Hemorrhagic encephalitis produced by selective non-occlusive intracarotid BCNU injection in dogs

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A selective non-occlusive technique was developed for administration of BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) into the internal carotid artery of the dog, and the neuropathological effects in the brain were studied. One out of three dogs showed ipsilateral hemorrhagic necrotizing encephalitis at doses of 102 mg/sq m, and all of three dogs showed similar but more severe pathology at doses of 215 to 232 mg/sq m. This study and previous studies in the dog and monkey suggest definite thresholds above which cerebral toxicity occurs when BCNU is administered via the intracarotid route. Greater dilution of drug in the larger territory of supply of the human internal carotid artery allows somewhat higher doses in man. The pathology of the lesion induced by BCNU suggests a primary vascular injury as a pathogenic mechanism, consonant with similar findings following high-dose systemic BCNU administration in man. Investigators conducting ongoing and future trials of intracarotid BCNU in the human for the treatment of intracranial neoplasms should be especially vigilant for a similar toxic effect.

KEY WORDS • encephalitis • intra-arterial infusion • carmustine • toxicity • drug therapy

The drug BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea)* is a cell-cycle nonspecific lipid-soluble nitrosourea compound with proven oncolytic activity3,23 that readily penetrates the blood-brain barrier.6,15 Systemic administration is limited by side effects, chiefly, delayed bone marrow suppression3,5,21 and pulmonary fibrosis.8,16,19 Intracarotid infusion of BCNU gives higher drug levels in the infused brain area, compared with intravenous infusion.14 Taken together, these facts provide a rationale for infusing BCNU intra-arterially into the blood vessel supplying a tumor-bearing region of the brain, and such trials are being conducted in the human for both metastatic15,22,24 and primary1,12 brain malignancies.

The present study of the effects of intracarotid BCNU infusion in dogs was undertaken as a preliminary to such trials in the human. The need for a more detailed examination of brain morphology after the intracarotid administration of BCNU has been suggested by previous studies.4,7 Because of previous reports of ocular toxicity of BCNU to the cornea and retina,7 an experimental design was devised in the dog that delivered virtually all of the drug to the cerebral circulation without occluding the external carotid artery (ECA), thus preserving the ECA contribution to the ophthalmic artery in the dog.

Materials and Methods

Nine mongrel dogs, weighing between 16 and 25 kg each, were divided into three groups of three dogs each. The first group received only the carrier solution of 10% ethanol, and served as a control group. The second and third groups received intracarotid BCNU in doses of 4 mg/kg and 8 mg/kg, respectively. Each dog was anesthetized with intravenous sodium pentobarbital (25 mg/kg), and spontaneous respiration was maintained.

A technique was devised for transfemoral non-occlusive catheterization of the internal carotid artery (ICA), as follows. A Cordis sheath† was inserted into

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* Carmustine supplied by Bristol Laboratories of Canada, 411 Roosevelt Avenue, Ottawa, Ontario, Canada.

† F5 Cordis sheath manufactured by Cordis Corp., Miami, Florida.
the femoral artery using the Seldinger technique, and a continuous-drip infusion attached. A No. 4.1 French polyethylene catheter with a tapered and slightly bent tip, along with a 21-mm Teflon-coated guide wire, offered the best combination for selective ICA catheterization. The tip of the catheter was carefully manipulated into the left carotid bulb, without occluding the ostium of the vessel. This was followed by drip-infusion angiography, which demonstrated a laminar flow effect in the ICA (Fig. 1). The rate of infusion of BCNU was selected following drip-infusion angiography to ensure no reflux into the external carotid circulation.

The BCNU was prepared immediately before administration. A clear and colorless solution of 3.3 mg BCNU in 10% ethanol was obtained. Infusion of this solution into the left ICA was performed over 15 minutes using a pressure pack. Infusion volume depended on the animal’s weight and the intended dose: it varied between 30 and 60 cc. A second infusion angiogram was performed immediately after drug administration to ensure correct positioning of the catheter without occlusion of the ICA, as well as patency of the vessel after injection. Five dogs (Dogs 1, 2, 7, 8, and 9), including those that received 8 mg/kg BCNU, had follow-up angiography 3 or 4 days after drug administration to confirm continued patency of the ICA. The ICA was patent in all follow-up angiograms so the procedure was therefore deemed unnecessary in the remaining dogs (Dogs 3, 4, 5, and 6).

