Cancer Chemother Pharmacol (1988) 22: 65–68

Cancer Chemotherapy and Pharmacology

Salivary concentrations of hexamethylene bisacetamide *(HMBA)* in patients receiving 5-day continuous infusions*

Barbara A. Conley[1, 2], Merrill J. Egorin[1, 2], Eleanor G. Zuhowski[1], Victoria J. Sinibaldi[1], and Douglas E. Peterson[3]

[1] Division of Developmental Therapeutics, University of Maryland Cancer Center
[2] Division of Medical Oncology, Department of Medicine, University of Maryland School of Medicine and
[3] Department of Oral Diagnosis, University of Maryland Dental School, Baltimore, MD-21201, USA

Summary. Salivary and plasma concentrations of hexamethylene bisacetamide (HMBA) were studied to determine (1) how the concentrations of HMBA achieved in saliva compared with those required to induce differentiation in vitro, and (2) whether saliva might substitute for plasma as a biologic fluid on which to base dosage adjustment. Plasma and expectorated saliva were collected concomitantly from 16 patients receiving 5-day continuous infusions of HMBA. The concentrations of HMBA in each fluid were determined by gas chromatography. The patients displayed a range of periodontal disease from gingivitis to complete edentia, but periodontal disease status did not appear to influence the salivary behavior of HMBA, which mirrored that of the drug in plasma, with concentrations of HMBA increasing in both fluids during the first 12–16 h of infusion. Between 24 and 120 h, HMBA concentrations in saliva and plasma remained constant. In some patients, salivary HMBA concentrations lagged behind those in plasma during the first 6–8 h of infusion, but after that the salivary HMBA concentrations approximated those in the plasma. Salivary concentrations of HMBA between 0.96 and 2.56 m*M* were associated with nontoxic plasma concentrations of HMBA. Therefore, in patients with restricted venous access, saliva might be a suitable substitute for plasma if adaptive control-dosing schemes for HMBA are employed. Moreover, the concentrations of HMBA in saliva bathing the oral cavity are quantitatively comparable to those required for induction of cell differentiation in vitro.

Introduction

Hexamethylene bisacetamide (HMBA, NSC 95580) induces in vitro morphological and functional differentiation of murine and human leukemic and solid tumor cell lines [7, 12, 15, 17, 19, 22–27, 30, 33]. Among the class of agents that have the potential for inducing the differentiation of tumor cells and that represent an exciting and novel approach to the chemotherapy of neoplasia [1, 3, 21, 29, 31, 32], HMBA has a number of characteristics which render it of great potential clinical use [4]. HMBA was selected for introduction into clinical trials because, of a series of bisacetamides tested, it possessed the maximal differentiation potency [24–27, 33]. In addition, clinical and pharmacokinetic studies have documented the ability to achieve and maintain concentrations of HMBA in plasma equal to those required for the induction of differentiation in vitro [9, 20, 28, 34]. However, adverse effects have been associated with its administration to humans [9, 20, 28, 34]. At HMBA dosages greater than or equal to 33.6 g/m^2 per day, metabolic acidosis and neurotoxicity occur as dose-limiting toxicities, and platelet count suppression, although not always dose-limiting, also occurs [9, 20, 28, 34]. In view of our documentation of the relationship between plasma concentrations of HMBA and these toxic consequences [9], we have carefully characterized the pharmacokinetics of HMBA and its metabolites [5, 9–11] and have used these studies to develop an adaptive control algorithm for dosage adjustment [13, 14]. This strategy, which relies on knowledge of plasma concentrations of the drug, allows careful control of such concentrations and maintenance of maximally tolerable, nontoxic concentrations of HMBA. The investigation of salivary concentrations of HMBA and their relationship to plasma concentrations was undertaken in part to see if salivary samples could substitute for plasma samples as a basis for dosage adjustment. The second major impetus for these studies was our belief that HMBA might play a role in the treatment of premalignant lesions, such as leukoplakia, and that knowledge of its behavior in saliva would be important data to elicit before clinical trials in leukoplakia could be undertaken.

