

Acta Medica Okayama

Volume 28, Issue 1

1974

Article 6

FEBRUARY 1974

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Abstract

By injecting ¹³¹I-Lipiodol into lymphatics of the dorsum of dog feet, the distribution of ¹³¹I in the lymph nodes and other principal organs as well as its histological effect were studied periodically after the injection for the period of two months. The characteristic feature of ¹³¹I distribution was the fact that ¹³¹I was accumulated into lymph nodes markedly higher than in any other organs and it was retained there over a long period of time. Histological examinations of the lymph nodes revealed a marked lymphocytopenia, the loss of germinal center, practically complete loss of lymphoid elements already 5 days after injection, and marked fibrosis. In the lung a considerable ¹³¹I-distribution could be seen in early stage, but with lapse of time it decreased rapidly. The distribution in other organs such as liver, spleen, bone marrow, kidney, ureter, bladder, thyroid gland, pancreas, testicles and small and large intestines was negligible in amount, and any specific histologic effect of irradiation could not be recognized in these organs including the lung. From these results, the authors concluded that ¹³¹I-Lipiodol has a selective activity on lymph nodes by injecting it via lymphatics and it is a safe method in clinical application to treat the patients bearing malignant lymphoma or metastatic lymph nodes.

Acta Med. Okayama 28, 59—67 (1974)

EXPERIMENTAL STUDIES OF THE ENDOLYMPHATIC RADIOTHERAPY

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Received for publication, July 9, 1973

Abstract: By injecting ^{131}I -Lipiodol into lymphatics of the dorsum of dog feet, the distribution of ^{131}I in the lymph nodes and other principal organs as well as its histological effect were studied periodically after the injection for the period of two months. The characteristic feature of ^{131}I distribution was the fact that ^{131}I was accumulated into lymph nodes markedly higher than in any other organs and it was retained there over a long period of time. Histological examinations of the lymph nodes revealed a marked lymphocytopenia, the loss of germinal center, practically complete loss of lymphoid elements already 5 days after injection, and marked fibrosis. In the lung a considerable ^{131}I -distribution could be seen in early stage, but with lapse of time it decreased rapidly. The distribution in other organs such as liver, spleen, bone marrow, kidney, ureter, bladder, thyroid gland, pancreas, testicles and small and large intestines was negligible in amount, and any specific histologic effect of irradiation could not be recognized in these organs including the lung. From these results, the authors concluded that ^{131}I -Lipiodol has a selective activity on lymph nodes by injecting it via lymphatics and it is a safe method in clinical application to treat the patients bearing malignant lymphoma or metastatic lymph nodes.

Since the lymphography was technically established by KINMONTH in 1952 with the advent of a good oily contrast medium, it has been markedly advanced in the past few years. This method has come to the use widely in various fields of medicine and its diagnostic significance has been generally recognized (1, 2). Furthermore, the lymphography with radioisotopes, *i. e.* endolymphatic radiotherapy, is also being applied to the treatment for metastatic lymph nodes and malignant lymphoma, and its usefulness has become gradually recognized (3). However, scarcely seen are basic studies on the reliability of endolymphatic radiotherapy for man, the findings on the distribution of isotope in organs and its effects. Considering the importance of this method for the treatment of malignant lymphoma, we have conducted some experiments using dogs first, before its clinical application.

The purpose of this study is to confirm the significance and reliability of clinical application of endolymphatic radiotherapy by studying the quan-

titative uptake and the effect of irradiation on lymph nodes and other organs when ^{131}I -Lipiodol is injected directly into the lymph vessels.

MATERIAL AND METHOD

Fifteen grown up dogs were anesthetized by intraperitoneal injection of sodium pentobarbital dissolved in distilled water (350mg/20ml). Since there was a considerable difference of anesthetic effect on individual animals, additional anesthesia was done when necessary. According to KINMONTH, after shaving hairs and sterilizing on the dorsal side of both paws, 0.5 ml patent blue was injected subcutaneously, the skin was cut open 1.5-2 cm in lengths along the lymph vessel stained blue, a lymphography needle was then inserted into the exposed lymph vessel. By confirming the flow of saline solution into the lymph vessel, radioisotope was injected. The radioisotope used was Iodinated Triolein ^{131}I -Lipiodol U. F. for intralymphatic injection (abbreviation: ^{131}I -Lipiodol), a product of The Radiochemical Centre, England. It contains a specific radioactivity of 10mCi/ml. To one paw was injected 2.5mCi or 5mCi ^{131}I -Lipiodol dissolved into Lipiodol Ultrafluide, a conventional contrast medium, not containing ^{131}I , by adjusting the final volume to 2.5 ml. In order to minimize the irradiation to the operator an automatic electric injector was used at the speed of 1 ml/10 minutes.

