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ANALYSIS OF ORGANIC ACIDS IN THE HEARTS OF PATIENTS WITH IDIOPATHIC CARDIOMYOPATHY BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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SUMMARY

Organic acids in the hearts of patients with idiopathic cardiomyopathy, obtained by biopsy, were studied using gas chromatography—mass spectrometry. The profiling of organic acids was compared among eight cases of hypertrophic cardiomyopathy, three cases of congestive cardiomyopathy, and nine cases of other heart diseases, which were regarded as controls.

It was found that almost all organic acids, especially deoxyaldonic acids of 2-deoxytetronic acid, 2,3-dideoxypentonic acid, 3-deoxy-2-C-(hydroxymethyl)tetronic acid, 3-deoxyerythropentonic acid and 3-deoxy-2-C-(hydroxymethyl)erythropentonic acid, were accumulated in large amounts in the heart in congestive cardiomyopathy, while these acids were decreased in hypertrophic cardiomyopathy. It was therefore suggested that deoxyaldonic acid metabolism in the heart in congestive cardiomyopathy is quite different from that in hypertrophic cardiomyopathy.

INTRODUCTION

The cause of idiopathic cardiomyopathy (ICM) has not been clear, and few morphological and biochemical studies have been performed [1, 2]. The paucity of research might be related to difficulties in producing an

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experimental animal ICM that is the same as human ICM, and of finding out which compounds are characteristic in ICM.

Gas chromatography—mass spectrometry (GC—MS) has the capability of analyzing many compounds simultaneously, and is ideally matched to a wide-range survey of metabolism, as, for example, which compounds are characteristic in the heart. Moreover, it has already been reported that organic acids in the heart muscle have been analyzed by this technique [3, 4].

The present study was therefore undertaken to examine organic acids in the heart of patients with hypertrophic cardiomyopathy (HCM) and congestive cardiomyopathy (CCM) by the use of GC—MS, to find out whether or not biochemical changes in the organic acid metabolism occur in the hearts of patients with ICM.

MATERIALS AND METHODS

Chemicals

Reagents of lactic acid, glycolic acid, glyceric acid, palmitic acid and stearic acid were commercial products. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Tokyo Kasei Co. (Tokyo, Japan). All other reagents were of the highest purity available commercially.

Gas chromatography and gas chromatography—mass spectrometry

A Shimadzu GC-6A gas chromatograph with dual flame ionization detectors was used. A glass coiled column (2 m × 3 mm, I.D.) was packed with 3% OV-17 on Gas-Chrom Q (80—100 mesh). The column oven was maintained isothermally at 80°C for 2 min and then programmed at 6°C/min until 290°C. Peak areas and retention times were determined with an on-line Shimadzu Chromatopac 4-B computer. For identification of the compounds, a JEOL JMS-D 100 GC—MS system with an on-line JMA 2000 data acquisition system was used.

Mass spectra were recorded at an ionizing voltage of 75 eV with a 300-μA trap current, and an ion source temperature of 280°C. The magnet of the mass spectrometer was scanned repetitively over field strengths from m/z 50 to m/z 700 every 5 sec.

Sample preparation

A few milligrams of heart samples were obtained from patients with heart diseases by biopsy during cardiac catheterization. Eight samples were obtained from the right heart ventricle of patients with HCM, who ranged in age from 19 to 64 years (mean 49 years) and included seven males and one female. Three samples were from patients with CCM, including a 15-year-old male, a 38-year-old male and a 35-year-old female. Nine samples were obtained from controls, who ranged in age from 17 to 63 years (mean 49 years) and included five males and four females. The diagnosis of the controls was as follows: atrial septal defect, hypertensive heart diseases, sick sinus syndrome, ventricular premature contraction, and atrioventricular block.

