Title	131I-toluidine blue Oのラットおよび犬の副甲状腺へのとりこみ(左心室内および静脈内投与の比較)
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TISSUE CONCENTRATION OF ^{131}I-TOLUIDINE BLUE O IN RATS AND DOGS WITH SPECIAL REFERENCE TO THE PARATHYROID CONCENTRATION BY INTRACARDIAC AND INTRAVENOUS ADMINISTRATION

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^{131}I-toluidine blue Oのラットおよび犬の副甲状腺へのとりこみ（左心室腔内および静脈内投与の比較）

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臨床上，副甲状腺を青染するために用いられている色素 toluidine blue O (T.B.O) が副甲状腺シンチグラム上描出上可能性があるか否かを検討するため ^{131}I-T.B.O を用いて次の動物実験を行なつた．

^{131}I-toluidine blue O (^{131}I-T.B.O) のラット左心腔内投与群（group Ⅰ）と頚静脈内投与群（group Ⅱ）について副甲状腺，甲状腺，頚筋，および膵，肝，肺，心筋へのとりこみを投与30分後において比較した．

group Ⅰでは group Ⅱに比し，副甲状腺へのとりこみは，甲状腺および頚筋より有意に高率であつた．

左心室内投与の場合でも緩徐な注入の場合は頚静脈投与群（group Ⅱ）と同様の傾向を示した．

色素としての T.B.O を ^{131}I-T.B.O に過量に加えた場合（Carrier group）では有意な変化は見られなかつた．

動物による差を見る意味で，ラットの他に，犬についても同様な実験を行なつたが，左心室腔投与群（groupⅢ）と静脈内投与群（group Ⅳ）との間に差を見なかつた．

膵に関しては groupⅠ，Ⅱ，ⅢおよびⅣ群の何れにおいても，肝より高いとりこみはなかつた．

Sission and his associates[11](1962) demonstrated that cobalt 57-labeled vitamin B_{12} was well concentrated in canine parathyroid glands. By autoradiographic methods, Potchen[10](1963) demonstrated

a high concentration of ^{3}H-methionine in parathyroid tissue of the rat. Klopper and Moe[6] (1966) reported a succeseful "in-vivo" blue coloration of the parathyroid and pancreas following an intravenous infusion of the dye: toluidine blue (T.B.) in dogs and patients. When the dye is infused intravenously in dosis of 5 mg per kg body weight, parathyroid glands are usuelly well stained but occasionally accompanied by arrythmia and transient myocardial damage. On the other hand, 1 ml of 0.1% toluidine blue O (T.B.O) solution administered into the inferior thyroid artery does not have any harm to the myocardium, but parathyroid coloration is not necessary obtained. In addition, the assessment of tissue concentration of the dye through colorimetric assay of the extirpated tissue specimen is subject to error due to protein binding and the conversion of T.B. to leucoform.

For these reasons, basic studies for the possibility of scintigraphic visualization of the parathyroid and pancreas using radioisotope labeled T.B. have been attempted.

Brien and Keaveny[5] (1968) used ^{131}I-T.B. in dogs, with the resulting parathyroid-thyroid ratio of 2.97: 1. Archer and his coworkers[1] (1969) used ^{125}I-T.B. in rats and obtained parathyroid-thyroid ratio of 3: 1, at 5 minutes after intravenous injection.

In their later report (1972)[2], they confirmed that parathroid-thyroid ratio of dye concertration was 3: 1, and parathyroid-pancreas ratio was 2: 1 at 5 minutes after injection. After 15 minutes, dye concentration in the parathyroid had fallen to levels as low or lower than those of pancreas and thyroid.

They concluded that a gamma-emitting analogue of T.B. would most likely be unsuitable for use in external scanning for localization of these glands.

Lindenauer and his cowerkers[8] (1969) infused ^{35}S-T.B. in dogs over thirty minutes. The concentration of ^{35}S-T.B. in the parathyroid varied directly with the dose and inversely with time. The parathyroid-thyroid ratio was 2: 1 to 5: 1 and was greater at the end of the infusion than one hour later. Larose and his coworkers[7] (1970) used ^{125}I-T.B. in rats and reported both parathyroid-thyroid ratio and pancreas-liver ratio to be less than 2: 1.

