EVALUATION OF IMINODIACETIC ACID DERIVATIVES OF ORTHO AND META AMINO HIPPURIC ACID ANALOGS AS RENAL FUNCTION AGENTS

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SUMMARY

Iminodiacetic acid derivatives of meta amino hippuric acid (MAHIDA) and ortho amino hippuric acid (OAHIDA) have been synthesized in the course of our continuing investigation of ^{99m}Tc-labeled hippuric acid analogs for application for renal function measurements. The two derivatives yielded stable ^{99m}Tc complexes using Sn(II) reduction method and are excreted rapidly via the GU tract as shown in animal biodistribution studies. Clearance of these two complexes is lower than that of the analog formed with para amino hippuric acid (PAHIDA) previously reported. Some aspects of structure activity relationship associated with PAHIDA point to the fact that para derivative has optimal renal excretory characteristics.

Key words: Aminohippuric Acid derivatives ^{99m}Tc, Renal Agents

INTRODUCTION

^{99m}Tc labeled renal function agents offer considerable advantage over the currently available ^{131}I-orthoiodo hippuric acid (OIH) or ^{123}I-OIH for routine clinical applications. With this in view, a number of investigations have focused recently on the development of several ^{99m}Tc labeled agents such as Tc(V)ON_2S_2 and Tc(V)ON_3S (1-3) complexes. Among the latter series the agent ^{99m}Tc-MAG_3 (mercaptoacetyl triglycine) is reported as having potential clinical utility for application as a renal function agent (4). A p-amino hippuric acid (PAH) analog, p-(bis-carboxymethyl) amino-methyl carboxyamino) hippuric acid (5) (PAHIDA,Fig.I) was synthesized and labeled with ^{99m}Tc. It has been shown to be rapidly excreted in mice and rats but its renal clearance is slower than that of OIH in rats and dogs (5,6). The present report describes the synthesis of two aminohippuric acid analogs, OAHIDA and MAHIDA and the comparison of the

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biodistribution characteristics of their ^{99m}Tc complexes with that of ^{99m}Tc-PAHIDA for evaluation as renal agents.

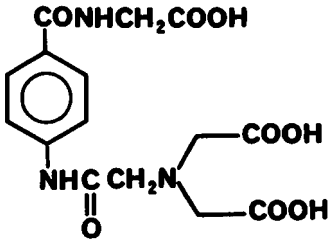
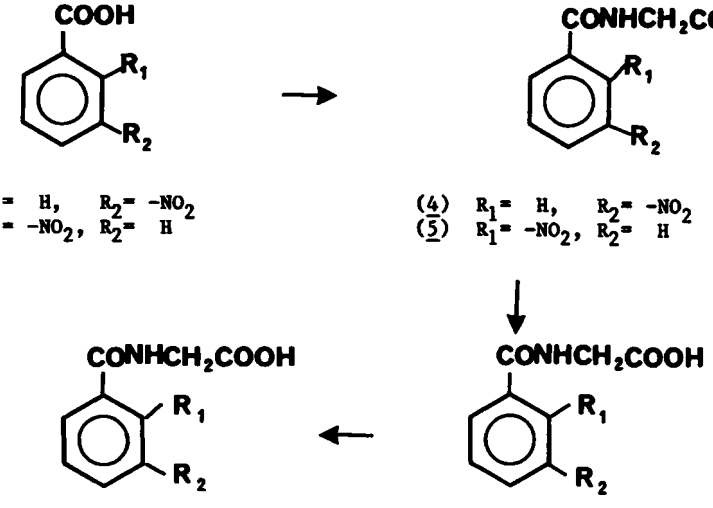


Figure 1.PAHIDA

MATERIALS AND METHODS

Proton magnetic resonance spectra were obtained with a varian XL-200 spectrometer with tetramethylsilane as an internal standard. Melting points were determined with an electrothermal melting point apparatus and are uncorrected. Elemental microanalysis ws performed by Schwarzkoff Microanalytical Laboratory, New York. The HPLC analyses of the complexes were carried out with a DuPont Model 850 liquid chromatography unit using Zorbax C-8 Column with UV monitoring. The system was run in an isocratic mode at 8% aqueous CH_3CN. The synthesis of MAHIDA and OAHIDA was carried out according to Scheme 1 and details are given below.



