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Penetration of Brain and Brain Tumor. VI. Radioactive Scanning Agents[1]

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Radioisotopic scanning in the diagnosis of brain tumors has become a routine clinical procedure. Many types of localizing agents have been used and the ever present questions remain: which agents are most effective, how can they be compared objectively and what are the biological and biochemical factors responsible for a compound's usefulness in scanning? This latter question is a key one in understanding how scanning agents work and in placing this diagnostic procedure on a more rational and less empirical base. In the past, compounds have been largely selected for such use on the basis of the nuclear properties of the isotope and not for any biological reasons. It seems highly pertinent to determine why such agents work and recent studies (1, 2), as well as those described here, are a beginning in understanding their mechanism of action.

A second reason for this current study, is that with the development of scintillation cameras and high speed scanners the use of very short lived isotopes in large doses, has become an obtainable objective. Large amounts of such tracer activity allow (a) more rapid procurement of the diagnostic picture, (b) lower radiation dose to the patient than is obtained with isotopes of longer half-life and (c) utilization of the increased information content for finer picture resolution. For these reasons, short lived isotopes are already of increasing importance in diagnosis. The development of useful compounds of such isotopes, requires that we follow both concomitant approaches; an understanding of how agents function in biological systems and the empirical determination of the localization in cerebral neoplasms of compounds containing these elements.

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This research was undertaken to assess the utility of mice with subcutaneously transplanted brain tumors, for evaluating and comparing compounds as scanning agents, with potentially useful short lived nuclides. In a previous study in these laboratories, a series of copper-64 chelates were compared in such tumor-bearing mice (3). The results did show major differences in the concentration of copper-64 in various tissues, depending upon the chelate used. From recent work using carbon-14 labelled chelate and copper-64, it has become apparent that the two moieties do not remain as an entity under *in vivo* conditions (4). However, the chelate does modify the localization of copper-64. It was on the basis of these studies that copper-64 diethylenetriaminepentaacetic acid (DTPA) chelate has been used as a scanning agent in man.

The isotope selected for evaluation in this study may be divided into three groups. In the first group are those nuclides which are currently being used routinely in many centers. They include ^{99m}Tc as the pertechnitate, ^{64}Cu as its DTPA chelate, ^{74}As as the arsenate/arsenite mixture and ^{197}Hg as 1-chloromercuri-2-methoxy-3-ureidopropane (chlormerodrin or neohydrin). In the second category are ions which are normal intracellular components. The rationale for screening such substances is that normal brain appears to be less permeable than brain tumors to certain ions. Also, with the neoplasm's more rapid metabolic rate, such components may achieve high levels in tumors and thereby be effective scanning agents. Included in this group are ^{32}P as the phosphate anion and ^{42}K as the cation. In the third group are compounds of elements which are either short lived isotopes, themselves, or have nuclides which are short-lived isotopes. Those which have been screened are ^{18}F, both as the fluoride and fluoroborate anions, ^{51}Cr as the chromate anion, ^{72}Ga as gallium chloride and ^{56}Mn as potassium permanganate.

Mice bearing subcutaneously transplanted brain tumors have shown some utility in the study of the distribution of alkylating agents (5) and in the concentration of boron compounds in neoplasms for neutron capture therapy (6). However, it had remained questionable whether such a system would be useful for evaluating scanning agents.

METHODS

The isotopes used for this study were obtained either from commercial sources (^{99m}Tc, ^{51}Cr, ^{42}K, ^{32}P and ^{197}Hg)[1] and (^{74}As)[2], or were prepared in this laboratory utilizing the nuclear reactor at the Massachusetts Institute of Technology.

The animals used in this study were C3H mice bearing subcutaneously transplanted ependymomas. The tumors, whose generation has been previously described (6), were readily transplanted in the region of the scapula. Within seven days, they were of sufficient size to be used in the distribution experiments and yet, not so large as to be highly necrotic.

Each mouse, held in a special holder, was intravenously injected through the tail vein with a standard dose of 10μc/20 gram mouse in a volume ranging

[1]Iso Serve Inc., a division of Cambridge Nuclear Corp.

[2]Abbott Laboratories.

