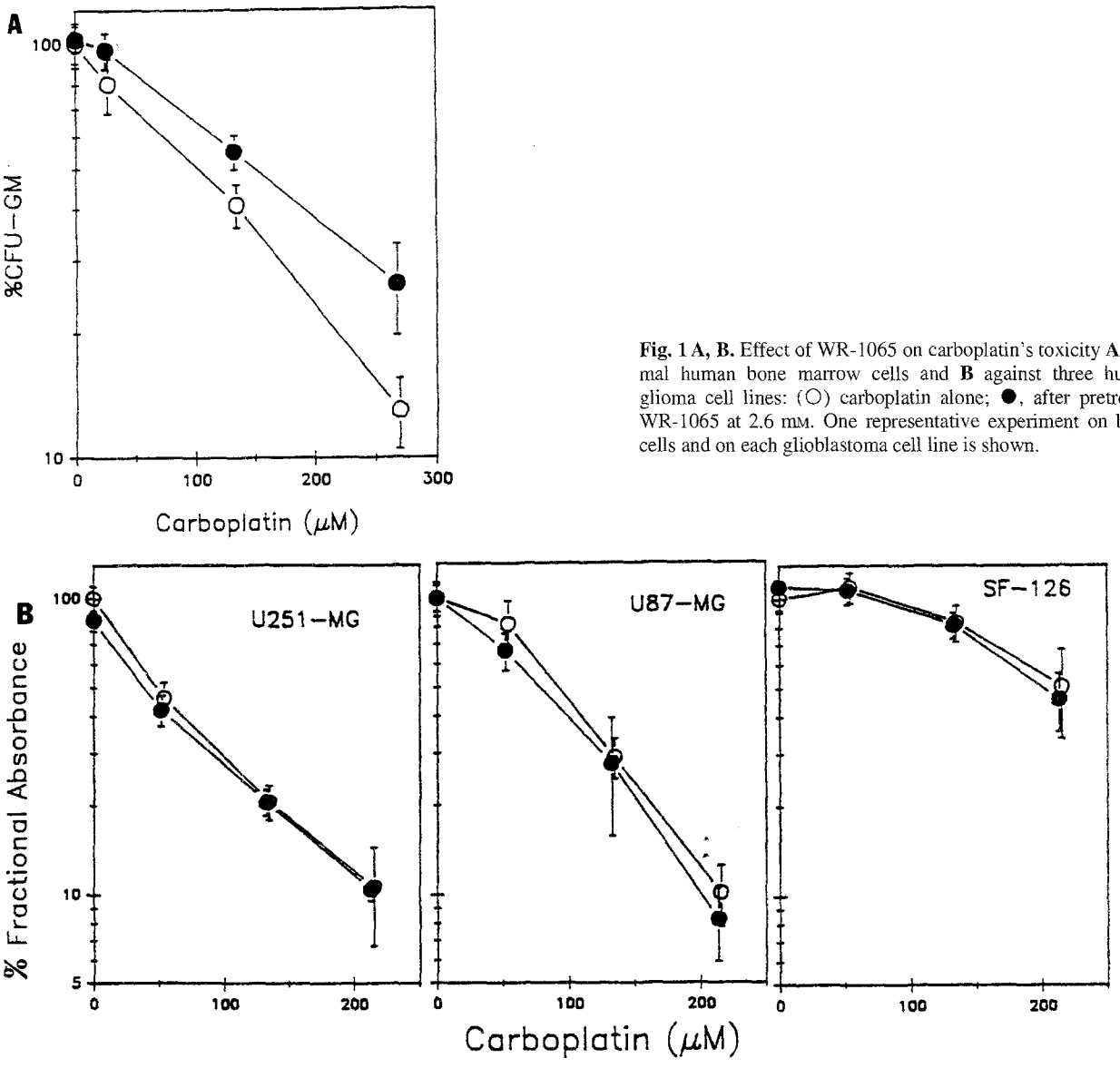

Fig. 1 A, B. Effect of WR-1065 on carboplatin's toxicity **A** against normal human bone marrow cells and **B** against three human-derived glioma cell lines: (○) carboplatin alone; ●, after pretreatment with WR-1065 at 2.6 mM. One representative experiment on bone marrow cells and on each glioblastoma cell line is shown.

experiment was performed three times with cell lines U-87 and SF-126 and twice with cell line U251-MG.

Data analysis. The influence of WR-1065 on the fractional CFU-GM and on the fractional absorbance of glioma cells in response to various doses of carboplatin was determined using the linear model procedure in SAS [8]. Separate regression lines were fitted to describe the response to various doses of carboplatin with and without WR-1065, and the slopes an intercepts were compared to determine if the differences were statistically significant.

