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SELENIUM LABELLED TOLUIDINE BLUE AS AN AGENT FOR PARATHYROID SCANNING

Theoretic considerations

by

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Radioisotope scintillation scanning was first introduced as a medical diagnostic aid in the middle nineteen fifties. Since that time it has become a useful technique for the investigation of disorders of many organs, particularly brain, thyroid, lungs, kidney, liver and bone. Two organs in which scintillation scanning has met with less success are the parathyroids and pancreas mainly because of the lack of a radiopharmaceutical which localizes sufficiently selectively in these organs.

Currently, the radiopharmaceutical used for scanning the pancreas and parathyroids is ^{75}Se selenomethionine, a ^{75}Se analogue of the natural sulfur containing amino acid methionine. First introduced by POTCHEN in 1963 this compound has been used by a number of workers for scanning both pancreas and parathyroids. The results, however, have been far from encouraging. MCGEOWN et coll. (1968) having reviewed the literature of parathyroid scanning and reported a further eight cases concluded 'we think that selenomethionine-^{75}Se is not sufficiently selectively concentrated in the parathyroid glands, and that real progress in the scanning of these glands will depend upon the discovery of a more suitable radioactive compound'. GARROW & SMITH (1968) considered a theoretic model of the process whereby selenomethionine is con-

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centrated in the parathyroids and concluded that 'it is not to be expected that the normal parathyroids will ever be demonstrable by the selenomethionine scanning technique' even though the main difficulty in surgery of the parathyroids lies in the necessity to detect and identify not only the tumor or tumors but the remaining normal glands (DAVIES 1959). In 1967 POTCHEN et coll. described 40 cases, in only 24 of which, the scan correlated with the surgical findings. They described the results as 'encouraging, albeit frustrating'. Similarly DI GIULIO & MORALES (1969) found that ^{75}Se-methionine scanning identified the abnormal gland in only 13 of 23 patients with surgically proven parathyroid adenoma. They concluded that scanning was of little diagnostic use in patients with small adenomas (less than 2 g) and in patients with hyperplasia or with adenomas or hyperplastic glands lying in the thorax. Pancreatic scanning has proved somewhat more successful but there again many difficulties arise. Selenomethionine is also taken up by the liver, which due to its greater size, accumulates more radioactivity than the pancreas. When the two organs overlap, pancreatic identification is either interfered with or completely blocked. SODEE (1964) reported a series of 251 pancreatic scans and despite difficulties of interpretation, successfully predicted carcinoma in 24 of 26 proven cases. In 151 cases later diagnosed as normal, the scan interpretation was also normal in 146. MELMED et coll. (1968) also reported a high correlation between the scan and the ultimate diagnosis. They correctly predicted the result in 37 out of 40 consecutive cases both normal and pathologic.

Despite these good results the interpretation of pancreatic scans presents many difficulties even to experienced clinicians, particularly when there is a generalized reduction in uptake of the radioisotope (AGNEW et coll. 1969). Attempts at improving the results have ranged from methods aimed at increasing uptake by pancreatic stimulation (KING et coll. 1966), to scanning liver and pancreas with two different isotopes and using electronic devices to subtract the liver scan from the superimposed liver-pancreas scan, to give an image of the pancreas alone (COTTRALL et coll. 1968). Another technique employed has been the direct injection of selenomethionine into the pancreatic artery (REUTER & COHN 1969) a method which has been shown to improve the pancreas/liver ratio of radioactivity (KUPIC & KASENTER 1969).

Nevertheless, the limitations of selenomethionine as a pancreatic scanning agent have prevented it from achieving its hoped for potentiality. Indeed, CHARLSWORTH et coll. (1970) have shown that with present methods, small, and hence resectable, tumors of the pancreas cannot be detected.

Toluidine blue. KLOPPER & MOE (1966) observed that when toluidine blue was administered intravenously to dogs it concentrated in the parathyroids, pancreas and gastric corpus, staining these organs a distinct blue color. This

observation has later been confirmed (HURVITZ et coll. 1967, KEAVENY & FITZGERALD 1968).