Mortenson and McRae[9] (1970) demonstrated in dogs that a very high concentration of toluidine blue O was obtained in the parathyroid; parathyroid-thyroid ratio of 30: 1, parathyroid-neck muscle ratio of 42: 1 and parathyroid-blood ratio of 85: 1.

In all of these studies, the dye solution and radioisotope labeled T.B. was injected intravenously. Because of these conflicting results, the present animal studies were undertaken to confirm whether or not ^{131}I-T.B.O is a promising scanning agent. Studies included varying the route of administration, and carrier dose.

Materials and Methods:

Prearation of ^{131}I-T.B.O

Toludidine blue O labeled with ^{131}I (^{131}I-T.B.O), which was prepared by Dainabot Radioisotope Lab., was identified by thin-layer chromatography. Preparation will be described in detail.

25mg of T.B.O (E. Merck) was dissolved in 3 ml of distilled water. 10 mg of KIO_3 was dissolved in 1 ml of distilled water. 0.1 ml of KIO_3 solution and 100 mCi of radioactive iodine (Na ^{131}I) were added to T.B.O solution. Two or three drops of 6 N Hcl were added and the mixture solution was incubated overnight.

The reaction mixture was then passed through the Dowex 1×8 anion exchanger. ^{131}I-T.B.O solution

was sterillized by Millipore filter. The final yield was 70 to 80 percent.

Free iodine was accounted for no more than 3% of the total radioactivity of the ^{131}I-T.B. solution. The specific activity was 1 to 2 mCi per mg of dye and radioactive concentration was approximately 500 μCi per ml of the solution.

Adult rats of Wistar strain, weighing 200–250gm, and adult mongrel dogs of 8–15 kg body weight were used. At least one week prior to the experiment, KI was administered to the rats and KI with dessicated thyroid was administered to dogs. This minimized the thyroid uptake of free iodine were used.

All experiments were carried out in animals anesthesized with Nembutal.

In the rats, 100 μCi of ^{131}I-T.B.O solution was given in a volume of less than 0.5 ml, the dose of T.B.O contained was 0.36–1.0 mg per kg body weight. In the dogs 150–500 μCi of ^{131}I-T.B.O was administered.

Rats and dogs were divided into groups depending upon the route of administration: Group I, consisting of 18 rats, was injected percutaneously into the left ventricle; Group II was consisted of 12 rats in which injections were made into the surgically exposed external jugular vein; Group III was consisted of 3 dogs and dye solution was injected into the left ventricle percutaneously; Group IV was consisted of 4 dogs which received intravenous injection into hhe antecubital vein.

In Group II and IV, the time of injection required 15 to 60 seconds. Except for one dog of Group IV, in which ^{131}I-T.B.O was dissolved in 500 ml saline solution and infused over 30 minutes. As a Carrier Group, an excess dose of up to 10 mg per kg body weight of T.B.O was injected in conjunction with the ^{131}I-T.B.O solution. This group consisted of 2 rats in which ^{131}I-T.B.O was injected into the left ventricle and one dog in which ^{131}I-T.B.O was administered intravenously.

Thirty minutes after the administration of ^{131}I-T.B.O, the animals were exsanguinated and the whole parathyroids, a piece of theyroid, neck muscle, lung, heart muscle, liver and pancreas were removed.

The parathyroids were removed from the thyroid using a pair of microscissors with the aid of a dissecting stereomicroscope. The specimen was confirmed microscopically that the thyroid tissue was not included in the specimen. (Fig. 1) The thyroid specimen was removed from the portion as remote from the parathyroid as possible.

All the specimens were placed within a wet chamber immediately after removal and weighed as

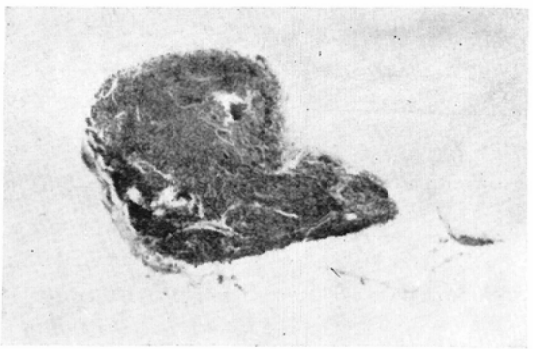

Fig. 1. extirpated parathyroid of the rat showing no piece of thyroid tissue.

quickly as possible. The wet weight of parathyroid per rat was 0.1–0.3 mg and in the dog, around 20 mg. In both Group I and Group II, tissue specimens from 2 rats were pooled and weighed because of their small sample weights.