Materials and methods

Patient selection and evaluation. All patients entered into this study had histological proof of malignant disease for which conventional chemotherapy had proven ineffective and for which no other investigational therapy with established efficacy was available. All patients had a minimal life expectancy of a least 12 weeks and a Karnofsky performance status of at least 60%. They were at least 4 weeks removed from previous radiotherapy and chemotherapy (8 weeks for agents with delayed hematological toxicity, such as mitomycin C or nitrosoureas). All patients had adequate bone marrow function (WBC count $>3{,}500/\mu l$ and plate-

* Supported in part by contract NO1-CM47734 awarded by the National Cancer Institute, Department of Health and Human Services

Offprint requests to: Merrill J. Egorin, University of Maryland Cancer Center, 655 West Baltimore Street, Baltimore, MD 21201 USA

let count >100,000/μl), adequate liver function (bilirubin less than 1.5 mg/dl), and adequate renal function (creatinine clearance ≥ 40 ml/min). No patient with unstable medical or psychiatric disease nor with cerebral metastases or history of seizure disorder was admitted to the study.

Baseline and follow-up studies were as described in a previously published phase I trial of HMBA carried out at our institution [9]. Periodontal disease status was determined with standard criteria [8]. Before entry into this study, the investigational nature of the treatment was explained to each patient and an informed consent, approved by the Institutional Review Board, was signed.

Drug schedule and administration. HMBA was supplied by the Investigational Drug Branch of the National Cancer Institute (Bethesda, Md). Each 500-ml bottle contained 25 g HMBA as a solution in 0.5 W. HMBA was given as a 5-day continuous infusion through a free-flowing peripheral or central venous catheter. The rate of HMBA infusion was controlled by a Travenol Flo-Gard 8000 volumetric infusion pump (Travenol Laboratories, Inc., Deerfield, Ill). The starting dose was 24 g/m^2 per day. If necessary, doses were adjusted daily to achieve plasma steady-state HMBA concentrations of 1.5–2.7 m*M*. Dosage modification was accomplished with an M.A.P.-Bayesian adaptive control algorithm developed with data from our previous phase I studies of HMBA (9, 13, 14).

Sample acquisition. Heparinized blood samples were obtained before and at 30 min, 1, 2, 4, 6, 12, 16, 24, 48, 72, 96, and 120 h into the infusion. Blood samples were centrifuged at 1000 *g* for 10 min, and the resulting supernatant plasmas were immediately removed and stored at 0–4° C until analyzed. Unstimulated, expectorated saliva samples were collected concomitantly with blood samples and were stored similarly. There were no consistent relationships between sample collection and time of day, meals, or fluid ingestion.

