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STRUCTURALLY MODIFIED FATTY ACIDS - CLINICAL POTENTIAL AS TRACERS OF METABOLISM.

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ABSTRACT

Recently 15-p-iodophenyl-betamethyl-pentadecanoic acid (BMPPA) was proposed für myocardial scintigraphy, as possible probe of metabolic processes other than ß-oxidation. In 19 patients (CAD/15, St.p. MI/7; control 4) myocardial scintigraphy was done after i.v. BMPPA (2-4 mCi). Data were collected (LAO 45°/14; anterior/5) for 1oo minutes (min) in the fasted patients.

From heart (H) and liver (L) organ to background (BG) ratios were calculated, and the elimination (E) behaviour was analyzed from BG (V. cava region) corrected time activity curves. In 1o patients plasma and urine were examined.

By $CHCl_3$ /MeOH extraction of plasma samples (9o min. pi) both in water and in organic medium soluble catabolitis were found. TLC fractionation showed, that those were comigrating, compared to standards, with bencoic acid, BMPPA and triglyzerides. In urine (0-2h pi: 4.1 % dose) hippuric acid found.

The mean t-max of BMPPA was at 15 min in the heart and at 9 min in the liver ($p < .01$) with H/BG and L/BG ratios if 1.8 and 2.1, resp. The elimination of BMPPA was slower from the heart than from the liver ($p < o.o1$). It was biexponential from the liver in all ($\bar{x}$: t/2 I: 11.4 min; t/2 II: 92 min; t/2 I uncor.: 38 min); with the size if phase I smaller than that of phase II ($\bar{x}$: I/II: o.57). In the heart BMPPA turnover was biexponential in 11 patients ($\bar{x}$: t/2 I: 13.8 min; t/2 II: 187 min; t/2 I uncor.: 65 min; I/II: o.34), but monoexponential in 8 patients ($\bar{X}$: t/2: 218 min.).
In 13 diseased regions (MI/7) BMPPA uptake was reduced, and the E behaviour mostly abnormal as compared to the respective normal region.

We conclude: BMPPA ist a useful agent fo. myocardial scintigraphy. Its longer retention in the heart compared to unbrached radioiodinated fatty acids may facilitate SPECT studies. E behaviour and plasma analysis indicate the metabolic breakdown of BMPPA. Yet, the complexicity of the supposed mechanism may impede curve interpretation in terms of specific metabolic pathways.

INTRODUCTION

Several radioiodinated fatty acid analogues have been developped for myocardial scintigraphy (1-4). In clinical studies with radioiodinated alipahtic and aromatic fatty acids the uptake pattern and the myocardial release rate of these compounds was evaluated, which was believed to give some hint on myocardial fatty acid metabolism (5-9). Yet, while there ist evidence that these compounds may serve as metabolic substrates, being subsequently utilized by the myocardium comparable to natural fatty acids (1o,11), there is controversy concerning the interpretation of myocardial turnover rates in terms of specific metabolic pathways (8,11 - 14). In addition the generation of free iodide in studies with an omego-iodo branched chain alkyl fatty acid seemed to indicate the occurance of chemical or enzymatic deiodination, since it was believed that in ß-position methylated fatty acids are not prone to metabolic cleavage (15,16). However, species specific differences might exist, since the behaviour of this compound appeared different in rats and dogs (15).
However, iodine binding to aliphatic compounds ist rather unstable, which might facilitate unspecific deiodination of such fatty acids, in contrast iodine binds strongly to an aromatic molecule.

Recently, 15-p-iodophenyl-ß-methyl-pentadecanoic acid (BMPPA) was proposed for myocardial scintigraphy, a compound where unspecific deiodination is unlikely to occur (17). According to previons suggestions, this compound beeig methylated in the ß-position, should serve as indicator of metobolic processes other than ß-oxidation (15,16). In rat experiments BMPPA exhibited a high myocardial uptake.
In addition the modification in its molecular structure resulted in a prolonged myocardial residence time. In heart extracts radioactivy was mainly found in the triglyzeride and phospholipid fraction. In further studies in hypertensive rats a disparity was seen in the uptake pattern of a perfusion tracer and this compound being normal and abnormal, respectively (18). These animal experiments support the clinical application of BMPPA for myocardial scintigraphy.