Radioactivity of each tissue specimen was counted in well-type NaI (Tl) scintillation detector. The tissue-blood ratio (cpm per mg of the wet tissue versus cpm per μl of the whole blood) was obtained. Parathyroid-thyroid ratio, parathyroid-neck muscle ratio, pancreas-liver ratio and lung-heart muscle ratio were calculated.

A linear scanning and area scanning of the whole body using 5×2 inch dual rectilinear scanner, (Toshiba, Type ROA-107) was carried out in one dog of Group IV immediately following the infusion of ^{131}I-T.B.O

Results

Results of mean tissue-blood ratios obtained in animals of Group I to Group IV are presented in Table 1.

Table 1 tissue spectmen-blood ratio (mean values)

	parathyroid	thyroid	neck muscle	pancreas	liver	lung	heart muscle
Group I	41.5	10.9	1.9	7.8	12.0	8.1	7.1
Group II	5.8	9.5	4.3	28.9	57.8	36.3	24.6
Group III	24.4	62.5	4.6	19.2	21.4	19.2	13.8
Group IV	8.3	15.1	4.4	7.5	7.9	7.8	1.8

The parathyroid-thyroid ratios, parathyroid-neck muscle ratios, pancreas-liver ratios and lung-heart muscle ratios abtained in Group I to Group IV are shown in Fig. 2.

The parathyroid-thyroid ratio in Group I was 3.8±2.2, which was significantly higher than those obtained in Group II, Group III and Group IV; 0.6±0.3 in Group II, 0.4±0.2 in Group III and 0.6±0.3 in Group IV.

The concentration of ^{131}I-T.B.O in neck muscle was low in all the groups.

The concentration of ^{131}I-T.B.O in pancreas of all four groups was lower than that in the liver. Pancreas-liver ratios were 0.7±0.4 in Group I, 0.5±0.2 in Group II, 0.9±0.4 in Group III and 0.9±0.5 in Group IV.

Similar lung-heart muscle ratios were noted in Groups I-III (1.7±1.1 in Group I, 1.5±0.7 in Group II and 1.4±0.5 in Group III), whereas, in Group IV the ratio was 4.3±1.5.

No difference in the lung-heart muscle ratio was found between the group of dogs with rapid intravenous injection and that with a prolonged infusion over 30 minutes.

No significant difference was noted in terms of parathyroid-thyroid ratios, parathyroid-neck muscle ratios, pancreas-liver ratios and lung-heart muscle ratios either with carrier (Carrier Group) or without carrier (Group I).

In the rat (Carrier Group) in which a fairly large dose of T.B.O was administered, no particular blue