Within the 1st week after drug administration, the dogs were sacrificed by pentobarbital overdose, and the brains were removed, along with the ipsilateral eyes in the group that had received 4 mg/kg BCNU. A specimen of lung was obtained in one dog that was severely ill following a 4 mg/kg BCNU injection. All specimens were fixed in 10% formalin. The brains were allowed to fix for 1 to 2 weeks, and were then sectioned coronally. Following paraffin embedding and sectioning in 8-μm slices, sections were stained with hematoxylin and eosin, Kluver-Barrera, and Martius’ scarlet blue stains.

Results

The findings are summarized in Table 1.

Clinical Findings

The control dogs receiving only 10% ethanol were clinically normal. All dogs receiving BCNU had systemic reactions, most commonly lethargy and dehydration. They were less active and did not feed well.
BCNU-induced hemorrhagic encephalitis in dogs

### TABLE 1
Summary of findings in nine dogs with intracarotid injection of BCNU

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight (kg)</th>
<th>Surface Area (sq m)*</th>
<th>Infusion</th>
<th>Dose (mg/sq m)</th>
<th>Clinical Follow-Up</th>
<th>Follow-Up Angiogram</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>0.641</td>
<td>10% eth-anol</td>
<td>10% eth-anol</td>
<td>normal</td>
<td>normal</td>
<td>normal brain &amp; ICA</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>0.668</td>
<td>10% eth-anol</td>
<td>10% eth-anol</td>
<td>normal</td>
<td>normal</td>
<td>normal brain &amp; ICA</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.641</td>
<td>10% eth-anol</td>
<td>10% eth-anol</td>
<td>normal</td>
<td>nil</td>
<td>normal brain &amp; ICA</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>0.668</td>
<td>4 mg/kg BCNU</td>
<td>4 mg/kg BCNU</td>
<td>lethargy; dehydration</td>
<td>nil</td>
<td>normal brain; normal lt eye; normal ICA</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>0.668</td>
<td>4 mg/kg BCNU</td>
<td>4 mg/kg BCNU</td>
<td>diarrhea; vomiting; dehydration</td>
<td>nil</td>
<td>lt hemisphere edema; hemorrhagic necrotizing encephalitis, chiefly centrum semiovale; normal lt eye; normal ICA</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>0.668</td>
<td>4 mg/kg BCNU</td>
<td>4 mg/kg BCNU</td>
<td>lethargy; dehydration; seizure</td>
<td>nil</td>
<td>lt hemisphere edema; hemorrhagic necrotizing encephalitis; normal ICA</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>0.744</td>
<td>8 mg/kg BCNU</td>
<td>8 mg/kg BCNU</td>
<td>lethargy; dehydration</td>
<td>normal</td>
<td>massive lt hemisphere edema with hemorrhagic necrotizing encephalitis, centrum semiovale; fibrointimal hyperplasia of ICA</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>0.864</td>
<td>8 mg/kg BCNU</td>
<td>8 mg/kg BCNU</td>
<td>lethargy; dehydration</td>
<td>normal</td>
<td>massive lt hemisphere edema; hemorrhagic necrotizing encephalitis; normal ICA</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>0.769</td>
<td>8 mg/kg BCNU</td>
<td>8 mg/kg BCNU</td>
<td>dehydration</td>
<td>normal</td>
<td>massive lt hemisphere edema; encephalitis involving centrum semiovale &amp; deep gray matter, but sparing cerebral cortex</td>
</tr>
</tbody>
</table>

* Based on: SA = K × W^{2/3}/100, with K = 10.1 for the dog.\(^{11}\)

for the first few days after injection. There was no obvious hemiplegia, in spite of the neuropathological findings in some of them.

**Pathology**

The dogs receiving 10% ethanol only had grossly and microscopically normal brains.

In the group of dogs that received 4 mg/kg of BCNU, two were normal and one showed a distinct gross and microscopic lesion, similar in pattern but less severe than that seen in the 8-mg/kg BCNU group of dogs. Ipsilateral ocular histological examination in the 4-mg/kg BCNU group disclosed no vasculitis or other lesions.

