SYNTHESIS AND EVALUATION OF POTENTIAL TUMOR LOCALIZING RADIOPHARMACEUTICALS: TECHNETIUM-99m IMINODIACETIC ACID DERIVATIVES OF SULFANILAMIDES

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Summary

To exploit the tissue affinity of compounds for radiopharmaceutical purposes, attachment of chelating groups is usually necessary to facilitate technetium-99m binding and transport.

The chelating group, iminodiacetic acid, previously used to modify lidocaine for hepatobiliary radiopharmaceuticals, was attached to several antibiotic sulfanilamides known to concentrate in certain transplanted animal tumors.

These iminodiacetic acid derivatives were labelled with technetium-99m by the stannous chloride reduction method.

Biodistribution of the ^{99m}Tc labelled compounds in tumor bearing rats revealed no specific concentration in the tumors and localization mainly in the excretory organs.

The results indicate that, as in the case of lidocaine, conversion of the sulfanilamides into iminodiacetic acid derivatives and chelation with technetium leads to an altered biodistribution and loss of biological specificity.

Key Words: Radiopharmaceuticals, Technetium-99m, Iminodiacetic Acid, Tumors.

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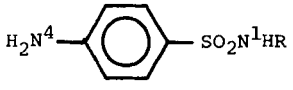
Introduction

Although gallium-67 citrate is currently the clinical agent of choice for tumor localization (1), its lack of specificity makes the search for better agents worthwhile.

Attempts are being made in these laboratories to develop tumor localizing agents of technetium-99m in order to exploit its superior radiation characteristics. The approach has been to incorporate an iminodiacetic acid (IDA) group into structures of compounds known to concentrate in tumors. This chelating group has been found to be a suitable ligand for technetium (2).

Certain sulfanilamides, such as sulfadiazine (R = 2-pyrimidyl) have been found to concentrate selectively in the Walker carcinoma and Yoshida sarcoma growing in rats (3).



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These studies indicated that the mechanism of selective deposition is quite specifically related to structure. The primary amine group does not appear essential and the pyrimidine moiety may be replaced by other groups such as pyridine without loss of activity (4).

Two series of IDA derivatives of the sulfanilamides, sulfadiazine (R = 2-pyrimidyl), sulfamerazine (R = 4-methyl-2-pyrimidyl), sulfamethazine (R = 4,6-dimethyl-2-pyrimidyl), sulfapyridine (R = 2-pyridyl) and sulfathiazole (R = 2-thiazolyl) were prepared:

i) N^4-carbonylmethyl iminodiacetic acid derivatives, which recent studies have suggested are better suited for incorporation into technetium radiopharmaceuticals than is IDA itself, and

ii) N^4,N^4-bis(carboxymethyl) derivatives. Although other IDA derivatives of this type have been found to form weaker chelates with technetium than derivatives of type i), (2), they represent the simplest modification of structure I.

Preparation and Characterisation of the Ligands

Elemental analyses were performed by the Australian Microanalytical Service, Port Melbourne, Victoria and infrared spectra were run on a Perkin-Elmer 21 double beam instrument as KBr discs.

N^4-Carbonylmethyl Iminodiacetic Acids. To nitrilotriacetic acid anhydride (0.04 mole) in 60 mL of anhydrous pyridine (5) was added 0.038 mole of the respective sulfanilamide (ICN Pharmaceuticals), and the mixture was heated at 100°C for 2 hours. The pyridine was removed under vacuum, the brown oil product taken up in 40 mL 1.5N NH_4OH and the pH adjusted to 2.0 with conc. HCl. An amorphous precipitate initially formed, and was converted into an easily filtered granular product after prolonged stirring (24h). The precipitate was then filtered off, washed with water and acetone, recrystallized from 50% acetone/water and dried in vacuum over P_2O_5. Yields, melting points, analyses and infrared data are given in Table 1. Compared with the infrared spectra of the parent sulfanilamides I (which show two sharp bands for $\gamma(N^4\text{-H})$ in the range 3340-3450 cm^{-1} (6)) the spectra of compounds in Table 1 show a small broad band in the region 3420-3450 cm^{-1} which may be the residual $\gamma(N^4\text{-H})$. The $\gamma(N^1\text{-H})$ of the amide group remains virtually unchanged at 3240 cm^{-1} and in the carbonyl region the spectra show only one band in the range 1690-1695 cm^{-1}, with a shoulder at approx. 1740 cm^{-1} (Table 1).