[3]Gammacord AG-1.

from 0.1 to 0.5 ml. The animals were sacrificed at the following time intervals after injection: 5, 15, 30, 120, 240 minutes. Samples of brain tumor, muscle, skull and scalp were excised, weighed and counted in a well counter[3]. Measured volumes of whole blood were pipetted and counted as well. From these values, cpm/gram of tissue were obtained for all the above tissues and the uptake ratios of tumor: brain, tumor: blood and tumor: muscle were calculated. In Table I are listed the average values and standard deviations of these ratios for the five animals at each time interval; Figure 1, shows these values plotted as a function of time. For this preliminary study, uptake ratios were a useful means of determining which moieties are promising. The calculation of absolute per cent dose of these desirable compounds in larger animals is underway.

RESULTS AND DISCUSSION

The validity of using a subcutaneous, transplantable murine ependymoblastoma as a model of a spontaneous intracranial neoplasm in man, for the comparison of scanning agents, has been a continuing source of concern. The results of the present study in part support this concern. In Table I and Figure 1, the data for ^{64}Cu DTPA and ^{74}As appear to indicate that the former should be the better localizing agent. In actual fact ^{64}Cu DTPA has given generally somewhat poorer visualization of focal lesions in man than has ^{74}As (7). In lieu of a better model system, however, the judicious assessment of the data may allow useful predictions. The actual scan is not merely a portrayal of tumor to brain uptake ratio alone, but it is a composite of the radioactive content of blood, brain, tumor, muscle, scalp, skull and meninges. Each of these tissues contributes to the overall count and its significance depends upon the relative concentration of the tracer, as well as the mass of the particular tissue, which is seen by the scanning detector. As a requirement for potential usefulness, it would seem essential for the nuclide to show a propensity for tumor compared with neighboring normal tissues. High levels of radioactivity in blood or muscle may effectively mask the presence and location of a neoplasm, thus tumor to blood and tumor to muscle ratio becomes greater than would be desirable. Additionally, since the goal is the use of short lived isotopes, radioactive decay becomes an important limitation. The high tumor levels, relative to other tissues, must be attained after a short time interval, in order for the isotope to have any practical utility. It is for this reason that the animals were killed at times, five, 15 and 30 minutes after the intravenous injection, as well as, at 120 and 240 minutes and sacrifice times greater than four hours were considered of no practical value.

From these data in Table I and Figure 1, certain observations can be made. One is that the initial blood concentrations were invariably higher than all other tissues. This is certainly to be expected, since the agents were administered intravenously. However, with the passage of time, the concentration of isotopes in different tissues varied considerably. In the case of ^{32}P as phosphate, within 30 minutes, the levels in tumor exceed those of blood, indicating either binding or metabolic incorporation into the tumor cell. In view of the role of phosphorus in nucleic acid metabolism, incorporation into such rapidly dividing cells is not unexpected. This observation and the fact that levels in normal brain and muscle

