JOURNAL OF NUCLEAR MEDICINE 5:895-900, 1964

Peripheral Appearance of Radiolabeled Albumin and an Alkylating Agent During Regional Brain Perfusion[1]

Richard P. Spencer, M.D., Ph.D., James B. D. Mark, M.D. and Stevenson Flanigan, M.D.[2]

New Haven, Connecticut

The technique of regional perfusion was introduced in order to deliver a cytotoxic agent to one area of the body while restricting the amount being deposited elsewhere. (1) There are two principal questions raised in the use of such perfusions: a) how much of the cytotoxic agent binds to the locale under study, (2) and b) how much escapes to the general circulation. (3) We wish to present data on these problems, gathered during two brain perfusions on one patient, and a single perfusion of a second patient. During one brain perfusion, radioiodinated human serum albumin (^{131}I) was included in the perfusing fluid, and during the other two perfusions, triethylene thiophosphoramide-S^{35} (Thio-TEPA) was present.

MATERIALS AND METHODS

The first patient was a 53-year-old Caucasian male who entered the Grace-New Haven Community Hospital with symptoms which were clinically consistent with a space-occupying lesion in the right cerebral cortex. Included in the diagnostic work-up was an ^{203}Hg-chlormerordin brain scan. The scan was performed two hours after the intravenous injection of 700μc of the compound; it revealed a localization of the radioisotope on the right side (Fig. 1). The diagnosis of a glioblastoma was made histologically after surgical removal of the greater bulk of the tumor. A course of 5,400 r (tumor dose) x-irradiation was administered to the head (11.4 mm Cu half-value-layer, 100 cm target-to-skin distance, 100 r/minute, 200 r/day $\times$ 27). Some three months later, because of recurrent symptoms, a regional perfusion with Thio-TEPA was performed. The right common and external carotid arteries and the jugular vein were temporarily clamped and polyethylene catheters introduced above the point of occlusion. (4) The pump system was primed with 600 ml of compatible whole blood; 50 μc of ^{131}I-labeled human serum albumin were introduced into the blood. The uni-

[1]Supported by USPHS Research Grants Nos. CA 6519 and HD 00411.

[2]From the Departments of Radiology and Surgery, Yale University School of Medicine, New Haven, Connecticut.

lateral carotid-jugular system was perfused for 20 minutes at 100 ml/minute. Samples were withdrawn from the pump system and from an arm vein at intervals after the perfusion was begun. Included in the perfusing fluid was 35 mg of Thio-TEPA. The patient had remarkable alleviation of symptoms following the perfusion. Subsequently, a gradual progression of motor difficulties was noted, particularly an inability to raise his legs. Five months after the initial brain perfusion, a second was performed, and again there was symptomatic relief. This time, with the injection of the 35 mg of stable Thio-TEPA into the perfusing fluid, 62 μc of triethylene thio-phosphoramide-S^{35} (Amersham, specific activity 0.044 mc/mg, dissolved in sterile saline and filtered through a Millipore 0.45 micron filter) were introduced. Again, samples were taken from the pump system and from an arm vein.

The second patient was a 51-year-old Caucasian male who entered the hospital because of loss of memory and occasional disorientation. Evaluation of the patient included an ^{203}Hg-chlormerodrin brain scan (650 μc) which revealed localization in the left temporal cortex. A diagnosis of glioblastoma was made on the basis of clinical and diagnostic findings. A course of x-ray treatments was administered (5,000 r, tumor dose, 200 r/day $\times$ 25, characteristics as before). Four months later, because of worsening symptoms, a decompression and biopsy of the tumor was performed. A regional brain perfusion with 35 mg Thio-TEPA (including 53 μc of the radiolabeled material) was then undertaken.

In analysis of the samples of ^{131}I-albumin, gamma emissions were quantitated by means of a scintillation well. The triethylene thiophosphoramide-S^{35} was estimated by liquid scintillation counting, using 0.05 ml of plasma in a mixture of toluene and phosphors (each vial contained 3 ml of methanol plus 10 ml of toluene, which contained 15.2 gm of 2,5-diphenyloxazole and 0.189 gm of 1,4-bis-2-(5-phenyloxazolyl)-benzene per 8 pints). Due to the presence of the plasma, there was considerable quenching of light emission; however, this was essentially the same in all vessels and no correction to 100 per cent efficiency was applied. Samples were also counted in the toluene solution after 0.1 ml had been applied to a 5 cm strip of Whatman No. 1 paper and dried. The same magnitude of activity was obtained as with the direct counting procedure.