Results

WR-1065 alone did not alter the growth of CFU-GM (Fig. 1 A). Cell kill from carboplatin followed first-order kinetics in the three samples (7.4%, 13.5%, and 27.5% survival at 270 μM). Carboplatin's toxicity to myeloid progenitor cells, however, was significantly decreased by pretreatment with 2.6 mM WR-1065 ($P < 0.02$). The dose-protection ratio from WR-1065 in cells treated with 270 μM carboplatin was 1.2 in one experiment, and 1.5 in the two others. WR-1065 (2.6 mM) alone did not influence the spontaneous growth of the three glioma cell lines, nor did it have a protective effect (Fig. 1 B).

Discussion

WR-1065 reduced the in vitro cytotoxic effect of carboplatin against human hematopoietic stem cells without reducing the sensitivity of three human glioma cell lines. In laboratory [3] and clinical [6] studies WR compounds have protected bone marrow cells against cytotoxic drugs. On cancer cells, however, WR compounds had a protective effect in some studies [11] and in other studies a sensitizing effect [3] not explained by a modification of the drug–DNA interaction. These conflicting results could be due to two potentially opposite actions of the WR compounds: direct protection of DNA [3], and reduction of intracellular reduced glutathione, which could decrease intracellular resistance to platinum compounds and thereby increase their cytotoxicity [9]. The selective protection of normal tissues

by WR compounds in vivo is probably due to a higher achievable concentration of WR compounds in normal cells than in hypoxic and more acidic cancer tissues [13]. Animal studies have shown very low concentrations of WR-1065 in brain after treatment with WR-2721 [12]. Because the blood–brain barrier is usually intact in the zone surrounding the tumor, where most recurrences occur, it is unlikely that WR-1065 would reach this zone and protect infiltrating tumor cells from the cytotoxic effect of chemotherapy. However, the blood–brain barrier is frequently altered in the center of malignant brain tumors, so it is important to determine that WR compounds do not protect tumor cells exposed to the agent in unresected permeable regions.

We conclude that WR-1065 decreases the bone marrow toxicity of carboplatin in vitro without decreasing its antitumor activity against glioma cells. The reduced hematotoxicity should allow a higher dose of carboplatin and a consequent increase in tumor cell kill [4]. These results provide an experimental basis for further in vivo and clinical studies of carboplatin and WR-2721 for the treatment of malignant brain tumors.

Acknowledgements. The authors wish to thank Kathleen Lamborn for the statistical analysis and Stephen Ordway for editorial assistance.

References

1. Bigner DD, Bigner SH, Ponten J, Westermark B, Mahaley MS, Ruoslahti E, Herschman H, Eng LF, Wikstrand CJ (1981) Heterogeneity of genotypic and phenotypic characteristics of fifteen permanent cell lines derived from human gliomas. J Neuropathol Exp Neurol 40: 201
2. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47: 936
3. DeNeve WJ, Everett CK, Suminski JE, Valeriote FA (1988) Influence of WR2721 on DNA cross-linking by nitrogen mustard in normal mouse bone marrow and leukemia cells in vivo. Cancer Res 48: 6002
4. Doz F, Berens ME, Dougherty DV, Rosenblum ML (1991) Comparison of the cytotoxic activities of cisplatin and carboplatin against glioma cells lines at pharmacologically relevant drug exposures. J Neurooncol (in press)
5. Fauser AA, Messner HA (1978) Granuloerythropoietic colonies in human bone marrow, peripheral blood and cord blood. Blood 52: 1243
6. Glover D, Glick JH, Weiler C, Fox K, Guerry D (1987) WR-2721 and high-dose cisplatin: an active combination in the treatment of metastatic melanoma. J Clin Oncol 5: 574
7. Rutka JT, Giblin JR, Dougherty DV, Liu HC, McCulloch JR, Bell CW, Stern RS, Wilson CB, Rosenblum ML (1987) Establishment and characterization of five cell lines derived from human malignant gliomas. Acta Neuropathol (Berl) 75: 92
8. SAS Institute, Inc. (1987) SAS/STAT™ Guide for personal computers, Version 6 edition. SAS Institute, Cary, NC
9. Schor NF (1988) Mechanisms of synergistic toxicity of the radioprotective agent, WR2721 and 6-hydroxydopamine. Biochem Pharmacol 37: 1751
10. Siddik ZH, Jones M, Boxall FE, Harrap KR (1988) Comparative distribution and excretion of carboplatin and cisplatin in mice. Cancer Chemother Pharmacol 21: 19
11. Smoluk GD, Fahey RC, Calabro-Jones PM, Aguilera JA, Ward JF (1988) Radioprotection of cells in culture by WR-2721 and derivatives: form of the drug responsible for protection. Cancer Res 48: 3641
12. Utley JF, Seaver N, Newton GL, Fahey RC (1984) Pharmacokinetics of WR-1065 in mouse tissue following treatment with WR-2721. Int J Radiat Oncol Biol Phys 10: 1525
13. Yuhas JM, Spellman JM, Culo F (1980) The role of WR-2721 in radiotherapy and/or chemotherapy. Cancer Clin Trials 3: 211