TABLE I

Isotope	*5 min*			*15 min*			*30 min*			*120 min*			*240 min*		
	T/Br	*T/Bl*	*T/M*	*T/Br*	*T/Bl*	*T/M*	*T/Br*	*T/Bl*	*T/M*	*T/Br*	*T/Bl*	*T/M*	*T/Br*	*T/Bl*	*T/M*
Tc^{99m}	6.1 ± 2.0	0.2 ± 0.1	1.2 ± 0.2	12.0 ± 3.3	0.3 ± 0.1	2.8 ± 0.4	11.6 ± 2.6	0.3 ± 0.0	2.4 ± 0.3	11.7 ± 2.5	0.4 ± 0.1	3.1 ± 1.2	14.8 ± 3.5	0.5 ± 0.1	3.8 ± 1.2
Cu^{64}	4.8 ± 1.8	0.2 ± 0.1	1.7 ± 0.8	14.4 ± 1.7	0.4 ± 0.1	3.2 ± 0.6	16.7 ± 8.5	0.7 ± 0.5	2.5 ± 0.6	29.6 ± 13.7	2.1 ± 0.5	6.5 ± 2.2	16.6 ± 2.9	1.9 ± 0.2	6.8 ± 1.1
As^{74}	9.1 ± 2.7	0.3 ± 0.1	1.5 ± 0.4	11.0 ± 1.1	0.5 ± 0.0	1.9 ± 0.4	8.2 ± 3.3	0.5 ± 0.1	1.7 ± 0.4	3.2 ± 0.5	0.9 ± 0.1	1.0 ± 0.6	4.1 ± 0.7	0.6 ± 0.1	1.5 ± 0.3
Gr^{51}	5.3 ± 1.9	0.1 ± 0.0	1.4 ± 0.9	6.4 ± 1.4	0.2 ± 0.0	1.4 ± 0.4	7.2 ± 2.0	0.2 ± 0.0	1.8 ± 0.5	7.9 ± 2.7	0.2 ± 0.1	2.0 ± 0.1	12.4 ± 3.1	0.3 ± 0.0	2.1 ± 0.7
K^{42}	2.9 ± 0.9	0.8 ± 0.2	0.1 ± 0.0	2.7 ± 1.0	1.1 ± 0.3	0.1 ± 0.0	3.9 ± 1.5	2.2 ± 0.8	0.2 ± 0.1	3.7 ± 1.1	2.6 ± 1.2	0.4 ± 0.2	3.1 ± 0.5	4.0 ± 1.2	0.4 ± 0.1
Ga^{72}	3.7 ± 0.8	0.1 ± 0.0	1.7 ± 0.6	6.6 ± 2.6	0.2 ± 0.1	2.4 ± 1.2	9.8 ± 3.5	0.2 ± 0.0	3.4 ± 0.9	15.5 ± 5.4	0.4 ± 0.1	2.0 ± 0.6	20.6 ± 11.3	1.0 ± 0.7	2.8 ± 1.3
Hg^{197}	5.5 ± 1.4	0.1 ± 0.0	2.0 ± 0.5	10.9 ± 2.4	0.2 ± 0.0	2.8 ± 1.1	17.0 ± 6.1	0.4 ± 0.1	3.6 ± 0.7	17.9 ± 2.2	0.5 ± 0.1	7.3 ± 1.1	16.4 ± 5.0	0.6 ± 0.2	3.8 ± 1.4
F^{18}	8.7 ± 2.9	0.4 ± 0.1	0.8 ± 0.2	8.2 ± 0.2	0.7 ± 0.1	1.0 ± 0.1	6.5 ± 1.9	1.2 ± 0.2	1.3 ± 0.2	2.4 ± 1.2	0.9 ± 0.6	0.9 ± 0.4	1.7 ± 0.5	1.0 ± 0.4	1.3 ± 0.9
KBF_4^{18}	3.4 ± 1.7	0.2 ± 0.1	0.9 ± 0.5	5.5 ± 2.1	0.4 ± 0.0	1.8 ± 0.5	7.3 ± 1.2	0.4 ± 0.1	2.0 ± 0.2	10.9 ± 2.1	0.4 ± 0.0	2.5 ± 0.4	11.8 ± 2.7	0.5 ± 0.0	2.2 ± 0.6
Mn^{56}	3.4 ± 0.8	0.4 ± 0.2	2.3 ± 0.4	3.2 ± 0.8	0.9 ± 0.5	2.8 ± 0.9	3.7 ± 0.8	0.8 ± 0.2	3.1 ± 0.9	3.2 ± 0.3	3.4 ± 0.5	2.7 ± 0.7	4.1 ± 1.0	8.2 ± 4.2	3.8 ± 2.1
P^{32}	7.5 ± 2.8	0.6 ± 0.2	1.5 ± 0.1	7.9 ± 3.2	0.9 ± 0.6	0.9 ± 0.3	10.1 ± 2.0	1.7 ± 0.4	1.4 ± 0.4	12.6 ± 5.0	3.5 ± 0.8	1.3 ± 0.2	8.1 ± 2.4	2.9 ± 1.0	1.1 ± 0.6