RESULTS

The ^{203}Hg-chlormerodrin brain scan in the first case revealed a large right-sided uptake of the radioisotope, superior to the ear (Fig. 1). At the time of operation, this was noted to correspond to the location of the tumor.

As determined by samples of blood from the pump system, drawn at 3 and 13 minutes after the start of the perfusion (fluid labeled with albumin-^{131}I), there was a rapid decrease in radioactivity. Assuming that first order kinetics were applicable between these two points, the half-time for loss of radioactivity from the pump system was 15 minutes. This was a rate greater than the 22 to 34 per cent loss in 30 minutes reported by Woodhall and co-workers. (3) Radioactivity was present in the peripheral (arm) venous blood in both the 3 and 13 minute samples.

Use of triethylene thiophosphoramide-S^{35} was expected to result in an even more rapid disappearance of radioactivity from the recirculating fluid, (2) due to the combination of binding of the alkylating compound to the tissues, as well as its "leak" into the peripheral circulation. Results from the first case are shown in Fig. 2 (where counts per 0.05 ml plasma, divided by 10,000, are plotted as a function of the time following the start of the perfusion). The curve approximates an hyperbola of the form:

$$R \cdot T = c \qquad (1)$$

where R is the radioactivity per unit of blood, T is the time in minutes, and c is a constant (in this case, 17.1, as determined from the mean of the 6 plotted points). To describe the data in terms of a series of exponents would require at least three compartments with half-times on the order of 1.2, 3.0 and 6.7 minutes.

To determine the closeness of fit of the experimental data to an hyperbola, equation (1) can be recast into the form:

$$R = \frac{1}{T} \cdot c \qquad (2)$$

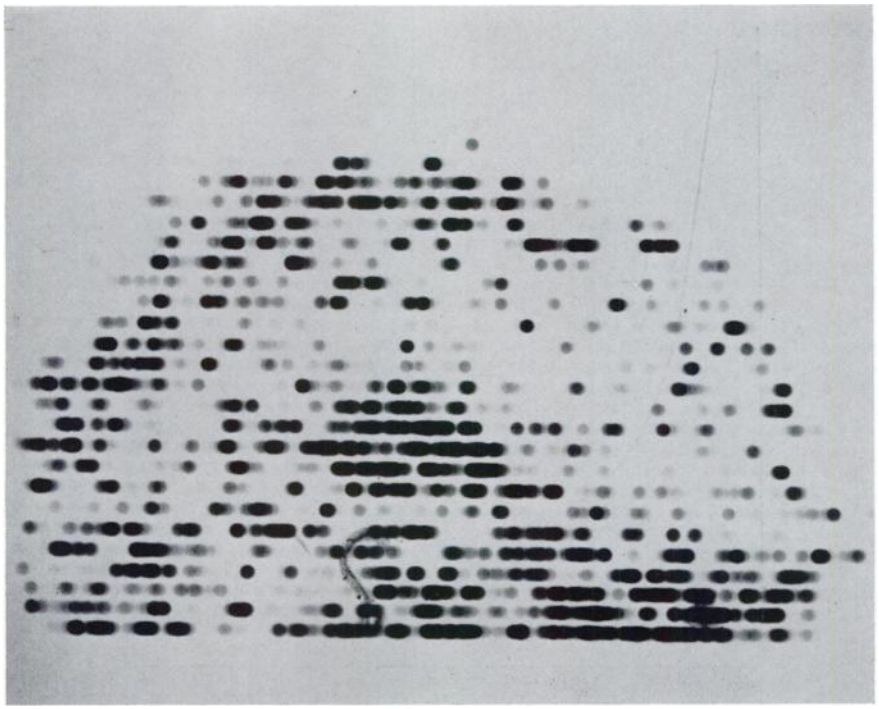

Fig. 1

Fig. 1. ^{203}Hg-chlormerodrin brain scan. In addition to usual accumulation in facial musculature, there is localized uptake on right, above ear.