To see the effect of ^{131}I on the lymph nodes, ^{131}I -Lipiodol and Lipiodol without ^{131}I were injected into the dorsal lymph vessel of the left and right paws respectively, and the both popliteal lymph nodes were compared with each other. The amounts of ^{131}I -distribution in the following organs were measured with a well-type scintillation counter by sacrificing the animals at intervals of 1, 2, 4 and 8 weeks after injection, *i. e.* retroperitoneal, popliteal and cervical lymph nodes, lung, liver, spleen, thyroid, pancreas, kidney, small and large intestines and testicles. For histologic examinations, various organs were fixed with 10% formalin and stained with H-E solution. The amount of ^{131}I flown into blood was estimated when ^{131}I -Lipiodol is injected into the dorsal lymph vessel of the dog paw; by the thoracic duct cannulation, lymph was collected hourly for the first six hrs and was fractionated for the additional six hrs, and the radioactivity was measured on each lymph sample.

RESULTS

1. ^{131}I -distribution in various organs :

Table 1 shows the ^{131}I -distribution in organs in terms of percentage to the doses administered, 2.5 and 5mCi of ^{131}I -Lipiodol, indicating that the quantitative uptake by the popliteal and retroperitoneal lymph nodes is 5.4% and 3.9% per 1 g one week later, respectively. This figure is markedly higher than that in other organs, and at 4 weeks the concentration persists as high as 4.9% and 3.3% respectively. This finding suggests that the endolymphatic radiotherapy with ^{131}I acts most selectively on the lymph

TABLE I ^{131}I -DISTRIBUTION IN VARIOUS ORGANS AFTER THE INTRALYMPHATIC ADMINISTRATION OF ^{131}I -LIPIODOL IN DOGS

weeks after administration	1		2		4		8	
	A**	B***	A	B	A	B	A	B
organs								
cervical l. n. *	0.006%	0.018%	0.005%	0.022%	0.006%	0.006%	0.001%	0.002%
thyroid	0.004	0.037	0.010	0.014	0.008	0.014	0.002	0.004
lung	0.547	51.382	0.160	14.240	0.013	0.754	0.004	0.326
liver	0.013	4.342	0.003	1.296	0.004	0.800	0.003	0.798
spleen	0.020	0.440	0.004	0.184	0.003	0.033	0.002	0.032
pancreas	0.011	0.242	0.003	0.096	0.003	0.030	<0.001	0.002
kidney	0.014	0.756	0.004	0.320	0.002	0.056	<0.001	0.004
small intestine	0.005	—	0.003	—	0.002	—	<0.001	—
large intestine	0.004	—	0.003	—	0.002	—	<0.001	—
retroperitoneal l. n.	3.993	7.986	3.246	7.141	3.334	3.434	2.772	5.821
popliteal l. n.	5.484	9.326	5.388	6.004	4.982	3.986	1.239	1.487
testicle	0.004	0.009	—	—	—	—	<0.001	0.001

* l. n.: lymph node

** A: percentage of doses distributed in one g tissue to the doses administered.

*** B: percentage of doses distributed in the whole organ to the doses administered.

—: not examined.

nodes and yields the most effective result. Relatively higher concentration (0.5%) as compared to other organs were observed in the lung one week later. In contrast to the lymph nodes, the concentrations in the lung are decreased rapidly to 0.1% and 0.01% at 2 weeks and 4 weeks later respectively. ^{131}I -Lipiodol was found extremely low (0.004%) in the thyroid, and in the liver, pancreas, kidney, large and small intestines and testicles the activity was almost negligible.

2. Cumulative lymph radioactivity of ^{131}I collected from dog thoracic duct (expressed in percent):

Fig. 1 shows the results of estimation of ^{131}I released into blood via the thoracic duct at the venous angle of dogs. About 35–45% of the dose administered is released within six hrs, and by 12 hrs the amount reaches 65%. However, as shown in Fig. 2, retroperitoneal lymph nodes in dogs are far less in the numbers than that in man, making this result rather difficult to be applied immediately to man.

