BIOLOGICAL BEHAVIOR OF $K^{56}MnO_4$: ITS ACCUMULATION IN NORMAL TISSUES AND PATHOLOGICAL BRAIN

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The binding of ^{56}Mn-permanganate to subcutaneously transplanted ependymoblastoma in C3H mice, associated with low levels in normal brain, muscle and blood, has been the subject of recent reports (*1,2*). During one of these studies (*2*), it was observed that this anion or its metabolic product was trapped largely in the pulmonary endothelium after intravenous administration. This prompted the consideration that this selective accumulation was the result not of an active uptake by the lung but instead of a simple filter action by the capillaries of the organ (*3*).

The present study was designed to evaluate this hypothesis by determining the biological behavior of $^{56}MnO_4^-$ after its injection by different parenteral routes. The accumulation of this anion has also been studied in rabbits with cerebral necrotic lesions and edematous areas peripheral to this zone.

METHODS

As in the previous studies (*1,2*), the isotope we used was ^{56}Mn (half-life, 2.59 hr) produced by thermal-neutron activation of stable potassium permanganate.

Twenty-six albino rabbits weighing 1.5–2 kg were used to assay the ^{56}Mn distribution in tissues. The animals were divided into two groups: one group of 16 was used to study isotope accumulation in normal tissues, and a second group of ten animals was used to investigate concentrations of this nuclide in cerebral lesions produced by freezing. The first group of 16 animals was divided into subgroups. In Subgroup A, consisting of four animals, the ear vein was injected. In Subgroup B with six animals the isotope was administered via the portal vein. In Subgroup C the four animals were injected through a catheter placed in the abdominal aorta at the level of both renal arteries. Finally, in Subgroup D two animals was injected through a catheter in the right common carotid artery. Under light anesthesia with 60 mg Diabutol and 2.5 mg Thorazine, the 14 animals of Subgroups A, B and C were subjected to a laparotomy with ligation of the common biliary duct. A catheter was also placed in the urinary bladder. The isotope was then administered, and a total collection of both urine and bile was made for assay.

The second group of ten rabbits was divided into two equal parts. One part received the isotope injection in the ear vein; the other was injected via the right common carotid artery over a 10-min period with a solution of the isotope in 20 cc of physiological saline. In the ten animals of this second group, a freezing lesion was produced over the right parietal convexity either 5 or 24 hr before isotope administration. The purpose of this lesion was an attempt to simulate conditions existing in a neoplastic brain. The lesion was produced by extradural application for 1 min of a cooled 250 gm lead probe tapering to an end 5 mm in diameter. The probe was cooled to −75°C by a mixture of acetone and dry ice.

The aqueous solution for administration contained approximately 500 μCi ^{56}Mn/ml with a chemical concentration of 0.92 mg $KMnO_4$/ml. All animals were injected with 0.3 μCi/gm. The two groups of animals were sacrificed by barbiturate overdose 1 hr, 2 hr and 4 hr after isotope administration. Blood samples were taken immediately before the animal's death; after autopsy, bile and tissue samples were removed, weighed and counted. From this data the percent total dose per gram was calculated for all the key tissues, and tissue ratios were determined.

RESULTS AND DISCUSSION

Table 1 summarizes the tissue-concentration data of ^{56}Mn when $K^{56}MnO_4$ was injected into rabbits.

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Stable, low-isotope concentrations in blood were attained 2 hr after administration regardless of the route of injection. Another important observation was that large quantities of isotope are eliminated rapidly through the biliary duct, and this excretion pathway is independent of the administration route. This fact undoubtedly accounts for the fast clearance of ^{56}Mn from the bloodstream. These findings corroborate those of Greenberg *et al* (*4*), who observed that the amounts excreted through the bile 24 hr after injection were between 75 and 90% of the total administered dose. Even at 4 hr after injection as much as 42% of the total dose appeared in the bile. The amount of isotope excreted in the urine was always negligible even after the administration of this nuclide in the renal artery.

Supporting the filter hypothesis are the striking differences between isotopic concentrations in lung, liver and kidney depending on the particular route of administration. As Table 1 shows, the lung has the highest amount of radioactivity when the isotope is injected in the ear vein. The liver shows appreciably greater levels than lung when the isotope is administered by the portal vein, and the kidney is higher than the two other organs when the animal is injected at the level of the renal arteries. The greater levels of activity in the lung of animals injected through the carotid artery are very similar to those in animals injected directly in the ear vein and are easily understood anatomically. The low activity in the brain can be explained by the blood-brain barrier which inhibits the passage (into the CNS) of this anion or products derived from it. As Table 2 also shows, the ratios between the tissues