A plot of R as a function of reciprocal time $(\frac{1}{T})$ should yield a straight line. That this is so is shown in Fig. 3. Data from both cases are given in the figure. The lines were drawn by the method of least squares.

Following perfusion of triethylene thiophosphoramide-S^{35} into the brain, radioactivity was detectable in the peripheral blood in the first sample drawn (1 minute later). At five minutes, the ratio; activity per unit plasma in the periphery/activity per unit plasma in the pump; was 0.30. At 15 minutes, the activity from both locations had fallen, but the decrease in peripheral activity was less than that from the pump side, so that the ratio had risen to 0.70. Radioactivity was still present in the last peripheral sample drawn (20 minutes after the start of regional brain perfusion). From the intercept on the R axis of Fig. 3, it can be calculated that at infinite time, the radiolabel would have distributed itself into a plasma volume (the total of actual plasma, plus tissue) of 3,000 to 4,000 ml. This result was obtained by comparing the counts/minute of the standard with that expected in 1 ml of plasma at "infinite" time.

DISCUSSION

During the infusion with triethylene thiophosphoramide-S^{35}, the magnitude

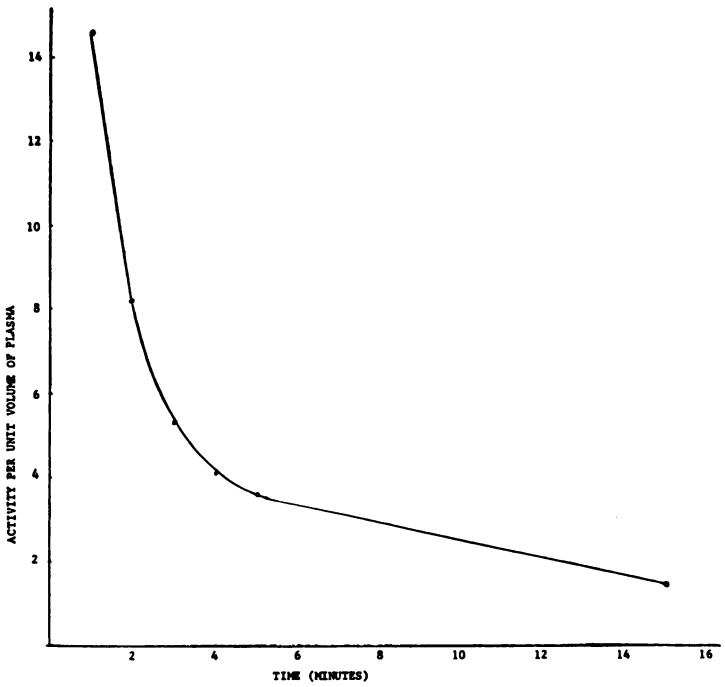

Fig. 2

Fig. 2. Radioactivity (triethylene thiophosphoramide-S^{35}) in perfusing fluid as function of time (data from first case).

of the decrease in the radioactivity from the 600 ml perfusate was unexpected. At one minute after the start of the perfusion with triethylene thiophosphoramide-S^{35}, the radioactivity was distributed approximately as follows:

Present in plasma in the pump circulation	60%
Present in plasma in the peripheral blood	14%
Therefore, bound to brain, formed elements of blood, or elsewhere	26%

The exact locale of the "bound" fraction cannot be stated with certainty, and a small peripheral component is undoubtedly present. In addition, much of the radiolabel may not be present as the original alkylating agent, since such compounds are unstable *in vivo*. (2)

For the albumin-^{131}I only limited data are available; based on determinations at 3 and 13 minutes, the half-time of the radioactive iodine within the pump circulation was on the order of 15 minutes. In this case, then, the collateral circulatory loss was considerable in comparison with reported experiences (or perhaps more bound to the tumor in this case).

