Eur J Cancer Clin Oncol, Vol. 20, No. 2, pp. 287-289, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Letter to the Editor

Effects of *p*-(3,3-Dimethyl-1-triazeno)benzoic Acid Potassium Salt on Leukemic Infiltration of Brain and Liver in Mice Bearing P388 Leukemia*

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A COMMON difficulty encountered in the treatment of leukemias is the involvement of the central nervous system. Meningeal and cerebral metastases in human acute lymphoblastic leukemia subsequently offer serious therapeutic problems, since cerebral reservoirs of tumor cells are poorly accessible to cytotoxic antineoplastic drugs [1]. The dimethyltriazene *p*-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt (DM-COOK) has been shown to possess selective antimetastatic properties in mice bearing Lewis lung carcinoma, a solid metastasizing tumor, preventing the formation of pulmonary metastases with a mechanism different from cytotoxicity for tumor cells [2]. We thought it therefore worthwhile to examine the possible occurrence of antimetastatic effects of DM-COOK in leukemic mice.

BDF 1 mice, implanted i.p. with 10^6 P388 lymphocytic leukemia cells, were used for this study. When treated i.p. daily on days 1–7 from tumor implantation with DM-COOK, 100 mg/kg/day was found to be the optimal dosage, increasing the survival time of the treated mice to 21.5 ± 0.8 days as compared with 12.3 ± 0.3 days for controls [3]. The effects of the treatment with 100 mg/kg/day of DM-COOK have been examined in groups of 5 mice, treated as above, which were killed on day 8 and whose brains and livers were collected, fixed in buffered picric acid–formaldehyde [4] and processed with conventional techniques for light microscopy. Sections were cut at 7 μm and stained with hematoxylin and eosin; double-blind examinations were made on 5 sections per organ. A severe lymphocytic infiltration was observed in the organs of untreated tumor-bearing animals. In the cerebral parenchyma a lymphocytic perivascular infiltration, more severe around the meningeal blood vessels, adjacent to subarachnoid spaces was noted; in 60% of the animals large lymphoblastic nodules were also present. The livers were severely damaged, showing metastatic nodules, a lymphocytic infiltration around the portal spaces and large necrotic areas. These neoplastic lesions were absent in all of the treated animals.

These results are in agreement with previous observations on the mechanism of the antileukemic action of DM-COOK. In mice implanted i.p. with P388 leukemia and TLX5 lymphoma, DM-COOK increases the survival time of animals, but no reduction either of the number of peritoneal tumor cells or of their viability, determined by *in vivo* bioassay, is observed. *In vivo* bioassays of the brains of the same treated animals, on the other hand, reveal a marked reduction or the virtual absence of clonogenic tumor cells [3, 5, 6]. These data, together with those presently reported, indicate that the early treatment of leukemic mice with DM-COOK abolishes the systemic spread of leukemic cells from the peritoneal site of implantation. DM-

Accepted 22 June 1983.
*Supported by Italian National Research Council, Special Project 'Control of Neoplastic Growth', contract n.82.00320.96, and by Grants from the Italian Ministry of Education and from the University of Trieste.
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COOK thus clearly exerts selective antimetastatic effects with a mechanism unrelated to cytotoxicity for leukemic cells in mice, as already observed in the solid metastasizing tumor, Lewis lung carcinoma [2]. The lack of cytotoxicity for tumor cells is also paralleled with a low systemic toxicity and with the absence of myelotoxicity, as indicated by normal leukocyte and thrombocyte counts in treated animals (unreported results which will be published in detail elsewhere).

The ability of DM-COOK to prevent leukemic cerebral dissemination in mice appears to have potential therapeutic applications. These results are of interest since, in spite of the fact that selective antimetastatic treatments have been developed for solid tumors [7], and that the metastatic behavior of leukemias in animals and man has also been investigated [8–14], to our knowledge no report has appeared in the literature on agents capable of selectively preventing the dissemination of leukemic cells *in vivo*. The only existing reports have been aimed at reducing regional (lymphonodal) leukemic spread by means of specialized forms of local delivery of cytotoxic antineoplastic drugs [15–18]; the effects of the i.p. treatment of a meningeal animal leukemia with the lipophylic cytotoxic nitrosourea, methyl-CCNU, was examined in one report [19].

Further research involving the use of DM-COOK and other drugs which may prove to act as selective antimetastatic agents in mice bearing P388 and other transplantable mouse leukemias is currently underway in our laboratory, with the aim of investigating the therapeutic usefulness of the combination of antimetastatic treatment started before leukemic systemic (cerebral) spread, with subsequent cytotoxic chemotherapy. A possible synergism of the antimetastatic and cytotoxic treatment in mice might provide indications for the clinical evaluation of antimestatic drugs as adjuvants for the treatment of leukemias in man.

Acknowledgement—The authors are indebted to Dr. L. Lassiani for the generous supply of *p*-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt (DM-COOK).

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