TABLE 1

N^4-Carbonylmethyl-iminodiacetic Acid Derivatives of the Sulfanilamides

No[a]	R	%[b]	MP (°C)	FOUND %C	%H	%N	REQUIRED %C	%H	%N	γ (C =O) cm^{-1}	γ (N^1-H) cm^{-1}
1a	2-pyrimidyl	66	189-190	45.51	4.03	16.34	45.39	4.05	16.54	1693	3420
1b	4-methyl-2-pyrimidyl	57	214-215	46.80	4.40	15.94	46.68	4.38	16.01	1692	3420
1c	4,6-dimethyl-pyrimidyl	55	200-201	47.56	4.80	15.22	47.89	4.69	15.51	1693	3430
1d	2-pyridyl	40	135-136	48.31	4.42	13.10	48.34	4.30	13.26	1695	3430
1e	2-thiazolyl	60	165-166	41.85	3.89	13.14	42.05	3.76	13.08	1691	3450

[a]Compound number, [b]Yield

N^4,N^4-Bis(carboxymethyl) Derivatives. The respective sulfanilamide (0.022 mole) was suspended in 40 mL of water and the pH adjusted to approx. 9 with 5N NaOH. Neutralized bromoacetic acid (0.05 mole) was then added dropwise while the reaction mixture was stirred and kept at approx. 70°C. Sodium hydroxide (2N) was added dropwise to keep the pH near 9 until all the bromoacetic acid had been added and there was no further significant decrease in the pH (about 2h). The pH was then adjusted to 2.0 with conc. HCl and the mixture stirred for several hours until a solid product was obtained. The precipitate was then filtered off, washed with water and acetone, and recrystallized from 50% acetone/water. Yields, melting points, analyses, and infrared data are given in Table 2.

The infrared spectra of the compounds in Table 2 show one strong band in the carbonyl region in the range 1720-1726 cm^{-1} and compared with the parent sulfanilamides, an absence of any bands due to $\gamma(N^4\text{-H})$. Bands due to $\gamma(N^1\text{-H})$ became very broad and small and could not be resolved from the acid OH bands.

Technetium-99m Labelling and Biodistribution Studies

The stannous chloride method was used to label ligands selected from Table 1. Because the directly carboxymethylated compounds (Table 2) did not form soluble complexes with stannous ion, they could not be labelled with technetium by this method.

The general labelling method was as follows; twenty milligrams of the ligand was dissolved in sodium hydroxide (1N) and the solution adjusted to pH 5 - 6. Stannous chloride dihydrate in .01N HCl was added (1 - 2 mg) and the solution readjusted to pH 5 - 6. The tin-ligand solution was Millipore filtered (0.22μ) into a serum vial and nitrogen purged. Pertechnetate was then added and the solution mixed for several minutes.