The biopsied heart specimens were immediately frozen in dry ice—acetone and kept until analysis. The extraction procedure followed was that described

in ref. 4. The samples were thawed and rinsed in cold saline solution and then homogenized. Then 20 μg of heptadecanoic acid per 1 mg of protein (which was determined by the Bio-Rad protein assay method) were added to the homogenates as an internal standard. Centrifugation was performed at 25,000 *g* for 15 min. The supernatant collected was concentrated in order to remove the ethanol. Organic acid fractions were obtained by extraction with an equal volume of diethyl ether and ethyl acetate twice at pH 1 with 2 *N* hydrochloric acid. Organic solvent extracts were dried under a nitrogen stream.

The samples were trimethylsilylated by adding 200 μl of BSTFA to the residue. The mixtures were then heated to 60°C for 1 h. Aliquots of the samples were subjected to GC and GC—MS for analysis.

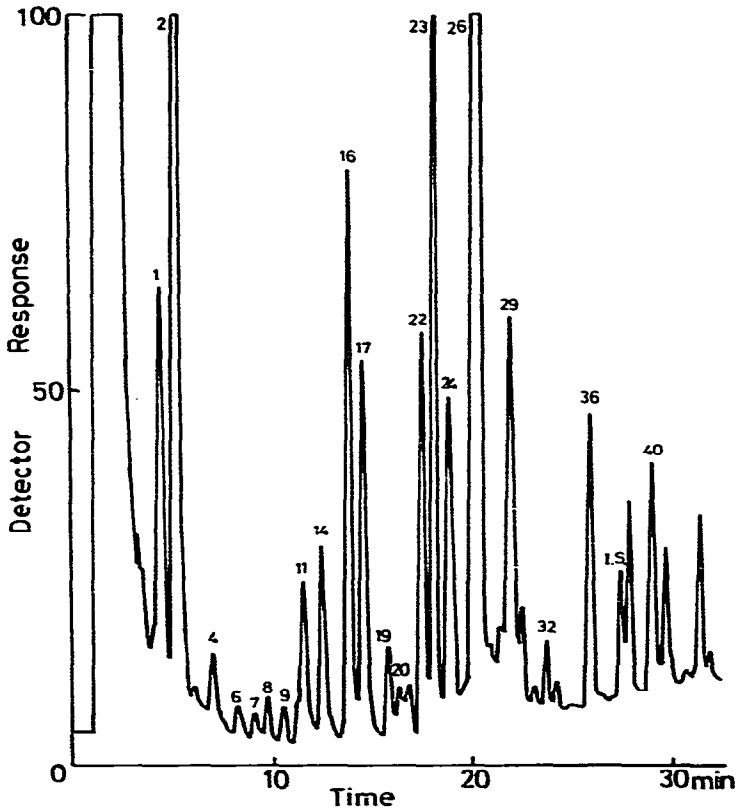

Fig. 1. Gas chromatogram of trimethylsilyl (TMS) derivatives of organic acids in heart biopsy of a patient with heart disease (hypertensive heart disease). Identified and tentatively identified compounds were as follows: 1 = lactic acid; 2 = glycolic acid; 4 = 3-hydroxypropionic acid; 7 = glycerol; 9 = 2-methylglyceric acid; 11 = glyceric acid; 16 = 2-deoxytetronic acid; 17 = 3-deoxy-2-C-(hydroxymethyl)tetrono-1,4-lactone; 20 = 2,3-dideoxypentonic acid; 22 = 3-deoxy-2-C-(hydroxymethyl)tetronic acid; 23 = 3-deoxypentono-1,4,-lactone; 24 = 3-deoxyerythropentonic acid; 26 = 3-deoxy-2-C-(hydroxymethyl)pentono-1,4-lactone; 28 = 3-deoxy-2-C-(hydroxymethyl)erythropentonic acid; 36 = palmitic acid; 40 = stearic acid.

RESULTS

The profile of organic acids in human heart obtained by biopsy is shown in Fig. 1. Over 40 peaks were detected on the gas chromatogram. The peak which appears between peaks 36 and 40 is the internal standard, heptadecanoic acid. When the profile was compared with that obtained from rat heart muscle [4] the profiles were found to be quite similar to each other.