The aim of the present study was to evaluate the clinical feasibility of BMPPA for myocardial scintigraphy in man. We further attempted to evaluate if BMIPPA ist metabolically degraded by human tissue.

PATIENTS AND METHODS

19 Patients (Controls, n=4; coronary artery disease (n=15,St.p. Mi, n=7) who underwent coronary angiography because of chest pain were studied. Myocardial scintigraphy with BMPPA was done in the fasted patients using either a LFOV gamma-camera (n=4) or a mobile gamma-camera (n=15), having the collimator in the LAO 45° (n=14) or in the anterior projection (n=5). After i.v. injection of 2-5 mCi I-123 BMPPA data were accumulated in a 64 x 64 word matrix for 1oo minutes with a frame rate of one/minute.

Besides visual interpretation of scintigrams background (v. cava region) corrected time activity curves of heart (gobal and regional) and liver were analyzed, which were fitted as appropriate with either a biexponential or a monoexponential function. Parameters evaluated were: activity peak time (t-max), target to background ratios, and the elimination half time of the initial (t/2 I) and second (t/2 II) phase in minutes. In addition the contribution of each phase on the elimination curve was calculated by a component ratio (counts phase I/counts phase II at t-max).

In addition to scintigraphy I-123 radioactivity was analyzed in plasma (5 minutes p.i. and 9o minutes p.i.) and urine (o-2 hours p.i.) samples for the occurance of eventual arising I-123 BMPPA metabolites or degradation products. Untreated and acidified (HCl, pH≃ 2) samples (o.5 ml) were extracted with chloroform / methanol (2:1 v/v; 2.5 ml) and phases separated by centrifugation. I-123 Radioactivity in the respective phases (organic, aqueous, solid phase) was counted in a well - scintillation counter. The organic phase was concentrated by a stream of nitrogen in a water bath and subsequently dissolved in chloroform / methanol.
Thereafter its radioactivty distribution was assayed by thin layer chromatography (TLC) using silica gel plates and chloroform / acetic acid (9:1 v/v) as eluent.

RESULTS

Following a rapid decrease in I-123 radioactivity in blood, with the lowest values beetween 15 - 2o minutes after tracer administration, there was a slight increase in I-123 blood activity.
Due to BMPPA degradation both in water and in organic medium soluble catabolites were found in plasma samples. Initially most of the activity was extracted into the organic phase (5 min. p.i.: 76.7 ± 3.9 %), thereafter decreasing (90 min.: 32.2 ± 8.7 %), while activity in the aqueous phase increased (5 min p.i. : 3.6 ± 1.9 %; 9o min. p.i. : 4o.3 ± 8.1 %). Activity in the aqueous phase resembeld in its behaviour weak acids since in acidified samples most of the activity was extracted into the organic phase (Table 1). By TLC fractionation 2 - 3 activity peaks were seen, which were comigrating, as compared to standards, with BMPPA, triolein and bencoic acid (Table 2).

Urinary excretion was somewhat lower as compared to previous findings with the unmethylated compound (excretion % dose: o-2 hours p.i. :4.1 ± o.9 % 2-16 hours p.i. 1o.5 ± 2.2 %). In urine analyzed by TLC, as indicator of BMPPA degradation an activity peak was found comigrating with the o-iodo-hippuric acid standard.

As total extraction of BMPPA in the liver exceeds that that in the heart, BMPPA degradation products formed in the liver will contribute most of the I-123 radioactivity found in plasma samples. However, besides the main source of BMPPA catabolism, these findings hint toward the metabolic usage and degradation of I-123 BMPPA by human tissue.