This dye has also been used to locate and identify parathyroid glands at surgery (KLOPPER & MOE 1966, KEAVENY & FITZGERALD 1968, YEAGER & KREMENTZ 1969) and to detect a tumor of the pancreas (KEAVENY et coll. 1971).

It soon became obvious, that if a suitable radioactive label could be incorporated within the toluidine blue molecule, without altering its ability to concentrate in the organs mentioned, a desirable scanning agent could be produced. The first attempt at producing a radioactive form of toluidine blue was by BRIEN & KEAVENY (1968) who labelled the dye with ^{125}I and achieved a parathyroid to thyroid ratio of 2.97 in a dog 20 minutes after injection. Further work, however, failed to improve this ratio which was approximately the same as that obtainable with selenomethionine (BRIEN, unpublished observations). ARCHER et coll. (1969) also prepared ^{125}I toluidine blue and measured its concentration in various organs in the rat. They found a parathyroid to thyroid ratio of 3.0 after five minutes but reported that the parathyroid uptake fell rapidly thereafter. They concluded that the labelled dye did not remain in the parathyroids for a sufficiently long period to render it a useful agent for external scintillation scanning.

Another attempt to produce a labelled version of toluidine blue using $^{99}Tc^{m}$ pertechnetate, failed because the material obtained was in an aggregated form and over 70 per cent of the injected dose accumulated in the liver (YEH et coll. 1968).

Since the molecule of toluidine blue contains a sulfur atom within the ring structure of the molecule, it was though that replacement of this sulfur atom by a radioactive selenium atom, could result in a molecule with the requisite biologic and tinctorial properties. ^{75}Se has an adequately long half-life (121 days) and emits only gamma rays, the principal energies being 140 keV, 270 keV and 280 keV. These energies are all suitable for use with standard scintillation scanners.

Calculation of theoretic specific activities and gland concentration of ^{75}Se toluidine blue. In order to evaluate the usefulness of ^{75}Se toluidine blue as a scanning agent for the parathyroids, the following calculations were made.

Molecular weight of ^{75}Se toluidine blue is 300; molecular weight of selenium is 79.

Specific activity of ^{75}Se after four weeks irradiation is 6.2 mCi/g (Radiochemical Manual). Specific activity of ^{75}Se toluidine blue is $\frac{79 \times 6.2}{300} = 1.63$

mCi/g. If the tissue concentration in the parathyroids after a 100 mg/kg dose is 90 μg toluidine blue/g parathyroid (KANG & DI GIULIO 1968) then the radioactive concentration is

$$\frac{1.63 \text{ mCi}}{\text{g}} \times \frac{90 \ \mu\text{g}}{\text{g}} = 0.15 \ \mu\text{Ci/g tissue.}$$

Alternatively, if ^{75}Se labelled toluidine blue can be synthesized using commercially available ^{75}Se with a specific activity of 1 mCi/g selenium (Radiochemical Centre, Amersham) then the specific activity of the ^{75}Se toluidine blue could be

$$\frac{1 \times 79}{300} = 0.26 \ \mu\text{Ci/g}$$

and the tissue concentration in the parathyroids would be

$$\frac{260 \ \mu\text{Ci}}{1\,000 \ \mu\text{g}} \times \frac{90 \ \mu\text{g}}{\text{g}} \text{ or } 23.4 \ \mu\text{Ci/g.}$$

Although there is evidence to suggest that radioactivation might prove a simple and convenient method of preparing ^{75}Se labelled toluidine blue from the non-labelled molecule (MCCONNELL et coll. 1962, BADIELLO & BRECCIA 1966) the length of time required to irradiate the material to an adequately high specific activity would appear excessively long. It is suggested therefore that the more practical approach would be ab initio synthesis of the molecule using relatively high specific activity ^{75}Se.

