THE FATE OF FOUR ^{75}Se-LABELED AMINO ACIDS: STUDIES OF SOME UNSUCCESSFUL PANCREAS-SCANNING AGENTS

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The Radiochemical Centre developed a number of alpha-amino acids labeled with ^{75}Se in the hope of finding a pancreas-scanning agent superior to L-selenomethionine. Six were submitted for clinical trial and we tested four of these: D-selenomethionine and three derivatives of selenocysteine. Dynamic scintillation camera studies showed that liver uptake was substantial in all cases but that the time course of accumulation and removal differed between agents. Plasma radioactivity curves demonstrated differences in initial removal rates. Two of the agents showed evidence of incorporation of activity into protein and release into the circulation just as with L-selenomethionine. Whole-body counter measurements showed that the retention of activity from the agents exhibiting early plasma protein incorporation was similar to that of L-selenomethionine. Much of the activity from the other two agents was excreted faster but a small proportion was retained for a long time. Although the cumulated activity and absorbed dose were substantially less for at least two of the agents than for ^{75}Se-L-selenomethionine, the lack of sufficient pancreatic concentration renders them inferior as scanning agents.

To date the only radiopharmaceutical in general use for pancreas scintigraphy has been ^{75}Se-L-selenomethionine. Although it has been used for many years, the substantial uptake by liver often interferes with the pancreatic image. Biologic retention of the radioactivity is long, and this, coupled with the long physical half-life of ^{75}Se, limits the activity that can be administered. The search therefore continues for an alternative agent. Animal experiments suggest that some other amino acids have a higher pancreas–liver concentration than methionine (*1*) and these might be useful if they could be suitably labeled. However, inter-species differences in pancreatic physiology are considerable and, in any case, the behavior of an amino acid may be modified by incorporation of a gamma-ray emitting label. Techniques which do not require external detection and which can therefore use beta-ray emitting labels have been developed for assessing the value of pancreas-scanning agents in man (*2*) but obviously the final test must be on the basis of pancreas visualization.

The Radiochemical Centre, Amersham, prepared a number of alpha-amino acids labeled with ^{75}Se in the hope of finding a substance that would show greater specificity for the pancreas than L-selenomethionine or more rapid elimination from the body or both. Animal experiments sustained this hope and also demonstrated that toxicity was no greater than that for L-selenomethionine. The agents were therefore submitted for clinical trial in several hospitals and we participated in these trials.

It became clear that the hope of finding a superior pancreas-scanning agent was not being realized. Nevertheless, much information was obtained about the agents tested. It was considered to be of sufficient interest to justify the present communication, which might help in developing more agents, and in promoting a general understanding of amino acid analog metabolism.

MATERIALS AND METHODS

Six experimental scanning agents were produced for clinical trial by the Radiochemical Centre and we were allocated four of these (Table 1). The identity of the agents was not known to us at the time of the trial. For comparison, some results are also presented here for the standard agent ^{75}Se-L-selenomethionine.

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TABLE 1. CODE NUMBERS AND IDENTITY OF AGENTS STUDIED

SCD 6	Se-(2-aminoethyl)-L-selenocysteine $NH_2CH_2CH_2SeCH_2CH(NH_2)COOH$
SCD 7	Se-methyl-L-selenocysteine $CH_3SeCH_2CH(NH_2)COOH$
SCD 8	Se-ethyl-L-selenocysteine $CH_3CH_2SeCH_2CH(NH_2)COOH$
SCD 10	D-selenomethionine $CH_3SeCH_2CH_2CH(NH_2)COOH$

The subjects examined were patients who had been referred for pancreas scanning but for whom the result of the scintigraphy was not of utmost importance. Only one patient (No. 4) had a final diagnosis of pancreatic disease; liver function was impaired in Patient No. 1.

At least two patients were examined with each agent although not all tests were carried out in each case. Had any agent shown promise more patients would have been examined, but in the absence of pancreas visualization such extension did not seem justified.