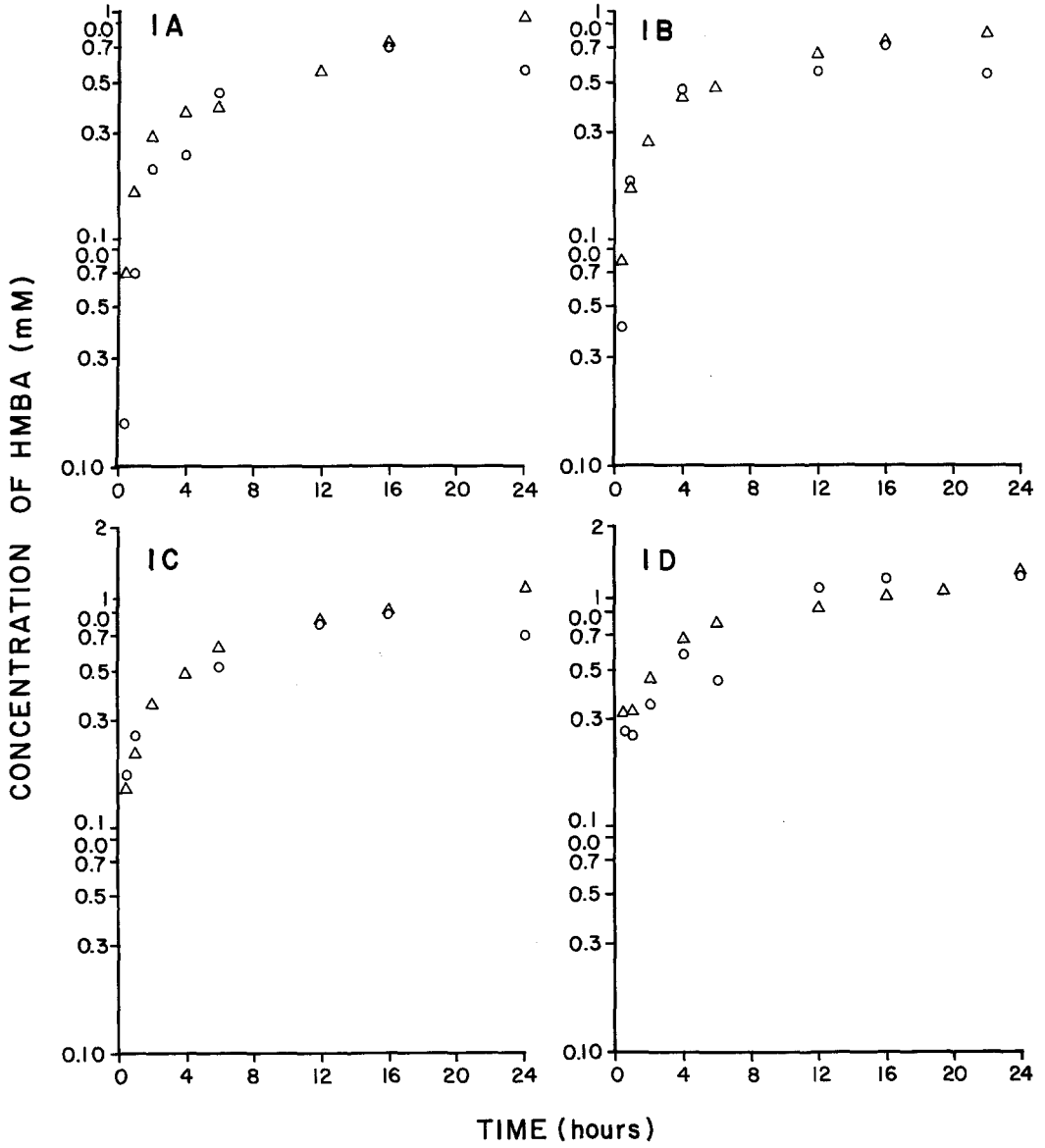

Fig. 1. Concentrations of HMBA in plasma (△) and saliva (○) of four representative patients during the first 24 h of 5-day infusions of HMBA. Concentrations of HMBA in each fluid were determined by gas chromatography as described in *Material and methods.* Points represent the means of duplicate determinations

Gas chromatographic analysis of HMBA. The method of analysis was a modification of those methods previously described [9, 18]. Briefly, 100 µl plasma or saliva was mixed with 5 µl 1 mg/ml pentamethylene bisacetamide internal standard and 500 µl absolute methanol in polypropylene microcentrifuge tubes. The mixture was then centrifuged at 12,000 *g* for 5 min. A 1.5-µl portion of the resulting supernatant solution was injected onto the gas chromatograph.

The gas chromatographic system used a Hewlett-Packard 5840A gas chromatograph and a 6-foot × 2 mm (inner diameter) glass column packed with 3% SP-2250DB on 100/120 mesh Supelcoport (Supelco, Bellefonte, Pa). The oven temperature was maintained at 220° C, the injection port temperature was 255° C, and the detector temperature was 275° C. A nitrogen-phosphorus selective detector was used with a hydrogen flow rate of 3 ml/min, an air flow rate of 90 ml/min, and a bead voltage of 16–18 V. Nitrogen (30 ml/min) was used as a carrier gas. Concentrations of HMBA were determined by comparison of the area of the HMBA peak with that of the internal standard peak in each sample. Under these conditions, excellent separation of HMBA and internal standard was obtained, and no endogenous substances in plasma or saliva interfered with the determinations. The quantitative aspects of this assay in our laboratory have previously been published [9].