The brains of the animals receiving 8 mg/kg of BCNU showed stereotyped lesions in the left cerebral hemisphere, ipsilateral to the carotid injection. On gross examination, the brains contained hemorrhagic necrotizing multifocal lesions undergoing early cavitation. There was edema of the entire hemisphere, with a left to right shift and cingulate herniation due to mass effect (Fig. 2). Obstruction of the third ventricle due to the mass effect caused mild to moderate hydrocephalus in some brains.

Microscopically, the most consistent feature was a necrotizing arteriolitis, present in small and medium-sized arterioles. Intramural infiltration of neutrophils and mononuclear cells caused complete obliteration of recognizable wall architecture (Fig. 3). Perivascular diapedetic hemorrhage was present in areas of frank angionecrosis, becoming confluent in the larger areas of grossly visible hemorrhage. Neural parenchymal necrosis was manifest by loss of tissue architecture, cytolysis, and karyorrhexis, and an inflammatory infiltrate of both neutrophils and macrophages. The white matter was consistently more affected than the gray matter. In less severely affected areas of the hemisphere, only edema of white matter and pallor of

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**Fig. 2.** Gross appearance of the brain in a dog after receiving 8 mg/kg of BCNU. Multifocal hemorrhagic necrotizing lesions are present in the centrum semiovale and region of the internal capsule (IC) and anterior commissure (AC). There is early cavitation. Surrounding edema is manifest grossly by mass effect, with left to right herniation of the cingulate gyrus (black arrow) beneath the midline position of the falk cerebri in the longitudinal cerebral tissue (white arrow). C = caudate nucleus, S = septal nuclei.
FIG. 3. Photomicrograph demonstrating necrotizing arteriolitis, with relative preservation of the intervening neural parenchyma. There is complete necrosis of the arteriolar walls, manifest by an intramural neutrophilic and mononuclear cell infiltration, total loss of normal wall architecture, and perivascular diapedetic hemorrhage. H & E, × 175.

FIG. 4. Low-power microscopic field of less severely damaged tissue showing pallor and rarefaction of white matter by edema fluid, occasionally forming lakes splitting the tissue (below). The cortex (above) is normal. Asterisks indicate cortical-white matter junction. H & E, × 60.

myelin staining was demonstrable (Fig. 4). As more severely affected tissue was approached under the microscope, arteriolonecrosis appeared, while the surrounding neural parenchyma was still preserved. Finally, these areas merged geographically into the areas of complete hemorrhagic necrosis.

Mild fibrointimal hyperplasia was noted in the ICA removed from a dog that had received 8 mg/kg of BCNU. Unfortunately, the eyes in the 8-mg/kg group were not examined. The lung of one dog receiving 4 mg/kg of BCNU showed severe hemorrhagic necrotizing bronchopneumonia.

Discussion

Infusion Technique

Previous studies of this nature used selective occlusion of the ECA in the neck and drug delivery through a puncture of the common carotid artery. In the dog, that method avoids the external ophthalmic artery. The infusion technique of the present study is unique in that the sinus of the ICA could be selectively catheterized via the transfemoral approach with continued patency of the ECA. The presence of a femoral sheath allows for withdrawal and reshaping of the catheter tip when necessary to ensure stability at the sinus. The choice of catheter placement in the carotid bulb was dictated by the fact that the ICA distal to the bulb is about 1 to 2 mm in diameter (Fig. 1 left). An attempt to advance beyond the sinus would lead to occlusion of the vessel. There was no damage done to this vessel, as shown by repeat angiography. The lack of reflux of BCNU into the external carotid artery was assured by the presence of laminar flow and contrast infusion angiography before, during, and after the BCNU injection.