Scintigraphic findings appeared comparable to previous findings by using unbranched radioiodinated fatty acid analogues for myocardial scintigraphy. However, in contrast to the latter, scintifotos with sufficient quality could be obtained up to 9o - 12o minutes after tracer injection. The regional myocardial uptake of I-123 BMPPA was reduced in infarcted regions (6/7), but also in noninfarcted regions supplied by stenosed vessels (7/25).

Surprisingly, the metabolic usage of BMPPA appared different for heart and liver, as indicated by the respective time activity curves. BMPPA uptake and elimination was lower and faster from the liver than from the heart, respectively (Table 3). The time activity curve from the liver fitted a biexponential function, wherewas from the heart it was monoexponential in 8 patients but biexponential in the remaining 11 patients (Table 4)

The regional myocardial turnover was abnormal from all diseased regions- (from those with a reduced as well as from those with a normal regional BMPPA uptake) - showing and /or a prolonged initial elimination half time and a reduced component ratio.

CONCLUSION

Our data show that I-123 BMPPA can be used for myocardial scintigraphy in man. Its longer myocardial retention as compared to unmethylated radio-iodinated fatty acid analogues may facilitate SPECT studies.

This 3-methyl branched chain fatty acid ist prone to metabolic usage and degradation, an item not discussed previosly. The theoretically required steps for its degradation are more complex than those involved in the degradation of unbranched fatty acids.

Several pathways for the oxidative degradation of I-123 BMPPA by human tissue appear possible. It may involve ATP-dependent carboxylation of the methylgroup - catalyzed by biotin-, then followed by acetic acid cleavage, the resulting 15-pIPPA-CoA may then undergo ß-oxidation, where finally hippuric acid is formed by condensation of bencoic acid with glycine. As another possiblity it may include α- oxidation, followed by propionyl-CoA cleavage of the subsequently activated compound, then ß-oxidation of the resulting even numbered phenylated compound may proceed, where finally phenylaceturic acid will arise, formed by the condensation of phenylacetic acid and glycine. (19)

Possible the above mentioned complexicity of BMPPA metabolism may be in favour for BMPPA as compared to other radioiodinated straight chain fatty acid analogues, making it more susceptible for recognizing patients with heart disease, however, limiting myocardial time activity curve interpretation in terms of specific metabolic pathways.

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TABLE 1

DISTRIBUTION OF I-123 RADIOACTIVITY IN PLASMA SAMPLES FOLLOWING CHLOROFORM/METHANOL EXTRACTION (pH ≈ 7; pH ≈ 2) AFTER I.V. INJECTION OF I-123 BMIPPA IN 10 PATIENTS.

		AQUEOUS PHASE	ORGANIC PHASE	SOLID PHASE
5 MIN	pH 7	3.6 ± 1.9	76.7 ± 3.9	19.8 ± 4.2
10 MIN	pH 7	15.7 ± 9.5	58.9 ± 7.3	25.4 ± 6.6
20 MIN	pH 7	39.1 ± 5.8	33.9 ± 6.3	27.5 ± 2.5
30 MIN	pH 7	41.6 ± 6.4	30.2 ± 8.9	28.2 ± 4.5
60 MIN	pH 7	38.9 ± 8.8	34.0 ± 9.9	27.0 ± 3.5
90 MIN	pH 7	40.3 ± 8.1	32.2 ± 8.7	27.5 ± 3.4
5 MIN	pH 2	0.9 ± 0.7	69.4 ± 9.9	29.7 ± 13.1
10 MIN	pH 2	2.3 ± 1.6	76.0 ± 8.8	23.8 ± 10.7
20 MIN	pH 2	3.6 ± 1.1	78.9 ± 6.5	17.4 ± 6.4
30 MIN	pH 2	3.4 ± 0.5	80.0 ± 9.6	16.7 ± 9.5
60 MIN	pH 2	2.9 ± 0.7	80.5 ± 6.5	16.5 ± 6.3
90 MIN	pH 2	2.8 ± 0.6	81.5 ± 5.7	15.6 ± 5.6

TABLE 2

TLC ANALYSIS OF I-123 RADIOACTIVITY DISTRIBUTION IN PLASMA SAMPLES AFTER CHLOROFORM / METHANOL EXTRACTION (PH - 2; ORGANIC PHASE) FOLLOWING I.V. I-123 BMIPPA.