(2) R_1= H, R_2= $-NO_2$
(3) R_1= $-NO_2$, R_2= H

(4) R_1= H, R_2= $-NO_2$
(5) R_1= $-NO_2$, R_2= H

(8) R_1= H, R_2= $-NHCOCH_2N(CH_2COOH)_2$
(9) R_1= $-NHCOCH_2N(CH_2COOH)_2$, R_2= H

(6) R_1= H, R_2= $-NH_2$
(7) R_1= $-NH_2$, R_2= H

Scheme 1

m-Nitrohippuric acid (4) : A suspension of m-nitrobenzoic acid (6.685 g) in 40 ml of benzene was refluxed with 4 ml of thionyl chloride for 4.5 hrs. The clear solution was flash evaporated and yellow liquid was added dropwise to a solution of glycine (3.75g) and sodium carbonate (5.3g) in 40 ml of H_2O. The mixture was stirred at room temperature for 4 hr and diluted with 50 ml of H_2O. Solution was filtered and filtrate was acidified using conc.HCl. Solid was filtered off and crystallized from ethyl acetate-pentane. Yield 4.5g (60%), m.p. 161-162°C., NMR(Me_2SO): 3.95 (d,2H $NHCH_2$), 7.75-8.70 (m,4H,aromatic), 9.30 (t,1H, NH). Elemental analysis: calc. for $C_9H_8N_2O_5$ C, 48.22, H, 3.59, N, 12.49; found C, 48.05,H, 3.62, N, 12.34.

o-Nitrohippuric acid (5): The desired hippuric acid was obtained in 56% yield as white crystals by following the procedure described for compound (4) and purified by crystallization from ethyl acetate-pentane, M.P. 192-194°C. H'NMR (Me_2SO, 200 MHz): 3.95 (d,2H, $NHCH_2$), 7.61-8.05 (m, 4H, aromatic), 9.05 (t,1H, NH) and 12.68 (b, 1H, COOH). Elemental analysis: calcd. for $C_9H_8N_2O_5$, C,48.11, H,3.59, N,12.49, Found C, 48.21, H, 3.49, N, 12.57.

m-Amino hippuric acid (6): A solution of m-nitro hippuric acid (4.48 g) in 100 ml of ethanol was hydrogenated at 30 psi pressure over 0.2 g Pd/C. The reaction was completed after two hrs. The catalyst was removed by filtration. Evaporation of the filtrate and crystallization from alcohol yielded 2.31g (60%) of 6 as white crystals. M.P. 196-197°C. NMR (Me_2SO): 3.85 (d, 2H, $NHCH_2$), 6.62-7.12 (m, 4H, aromatic), 8.35 (t, 1H, NH).Anal. Calcd. for $C_9H_{10}N_2O_3$: C, 55.67, H,5.19, N,14,43; found C, 55.46, H, 5.19, N, 14.23.

o-Amino hippuric acid (7): o-Nitro hippuric acid using exactly same procedure as for compound 6 gave (7) in 89% yield. M.P. 137-138°C, NMR (Me_2SO): 3.80 (d, 2H, $NHCH_2$), 6.43-7.52 (m, 4H, aromatic), 8.32 (t, 1H, NH).Microanalysis: calcd. for $C_9H_{10}N_2O_3$, C, 55.67, H, 5.19, N, 14.43; found C, 55.56, H,5.24, N, 14.30.

m-((Bis-carboxymethyl)-aminomethyl carboxyamino) hippuric acid (8): To a hot solution of 0.485 g (2.5 mmole) of m-amino hippuric acid in 100 ml acetonitrile, 0.649 g (3.75 mmole) of freshly prepared nitrilotriacetic acid anhydride (7) (NTAA) in 5 ml of acetonitrile was added. After 3 hr refluxing

the reaction mixture was cooled and the separated solid was filtered off. Yield 0.548 g (60%), m.p. 181-182°C. NMR (Me_2SO): 3.50 (s.2H, -NHCO$\underline{CH_2}$N), 3.55 (s, 4H, N ($\underline{CH_2}$ COOH)$_2$), 3.86 (d,2H, CONH$\underline{CH_2}$COO), 6.70-8.52 (m, 4H, aromatic),8.79 (t,1H,$\underline{NH}$) and 10.40 (b,2H,2xCOO$\underline{H}$). Microanalysis: calcd for $C_{15}H_{17}N_3O_8$; C,49.05, H,4.67, N, 11.44, found C, 48.95, H, 4.72, N, 11.54.