TABLE 2

N^4,N^4-Bis(carboxymethyl) Sulfanilamides

No[a]	R	%[b]	mp (°C)	FOUND %C	%H	%N	REQUIRED %C	%H	%N	ν(C=O) cm^{-1}
2b	4-methyl-2-pyrimidyl	25	235-236	47.14	4.54	14.76	47.36	4.24	14.73	1725
2c	4,6-dimethyl-2-pyrimidyl	28	236-237	48.71	4.93	14.12	48.73	4.60	14.21	1725
2d	2-pyridyl	25	201-202	49.10	4.20	11.45	49.31	4.14	11.50	1720
2e	2-thiazolyl	47	199-200	41.82	3.83	11.59	42.04	3.53	11.31	1726

[a]Compound number, [b]Yield

Radiochemical purity was determined by paper electrophoresis using 0.05M phosphate buffer pH6, and Whatman No. 1 paper, 300V for one hour. The ^{99m}Tc compounds gave single peaks on electrophoresis that were distinct from pertechnetate. Technetium-99m chelates of 1a, 1c and 1e migrated 2.6, 2.8 and 4.9 cm respectively towards the anode, whereas pertechnetate, which was not detected in the samples but used as a standard, migrated 10 cm.

The compounds in Table 1 labelled with ^{99m}Tc were screened for tumor localizing ability by intravenous injection into specific pathogen free D.A. rats bearing a three-week old mammary carcimona (7), and imaging with a gamma camera. Of these compounds, the sulfadiazine, sulfamethazine and sulfathiazole carbonylmethyl iminodiacetic acid derivatives were selected for detailed bio-distribution studies. Rats received injections (2 MBq, 2 mg/kg complex) via the tail vein.

The rats were sacrificed 2 and 24 hours after injection and the distribution of radioactivity determined by well scintillation counting of the dissected organs. The results are shown in Table 3.

Although the tumor/muscle ratios are greater than unity at both time intervals (Table 4) the tumor/blood ratios are less than unity, indicating a lack of specific concentration in the tumor. This was confirmed by a lack of tumor delineation in the gamma camera studies.

The sulfadiazine and sulfamethazine derivatives are excreted primarily by the urinary route (59.2% and 59.7% at 2 hours in the urine respectively), whereas the sulfathiazole derivative was found in the intestine (59.3%; 2 hours) indicating hepatobiliary excretion.

TABLE 3

Biodistribution of ^{99m}Tc-chelates of N^4- Sulfanilamide Carbonylmethylminodiacetic Acids (Means of 3 animals)[a]

	h[b]	Liver	Kidneys	Muscle	Blood	Urine	GIT + Faeces	Tumor
Sulfadiazine - (1a)	2	1.8	4.32	5.14	3.13	59.2	14.9	0.41
	24	0.61	2.89	1.26	0.78	76.1	12.1	0.15
Sulfamethazine - (1c)	2	2.77	3.01	2.90	4.93	49.3	25.7	1.3
	24	0.73	2.28	0.48	1.55	59.7	30.1	0.23
Sulfathiazole - (1e)	2	1.59	2.50	2.48	2.48	19.8	59.3	0.36
	24	0.58	2.31	1.22	0.94	35.6	49.1	0.21

[a]Percentage of injected dose per organ. [b]Hours after injection.

TABLE 4

Tumor/Tissue Ratios (% per g tumor/% per g tissue)

	Tumor/Muscle	Tumor/Blood
Tc-99m-Ia	3.2[a] 2.9[b]	0.70 0.79
Tc-99m-Ic	6.9[a] 10.1[b]	0.58 0.43
Tc-99m-Ie	4.0[a] 4.0[b]	0.58 0.61

[a]Two hours and [b]24 hours after injection

Discussion

It can be concluded from the above results that the modification of the sulfanilamides by iminodiacetic acid and subsequent ^{99m}Tc labelling leads to an overwhelming perturbation in their tumor localizing properties. This phenomenon has been observed (8) with lidocaine based IDA compounds, where the biodistribution of C-14 labelled lidocaine and the technetium chelates were vastly different. This behaviour was attributed to the formation of Tc-chelates with two chelating ligands per atom of Tc thus forming a complex with a greatly increased molecular weight compared with the original lidocaine molecule (9). It is possible that the sulfanilamide carbonylmethyl-IDA compounds also exist in similar bis-complexes resulting in the observed loss of tumor localizing ability.

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