are lower than tumor, support the use of ^{32}P for probe counting of brain tumors (8) and indicate that the weaker β-emitting ^{33}P may be even more desirable. The intracellular ^{42}K ion also rapidly achieves higher levels in tumor than in blood or brain. However, the concentrations in muscle were appreciably higher than in all tissues examined and it may be worthwhile to consider the use of ^{43}K for determining the presence and location of lesions in muscle. ^{197}Hg, in the form of neohydrin, ^{99m}Tc as the pertechnitate anion and ^{51}Cr as chromate, all had very low tumor to blood ratios over the four hour evaluation period. Of the three, ^{197}Hg showed the very interesting property of a rapid attainment of a high tumor/brain ratio, within 30 minutes after injection. There is evidence that this compound is being fixed to tumor (9) and possibly this occurs through the sulfhydryl groups present in the neoplastic proteins. At any rate, this binding certainly would account for the utility of this compound as a scanning agent. In a similar way, but to a lesser extent, ^{99m}Tc achieves a high tumor: brain ratio, but in contrast with ^{197}Hg, levels in skull and scalp were comparable to those in tumor. If these findings are borne out in man, it would appear that of the two, ^{197}Hg-neohydrin is a more satisfactory agent from biological considerations. Initially the levels of ^{51}Cr chromate in tumor were greater than those in muscle and brain, but lower than those in skull and scalp. At longer time intervals after injection, the concentrations in scalp and tumor were comparable but appreciably lower than those in skull. Chromate would appear to be unsuitable as an agent for brain tumor localization, but the high skull levels may indicate its possible utility in the detection of bone lesions. ^{18}F as the fluoride anion is also a bone seeking moiety (10). Interestingly enough, within 5 minutes after injection, the radioactive levels in skull were three to five times those in blood and this ratio increased 25 fold during the four hour period. This binding to bone, even at such short time intervals effectively eliminates ^{18}F anion as a brain tumor scanning agent; but its high and rapid selectivity points to its potentiality as an agent for the study of bone abnormality and pathology (17). ^{18}F as the fluoroborate anion does not show such a marked propensity for bone as fluoride itself (12), but this substance is also cleared from the vascular supply and the levels achieved by skull are greater than those in blood and tumor. This may indicate, either that the BF_4^- anion *per se* is bound to bone, or that in the course of its metabolism, fluoride ion is liberated and thereby incorporated. In either case, these agents are not suitable as brain tumor localizers. In view of the desirable physical properties of ^{18}F, however, a compound containing fluoride which is bound selectively to tumor, would be very useful. Metabolic studies have indicated types of structures which are incorporated preferentially into tumor cells (13), but efforts to synthesize such fluorine-containing compounds in useful amounts have been unsuccessful to date. A recent report on the rapid incorporation of ^{18}F into steroids presents a feasible method for labelling a variety of compounds with ^{18}F (14).

The useful physical characteristics of 68 minutes gallium-68 has prompted its use in brain tumor scanning (15) (16). To compare the gallium ion in this test system, gallium-72 as the chloride has been used in place of gallium-68. The concentrations in skull and blood are appreciably higher than in tumor and on

