Brain-tumor imaging using radiopaque perfluorocarbon

A preliminary report

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Perfluorocarbon, a new tumor-seeking x-ray contrast agent, was injected into three rats with experimental brain tumors. After 1 to 3 days the rats were sacrificed, and the brains were removed and subjected to x-ray study. All showed dense radiopaque areas which correlated with the size and shape of the corresponding brain tumors. Conversely, none of the radiograms taken of the brain tumor in five rats receiving no perfluorocarbon (control animals) showed similar increased density. These findings suggest that perfluorocarbon may serve a useful role as a contrast medium for computerized tomography studies of brain tumors in man.

KEY WORDS • perfluorocarbon • contrast medium • imaging • rat brain tumor • experimental brain tumor

The detection of brain tumors using radiographic techniques has passed through various stages of development. Currently, cerebral angiography and computerized tomography (CT) are the most widely used methods of study. In both of these modalities, iodinated water-soluble contrast agents are injected intra-arterially or intravenously. However, when there is a break of the blood-brain barrier in a sizable region of the tumor, the contrast medium that escapes from the vascular system may not be sufficient to produce noticeable opacification of the tumor. If an adequate amount of the contrast medium does concentrate in the parenchyma of the tumor, the radiographic enhancement lasts for a short period of time, that is, a few seconds in the case of cerebral angiography, and about 2 hours in the case of CT scanning. In addition, water-soluble contrast agents produce an undesirable increase in the x-ray absorption of normal tissues which make their differentiation from the pathological tissues more difficult.

Because of these limitations, the search for different types of contrast media to identify neoplastic lesions in various organs has recently been renewed. Among the various radiopaque substances that have been tried, perfluorocarbon compounds were shown to be both safe and effective contrast agents in human and experimental animals.

Perfluorocarbon compounds are linear chains of six or eight carbons in which all of the hydrogen atoms bound to the carbon have been replaced by fluorine. Bromide substitution of one of the fluorine atoms produces perfluorohexyl bromide (PFHB) and perfluoroctyl bromide (PFOB), respectively. Efficacy and toxicity studies after intrabronchial and oral administration have shown that both these compounds are less toxic and more biologically inert than any known radiopaque agent. Furthermore, intravenous administration of PFOB emulsions succeeded in opacifying neoplastic lesions in the thigh and liver of rats and rabbits, pointing to the usefulness of this substance in tumor detection. In the present communication we report the radiographic identification of brain tumors in rats after intravenous injection of PFOB emulsions.

Materials and Methods

Brain tumors were developed in eight rats through intracerebral inoculation of murine erythroblastosis virus. The details of the technique are found elsewhere. The animals were selected for these experi-
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Emulsification of the PFOB was accomplished by pulse-blending and sonificating pure liquid PFOB with 5% pluronic F-68 in lactated Ringer's solution. Subsequently, filtration of the emulsion was performed to select the desired particle size, 0.5 μm or smaller. The technique for preparation of PFOB emulsions of small particle size has been described in detail by Long, et al.

Three of the eight animals were first anesthetized with intraperitoneal injection of pentobarbital (25 mg/kg), after which emulsified PFOB was administered through veins in the tail or the femoral veins. The concentration of the injected perfluorocarbon was 48% and the amount given was 1 ml/100 gm body weight. In order to evaluate the concentration of the medium at different times, the three animals were allowed to live 24, 48, and 96 hours, respectively, after the injection. In the remaining five animals no perfluorocarbon was administered; these were used as the control group. All eight animals were sacrificed through intracardiac injection of paraformaldehyde. Their fixed brains were removed from the cranial capsule for radiological and histological examination.

Because bone absorbs x-rays more efficiently than bromide, the skull could prevent the detection of small concentrations of PFOB within the tumor. Each brain was placed on Kodak industrial-type M film and exposed with an x-ray tube at a distance of 120 cm. The exposure factors were 400 mA, 1 second, and 44 kV.

Results

The radiograms of the brain of all three animals which received perfluorocarbon showed radiopaque images which correlated with the size and shape of the corresponding tumors in the brains. In the first animal, the tumor was rather large and it was associated with a dense and clearly identifiable radiopaque image (Fig. 1). In the second, the tumor in the right frontoparietal region was very small, yet the radiographic density was rather clear (Fig. 2). The third animal had a tumor with a hemorrhagic center. The radiographic density of only the tumor surrounding the hemorrhagic portion was increased as compared with the remaining brain parenchyma (Fig. 3).

Conversely, the radiograms of all five animals not receiving perfluorocarbon showed no increased radiopaque density in the tumor regions.
Discussion

Of the two perfluorocarbon compounds that have been used thus far as contrast agents, PFHB has given satisfactory results in outlining the tracheobronchial tree and the gastrointestinal tract. The tendency of this compound to evaporate and form gas, however, has made it unsuitable for systemic administration. This serious side effect is directly related to the high vapor pressure of the compound (90 torr). On the other hand, PFOB, with a vapor pressure of 14 torr, has been found to be well tolerated following oral, intrathecal, intrabronchial, and intravenous administration.

Intravenous injection was used to study the kinetics of PFOB in rats. It was found that this compound gradually concentrates in various organs, such as liver, spleen, and adrenal glands, over a period of a few days. No behavioral or other changes were observed in these animals if the dose was less than 15 mg/kg. Subsequent reports of selective concentration of PFOB in tumor parenchyma suggested a promising development in the field of tumor diagnosis by radiographic means. From these studies it became apparent that there are two different mechanisms of trapping of PFOB in the tumor. During the first few hours, opacification of the tumor is principally due to circulation and retention of the compound in the tumor vessels. After this initial period, the density of PFOB in the tumor gradually increases, reaching its peak between the 1st and 2nd week after injection. Subsequently, there is gradual diminution of the contrast medium in the tumor but in some cases it remained within the tumor parenchyma for 4 to 10 weeks.

Our experiments show clearly that PFOB is taken up by brain tumors. In the three rats injected with PFOB, the concentration in the brain tumors was sufficiently high to be detected easily on high-resolution fine-grain film. On the contrary, the tumors in the control group did not opacify and could not be distinguished from the surrounding normal tissues. Since the animals in the present study were not allowed to live more than 96 hours, it appears that both intravascular and parenchymal entrapment of the radiopaque medium were responsible for the tumor opacification. Our findings, although shown in a limited number of animals, reinforce those of the investigators cited above, and indicate that PFOB emulsions can be used in the diagnosis of brain tumors as well. Perfluorooctyl bromide, unlike commonly used water-soluble contrast media, has the unique property of remaining within the tumor for longer periods of time. Furthermore, the rate of uptake of this substance by tumor and its turnover in time may be significant in evaluating the various types of tumors.

In these experiments, we used industrial x-ray film and conventional radiography to visualize the tumors. The density resolution of this technique is known to be inferior to that of CT scanning, which is far more sensitive and will undoubtedly allow detection of perfluorocarbon in smaller concentrations. In view of our current results, we feel that further experimental work is needed in vivo. Larger animals and CT scans should be evaluated in the future to better establish the usefulness of this compound in humans.

Fig. 2. Left: Radiograph showing a small brain tumor in a rat brain, seen as an area of radiopaque density in the right frontoparietal region (arrows). The radiograph was taken 96 hours after injection of perfluorocarbon. Right: Anatomical specimen showing that the area of increased density (left) corresponds with the location of the tumor.

Fig. 3. Left: Radiograph of a rat brain tumor which contained a hemorrhagic center. The radiopaque image can be seen only at the periphery of the tumor; there is no increased density in the hemorrhagic center. Right: The anatomical specimen.
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References


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