Localized Changes in Blood-Brain Barrier Permeability following the Administration of Antineoplastic Drugs

Lindsay A. MacDonell, Pamela E. Potter, and Ronald A. Leslie

Departments of Pharmacology [L. A. M., P. E. P.] and Anatomy [R. A. L.], Dalhousie University, Halifax, Nova Scotia, Canada

ABSTRACT

The effects of four antineoplastic drugs on the permeability of the blood-brain barrier to Evans blue-albumin and to horseradish peroxidase were studied in cats. Extravasation of tracer in brain tissue was observed only rarely following the injection of methotrexate, cyclophosphamide, or vincristine. However, 5-fluorouracil (15 mg/kg) caused localized Evans blue-albumin exudation in various gray and white matter areas in 8 of 13 cats to which the Evans blue was administered 7 hr after the drug injection. Electron microscopy revealed that 5-fluorouracil stimulated pinocytic vesicular transport of peroxidase across brain capillary endothelial cells and possibly that it widened endothelial tight junctions. Barrier leakage was observed only when time periods longer than 7 hr elapsed between 5-fluorouracil injection and tracer administration, and extravasation occurred only once after a shorter time interval. These results suggest that changes in blood-brain barrier permeability observed 7 hr after 5-fluorouracil administration are reversible and of fairly short duration. Such changes may be relevant to the development of secondary intracranial tumors following antineoplastic chemotherapy.

INTRODUCTION

Recent observations suggest that the incidence of metastatic brain tumors is increasing as chemotherapy against primary and secondary extracranial neoplasms is becoming more effective (2, 14). Although this could merely reflect prolongation of survival time, the fact that most brain metastases originate as blood-borne emboli (14) led us to investigate the effects of some antineoplastic drugs on the permeability of the blood-brain barrier.

MATERIALS AND METHODS

Physiological Techniques. Cats weighing 2 to 4 kg were anesthetized by an i.p. injection of sodium pentobarbital (35 mg/kg). Maintenance doses of pentobarbital were administered via a femoral venous catheter, and this route was also used for the administration of other drugs. Arterial blood pressure was monitored continuously through a femoral artery catheter with a Statham P23AC pressure transducer and a Grass Model 7 polygraph. The arterial catheter also enabled withdrawal of arterial blood samples for measurement of arterial oxygen tension, arterial carbon dioxide tension, and pH on a Radiometer BMS MK2 Blood Micro System in conjunction with a Radiometer PHM71 MK2 Acid-Base Analyzer. The animals were artificially ventilated with a Harvard Model 675 respiratory pump in such a way that arterial blood gasses remained within normal limits. Rectal temperature was maintained at 37 ± 0.5° throughout the experiments. A 16-gauge Cathion needle was inserted into the cisterna magna of some animals, through which intracranial pressure was monitored with a Statham pressure transducer and Grass polygraph.

Antineoplastic drugs were administered via the venous catheter. At certain time intervals following drug injection, Evans blue (3 ml of a 2% solution in 0.9% NaCl solution per kg) was infused i.v. The infusion period was 5 to 8 min, in order to allow time for the Evans blue to bind to albumin as it was being administered (4). The EBA4 complex was allowed to circulate for 30 min, after which time blood was flushed from the cerebral circulation by aortal perfusion of 10% formalin at a pressure of approximately 90 mm Hg. Brains were then removed, cut into slices 1 to 2 mm thick, examined for blue staining, which, if present, indicated extravasation of the EBA complex, and photographed.

Microscopy. HRP (Sigma, type II; 100 mg/kg) was dissolved in 0.9% NaCl solution and injected i.v. 15 min after completion of the Evans blue infusion. After a further 15 min, the brains were fixed in situ according to the method of Palay and Chan-Palay (8). Excised blocks of tissue were treated with diaminobenzidine (5). After completion of processing for electron microscopy, ultrathin sections were stained with lead citrate (13) or examined unstained in a Zeiss EM10A electron microscope. Semithin sections (0.5 to 1.0 µm) were also taken and stained with 1% toluidine blue in 1% sodium borate for light microscope examination.