Results

Pharmacokinetic studies were carried out on 16 patients. The behaviour of HMBA in saliva mirrored that of the drug in plasma (Fig. 1). Concentrations of HMBA in both body fluids increased during the first 12–16 h of the infusion. Thereafter both salivary and plasma HMBA concentrations remained constant. In some patients there was a disparity between the salivary and plasma concentrations of HMBA that were measured early in the infusion (Fig. 1). In these patients, salivary concentrations of the drug were less than those in plasma, but within 8 h the salivary concentrations of HMBA in all patients approximated those in plasma (Figs. 1 and 2). Quantitatively, salivary concentrations of HMBA between 0.96 and 2.56 m*M* were associated with clinically tolerated plasma concentrations.

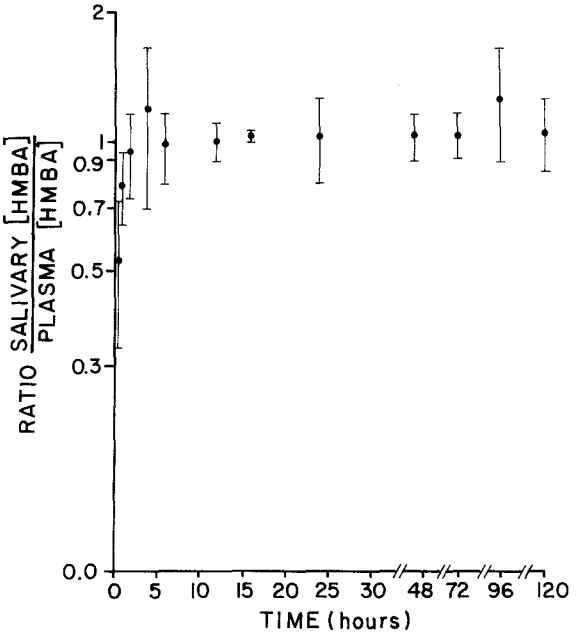

Fig. 2. Relationship of concentrations of HMBA in saliva to those in plasma during the 5-day infusion of HMBA. Points represent the means ± SE of 16 patients

Discussion

Our previous experience with the cellular differentiating agent HMBA raised two issues which led us to pursue the present salivary studies. Earlier phase I studies had shown that neurotoxicity and acidosis were not observed in patients with plasma drug concentrations <2 m*M* [9, 20, 28, 34] but that significant interindividual differences existed in the plasma HMBA concentrations obtained in a population of patients receiving the same dosage [9, 28]. This led us to implement a study in which the dosing of individual patients was adjusted to achieve a maximally tolerable plasma concentration, rather than using a population-based maximally tolerated dosage [13, 14]. An essential component of such a dosing scheme was the ability to determine plasma concentrations of HMBA rapidly and easily. The second reason for defining salivary concentrations of HMBA involved the potential use of this compound in premalignant or malignant lesions of the oral cavity. The results presented have important implications with regard to each of these issues. We have shown that saliva can serve as a reasonable substitute for plasma to assay and guide adaptive control dosing of HMBA. Possibly of more importance is the documentation of salivary HMBA concentrations of 1–2.6 m*M*, i.e., concentrations known to have in vitro activity, in patients receiving nontoxic doses. At present we are attempting to explore each of these observations in terms of the situations in which they were made.