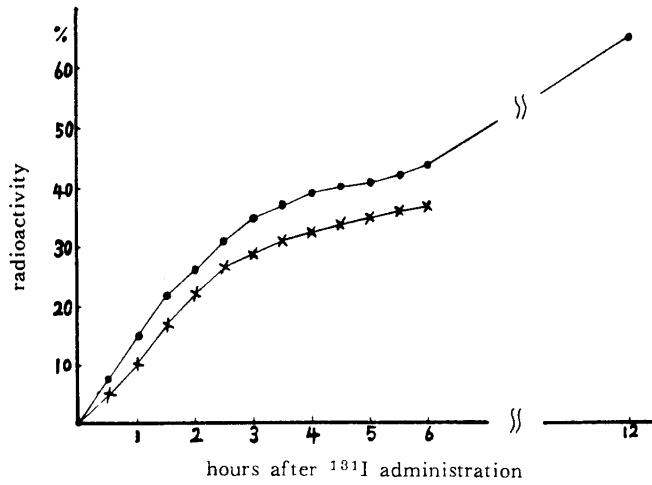


Fig. 1. Cumulative lymph radioactivity of ^{131}I collected from the thoracic duct after the intralymphatic administration of ^{131}I -Lipiodol in dogs. (expressed in percentage to the doses administered)



Fig. 2. Retroperitoneal lymphogram taken immediately after the intralymphatic administration of ^{131}I -Lipiodol in the dog

3. *Histological examinations to find the effect of ^{131}I -Lipiodol on lymph nodes and other organs:*

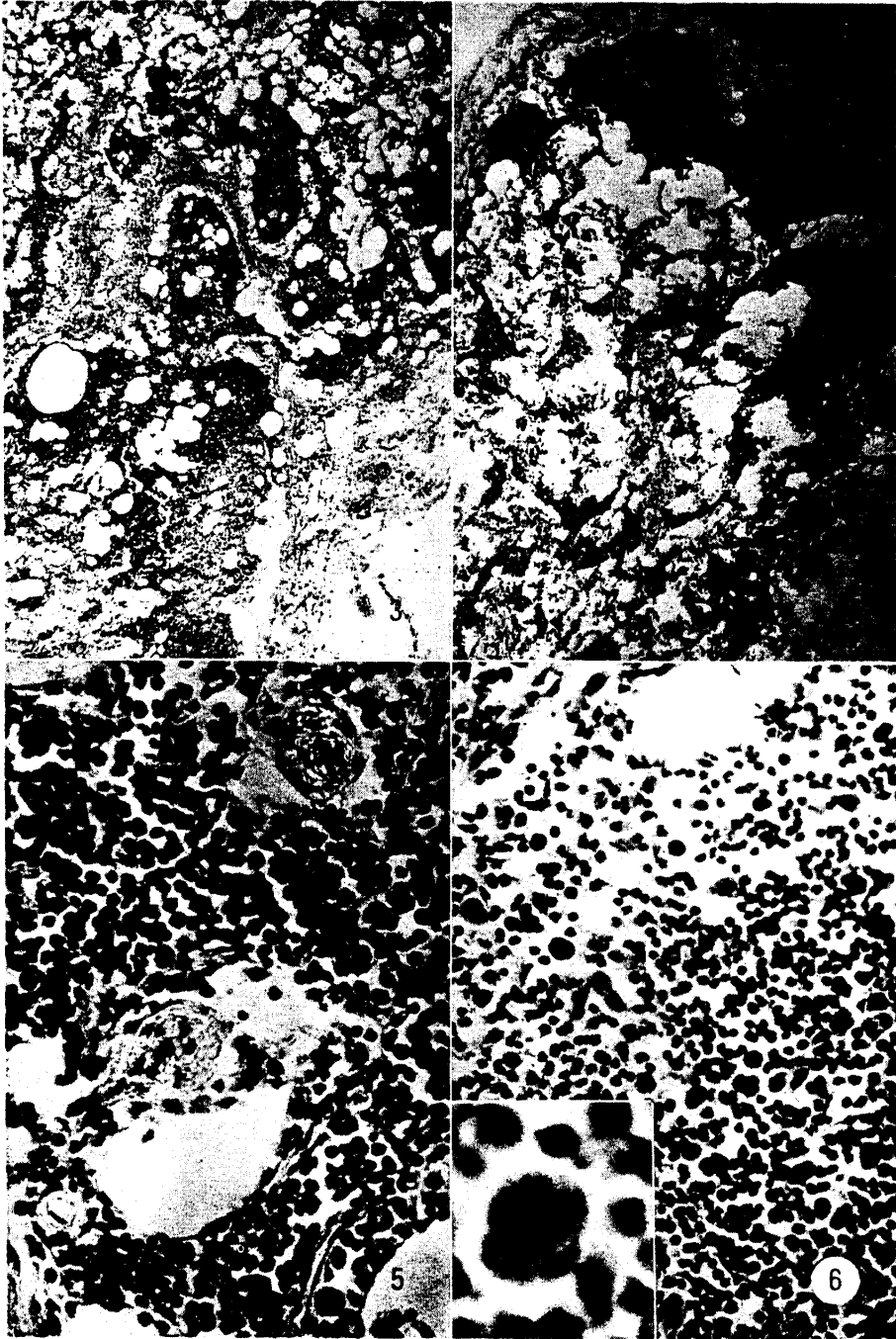
By injecting 2.5-5mCi ^{131}I -Lipiodol and, as a control, 2.5 ml Lipiodol Ultrafluide not containing ^{131}I into the dorsal lymph vessel of the left and right paws respectively, lymph nodes were compared histologically. Fig. 3 shows the lymphogram of popliteal lymph node 24-48 hrs after ^{131}I -injection, and Fig. 4 that taken 24-48 hrs after injection of Lipiodol alone. The contrast medium is extensively distributed within dilated sinusoids, and at a low magnification there appears no appreciable difference between the two pictures. At a high magnification, however, no damage to lymphocytes as seen in the Fig. 5 but reactions to injected contrast medium can be observed in the lymph nodes, into which Lipiodol Ultrafluide alone was injected. That is, multinucleated foreign-body giant cells, histiocytes, neutrophils and eosinophils are seen in the above lymph nodes together with lymphoid hyperplasia. In contrast, as seen in Fig. 6, destroyed lymphocytes, phagocytes ingesting nuclei, large mononuclear cells and neutrophils are markedly increased in the lymph nodes injected with ^{131}I ; and already by 24 hrs there can be observed a marked injurious effect on lymphocytes.