The patient was placed in the supine position and a scintillation camera (Nuclear Enterprises Scinticamera 4) was centered over the pancreas–liver area. In all cases a liver scan had been performed 1 or 2 days before using ^{99m}Tc-sulfur colloid and the same scintillation camera. After intravenous administration of the agent, a series of 10-min exposures was obtained on both Polaroid and x-ray film for 1 hr and data were recorded on a magnetic videotape system. Subsequently the tape was replayed, two rectangular areas were selected, and the counts from them were routed through ratemeters to a chart recorder. To obtain an estimate of the absolute activity within the selected area, calibration was carried out with ^{75}Se in two models giving good agreement.

Blood samples were obtained at intervals during the scintigraphy and plasma radioactivity was measured in a well scintillation counter. The exact amount of activity administered to the patient was determined by measuring the vial before and after injection. Counting standards were prepared from a known activity in a similar way, stable phenylalanine being added to minimize adsorption.

Urine was collected for 24 hr and its radioactivity was assayed.

Immediately after the 1-hr period of scintigraphy, radioactivity in the patient was assessed with a whole-body counter. The counter had two 12.5 × 9-cm crystals arranged in a shadow-shield scanning geometry and substantial lead shielding (*3*). Slit collimators were fitted and could be used to vary the sensitivity. Initially an opening of 2 cm was used; later when retained activity had diminished substantially, the slits were widened to 5 cm. The couch was scanned through the sensitive volume over the length of the patient at a speed of 2 cm/sec. When there was no appreciable retained ^{99m}Tc activity in the patient, an energy band of 110–500 keV was used. It had been shown that this minimized the effects of redistribution of activity upon counting efficiency. With ^{99m}Tc present, energy acceptance was limited to 210–500 keV. Phantom and patient studies showed that sensitivity variations were still acceptably small when the responses of the two detectors were added.

Whole-body retention of ^{75}Se was measured at intervals, according to the availability of the patients, up to 4 months after administration.

RESULTS

Scintillation camera display. In one patient (No. 3) the pancreas was visualized with agent SCD 7. The result was certainly no clearer than might have been expected with ^{75}Se-L-selenomethionine, and the count density was lower over the pancreas area than over the liver.

The kidneys could be seen on the scintigrams of two patients receiving SCD 6. This was in accord with the high proportion of administered activity found in the urine in the first few hours after injection.

All cases showed activity concentrated in the liver. The dynamic studies of this retention, obtained from replaying the tape recording through area selectors to ratemeters and a chart recorder, are shown in Fig. 1. A rectangular area was selected to approximate the liver and the result displayed on one channel. The second channel was devoted to an equal area situated lower in the abdomen and clear of any obvious concentration of activity. This second area gave some idea of "background" activity in blood and extravascular tissue. In order to facilitate the comparison between agents and patients, the curves have been smoothed and normalized to account for differences of activity injected and collimator sensitivity. The calibration procedure was used to indicate the proportion of the administered activity within the field of view. Although the unknown depth of the liver means that this calibration is only approximate, the technique provides the best available normalization procedure.

Curves were obtained for Patient No. 2 (SCD 6) but are not included because part of the record was lost. The satisfactory part of the record was similar to the trace from Patient No. 7 who also received SCD 6.

The results for SCD 7, 8, and 10 were all similar; namely, a rapid rise of activity in the liver to a peak