Given a reasonable high specific activity ^{75}Se toluidine blue, it still remains to be shown that sufficient radioactivity will be concentrated in a parathyroid gland to permit its demonstration by conventional scanning techniques. GARROW & SMITH (1968) determined that it was necessary for a point source to contain at least 0.5 μCi of ^{75}Se in order to be visible on scan with the conditions they used. Assuming that a 1 g adenoma effectively represents a point source, then a tissue radioactivity of 0.5 μCi/g would be necessary to permit demonstration of the gland. MCGEOWN et coll. (1968) using phantom tumors in a radioactive solution simulating an evenly distributed background, found that for a 1 ml 'tumor' a minimum activity of 0.16 μCi was necessary to allow detection when the background was one third this level. On the other hand DI GIULIO & BIERWALTES (1964) in similar experiments found a detection limit of 0.02 μCi in a 1 ml 'tumor', again with a background of one third the tumor level. These latter figures agree fairly well with their own data on the tissue activity of parathyroid glands excised after administration of ^{75}Se-methionine (0.005 to 0.043 μCi/g) and also with that of MCGEOWN et coll. (0.012 to 0.021 μCi/g).

Although this evidence from ^{75}Se-methionine phantom investigations appears to be somewhat conflicting, there is no doubt that a 1 g parathyroid adenoma

should be readily demonstrated at a tissue radioactivity concentration of 1 μCi/g.

According to the data of KANG & DI GIULIO (1968) a dose of 10 mg of toluidine blue per kg body weight results in a concentration of 90 μg of dye per gram of parathyroid tissue. In order to achieve a radioactive concentration of 1 μCi/g parathyroid the necessary specific activity of toluidine blue would be 1 μCi/90 μg or 0.011 μCi/μg. The total dose to a 70 kg man would then be $10 \times 70 \times 1\,000 \times 0.011 = 7\,700$ μCi.

This would appear to be an unacceptably high dose of radioactivity to administer in a diagnostic test considering that the generally accepted dose of ^{75}Se-methionine is 200 to 250 μCi.

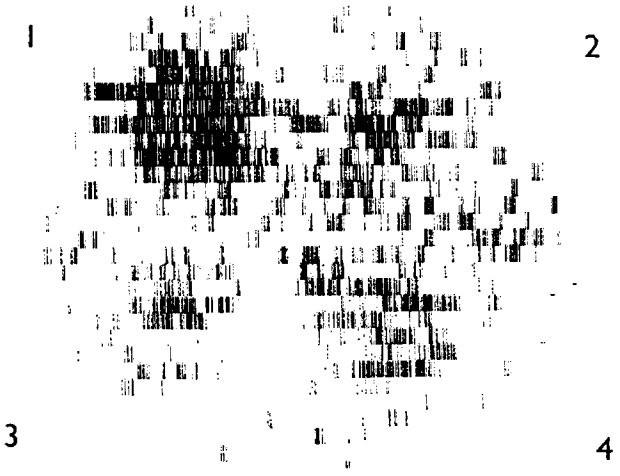
An alternative method of approach is to calculate the percentage of the administered dose which is concentrated in parathyroid tissue. Using the same data as above the dose to a 70 kg man is $10 \times 70 = 700$ mg. The parathyroid concentration is 90 μg/g tissue. Therefore the per cent dose/g tissue is

$$\frac{90 \times 100}{700 \times 1000} = 0.013\ \%/\text{g}.$$

This is about the same as that found after ^{75}Se-methionine (MCGEOWN et coll.).

Since toluidine blue has a much larger molecular weight than methionine (306 as against 117) the incorporation of one labelled atom of selenium into each molecule would result in a much higher specific activity for selenomethionine than for seleno-toluidine blue. Thus if the same percentage of the administered dose of each is incorporated into parathyroid tissue, a higher concentration of radioactivity would be expected in the case of selenomethionine. Thus it would appear, that on a molar basis, selenomethionine is in fact concentrated to a greater extent in the parathyroids, than is toluidine blue. However, the average parathyroid to thyroid ratio of radioactivity is about 3, after ^{75}Se-methionine administration (DI GIULIO & MORALES 1969, MCGEOWN et coll. 1968). After toluidine blue this ratio rises to 10 (KANG & DI GIULIO 1968). Since the background activity registered by the scintillation scanner would thus be decreased, the absolute level of activity necessary for demonstration of the parathyroids would also be decreased. This factor was taken into account by KANG & DI GIULIO when they estimated that for a 70 kg man 350 μCi of ^{131}I labelled toluidine blue would be sufficient to demonstrate a 0.3 g parathyroid at a tissue concentration of 0.05 μCi/g, this figure of 0.05 μCi/g being their measured level of radioactivity in excised parathyroid after ^{75}Se-methionine.