By five days after the ^{131}I -injection, as shown in Fig. 7, there is observed a marked decrease in lymphoid elements, the proliferation of interstitial cells and a marked fibrosis. Two weeks later the lymph node presents a picture like a fat marrow. By 4 and 8 weeks focal regenerations of lymphoid elements are seen as in Fig. 8. Other organs such as the thyroid, liver, spleen, bone marrow, kidney, ureter, bladder, pancreas, testicles, small and large intestines did not show any noteworthy changes, histologically.

DISCUSSION

Attempts at injecting radioisotopes into the lymph vessel for the treatment of malignant lymphoma and metastatic lymph nodes had been carried out quite early. In 1950 WALKER and in 1953 SHERMAN *et al.* (4, 5) conducted the interstitial or subcutaneous injection of radioisotope into animals, but, as the uptake of isotope into the lymph node was not sufficient, such a method has not been applied to clinical practices.

WALKER (4) tried for the first time isotope injection, yttrium colloid, directly into the rabbit lymph vessel, found the uptake of isotope into the lymph nodes in a high concentration and pointed to a possibility of its application in clinics. Since then, with the advent of ^{131}I -Lipiodol and ^{32}P -Lipiodol, this method has become clinically available (3). For the clinical application of endolymphatic radiotherapy, however, it is necessary to know the quantitative uptake of isotope into organs, the amount of radioactivity