o-((Bis-carboxymethyl)-aminomethyl carboxyamino) hippuric acid (9): The desired compound was obtained as a white solid by following the procedure for compound 8 in 81% yield, M.P. 182-183° C. NMR (Me_2SO,200 Hz): 3.40 (s,2H, NHCO$\underline{CH_2}$N), 3.51 (S,4H,-N($\underline{CH_2}$COOH)$_2$), 3.95 (d,2H,-CONH$\underline{CH_2}$COO$\underline{H}$), 7.18-8.40 (m, 4H, aromatic), 9.00(t, 1H, NH),11.40s, 1H, COOH) and 12.60 (b,2H, 2 x COOH),Microanalysis: calcd. for $C_{15}H_{17}N_3O_8$; C, 49.05, H, 4.67, N, 11.54 found C, 48.90, H, 4.58, N, 11.28.

Radiolabeling: A solution of 10 mg of the hippuric analog in 0.5 ml of 0.1 N NaOH was adjusted to pH 7 with 0.05 N HCl and saturated with nitrogen. $SnCl_2.H_2O$ (0.25) mg in 10 ul was then added and pH was again adjusted to 5.7. The solution was filtered through 0.22um Millipore filter and 3 ml of ^{99m}Tc-pertechnetate (200 uCi) in saline from Dupont's Technetium Tc99m Generator was added to form the corresponding ^{99m}Tc-OAHIDA and MAHIDA complexes.

Animal biodistribution: For biodistribution studies, male mice CD-1 (25-30g) were used and 0.1 ml of the complex preparation (5 uCi) was injected into tail vein. For rat biodistribution studies, Sprague-Dawley male rats (200-225 g) were used and they were also injected with 0.2 ml (10uCi) of the preparation via the tail vein. The animals were sacrificed at different time intervals, tissues were separated and weighed and counted. The % dose/organ was determined by comparison of tissue radioactivity with suitable diluted aliquots of the injected dose.

Single-injection clearances: These were obtained for Tc-99m complexes following the protocol previously described (5,8).

RESULTS AND DISCUSSION

The synthesis of meta and ortho analogs of aminohippuric acid starting with the corresponding nitrobenzoic acids (2,3) (Scheme 1) gave an over all yield of approximately 20% and they were characterized by the standard elemental analysis

and NMR methods.

The ^{99m}Tc complexes of both MAHIDA and OAHIDA were stable when formulated at pH 5.7 under the conditions described in experimental section. ITLC with solvent A, CH_3CN/H_2O,(3:1) gave an R_f value of 1 for the complex and for pertechnetate, and R_f=0 for the hydrolyzed form. In the solvent B system $CHCl_3$/EtOH, (3:1), R_f values are 0 and 1.0 respectively, for the Tc-99m complex and free pertechnetate. Radiochemical purity of the complexes was greater than 95% even at six hours after preparations.

Both preparations have been injected into mice and rats for biodistribution studies and the percent administered dose present at various time intervals in different organs are given in Table 1 and 2 for mice and rats, respectively and compared with PAHIDA biodistribution data performed in an identical strain of animals (5).

Table 1 BIODISTRIBUTION OF ^{99m}Tc-MAHIDA, OAHIDA & PAHIDA IN MICE*
Mean value ± 1 s.d.