The degree of ulceration associated with the periodontal disease status in these patients may have contributed to some variation in salivary levels of HMBA. The crevicular fluid within the space between the gingival epithelium and the tooth can serve as a means by which serum components can mix with whole saliva. Considerable data have been published documenting crevicular levels of antibotics given systemically [2, 6, 16] and have shown that a disparity between serum and crevicular antibotic concentrations may exist under certain conditions. At the time of whole saliva collection, the 16 patients in this study displayed periodontal disease states that ranged from gingivitis only to complete edentia. Knowing that an ulcerated periodontal epithelium could represent a site for the entry of systemically given agents into the oral cavity, we considered whether some of the variation in the data reported in this study might be attributed to interpatient differences in periodontal disease status. However, the fact that the salivary concentrations of HMBA in edentulous patients were reproducible and comparable to those in plasma reduces the likelihood of this explanation. Given the low molecular weight and relatively nonpolar nature of HMBA, free diffusion across cells and through salivary gland structures is possible. Alternatively, the potential exists for an efficient acinar transport system. These issues are intriguing and may merit further investigation.

Acknowledgements. We thank H. C. Spiker and B. Knickman for excellent secretarial assistance in the preparation of this manuscript and D. M. Stansbury for clinical assistance in oral assessment.

References

1. Block A (1984) Induced cell differentiation in cancer therapy. Cancer Treat Rep 68: 199–205
2. Britt MR, Pohlod DJ (1986) Serum and crevicular fluid concentrations after single oral dose of metronidazole. J Periodontol 57: 104–107
3. Calabressi P, Dexter DL, Heppner GH (1979) Clinical and pharmacological implications of cancer cell differentiation and heterogeneity. Biochem Pharmacol 28: 1933–1941
4. Chun HG, Leyland-Jones B, Hoth D, Shoemaker D, Wolpert-Defilippes M, Grieshaber C, Cradock J, Davignon P, Moon R, Rifkin R, Wittes RE (1986) Hexamethylene bisacetamide: a polar-planar compound entering trials as a differentiating agent. Cancer Treat Rep 70: 991–996
5. Callery PS, Egorin MJ, Geelhaar L, Nayar MSB (1986) Identification of metabolites of the cell-differentiating agent hexamethylene bisacetamide in humans. Cancer Res 46: 4900–4903
6. Cimasoni G (1983) Crevicular fluid updated. In: Myers HM (ed) Monographs in oral science. Karger, Basel, pp 103–123
7. Collins SJ, Bodner A, Ting R, Gallo RC (1980) Induction of morphological and functional differentiation of human promyelocytic leukemia cells (HL-60) by compounds which induce differentiation of murine leukemia cells. Int J Cancer 25: 213–218
8. Current procedural terminology for periodontics, 5th ed (1986) Chicago, American Academy of Periodontology
9. Egorin MJ, Sigman LM, Van Echo DA, Forrest A, Whitacre MY, Aisner J (1982a) Phase I clinical and pharmacokinetic study of hexamethylene bisacetamide (NSC 95580) administered as a five-day continuous infusion. Cancer Res 47: 617–623
10. Egorin MJ, Zuhowski EG, Cohen AE, Geelhaar LA, Callery PS, Van Echo DA (1987b) Plasma pharmacokinetics and urinary excretion of hexamethylene bisacetamide metabolites. Cancer Res (in press)
11. Egorin MJ, Zuhowski EG, Nayar MSB, Callery PS (1987c) Gas chromatographic analysis of metabolites of the cell differentiating agent hexamethylene bisacetamide. J Chromatogr 415: 148–155
12. Fibach E, Reuben RC, Rifkind RA, Marks PA (1977) Effect of hexamethylene bisacetamide on the commitment to differentiation of murine erythroleukemia cells. Cancer Res 37: 440–444