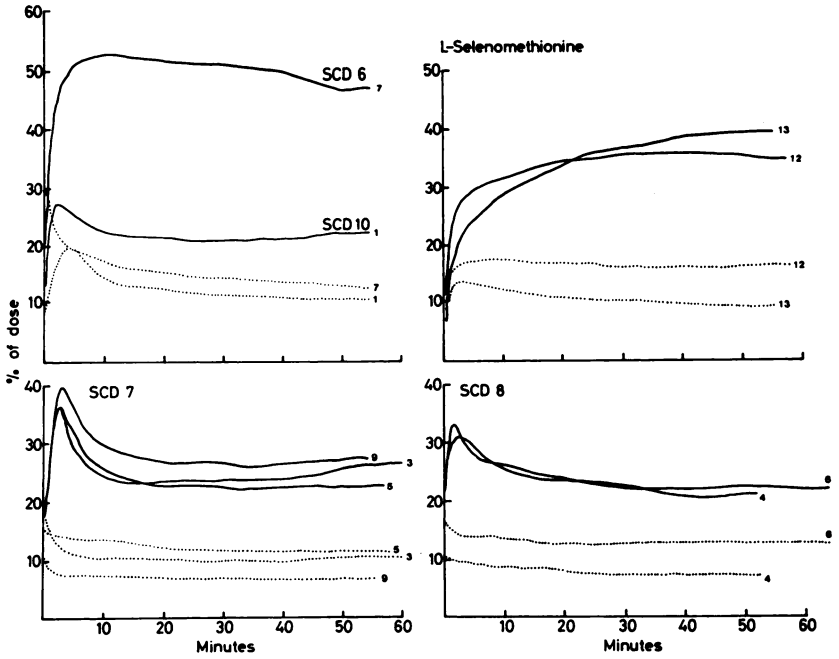

FIG. 1. Variation with time of activity in liver area (continuous lines) and equal area lower in abdomen (broken lines) derived from scintillation camera counting rates. Numbers relate to patient identification.

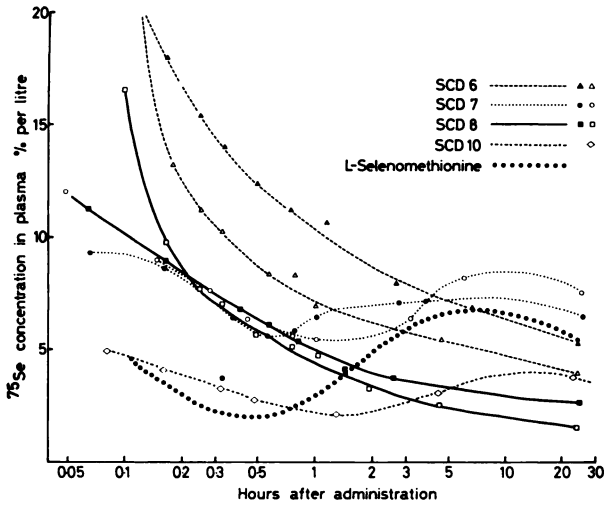

FIG. 2. Activity concentration in plasma at various times after injection of experimental agents. Curve for L-selenomethionine is mean of some of our results and those from Lathrop, et al (4).

of approximately 30% of the dose at about 3 min, followed by a fall over the next 10 min or so to a level about three-quarters of that of the peak. SCD 6 exhibited a plateau after the initial rapid liver uptake. This resembled the pattern with L-selenomethionine, although with the standard agent a gradual rise of activity persisted for 30–60 min.

In all cases the background curve was nearly flat. Evidently disappearance of activity from the plasma was more or less matched by uptake in tissues within the selected fields of view.

Plasma concentrations. The plasma concentration of ^{75}Se is plotted against time in Fig. 2. A logarithmic scale is used for the abscissa for clear presentation rather than in an effort to deduce mathematical relationships. The curve relating to L-selenomethionine is the mean of some of our own results and those from Lathrop, et al (*4*). Again patterns were similar for the same agents used in different subjects, with differences between the agents.

The initial plasma clearance was slower than that for L-selenomethionine for all agents except SCD 10.

Agents SCD 6 and 8 both exhibited a continuously falling plasma concentration of ^{75}Se throughout the observation period. The SCD 6 in particular left the circulation relatively slowly. The other two agents, SCD 7 and 10, resembled L-selenomethionine in showing a minimum concentration at ½–1 hr after administration, followed by a rise. It seemed most likely that the rise was due to the release of labeled protein into the plasma. Attempts were made to confirm this but satisfactory results were not obtained.

