IN VITRO-IN VIVO SCREENING OF DRUGS FOR USE IN REGIONAL BRAIN CANCER CHEMOTHERAPY*

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This laboratory has developed a technique that permits regional extracerebral perfusion of the head, with partial isolation of both the afferent and efferent cerebral circulation.15-17 Designed originally to permit localized cerebral hypothermia, the procedure also offers the opportunity for regional perfusion of brain tumors or tumors involving other structures of the head with blood containing one or more chemotherapeutic agents. Drugs must be selected specifically for regional perfusion that offer the greatest potential for inactivating cancer cells following a prolonged exposure of drug to tumor. Because regional perfusion may subject normal as well as pathological cerebral tissue to action of the drugs, any effects of high concentrations of drug upon normal cerebral tissue must be determined by perfusing each drug to the heads of experimental animals. The magnitude of permissible "leak" of drug through collateral vessels into any sensitive tissue of the general circulation during regional perfusion of the head must also be established. This paper presents a technique of in vitro-in vivo screening of drugs for obtaining information on one of these areas of investigation, i.e., the anti-cancer effect of drugs following varying drug-tumor exposure periods.

METHODS AND MATERIALS

Alkylating agents screened in this study were: methyl-bis (B-chloroethyl) amine (HN2); 2, 5-diethylenimino-3, 6-bis (tol-methoxyethoxy)-1, 4-benzoquinone (A139); triethylene thiophosphoramide (TSPA); 1, 6-bis (2-chloroethylamino)-1, 6-deoxy-D-mannitol dihydrochloride (BCM); triethylene melamine (TEM); N-3-oxapentamethylene-N', N'-diethylene thiophosphoramide (OPSPA); and H-1,3,2-oxazaphosphorine, 2-bis (2-chloroethyl) amino tetrahydro-2-oxide (3ytoxan). Amino acid analogs screened were: meta-, ortho-, and para-fluorophenylalanine; B-2-thienylalanine; phenylactic acid; d1-ethionine and methionine sulfoxide. Two experimental animal tumors were used for screening: the VX2 rabbit carcinoma (VX2) and the Walker rat carcinosarcoma 256 (WR256).

Suspensions of both types of tumor were obtained under sterile conditions in the following manner: Tumors from donor animals were stripped of surrounding tissues and capsules, and were minced by cross-cutting with scalpels. The minced tumor was next pressed by a pestle through a sieve with 500 micron openings while Ringer's solution was dripped through the sieve. The sieved tumor clumps were trapped in a second sieve with 74 μ openings and were used in preparing in vitro suspensions. In each set of experiments equal amounts of moist tumor clumps, 74-500 μ in diameter, were suspended in standard volumes of Ringer's solution as controls, or in various molar (M) concentrations of each drug dissolved in Ringer's solution as experimental suspensions.

Suspensions were incubated at 37°C. at room atmosphere for 1, 2, 3, 4, or 24 hours without continuous agitation. At the end of each incubation period, a standard amount of tumor suspension was withdrawn from the control and from each experimental suspension and was centrifuged at...
1,000 r./min. for 5 min. The supernatant material was decanted; the tumor clumps were re-suspended in 10 cc. of Ringer’s solution, recentrifuged and the supernatant material again was decanted. At the end of each incubation period the washed tumor clumps from the control and from each experimental suspension were re-suspended in 2 cc. (VX2) or 1 cc. (WR256) of Ringer’s solution and were transplanted through 16 gauge needles into separate host animals. Transplantations of VX2 were made into the anterior muscles of the thigh or abdominal subcutaneous tissue of 250 stock rabbits, and WR256 was transplanted subcutaneously into 250 locally inbred derivatives of Osborne-Mendel strain rats. No selection of sex was attempted.

Twenty-one days after transplantation of VX2 and 12 days after transplantation of WR256, the host control and experimental animals in a given set of experiments were sacrificed. Any tumors present were excised, stripped of surrounding tissues and weighed. The anti-cancer activity of drugs for each time interval of exposure was judged inhibitory when the weight of the experimental tumor was greater than 10 per cent but less than 50 per cent of the weight of the control tumor and was judged lethal when the experimental tumor did not grow or was less than 10 per cent of the weight of the control. Each experimental and control tumor, after having been weighed, was fixed in 10 per cent formalin and was stained with hematoxylin and eosin for histological examination.

In several experiments the above procedure was modified in that control and experimental incubations were carried out at 20° C. as well as 37° C. for each period of exposure. In such cases, for the control and each experimental suspension at each period of exposure, incubated tumor clumps were transplanted into anatomically similar areas of one animal.

RESULTS

It was determined in preliminary experiments that VX2 clumps would survive in vitro at 20° C. or 37° C. up to 48 hours, whereas WR256 clumps did not survive consistently beyond 2 hours in vitro. In general terms, the alkylating agents were more inhibitory against both tumors than were the amino acid analogs. In regard to the VX2, the minimum molar concentrations of drugs that were lethal to the tumor clumps during an exposure period of only 1 or 2 hours at 37° C. were: HN2, 0.000015; TEM, 0.00015; A139, 0.00015; TSPA, 0.0015; o-fluorophenylalanine, 0.0022; OPSPA, 0.0068; BCM, 0.00015; and thienylalanine, 0.10. Higher concentrations of phenyllactic acid, m-fluorophenylalanine, cytoxan*, methionine sulfoxide, and ethionine either required 3 to 24 hours to produce an anti-cancer effect or were completely ineffective when experimental preparations were compared with controls. In regard to WR256, the minimum molar concentrations of drugs that were lethal to the tumor clumps during an exposure of 1 to 2 hours at 37° C. were: HN2, 0.000015; TSPA, 0.000015; TEM, 0.000015; A139, 0.000015; BCM, 0.00015; thienylalanine, 0.10; and phenyllactic acid, 0.10. Higher concentrations of p-fluorophenylalanine, ethionine, and methionine sulfoxide did not produce this lethal effect against WR256. In the interest of brevity, representative data regarding only VX2 are shown in Table 1.

The anti-cancer effect of each drug was more pronounced against both tumors at 37° C. than at 20° C. incubation periods. The only controls incubated at both temperatures that showed any consistent difference in subsequent weights of tumor were the 24-hour VX2 controls; those incubated at 20° C. were consistently larger than those incubated at 37° C. The longer the period of exposure (at either 37° C. or 20° C.) the more inhibitory were the effective anti-cancer drugs. Also, when a range of effectiveness was obtained from noninhibitory to lethal, the higher the concentration of the drug, the greater was the inhibitory effect upon subsequent growth of tumor. Histological sections of tumors that grew following exposure to drugs revealed no obvious mitotic or morphological changes in the cells.

DISCUSSION

In the past, chemotherapy of human