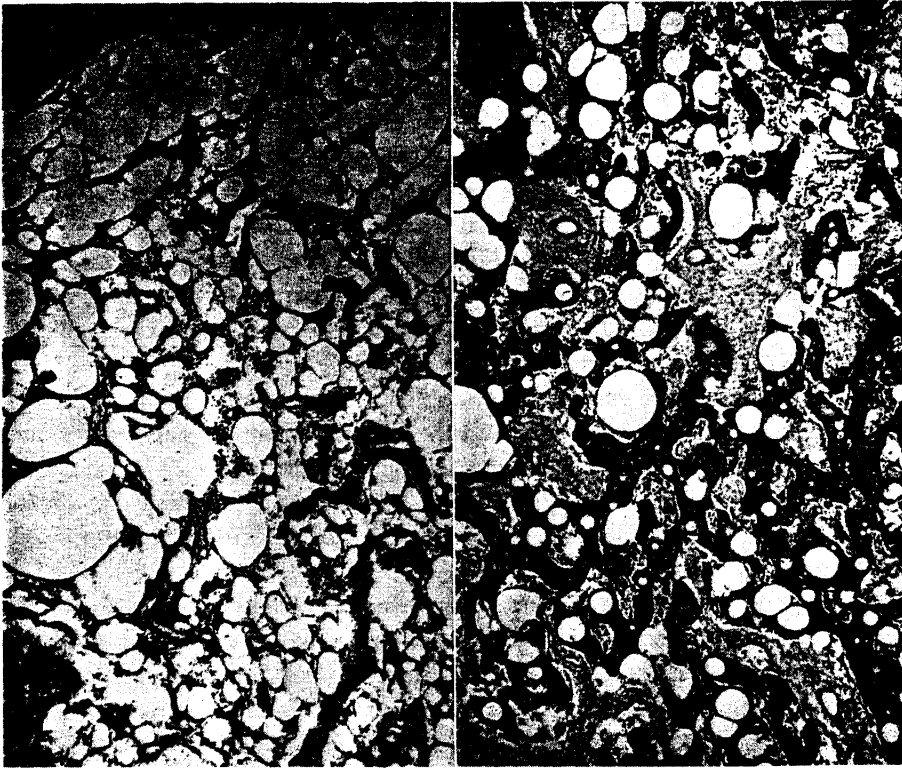


Fig. 7. Section of the popliteal lymph node 5 days after the ^{131}I -Lipiodol administration. H-E. $\times 40$.

Fig. 8. Section of the popliteal lymph node 4 weeks after the ^{131}I -Lipiodol administration. H-E. $\times 40$.

released into blood, and its effects on lymph nodes and other organs (6-11). In the experiments conducted with dogs we have found that 51% of the injected dose has reached lung while in the popliteal and retroperitoneal lymph nodes only about 17% is present one week after the injection. The uptake into the lymph nodes in terms of % per 1 g tissue, however, was 6-10 times as much as that in the lung. What is more, while in the the lung the radioactivity declines rapidly with lapse of time (2-8 weeks), in the lymph

Fig. 3. Section of the popliteal lymph node 24 hrs after the ^{131}I -Lipiodol administration. H-E. $\times 40$.

Fig. 4. Section of the popliteal lymph node 24 hrs after the administration of Lipiodol Ultrafluide not containing ^{131}I . H-E. $\times 40$.

Fig. 5. Section of the popliteal lymph node 24 hrs after the administration of Lipiodol Ultrafluide not containing ^{131}I . H-E. $\times 400$.

Fig. 6. Section of the popliteal lymph node 24 hrs after the ^{131}I -Lipiodol administration. H-E. $\times 400$. The large cell of higher magnification (lower right), shows the nucleophagia.

node it was sustained at a considerable high concentration. The uptake in the organs other than the lung by hematogenous dissemination is extremely low and negligible, and no irradiation effect can be suspected. These results seem to indicate that the radioisotope injected directly into the lymph vessel has its strong irradiation effect selectively on the lymph nodes.

The amount of ^{131}I -Lipiodol released in blood was estimated by the thoracic duct cannulation; six hrs after the injection 30-40% of ^{131}I was released into blood and at 12 hrs about 60%. Since the retroperitoneal lymph nodes of dogs are much less in numbers as compared with man, this result cannot be directly applied to man. One must, however, bear the above fact in mind in case we apply this technique clinically.

By the histologic examinations in the group administered with ^{131}I -Lipiodol, a marked lymphopenia and the disappearance of germinal centers were observed. By fifth day the lymph node lost practically all the lymphoid elements and presented an appearance of fat marrow. In contrast, various organs other than lymph nodes have shown not any findings suggestive of irradiation injury at all.

It is generally known that the lymph node is radiosensitive, and that the contrast medium injected is distributed homogeneously in the lymph node with malignant lymphoma and is retained for a long period of time. It could be concluded that the endolymphatic injection of ^{131}I -Lipiodol would be a good therapeutic method for malignant lymphoma, and that it is a safe method because it did not cause any side-effects demonstrable in our experiment.

Acknowledgement: The authors wish to thank Prof. K. HIRAKI for valuable advices and for reviewing the manuscript.

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