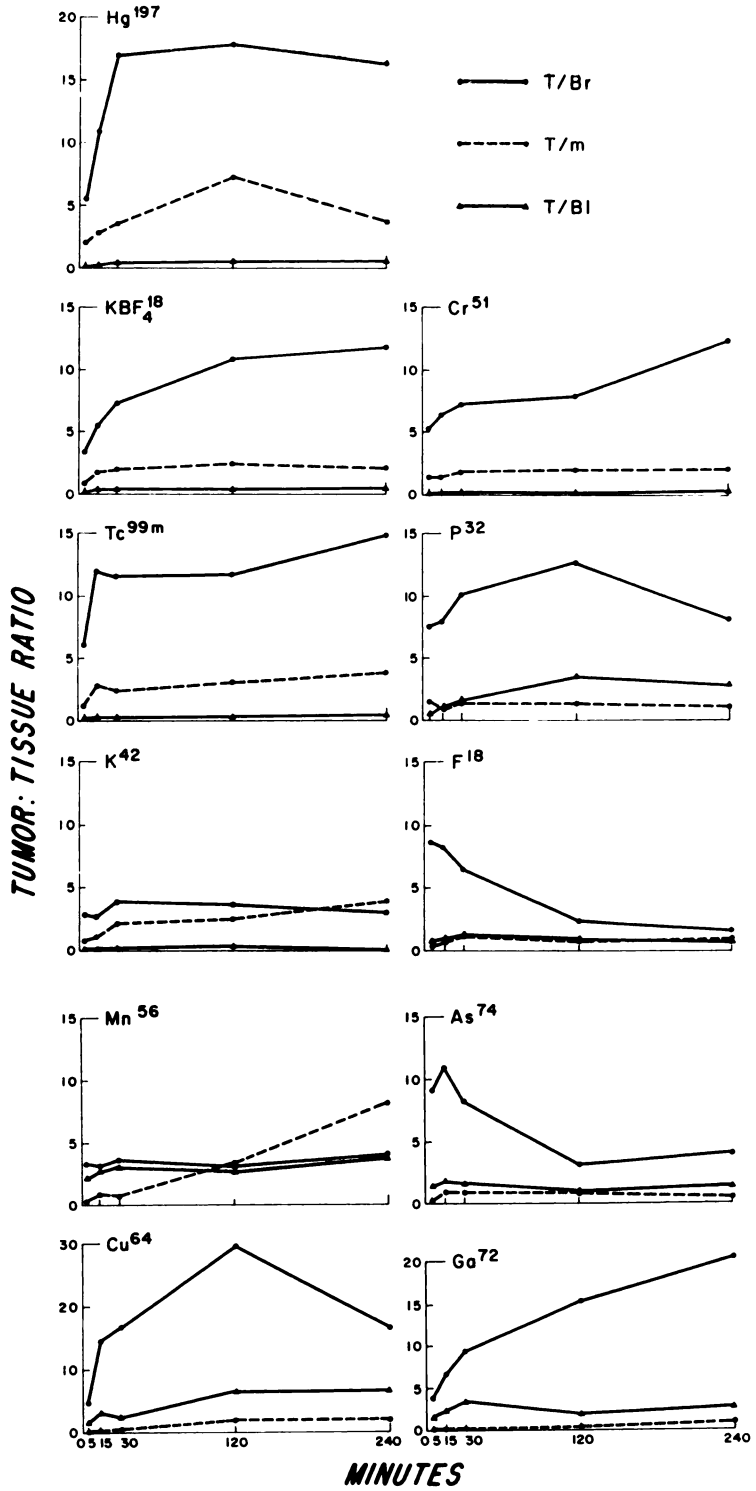

Fig. 1. Uptake Ratios of Isotopes as Function of Time. Tumor: Brain (T/Br); Tumor: Muscle (T/m) and Tumor: Blood (T/Bl). Hg^{197} as 1-chloromercuri-2-methoxy-3-ureidopropane; Tc^{99m} as the pertechnitate ion; K^{42} as the cation; Mn^{56} as the permanganate ion; Cu^{64} as the DTPA chelate; Cr^{51} as chromate; P^{32} as phosphate; F^{18} as the fluoride anion; As^{74} as a mixture of arsenate and arsenite and Ga^{72} as gallium chloride.

this basis, gallium would not appear to be useful for brain tumor detection, but has been considered for skeletal scanning (17). Manganese-56 as permanganate, on the other hand, does look extremely promising. While tumor:brain ratios ranged between three and four to one during the particular time sequence, the tumor: blood value gradually increased from 0.4 at 15 minutes to 3.4 at two hours and 8.2 at four hours. In contrast with ^{42}K, which shows higher levels in muscle than in tumor, permanganate has a tumor: muscle ratio of 2.3 to 3.8 in the time range studied. Levels in tumor were significantly higher than scalp, but comparable to those in skull. The fact that this anion, $^{56}MnO_4^-$, achieves appreciably higher concentrations in tumor than blood, is indicative that a tumor-binding mechanism is operative. Such a property is certainly highly desirable from a standpoint of tumor localization and further studies with manganese-56 will be reported shortly. From a consideration of positron emitting isotopes, ^{51}Mn and ^{52m}Mn, two short-lived nuclides, may prove to be useful scanning agents. Methods of producing these isotopes are currently being examined.

The test system which has been used, does have distinct limitations. The reason for the lack of analogy between this transplantable mouse tumor and an intracranial lesion in man has been considered. Although the difference may be one of species, it is more likely that the site of the neoplasm is the cause of this divergence. An intracranial mass produces physiological alteration in normal brain peripheral to the site of the lesion. Such changes undoubtedly influence the concentration of various agents in these adjacent areas (9) (18). The increased isotopic content in such sections results in a magnification of the apparent lesion size. Consequently, a more effective means for evaluating all brain tumor scanning agents would be by using an animal larger than the mouse, with a transplantable or chemically-induced intracranial cerebral tumor. From recent studies (19), such neoplasms in rats are possible and the development of the technique is underway in this laboratory. Until this test system is available, the use of subcutaneous tumors for assessing tumor-binding substances does have merit, even though it has definite limitations as a method for screening scanning agents.

SUMMARY

Three groups of potentially useful scanning agents (11 compounds) have been studied in mice with subcutaneously implanted ependymoblastomas. The results do not appear to be directly related to the scanning efficiency of a compound in man, but may have merit for elucidating substances which are tumor-binding.

^{56}Mn-labelled permanganate has shown levels in blood, muscle and normal brain, which are appreciably lower than in the neoplasm at times of two to four hours after administration. ^{18}F, though desirable from a physical standpoint, did not show any special propensity for neoplastic brain as the fluoride ion. However, in this form bone concentration were extremely high even five minutes after injection.

A better biological model for evaluating scanning agents is needed and new ways are being considered.

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