TABLE 1. AVERAGE PERCENT TOTAL DOSE/GRAM IN ANIMAL TISSUES IN RELATION TO ADMINISTRATION ROUTE OF $^{56}MnO_4^-$

Route of administration	Sacrifice time after injection (hr)	Lung	Liver	Spleen	Kidney	Muscle	Bone	Brain	Blood	Bile	Urine
Ear vein	2	2.6 ± 0.3	0.73 ± 0.02	0.12 ± 0.006	0.36 ± 0.008	0.007 ± 0.002	0.02 ± 0.008	0.004 ±	0.01 ± 0	16.5 ± 5.6	0.02 ± 0.003
Ear vein	4	2.8 ± 0.6	0.65 ± 0.008	0.09 ± 0.003	0.37 ± 0.01	0.01 ± 0	0.05 ± 0.006	0.008 ± 0.009	0.007 ± 0.002	26.3 ± 7.8	0.08 ± 0
Portal vein	2	0.22 ± 0.06	1.62 ± 0.006	0.03 ± 0.0003	0.26 ± 0.010	0.01 ± 0.0001	0.02 ± 0.0008	0.006 ± 0.0001	0.01 ± 0.0001	7.3 ± 4.4	0.008 ± 0.0002
Portal vein	4	0.09 ± 0.002	2.0 ± 0.06	0.27 ± 0.03	0.37 ± 0.06	0.01 ± 0.008	0.01 ± 0.006	0.001 ± 0.0001	0.01 ± 0.003	17.6 ± 11.9	0.007 ± 0
Renal artery	2	0.15 ± 0.002	0.85 ± 0.003	0.13 ± 0.008	6.7 ± 0.002	0.006 ± 0.001	0.06 ± 0.0002	0.004 ± 0.0001	0.01 ± 0	31.2 ± 0.5	0.006 ± 0
Renal artery	4	0.21 ± 0.006	1.06 ± 0.02	0.08 ± 0	3.0 ± 0	0.006 ± 0.001	0.02 ± 0.007	0.007 ± 0.0001	0.01 ± 0	42.3 ± 4.8	0.01 ± 0.006
Carotid artery	4	4.3 ± 0.5	1.5 ± 0.6	—	0.53 ± 0	0.003 ± 0.002	0.02 ± 0.0001	0.001 ± 0	0.008 ± 0.0002	—	—

TABLE 2. TISSUES RATIOS RELATED TO ROUTE OF INJECTION

Route of administration	Sacrifice time after injection (hr)	Lung-liver	Lung-kidney	Liver-lung	Liver-kidney	Kidney-lung	Kidney-liver
Ear vein	2	3.73	7.22	0.28	2.02	0.13	0.46
Ear vein	4	4.30	7.56	0.23	1.75	0.13	0.56
Portal vein	2	0.15	0.84	6.45	5.46	1.18	0.18
Portal vein	4	0.065	0.24	22.2	5.40	4.11	0.18
Renal artery	2	0.17	0.022	5.66	0.12	44.60	7.88
Renal artery	4	0.20	0.070	4.99	0.34	14.28	2.87
Carotid artery	4	2.86	8.11	0.34	2.83	0.12	0.35

TABLE 3. PERCENT TOTAL DOSE/GRAM IN RABBIT BRAIN AFTER COLD LESION IN RIGHT PARIETAL CORTEX

Route of administration	Time of sacrifice after injection (hr)	Time after cold lesion (hr)	Frontal lobe		Parietal cortex		Parietal		Occipital		Muscle	Skull	Blood
			Right	Left	Right	Left	Right	Left	Right	Left			
i.v.	1	5	0.076	0.009	0.10	0.009	0.042	0.008	0.063	0.006	0.014	0.05	0.07
i.v.	1	5	0.045	0.009	0.10	0.007	0.011	0.009	0.005	0.006	0.020	0.02	0.03
i.v.	1	5	0.004	0.0006	0.05	0.001	0.012	0.001	0.001	0.002	0.007	0.01	0.02
i.v.	4	24	0.002	0.001	0.027	0.001	0.029	0.002	0.024	0.0006	0.002	0.008	0.006
i.v.	4	24	0.74	0.024	0.17	0.008	0.092	0.008	0.011	0.024	0.001	0.01	0.006
r.c.	1	5	0.18	0.006	0.45	0.006	0.051	0.007	0.007	0.007	0.019	0.01	0.07
r.c.	1	5	0.002	0.003	0.12	0.002	0.054	0.004	0.014	0.003	0.018	0.02	0.06
r.c.	1	5	0.008	0.0006	0.020	0.001	0.021	0.001	0.002	0.001	0.005	0.01	0.01
r.c.	4	24	0.23	0.002	0.55	0.001	0.21	0.0005	0.15	0.001	0.002	0.004	0.007
r.c.	4	24	0.17	0.001	0.61	0.002	0.51	0.0009	0.067	0.014	0.001	0.01	0.002
r.c.	4	—	0.0005	0.001	0.0008	0.0007	0.002	0.0009	0.0005	0.0008	0.002	0.02	0.006
r.c.	4	—	0.001	0.001	0.002	0.001	0.003	0.001	0.001	0.004	0.003	0.02	0.01

i.v. = intravenous.
r.c. = right carotid artery.

vary according to the route of injection of ^{56}Mn. Only those animals injected in the carotid artery had ratios between tissues which were similar to those injected in the ear vein. These results support the suggestion that the $K^{56}MnO_4$ becomes particulate, possibly forming MnO_2 microparticles under *in vivo* conditions and is removed from the bloodstream by organs such as lung, liver and kidney in a manner similar to that of a filter. Aside from this passive uptake, an active transport phenomenon also appears to occur in the case of certain tissues. This is readily shown in the case of the liver and kidney since there is basically a high level of radioactivity in these tissues which appears to be independent of the route of administration. These observations are in agreement with those of Maynard and Cotzias (*5*), who found that tissues that are rich in mitochondria, such as liver and kidney, have a large manganese uptake, suggesting that this element may be essential for mitochondrial function.