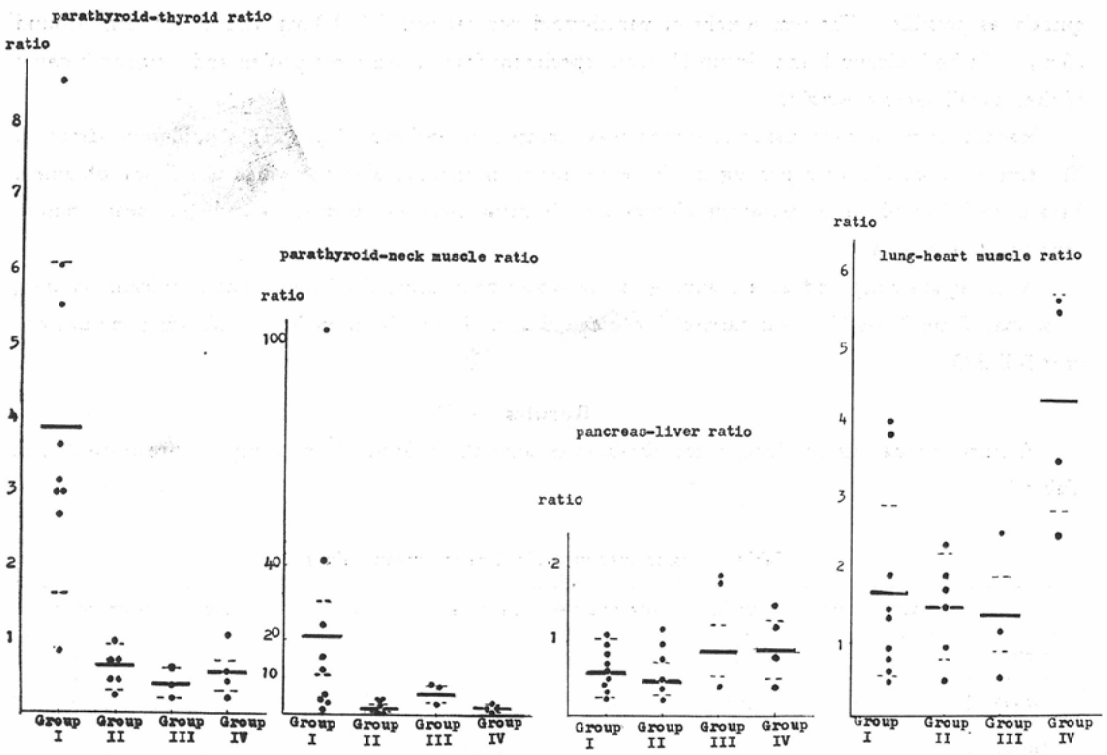

Fig. 2. The parathyroid-thyroid ratio, parathyroid-neck muscle ratio, pancreas-liver ratio, and lung-heart muscle ratio.

coloration of the parathyroids and pancreas was noted, while the gastric wall was stained in a slight bluish tint.

In the Carrier Group, receiving a drip infusion of 500 μCi of ^{131}I-T.B.O with 10 mg per kg of T.B.O no abnormal E.C.G. findings appeared over a period of 30 minutes.

In one dog in Group IV, a linear scanning was performed at the end of the infusion. An area of apparent uptake of radioactivity was not recognized in the neck region.

Discussion:

In our present studies, significantly higher parathyroid-thyroid ratios and parathyroid-neck muscle ratios of ^{131}I-T.B.O uptake were obtained only in Group I, when the animals were sacrifieed at 30 minutes after injection and pieces of the tissues were removed, weighed and the radioactivity were counted.

It is interesting why relative high parathyroid-thyroid ratios were observed only in Group I: injection of ^{125}I-T.B.O into the left ventricle of the rat.

Archer and his cowarkers[2] (1972) reported that the thyroid, parathyroid and neck muscle studied appeared to treat ^{125}I-T.B. very similarly to the unlabeled dye, though in both pancreas and stomach the uptake discrepancy between ^{131}I-T.B. and unlabeled T.B. was observed.

The addition of carrier T.B.O was studied with the hypothesis that the dye might be concentrated in the parathyroid only when it was administered in a dosage over a certain threshold, and this was not

proved although there were several clinical instances where 0.5 to 1.0 ml of 0.1% T.B.O solution occasionally produced distinct blue coloration of parathyroids when it was administered into the inferior thyroid artery.

We observed that an extremely slow intracardiac injection of 5 ml of diluted ^{131}I-T.BO in rats gave low parathyroid-thyroid ratios similar to results obtained in Group II. These results indicate that the dye should reach the parathyroid quickly following injection to obtain a high uptake in the parathyroid gland.

^{131}I-T.B.O was concentrated in the parathyroids in rats, but not in the dogs, when it was administered into the left ventricle. The basis of these species differences had not been well explained, though the quantitative studies of T.B. distribution in the dog differ significantly from those in rats in that there is maintenance of high parathyroid-thyroid ratios during three hours of observation (Kang and DiGiulio, (1968)[5], DiGiulio and Lindenaner, (1970)[4]).

Our animal experiments were done in the state of normally functioning parathyroid. It might be necessary to create hyperfunctioning parathyroid through EDTA administration, or renal injury, in order to obtain higher concentrations of ^{131}I-T.B.O.

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