Cerebral BCNU Histotoxicity

The susceptibility of brain tissue to damage by high concentrations of BCNU is suggested by several reports. Rhesus monkeys developed a hemiparesis and electroencephalographic (EEG) changes after intracarotid BCNU in doses of 172 mg/sq m, but tolerated doses of 12 to 115 mg/sq m well. Autopsy revealed no pathology that could account for the hemiparesis and EEG changes, but instead revealed severe liver damage. An ipsilateral necrotizing cerebral arteriolitis was produced by intracarotid BCNU in four dogs at doses of 2 to 4 mg/kg (67 to 125 mg/sq m). High doses of 1500 to 2850 mg/sq m administered systemically in the human can give rise to a necrotizing symmetrical encephalomyelopathy with prominent necrosis of blood vessels. Such pathology was not seen in human necropsy material after 1400 mg/sq m BCNU was administered systemically. These findings suggest that tissue concentrations in the brain and not merely the route of administration of the BCNU are important in the development of central nervous system (CNS) toxicity. In the monkey, intra-
BCNU-induced hemorrhagic encephalitis in dogs

carotid BCNU infusion gives roughly four times the drug concentration in the infused brain areas when compared with intravenous administration. This may explain why the doses per square meter necessary to produce CNS toxicity have been lower via the intracarotid route.

In the present study, doses of 102 mg/sq m (4-mg/kg BCNU group) led to CNS pathology in one out of three dogs, and doses of 215 to 232 mg/sq m (8-mg/kg group) led to CNS pathology in three out of three dogs. The lack of significant neuropathological findings in the monkey at 172 mg/sq m, with only transient clinical and EEG changes, may be accounted for by the larger territory of ICA supply (and hence greater BCNU dilution) in the monkey, as compared with the smaller territorial ICA supply in the dog. The human brain has a dominant ICA distribution and thus tolerates higher intracarotid doses of 200 to 300 mg/sq m. Still, the finding in the human of serious encephalomyelopathy after 1500 to 2850 mg/sq m of systemic BCNU, with similar pathology, suggests that the human brain is similarly susceptible to BCNU.

Ocular Toxicity

Comparison of the ocular toxicity of intracarotid BCNU in dog and man must similarly take into account the vascular anatomic differences. In the dog, both the ECA and the ICA supply the eye, whereas in man the eye is supplied by the ICA. Thus, a mitigating dilution effect of the larger ICA flow in man compared with the dog is lessened by the fact that the ICA is the only source of blood to the human eye. Ocular toxicity should therefore be relatively more prominent in BCNU infusion via the ICA in humans. This has been borne out, with ocular complications seen in four out of six patients reported in a preliminary study who received doses of 200 to 300 mg/sq m. Blindness was not reported at 100 mg/sq m in other trials.

Action and Distribution of BCNU

Tong and Ludlum reported that BCNU causes cross-linking of DNA guanine, but the ultimate biochemical mechanism of cytotoxicity is as yet unknown. The common feature of prominent angionecrosis in both human BCNU encephalopathy and in the present and a previous animal study, suggests a primary vascular injury as a pathogenic mechanism at the tissue level. BCNU is lipid-soluble, and crosses the blood-brain barrier within 1 minute in dogs. Although BCNU is rapidly metabolized to other compounds, radioactivity due to intravenous carbon-14 (14C)-labeled BCNU was found in man to equilibrate from plasma to cerebrospinal fluid (CSF) within 1 hour in a multi-species study by DeVita, et al. This short biological half-life contrasts with the longer time course of action and toxicity of the drug. This is probably due to tissue retention. Indeed, in the same study, the fate of 25% to 30% of 14C-BCNU radioactivity could not be determined after analysis of plasma, CSF, urine, feces, and exhaled CO2, but delayed retention and slow release of a metabolite of BCNU by body tissues could not be ruled out, as residual carcass radioactivity was not determined in that study. The fate of intracarotid BCNU that has passed through the cerebral circulation is likely to be similar, as the drug has a 5-minute serum half-life after ICA infusion, and has largely disappeared from the plasma in 60 minutes.

Theoretical considerations suggest that the superiority of intra-arterial over intravenous administration of a drug is dependent on a high level of tissue binding (blood-brain extraction). BCNU is thus a suitable agent for intracarotid infusion, the advantage being incurred through brain-tissue binding on the first pass through the cerebral circulation.

Tissue retention of BCNU should be studied in the human brain at autopsy of patients who have received intracarotid BCNU. The levels of BCNU and known metabolites should be compared in each hemisphere.

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