Seven control animals were treated in a similar manner, except that they were given injections of 1 to 2 ml of 0.9% NaCl solution containing no antineoplastic drugs.

Drugs. The antineoplastic drugs and their dosages used in this study were: 5-FU, 15 to 30 mg/kg; methotrexate, 0.64 mg/kg; cyclophosphamide, 42.85 mg/kg; vincristine, 0.05 mg/kg. In addition, 13 cats were treated with various doses of fluorocacetate (see Table 2).

RESULTS

Controls. Control experiments all demonstrated that the blood-brain barrier was impermeable to the tracers used

1 This work was supported by the Medical Research Council of Canada (MA-5779).
2 Present address: Department of Pharmacology, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.
3 To whom requests for reprints should be addressed.
Received February 28, 1978; accepted June 13, 1978.

* The abbreviations used are: EBA, Evans blue-albumin; HRP, horseradish peroxidase; 5-FU, 5-fluorouracil.

CANCER RESEARCH VOL. 38

2930
under the experimental conditions of this study. No extravasation of EBA was observed in the animals after maintenance under anesthesia and controlled ventilation for 6 to 7 hr (Table 1). Electron micrographs of capillaries from brains of HRP-treated animals showed the reaction product to be contained completely within the lumens of the capillaries (Fig. 1).

Antineoplastic Drugs. Preliminary experiments determined appropriate time intervals between antineoplastic drug injection and tracer administration. Drugs were injected i.v. into unanesthetized cats, after which the animals were observed for several hr for side effects such as vomiting, ataxia, incoordination, or lethargy. When any such symptoms developed, the animals were anesthetized, prepared as described above, and infused with Evans blue. If extravasation of EBA occurred in the brain, the time period between drug administration and infusion of Evans blue served as a guide for subsequent experiments. The most common side effect was ataxia which began to develop 6 to 7 hr after 5-FU administration.

5-FU. The results obtained from 5-FU-treated animals are shown in Table 1. Increased barrier permeability to EBA was observed, and this occurred most frequently 7 hr after the 5-FU injection in 8 of the 13 animals that received the 15-mg/kg dose. This was not accompanied by any significant changes in arterial blood pressure or in cerebrospinal fluid pressure.

Areas of extravasation of EBA were found in either gray or white matter of the cerebral hemispheres, the cerebellum, the inferior colliculi, and the brain stem. Blue staining was never widespread; sometimes it was localized to a single area of 1 to 3 mm diameter (Fig. 2), while in others it was more diffuse and involved more than 1 area.

In experiments in which HRP was also used as a tracer, light micrographs of areas showing macroscopic evidence of EBA exudation indicated that there was penetration of HRP into the surrounding tissue of a few vessels in every such area. Fig. 3 shows HRP leakage around 2 of the 5 capillaries in the field.

With the electron microscope, HRP reaction product was observed in basement membranes (Figs. 4 and 5), within endothelial cells (Fig. 5), and in intercellular spaces between endothelial cells (Fig. 4) of capillaries that had become permeable. It was not possible to see the tracer filling any intercellular spaces, all the way from the lumen to the basement membrane. The reaction product found within endothelial cells could be seen to be enclosed in pinocytotic vesicles. Occasionally, such a vesicle appeared to be extruding its contents into a basement membrane (Fig. 5) or space between adjacent endothelial cells. Perivascular tracer was largely confined to the extracellular spaces, and it penetrated for distances of several μm into the brain parenchyma (Fig. 4).

Of 4 animals studied at 5.5 to 6.5 hr after 5-FU administration, only 1 showed barrier breakdown. No extravasation of tracers was found in 4 animals after 8.5 to 19.5 hr (Table 1). The 30-mg/kg dose of 5-FU (Table 1) used in 3 animals caused no more widespread changes in barrier permeability than did the 15-mg/kg dose.