Another factor which needs to be taken into account is the fact that the permissible dose of ^{75}Se-methionine is limited by its long biologic half-life in man which has been variously reported as being as low as 23 and as high as



Scan of parathyroid phantom containing four mock 'tumors'. 1—1 μCi in 1 ml; 2—0.3 μCi in 0.3 ml; 3—0.5 μCi in 1 ml; 4—0.5 μCi in 0.5 ml.

144 days (SODEE 1964, PENNER 1966). Although there is to date no published data on the biologic half-life of toluidine blue in man the impression gained from clinical investigations is that the figure is of the order of days rather than weeks.

This is supported by the findings of MORTENSEN & MCRAE (1970) that no residual toluidine blue could be found in any organ of dogs who had received intravenous injections of the dye forty-eight hours previously. From the radiation dosimetry point of view, therefore, the acceptable dose of ^{75}Se labelled toluidine blue might well be considerably greater than that for ^{75}Se-methionine. The final answer to this question however will probably have to await the production of the labelled molecule for metabolic radiotracer investigations.

Experimental. In order to evaluate the level of activity which would be required and the minimum size of parathyroid detectable a number of experiments were carried out using a phantom model of the parathyroids. This phantom consisted of a circular dish 12 cm in diameter in which were placed four small flat bottomed test tubes arranged in a square of side 4 cm. These test tubes contained a solution of ^{75}Se of the following volumes and concentrations:

Tube	Volume	Concentration
1	1 ml	1 μCi/ml
2	0.3 ml	1 μCi/ml
3	1 ml	0.5 μCi/ml
4	0.5 ml	1 μCi/ml

The circular dish was filled with a solution of 100 ml of ^{75}Se at a concentration of 0.1 μCi/ml.

The phantom was scanned using a Nuclear-Chicago Pho-Dot scanner using a 19 hole collimator at a speed of 10 cm/min and a suppression factor of 80 per cent. The result is shown in the Figure. All four 'tumors' are clearly seen and some indication of their relative sizes can also be deduced.

Conclusion

If ^{75}Se labelled toluidine blue with a specific activity of 0.01 μCi/μg can be synthesized and if this compound retains the biologic properties of toluidine blue it should concentrate sufficiently selectively in the parathyroids to prove a useful scanning agent for these glands. Tumors down to 300 mg or less in size should be adequately demonstrated instead of the 1 to 2 g limit currently obtainable with ^{75}Se-methionine. Some indication of tumor size may also be possible.

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SUMMARY

The usefulness of radioactive labelled analogues of toluidine blue as scanning agents for pancreas and parathyroids is considered with particular reference to the minimum size of parathyroid adenoma likely to be demonstrated. It is concluded that a selenium labelled analogue of toluidine blue should prove a better scanning agent for parathyroids then selenomethionine and that with appropriate specific activities adenomas of 300 mg or less could be detected.

ZUSAMMENFASSUNG

Die Anwendbarkeit radioaktiv gezeichneter Analoge von Toluidin-Blau als Substanzen für das Scanning des Pankreas und der Parathyreoidea wird erwogen, besonders im Hinblick auf die geringste darstellbare Grösse des Adenoms der Parathyreoidea. Man kommt dazu, dass ein Selenium-gezeichnetes Analog von Toluidin-Blau sich mehr als Scanning-Substanz der Parathyreoidea eignet als Selenmethionin, und dass mit geeigneten spezifischen Aktivitäten Adenome von 300 mg oder weniger nachgewiesen werden können.

RÉSUMÉ

L'auteur étudie l'utilité d'analogues marqués radio-activement de bleu de toluidine utilisés pour la scintigraphie du pancréas et des parathyroïdes; il a étudié en particulier les dimensions minimales du plus petit adénome parathyroïdien que ces corps permettraient de mettre en évidence. Il conclut qu'un analogue du bleu de toluidine marqué au sélénium devrait être un meilleur agent scintigraphique pour les parathyroïdes que la sélénométhionine et que, avec des activités spécifiques appropriées, on pourrait détecter des adénomes de 300 mg ou moins.

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