STANDARD	Rf VALUES
BMIPPA	0.70 ± 0.04
I-BENCOIC ACID	0.59 ± 0.02
I-HIPPURIC ACID	0.15 ± 0.01
TRIOLEIN	0.85 ± 0.04

	Rf VALUES			
	SAMPLE 5 MIN P.I.	SAMPLE 90 MIN P.I.		
#1	0.73	0.61	0.77	0.92
#2	0.69		0.70	0.92
#3	0.67	0.67	0.84	0.96
#4	0.69		0.71	0.87
#5	0.69		0.67	0.89
#6	0.73	0.57	0.75	0.81
#7	0.75	0.60	0.80	0.91
#8	0.74	0.62	0.79	0.85

TABLE 3

DISTRIBUTION OF I-123 BMPPA IN HEART AND LIVER IN 19 PATIENTS ($\bar{x} \pm SD$).

	HEART	LIVER
T-MAX (MINUTES)	14.9 ± 3.40	8.80 ± 2.30
TARGET / BG RATIO AT T-MAX	1.8 ± 0.30	2.05 ± 0.30
TARGET / BG RATIO AT T-100 MIN	1.6 ± 0.30	1.45 ± 0.26

TABLE 4

ELIMINATION PARAMETERS OF I-123 BMPPA IN HEART AND LIVER ($\bar{x} \pm SD$).

	HEART	LIVER
MONOEXPONENTIAL TIME ACTIVITY CURVE	N=8	N=0
T/2 (MINUTES)	218.8 ± 102.5	----------
BIEXPONENTIAL TIME ACTIVITY CURVE	N=11	N=19
T/2 I (MINUTES)	13.8 ± 4.12	11.4 ± 4.40
T/2 II (MINUTES)	187.2 ± 59.8	91.5 ± 36.8
T/2 I' (MINUTES)	64.0 ± 13.0	37.5 ± 13.2
C-I / C-II	0.34 ± 0.11	0.57 ± 0.35

TABLE 5

REGIONAL MYOCARDIAL ELIMINATION PARAMETERS AFTER I.V. INJECTION OF I-123 BMPPA IN

A: 6 PATIENTS WITH CORONARY ARTERY DISEASE SHOWING A MONOEXPONENTIAL TIME ACTIVITY CURVE

	NORMAL REGION N=6	DISEASED REGION N=11
T/2 (MINUTES)	150.5 ± 45.6	§ 234.8 ± 107.1* §§ 417.6 ± 352.0*

§ "CHRONIC" ISCHEMIC (N=6); §§ INFARCTED REGION (N=5)

B: 9 PATIENTS WITH CORONARY ARTERY DISEASE SHOWING A BIEXPONENTIAL TIME ACTIVITY CURVE

	NORMAL REGION (+BEST VESSEL 3 VD) N=13	DISEASED REGION N=14
T/2 I (MINUTES)	11.2 ± 4.30	19.7 ± 12.7*
T/2 II (MINUTES)	153.7 ± 47.9	160.1 ± 64.2
T/2 I' (MINUTES)	62.4 ± 20.7	69.6 ± 22.0
C-I/C-II RATIO	0.36 ± 0.15	0.28 ± 0.14*

* $P < 0.01$: SIGNIFICANTLY DIFFERENT COMPARED TO THE RESPECTIVE NORMAL REGION.