Fluoroacetate. Thirteen animals were given injections of fluoroacetate in order to determine whether this metabolite of the antineoplastic drug could be responsible for the observed effects of 5-FU (Table 2). Each dose of fluoroacetate resulted in the development of cardiac arrhythmias some 2 hr after injection, but only the highest dose repeatedly caused abnormal barrier permeability to EBA.

Methotrexate. Six cats were treated with methotrexate and Evans blue was administered at times ranging from 4.5 to 7.0 hr after the drug injection (Table 1). Extravasation of EBA occurred in 1 of the 4 animals in which the tracer was infused 6 hr after the methotrexate. In this animal 4 discrete patches of blue were found in dorsal gray matter of the cerebral parietal and occipital lobes. One additional cat, not included in Table 1, was given a total of 9 injections of methotrexate over a period of 102 hr. This caused no abnormal barrier permeability to EBA.

Cyclophosphamide. Extravasation of EBA was observed in 1 of 6 animals treated with cyclophosphamide (Table 1). It appeared as diffuse blue staining of the posterior paleocerebellum, 4.5 hr after the drug injection.

Vincristine. Five experiments were performed with vincristine, in which Evans blue was administered 6.0 to 7.5 hr after the drug injection. Extravasation of EBA was observed in one of these (Table 1) and the pattern was similar to that seen with methotrexate. An additional animal, not included in Table 1, received 6 injections of vincristine over a period of 72 hr, but no blue staining of brain tissue occurred.

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Time after injection(a) (hr)</th>
<th>No. of animals</th>
<th>Results(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.9% NaCl solution)</td>
<td>6.0-7.0</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>5-FU</td>
<td>15</td>
<td>5.5-6.5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5-FU</td>
<td>15</td>
<td>7.0</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>5-FU</td>
<td>15</td>
<td>8.5-19.5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5-FU</td>
<td>30</td>
<td>7.0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.64</td>
<td>4.5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.64</td>
<td>6.0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.64</td>
<td>7.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>42.85</td>
<td>4.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>42.85</td>
<td>4.5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>42.85</td>
<td>6.5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.05</td>
<td>6.0-6.5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.05</td>
<td>7.5</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

\(a\) Length of time between antineoplastic drug injection and tracer administration.

\(b\) + or −, extravasation or no extravasation of EBA, respectively.

Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. of animals(a)</th>
<th>Results(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoacetate</td>
<td>0.15-0.20</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Fluoacetate</td>
<td>1.00</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Fluoacetate</td>
<td>2.00</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

\(a\) Evans blue was infused 2 to 3 hr after fluoroacetate injection.

\(b\) As for Table 1.
L. A. MacDonell et al.

Methotrexate, cyclophosphamide, and vincristine had no significant effect upon arterial blood pressure or intracranial pressure.

**DISCUSSION**

**Controls.** The lack of extravasation of EBA in brain tissue of control animals would indicate that the experimental conditions of anesthesia and controlled respiration for 6 to 7 hr did not cause breakdown of the barrier in experimental animals to the dye-albumin complex. The presence of endothelial zonulae occludentes preventing extravasation of HRP and the paucity of transendothelial vesicular transport in the control vessels is in accordance with the observations of other investigators (1, 12).

5-FU. The localized increases in blood-brain barrier permeability caused by 5-FU occurred most consistently 7 hr after the administration of the drug. Barrier permeability was less frequent after shorter time periods and absent in the 4 experiments involving 8.5- to 19.5-hr lapses after the 5-FU injection. This suggests that the increased barrier permeability is reversible and lasts only 7 to 8 1/2 hr.

The mechanism of blood-brain barrier breakdown after 5-FU administration has not been firmly established by the present investigation. Increased vesicular activity has been shown, and peroxidase reaction product was observed within extracellular channels between endothelial cells. In no instance, however, was tracer seen to fill the entire length of such channels, so it cannot be stated with certainty that disruption of zonulae occludentes occurred.