13. Forrest A, Conley B, Egorin M, Zuhowski E, Bachur NR, Van Echo DA (1987a) Phase I trial employing adaptive control dosing of hexamethylene bisacetamide (HMBA). Proc Am Soc Clin Oncol 6: 39
14. Forrest A, Egorin MJ, Zuhowski E, Van Echo DA (1987b) Development and validation of unique adaptive control algorithm. Clin Pharmacol Ther 41: 178
15. Gazitt Y, Friend C (1980) Polyamine biosynthesis enzymes in the induction and inhibition of differentiation in Friend erythroleukemia cells. Cancer Res 40: 1727–1732
16. Gordon JM, Walker CB, Goodson JM, Solransky SS (1980) Sensitive assay for measuring tetracycline levels in gingival crevice fluid. Antimicrob Agents Chemother 17: 193–198
17. Hughes EH, Schut HAJ, Thorgeirsson SS (1982) Effects of hexamethylene bisacetamide on alpha-fetoprotein, albumin, and transferrin production by two rat hepatoma cell lines. In Vitro 18: 157–164
18. Kelley JA, Roth JA, Litterst CL (1985) Gas chromatographic determination of hexamethylene bisacetamide in plasma and urine. Anal Lett 18: 1043
19. Kloog Y, Axelrod J, Spector I (1983) Protein carboxyl methylation increases in parallel with differentiation of neuroblastoma cells. J Neurochem 40: 522–529
20. McGuire WP, Rowinsky EK, Ettinger DS, Grochow LB, Luk GD, Donehower RC (1987) Prolonged infusion of hexamethylene bisacetamide: Phase I and pharmacologic study. Proc Am Assoc Cancer Res 28: 193
21. Marks PA, Rifkind RA (1984) Differentiation modifiers. Cancer 54: 2766–2769
22. Palfrey C, Kimhi Y, Littauer UZ, Reuben RC, Marks PA (1977) Induction of differentiation in mouse neuroblastoma cells by hexamethylene bisacetamide. Biochem Biophys Res Commun 76: 937–942
23. Rabson AS, Stern R, Tralka TS, Costa J, Wilczek J (1977) Hexamethylene bisacetamide induces morphologic changes and increased synthesis of procollagen in cell line from glioblastoma multiforme. Proc Natl Acad Sci USA 74: 5060–5064
24. Reuben RC (1979) Studies on the mechanism of action of hexamethylene bisacetamide, a potent inducer of erythroleukemic differentiation. Biochim Biophys Acta 588: 310–321
25. Reuben RC, Wife RL, Breslow R, Rifkind RA, Marks PA (1976) A new group of potent inducers of differentiation in murine erythroleukemia cells. Proc Natl Acad Sci USA 73: 862–866
26. Reuben RC, Khanna PL, Gazitt Y, Breslow R, Rifkin RA, Marks PA (1978) Inducers of erythroleukemic differentiation. Relationship of structure to activity among planar-polar compounds. J Biol Chem 253: 4214–4218
27. Reuben RC, Rifkin RA, Marks PA (1980) Chemically induced murine erythroleukemia differentiation. Biochim Biophys Acta 605: 325–346
28. Rowinsky EK, Ettinger DS, Grochow LS, Brundrett RB, Cates AE, Donehower RC (1986) Phase I and pharmacologic study of hexamethylene bisacetamide in patients with advanced cancer. J Clin Oncol 4: 1835–1844
29. Sartorelli AC (1985) Malignant cell differentiation as a potential therapeutic approach. Br J Cancer 52: 293–302
30. Speers WC, Birdwell CR, Dixon FJ (1979) Chemically induced bidirectional differentiation of embryonal carcinoma cells in vitro. Am J Pathol 97: 563–584
31. Sporn MB, Roberts AB, Driscoll JS (1985) Principles of cancer biology. Growth factors and differentiation. In: Devita VT, Hellman S, Rosenberg SA (eds) Cancer: Principles and practice of oncology. JB Lippincott, Philadelphia, pp 19–65
32. Spremulli EN, Dexter DL (1984) Polar solvents: A novel class of antineoplastic agents. J Clin Oncol 2: 227–241
33. Tanaka M, Levy J, Terada M, Breslow R, Rifkind RA, Marks PA (1975) Induction of erythroid differentiation in murine virus infected erythroleukemia cells by highly polar compounds. Proc Natl Acad Sci USA 72: 1003–1006
34. Walsh TD, Fanucchi MP, Williams L, Lokos G, Stevens Y-W, Cassidy C, Young CW (1987) Phase I clinical and pharmacokinetic study of hexamethylene bisacetamide (HMBA) by 10 day continuous infusion. Proc Am Assoc Cancer Res 28: 188

Received November 7, 1987/Accepted January 28, 1988