	Time (min)	Blood	GI	Liver	Kidney	Urine
MAHIDA	15	1.6 ± 0.5	2.7 ± 0.5	3.2 ± 0.5	3.1 ± 0.3	
	60	0.5 ± 0.1	5.8 ± 1.1	1.1 ± 0.2	2.6 ± 1.5	57.6 ± 6.6
	240	0.2 ± 0.0	5.3 ± 1.5	0.8 ± 0.2	0.5 ± 0.8	
OAHIDA	15	4.8 ± 1.1	3.2 ± 0.5	6.4 ± 0.6	5.5 ± 1.1	45.0 ± 6.8
	60	1.4 ± 0.5	3.9 ± 0.3	3.6 ± 0.6	4.4 ± 0.1	53.1 ± 7.6
	240	1.0 ± 0.3	5.7 ± 1.5	3.6 ± 1.0	2.6 ± 1.6	61.6 ± 7.3
PAHIDA	15	3.3 ± 0.5	1.4 ± 0.5	0.9 ± 0.2	2.1 ± 0.2	58.5 ± 9.8
	60	0.6 ± 0.2	1.7 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	87.0 ± 8.6
	240	0.3 ± 0.1	1.6 ± 0.4	0.3 ± 0.1	0.8 ± 0.2	90.0 ± 7.0

*Six animals for each time interval

Both the meta and ortho derivatives showed good renal excretion though not to the same degree as PAHIDA. Results given in Tables 1 and 2 for these two derivatives also show greater degree of GI excretion than PAHIDA at all time intervals. Single injection clearance studies in rats of these labeled agents have yielded values of 0.46 ml/min/100g for ^{99m}Tc-MAHIDA and 0.67 ml/min/100g for ^{99m}-OAHIDA which are lower than those observed with ^{99m}Tc-PAHIDA, 1.23 ml/min/100g (5). Relatively larger renal fixation and GI excretion for these

Table 2. BIODISTRIBUTION OF ^{99m}Tc-MAHIDA, OAHIDA & PAHIDA IN RATS*

(Mean value ± 1 s.d.)

	Time (min)	Blood	GI	Liver	Kidney	Urine
MAHIDA	15	9.8 ± 2.2	5.1 ± 1.4	3.8 ± 1.4	4.4 ± 1.0	31.9 ± 8.3
	60	2.7 ± 0.3	5.4 ± 1.4	1.1 ± 0.1	3.9 ± 1.8	62.5 ± 7.0
	240	1.8 ± 0.3	6.0 ± 1.2	0.7 ± 0.3	4.4 ± 0.6	69.3 ± 9.8
OAHIDA	15	9.9 ± 0.3	4.7 ± 1.1	2.7 ± 0.9	7.3 ± 1.7	38.3 ± 9.4
	60	1.9 ± 0.6	2.8 ± 0.4	1.1 ± 0.2	5.3 ± 0.8	62.4 ± 4.4
	240	1.4 ± 0.2	4.8 ± 1.2	1.2 ± 0.2	6.7 ± 1.2	71.6 ± 5.9
PAHIDA	15	9.2 ± 1.1		1.8 ± 0.4	4.1 ± 0.4	42.0 ± 7.5
	60	2.5 ± 0.6		0.7 ± 0.1	3.2 ± 0.4	69.7 ± 3.0
	240	1.2 ± 0.2		0.4 ± 0.1	3.5 ± 0.4	79.0 ± 7.0

*Six animals for each time interval.

two analogs compared to PAHIDA would suggest that the para derivative has better renal excretory characteristics and that the amino group in the para position in the amino hippuric acid moiety is necessary for further development of various analogs for evaluation as renal radiopharmaceuticals.

REFERENCES

1. Fritzberg A.R., Klingensmith W.C., Whitney, W.P., et al.- J. Nucl. Med. 22:258 (1981)
2. Klingensmith W.C., Fritzberg A.R., Spitzer V.M. et al.- J. Nucl. Med. 25:42 (1984)
3. Fritzberg A.R., Kasina S., Eshima D. et al.- J. Nucl. Med. 27:111 (1986)
4. Taylor A Eshima D., and Alazraki N.- Eur. J. Nucl. Med. 12: 510 (1987)
5. Chervu L.R., Sundoro B.M. and Blaufox M.D.- J. Nucl. Med.25:111 (1984)
6. Summerville D.A., Packard A.B., Bartynski, B. et al.- J. Nucl. Med. 28:907 (1987)
7. Burns H.D., Sowa D.T. and Marzilli L.G.-J. Phar. Sci. 67: 1434 (1978)
8. Chervu L.R.and Blaufox, M.D.- Renal secretion and filtration studies. In studies of Cellular Function Using Radiotracers. Billinghurst M.W.,ed. CRC Press, Boca Raton, FL, 1982, p-168.

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