Effects of antimetastatic, antiinvasive and cytotoxic agents on the growth and spread of transplantable leukemias in mice

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The effects of cytotoxic (cyclophosphamide, CCNU, GANU), antiinvasive (vincristine, vinblastine) and antimetastatic (ICRF-159, DM-COOK) agents have been compared in mice-bearing P388 and L1210 leukemias, and TLX5 lymphoma. The drugs tested increase the survival time of the treated mice in a manner consistent with a cytotoxic action in the case of cyclophosphamide, CCNU, GANU, vincristine and vinblastine. Leukemic infiltration of the brain after i.p. tumor implantation has been determined by bioassay of this organ, and is reduced by treatment with all of the drugs tested, with the exception of ICRF-159. DM-COOK appears to increase the life-span of the treated animals by the inhibition of leukemic spread rather than by a cytotoxic action. The marked cytotoxicity of vincristine and vinblastine is sufficient to account for failure to detect any antimetastatic effects of these agents. The lack of antdispersative effect observed for ICRF-159 under the experimental conditions employed might be connected with the observation that the antimetastatic action of this drug on solid tumors is due to its effects on tumor blood vessels.

Introduction
Systemic metastases produced by solid malignant tumors have been the object of an increasing number of investigations performed in laboratory animals and humans. Such studies have provided new insights into the pathogenetic mechanisms of solid tumor spread, and correspondingly numerous therapeutic approaches, including the use of selective antimetastatic drugs, were tested, and proved to be successful in the laboratory animal [2, 5]. Leukemias, similar to solid malignant tumors, also disseminate into practically any organ of the host. Leukemic spread limits the usefulness of treatment with cytotoxic antineoplastic drugs, since these agents do not penetrate easily into privileged sites where reservoirs of tumor cells are established. This causes relapses after phases of drug-induced remission [18, 4]. Leukemic metastases have been less often investigated, both in terms of pathogenesis and treatment, in spite of the clinical relevance of the problems encountered in the treatment of leukemias [18].

The aim of the present investigation was therefore to examine, in mice bearing the transplantable leukemias P388 and L1210, and TLX5 lymphoma, the cytotoxic and antimetastatic effects on antileukemic activity of selective antimetastatic agents (ICRF-159 and DM-COOK), antiinvasive drugs (vincristine and vinblastine) and cytotoxic drugs (cyclophosphamide, CCNU and GANU). The effects of i.p. treatment with each drug have been determined in terms of prolongation of the survival time of mice carrying i.p. or i.c. tumor implants, and have been compared to the cytotoxicity of the drugs for tumor cells and to the in vitro dissemination of tumor cells into the central nervous system.
Materials and methods

Drug treatment

The drugs tested were obtained from the following sources: p-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt (DM-COOK) was generously supplied by Dr L. Lassiani, Institute of Medicinal Chemistry, University of Trieste; (±)-1,2-di(3,5-dioxopiperazin-1-yl)propane (ICRF-159, razoxane) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) were kindly provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, Bethesda, MD, U.S.A.; cyclophosphamide (CY) and 1-(β-D-glucopyranosyl)-3-nitrosourea (GANU) were a gift from Schering SpA, Milano, Italy; and vincristine (VCR) and vinblastine (VBL) sulphates were purchased from Eli Lilly Italia SpA. The solutions used were isotonic saline for GANU and CY, and 0.1% NaHCO₃ for DM-COOK; CCNU and ICRF-159 were administered as a suspension in olive oil or in isotonic saline containing 1% sodium carboxymethylcellulose, respectively. The drugs were administered i.p. in 0.1 ml (0.05 ml in the case of the oil suspension of CCNU) per 10 g of animal weight.

Tumor transplantation and evaluation

The tumor lines, originally provided by the National Cancer Institute, Bethesda, MD, U.S.A. (TLX5 lymphoma was obtained from the Chester Beatty Research Institute, London, U.K.) were maintained by weekly i.p. transplantation into syngeneic DBA/2 mice (CBA/Lac mice for TLX5 lymphoma) of 10⁵ tumor cells for L1210 lymphoid leukemia and TLX5 lymphoma, and of 10⁶ cells for P388 lymphocytic leukemia. For the reported experiments, similar inocula were performed i.p. or i.c. as indicated, using BD2F1 hybrid mice for P388 and L1210 leukemias. Female mice, weighing 18-20 g, purchased from Charles River, Calco Como, Italy, were used; CBA/Lac belong to a local conventional breeding colony.

The survival time of the animals was recorded, or the total number of peritoneal tumor cells was measured at sacrifice at the end of treatment by means of a Coulter Counter, mod. ZF, after careful collection of the tumor cells by repeated washing of the peritoneal cavity with isotonic saline. The presence of clonogenic tumor cells in the brain was also measured at the end of treatment by aseptically transplanting the whole minced brains i.p. (s.c. in the case of TLX5 lymphoma) into normal syngeneic mice. The survival time of the normal untreated recipients was subsequently recorded, which provides an indirect evaluation of the viability and number of tumor cells present in the bioassayed sample [12, 16].

Results and discussion

The data reported in table 1 show that all the compounds tested significantly increase the survival time of mice bearing transplantable leukemias. However, different responses are observed on the three tumor lines examined, and only DM-COOK, VCR, CY and GANU are active, although at different degrees, on each of the tumor lines used. The activity of CCNU is evident against TLX5 lymphoma and P388 leukemia, whereas that of VBL and ICRF-159 are limited to P388 and L1210 leukemias, respectively. Considering that DM-COOK exerts the greatest response on TLX5 lymphoma, ICRF-159 on L1210 leukemia and the other drugs on P388 leukemia, these relationships between the drugs and tumor lines were used in the subsequent experiments. The drug dosages used in the experiments of tables 2 and 3...