Urine activity. Varying amounts of activity from the different SCD agents appeared in the urine in 24 hr: approximately 50% from SCD 6 (Patients Nos. 2 and 7), 15% from SCD 7 (Patients Nos. 3 and 5), 5% from SCD 8 (Patients Nos. 4 and 6), and 13% from SCD 10 (Patient No. 1).

Whole-body retention. The whole-body counter results are plotted (Fig. 3). Some of the gaps between measurements were long but the interpolations appear plausible. They do not, however, justify detailed mathematical analysis. The curve for L-selenomethionine comes from the collected results of Lathrop, et al (*4*). The figures have been corrected for physical decay and therefore represent biologic elimination rather than remaining activity.

The rapid early loss by excretion of agents SCD 6 and 8 was followed by further elimination at a slower rate. Agents SCD 7 and 10 behaved similarly to

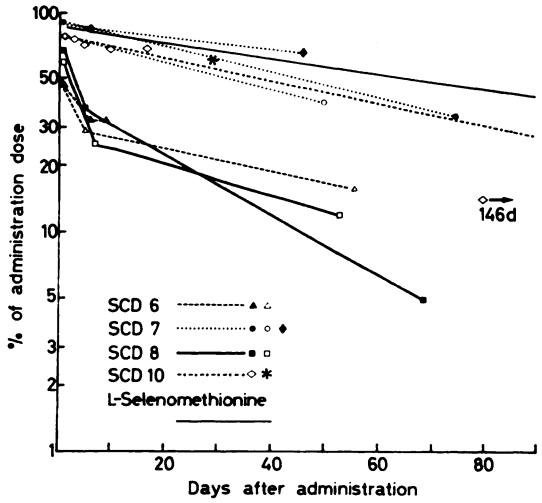

FIG. 3. Whole-body retention of ^{75}Se. Curve for L-selenomethionine is mean of 24 MIRD patients (Ref. 4).

L-selenomethionine in that a small loss on the first day was followed by a period of slow elimination. Biologic half-life is not a very meaningful conception for processes that cannot be described by a single exponential function, but the times for 50% elimination are given (Table 2). Of more value for dosimetric purposes is the cumulated activity over an infinite time after administration, physical decay being taken into account. The results of such estimations are also shown in Table 2. They were derived by measuring the area under the whole-body retention curve up to the last measurement made and assuming that subsequent loss was at the same rate as the slowest component of the mean L-selenomethionine curve (rate constant = 0.00315 days^{-1}, $T_{1/2}$ = 220 days). This method probably overestimates the integrated activity since faster functions of elimination were still likely to be significant at the time of our last measurements, particularly when these terminated early as in the case of Patient No. 2.

DISCUSSION

Although pancreas scanning based on ^{75}Se-L-selenomethionine has proved somewhat disappointing as a means of identifying pancreatic disease (*5*), the pancreas is nevertheless reliably identified in the majority of scans performed. Intravenously administered selenomethionine is assumed to be transferred from plasma to pancreas, liver, and other tissues by a process similar to that for natural methionine and it is then incorporated in newly synthesized protein. Clearly, other labeled amino acids may behave similarly but their rates of uptake from plasma and incorporation into protein differ from those of selenomethionine. The kinetic behavior resulting in an optimum concentration in the pancreas for scanning purposes is not known.

The substances examined in the present study are all alpha-amino acids and their transfer to tissues from plasma is therefore likely to have been mediated by an active transport process. The initial removal from plasma of SCD 6, SCD 7, and SCD 8 clearly has been slower than that of L-selenomethionine. This suggests that these agents have a lesser affinity for the transporting mechanisms. The slower tissue uptake was particularly obvious with the basic amino acid SCD 6 and was in accord with the relative transport rates of neutral and basic amino acids in the intestine (*6*).