The information from the two perfusion studies with thio-TEPA raises question as to the margin of benefit of carotid perfusion over carotid infusion. The clinical responses were of such a nature as to support the use of thio-TEPA

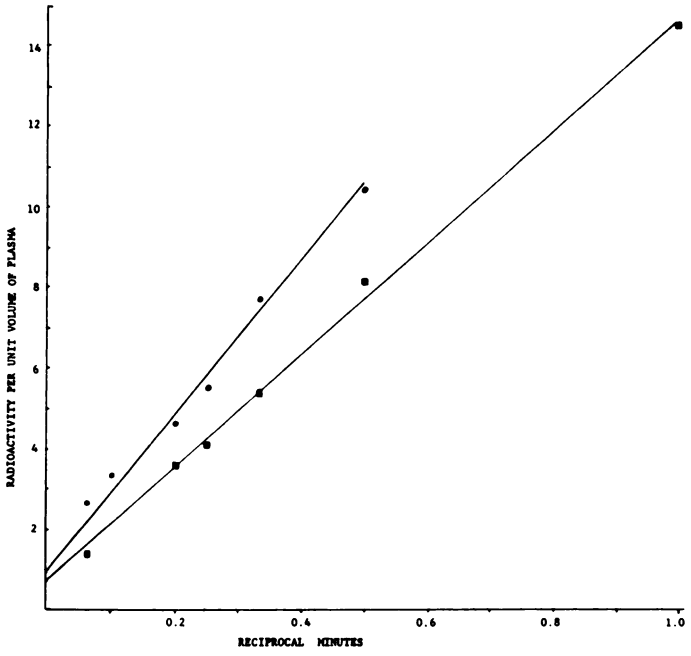

Fig. 3

Fig. 3. Radioactivity in perfusing fluid plotted as function of reciprocal time. Lines drawn by method of least squares. Squares: case 1; circles: case 2.

in glioblastoma multiforme. However, because of the rapid depletion of the therapeutic agent from the perfusate, prolonged recirculation may be unnecessary. On the other hand, the technique does permit exclusion of the external carotid circulation, and in the initial four minutes of perfusion there is probably opportunity for repeated passage of the drug. Even with a rapid fall-off in the concentration of the alkylating agent in the pump circulation, the tissues under treatment are experiencing a greater exposure to the drug than in the case of a simple infusion.

The significance of the hyperbolic description of the loss of radioactivity from a regional perfusion system is still obscure. We have observed that in analogue computer solutions of the distribution of radioactivity in models, one or more of the peripheral compartments have entry terms that closely approximate hyperbolas. Hence, the observation of a hyperbolic fit to the present data may be fortuitous, and dependent upon the large number of compartments involved. Austen and Racker (5) described studies of tumor perfusion, and included measurement of the erythrocyte loss from the isolated cerebral circulation as quantitated by means of ^{51}Cr-labeled red blood cells. From values given in their Fig. 25, we can calculate that the hyperbolic description also holds for loss of tagged cells during the period 10 to 35 minutes after the start of perfusion.

SUMMARY

During a regional brain perfusion, albumin-^{131}I added to the perfusing fluid rapidly decreased in activity and appeared in the peripheral circulation. When triethylene thiophosphoramide-S^{35} was present in the perfusing fluid, about 14 per cent appeared in the peripheral circulation within one minute. The concentration of radioactivity in the perfusing fluid, as a function of time, was described by an hyperbola. These results cast doubt on the efficacy of carrying regional brain perfusion beyond four or five minutes.

REFERENCES

1. Klopp, C. T.; Alford, T. C.; Bateman, J.; Berry, G. N. and Winship, T.: Fractionated intra-arterial cancer chemotherapy with methyl bis amine hydrochloride; a preliminary report. *Ann. Surg.* **132**:811, 1950.
2. Mahaley, M. S., Jr. and Woodhall, B.: An evaluation of plasma levels of alkylating agents during regional perfusions. *J. Surg. Res.* **1**:285, 1961.
3. Woodhall, B.; Reynolds, D. H.; Mahaley, M. S., Jr. and Sanders, A. P.: The physiologic and pathologic effects of localized cerebral hypothermia. *Ann. Surg.* **147**:673, 1958.
4. Aronson, H. A.; Flanagan, S. and Mark, J. B. D.: Chemotherapy of malignant brain tumors using regional perfusion. I. Technic and patient selection. *Ann. Surg.* **157**:394, 1963.
5. Austen, W. G. and Raker, J. W.: Monitoring losses during isolated tumor perfusion. *Cancer Chemotherapy Reports* **10**:61, 1960.