The effect of 5-FU cannot be secondary to abnormalities in arterial oxygen tension, arterial carbon dioxide tension, and blood pressure or in intracranial pressure (11) since all these variables remained within normal limits. Nor is it likely that cell cycle inhibition is related directly to the permeability changes, since endothelial cells have an estimated turnover time of 3 years or more (3).

Fluoroacetate is a potent metabolic inhibitor (10) that has been claimed to impair permeability barriers (9). Because some 5-FU can be metabolized to this toxic compound (6, 7), the experiments with fluoroacetate were performed. However, the dose required to open the blood-brain barrier consistently was approximately 10 times the median lethal dose for cats.

This, together with the fact that fluoroacetate but not 5-FU caused severe cardiac arrhythmias, indicates that the effects of 5-FU are not mediated by the metabolite fluoroacetate.

**Methotrexate, Cyclophosphamide, and Vincristine.** Only occasionally did extravasation of EBA occur following the administration of methotrexate, cyclophosphamide, or vincristine. Drug dosage was not varied, and the possibility exists that larger doses might have produced different results. Repeated doses of methotrexate and vincristine were, however, ineffective. There were no abnormalities in blood pressure, intracranial pressure, or blood gases to explain the occasional experiment in which extravasation of EBA occurred after these drugs.

Although the role of blood-brain barrier permeability in the development of cerebral metastases has not been investigated, the results of this study indicate that 5-FU can cause localized abnormalities in the blood-brain permeability barrier. Such an effect, which has not been reported previously, could possibly influence the penetration of blood-borne metastatic emboli into the central nervous system.

**ACKNOWLEDGMENTS**

We are indebted to Hanni Weideli and John Stevens for their technical assistance, and to Dr. J. A. Aquino for his helpful advice.

**REFERENCES**

Fig. 1. Electron micrograph of part of a capillary from a control experiment. The erythrocyte (RBC) has trapped some peroxidase (HRP) within the capillary lumen. Note the absence of HRP reaction product in the basement membrane (BM). Zonulae occludentes (arrowheads) appear to be preventing the passage of HRP between the adjacent endothelial plasma membranes. EC, endothelial cell. x 108,000.

Fig. 2. Macroscopic extravasation of EBA resulting in blue staining (arrowhead) in the left occipital lobe of an animal treated with 5-FU. Note the lack of intensity and the localized nature of the staining. x 3.5 (approximately).

Fig. 3. Light micrograph of a blue-stained area of the brain from a 5-FU-treated cat. The HRP has traversed the endothelium of 2 capillaries (arrowheads) of the 5 visible in the micrograph. The remaining capillaries appear to contain no HRP reaction product since the HRP has presumably been washed out by the perfusion fixation. x 600.
Fig. 4. Electron micrograph of part of a capillary from a 5-FU-treated cat. HRP reaction product is present in the basement membrane (BM) of both the endothelial cell and the pericyte (arrow) and in the extracellular spaces surrounding the myelinated axons (A) and other parenchymal cells. In this region the HRP has penetrated at least 3 μm into the parenchyma from the capillary lumen (L). × 23,500. Inset, HRP reaction product clearly visible in spaces between adjacent endothelial cells (arrowheads), although the plane of the section is such that it is impossible to see whether or not there is a continuous column of the tracer from the lumen (L) to the basement membrane. × 38,500.

Fig. 5. Electron micrograph of a capillary following 5-FU treatment. As well as HRP reaction product in the basement membrane (BM) and in parenchymal interstices, small pinocytotic vesicles containing the tracer (arrowheads) can be seen in the capillary endothelial cells. × 37,500. Inset, a pinocytotic vesicle (arrowhead) which could be extruding its contents into the basement membrane. L, lumen. × 74,000.
Localized Changes in Blood-Brain Barrier Permeability following the Administration of Antineoplastic Drugs

Lindsay A. MacDonell, Pamela E. Potter and Ronald A. Leslie


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/9/2930

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.