From these data it must be concluded that ^{56}Mn accumulates readily in three important organs—the lung, liver and kidney—after the injection of radioactive permanganate. Consequently, this nuclide or other manganese isotopes appear to offer the possibility for determining focal pathology in these organs by scanning. The localized lesions might well behave as "cold spots" analogous to the behavior of nonfunctional adenomas in the thyroid gland following the administration of ^{131}I. Also the rapid elimination of manganese by the bile points to its potential as a diagnostic tool for studying gallbladder physiopathology.

Because normal brain levels were invariably low regardless of the route of administration, it seemed pertinent to determine whether ^{56}Mn would concentrate in cerebral lesions and/or edematous areas peripheral to a lesion. For this purpose, ten rabbits were prepared bearing freezing lesions extending over the right parietal cortex.

The animals were injected with the isotope either 5 or 24 hr after the cerebral lesion was produced. Five of the animals were injected over a period of 10 min via the right common carotid artery with the isotope diluted in 20 cc of physiological saline. The other five animals were injected with the undiluted isotope in the ear vein. The purpose of the carotid injection was to determine whether there was any difference in the uptake of the isotope by the lesion in relation to both the proximity of the injection site and the concentration of the administered nuclide. The animals were sacrificed 1–4 hr after the injection of the isotope, and symmetrical anatomical sections of the cerebral hemispheres were removed surgically, counted and examined histologically. As seen in Table 3, the right cerebral hemisphere containing the lesion usually has much larger amounts of isotope than the left hemisphere. Also, generally speaking, the animals that were injected through the carotid artery had higher isotopic accumulation in their right brain than those injected by the intravenous route. Finally, those animals that were injected with ^{56}Mn 24 hr after the formation of the lesion had larger amounts of the nuclide in this area than those with a 5-hr old lesion. Table 4, which gives the ratios of frontal, parietal cortex, parietal

TABLE 4. RATIOS OF PERCENT TOTAL DOSE/GRAM OF RIGHT TO LEFT ANATOMICAL CEREBRAL REGIONS FOLLOWING COLD LESIONS IN RIGHT PARIETAL CORTEX

Route of administration	Time sacrifice after injection (hr)	Time after cold lesion (hr)	Frontal right-to-left	Parietal cortex right-to-left	Parietal right-to-left	Occipital right-to-left
i.v.	1	5	8.4	11.1	5.2	10.5
i.v.	1	5	5.0	14.3	1.2	0.8
i.v.	1	5	6.6	50.0	12.0	0.5
i.v.	4	24	2.0	27.0	14.5	40.0
i.v.	4	24	30.8	21.2	11.5	0.4
r.c.	1	5	30.0	75.0	7.3	1.0
r.c.	1	5	0.7	60.0	13.5	4.7
r.c.	1	5	13.3	20.0	21.0	2.0
r.c.	4	24	115.0	550.0	420.0	150.0
r.c.	4	24	170.0	305.0	567.0	4.8
r.c.	4	—	0.5	1.1	2.2	0.6
r.c.	4	—	1.0	2.0	3.0	0.2

i.v. = intravenous.
r.c. = right carotid artery.

and occipital regions from right and left hemispheres, readily shows the difference between the two sides. It was apparent that the larger isotope uptake in pathological brain resulted from its concentration in the necrotic area and that these areas were greater in animals with older lesions. Levels of ^{56}Mn in edematous tissue of the right hemisphere were also elevated. This edema was easily discernible because the high number of counts obtained from the tissue correlated well with the histological picture characteristic of an edematous state. Generally, this edema surrounds the necrotic area extending deeply into the ventricular wall and to the frontal lobe but penetrating only slightly into the occipital lobe. It was seen, however, that the extension and localization of the edema was not uniform or consistent from one animal to another, and this accounts for the variations in Table 3. An exception to the results with other animals is seen in the last intravenous rabbit in Table 3. This animal was ill at the time of sacrifice with a large edematous reaction extending into both hemispheres. This resulted in an abnormal increase in the radioactive uptake of the frontal and occipital lobes of the left hemisphere. From these results it can be concluded that the edematous tissue adjacent to the necrotic area is more accessible to ^{56}Mn circulating in the plasma (possibly as MnO_2 particles) than normal brain is. The necrotic zone itself is even more easily penetrated. These results suggest that radioactive permanganate may be a useful scanning agent for detecting brain lesions in man.

SUMMARY

Radioactive permanganate as ^{56}Mn has been found to concentrate in lung, liver, kidney and cerebral lesions at levels that depend on the route of administration. Consequently, radioactive permanganate may have potential as a scanning agent for these organs. Moreover, its rapid elimination in the bile may permit its use for determining pathology in the gallbladder.

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