In the part of the plasma activity curves indicating later time, SCD 7, SCD 10, and L-selenomethionine differ from SCD 6 and SCD 8 in that they show an increasing activity with time. This probably represents the appearance in the circulation of newly synthesized plasma protein into which the ^{75}Se amino acids have been incorporated. The whole-body retention data also appear to distinguish SCD 7, SCD 10, and L-selenomethionine from SCD 6 and SCD 8, presumably for similar reasons. Incorporation of the former three agents into proteins may involve preliminary conversion of SCD 7 (methylselenocysteine) and SCD 10 (D-selenomethionine) to selenocysteine and L-selenomethionine, respectively. The conversion of D-methionine to the L-stereoisomer occurs in the rat (*7,8*) and D-methionine can be utilized by man (*9*) although it is excreted in the urine more readily than the L form (*10*). Since we were unable to demonstrate any pancreatic uptake of SCD 10, although liver uptake was substantial, its presence as a contaminant of ^{75}Se-L-selenomethionine at an appreciable level would be unwelcome in the context of attempts to obtain satisfactory pancreas scintigrams.

TABLE 2. FACTORS RELATING TO WHOLE-BODY RETENTION AND ABSORBED RADIATION DOSE

Agent	Patient (No.)	Time to 50% elimination (days)	Cumulated activity for 1 μCi administered (μCi-days)
SCD 6	2	0.8	38
SCD 6	7	0.8	24
SCD 7	3	40	62
SCD 7	5	30	56
SCD 7	9	46	87
SCD 8	4	2.5	14
SCD 8	6	1.7	20
SCD 10	1	40	59
SCD 10	8	29	76
L-Selenomethionine (MIRD mean)		70	68

The contrast in the plasma activity and whole-body retention patterns of SCD 7, SCD 10, and L-selenomethionine when compared with SCD 6 and SCD 8 may not be due solely to protein incorporation. It has been shown that selenate ions disappear faster than sulfate ions from plasma and that ^{75}Se activity reappears protein bound after about 1 hr (*11*). These same characteristics were more marked with ^{75}Se-selenite, which also led to greater liver concentration in dogs. The latter finding was confirmed in man (*12*), 75% of the administered activity being retained in the body with an average half-life of 43 days. The differing patterns of plasma activity and whole-body retention that we have observed, therefore, may be due in part to differing proportions of the administered agents being metabolized to produce ^{75}Se in an inorganic form.

In spite of the differences in initial plasma disappearance rates, it is evident that substantial liver uptake of all the new agents was accomplished within a few minutes of their injection. Thereafter, a progressive increase in hepatic content was obvious only with L-selenomethionine and clearly it is in this continuing accumulation rather than in the initial uptake rate that L-selenomethionine notably differs from the other agents.

These observations suggest that in considering the characteristics of a possible new pancreas-scanning agent, the process of plasma-to-tissue transport must be distinguished from incorporation of the agent into tissue protein. Studies of the transport of various amino acids by the intestine (*13*) and by the Ehrlich ascites cell (*14*) indicate that the transport of methionine is particularly rapid and if this is also true of pancreatic tissue, few amino acid analogs are likely to show more rapid initial uptake rates than L-selenomethionine. This may not be the most important characteristic, however, of a successful scanning agent. From these studies, protein incorporation appears to be crucial in determining overall isotope accumulation in the liver and it seems inevitable that it will also be important in determining accumulation in the pancreas. Some evidence for this is provided by the observation that, following administration of ^{35}S-DL-methionine to mice, radioactivity of the TCA-soluble fraction of a pancreatic homogenate attained a maximum within 10 min, but in whole pancreatic tissue the maximum was not attained until 30 min (*15*).

In consequence, we suggest that unnatural amino acids that may be taken up by tissues but cannot then be incorporated into protein are unlikely to be more effective than L-selenomethionine as pancreas-scanning agents unless some hitherto unrecognized peculiarity of the amino acid uptake mechanism in the pancreas can be identified and utilized. Protein incorporation, rather than the plasma-to-tissue transfer rate, is probably the dominant factor in determining the effectiveness of labeled amino acids as pancreas-scanning agents.

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