Identification of the peaks was performed by comparing their mass spectra and retention times with those of laboratory samples or literature references [5, 6]. The mass spectrum obtained from peak 16 is shown as an example (Fig. 2). The molecular ion at *m/z* 336 was not detected but an ion at *m/z* 321 $(M-15)^+$ was found as a relatively small peak. Other fragment ions, at *m/z* 246 $(M-90)^+$, at *m/z* 233 $(M-CH_2OTMS)^+$, at *m/z* 231 $(M-15-90)^+$ and at *m/z* 205 $(CH_2OTMS \cdot CH{=}OTMS)^+$ were detected. The base peak was observed at *m/z* 73. This mass spectrum and the retention time were consistent with that of ref. 4. Peak 16 was, therefore, identified as 2-deoxytetronic acid tri-TMS. In this way, each peak was identified, and the identified compounds are shown in the legend of Fig. 1.

Gas chromatograms of specimens obtained from patients with HCM and with CCM are shown in Figs. 3 and 4, respectively. The peak of lactic acid (No. 1), which increases in ischemic myocardium, appeared as a relatively small peak. Peaks of 2-deoxytetronic acid (No. 16) and 2,3-dideoxypentonic acid (No. 20), which accumulate in ischemic-like conditioned rat heart muscle [7, 8], were observed as small peaks in Fig. 3. However Fig. 4 shows that these peaks were detected as relatively large peaks.

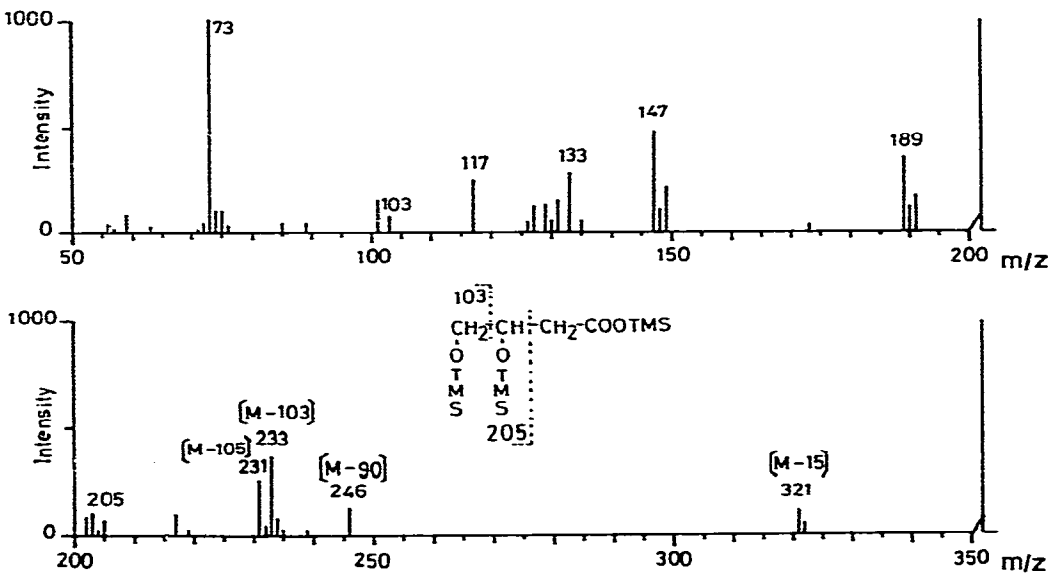

Fig. 2. Mass spectrum of the trimethylsilyl (TMS) derivative of 2-deoxytetronic acid, which was obtained from peak 16 in Fig. 1.

As it has been reported by Thompson et al. [9] that lactones are formed under acidic conditions during the extraction procedure, the lactones were therefore regarded as the corresponding acids. Peaks of deoxyaldonic acids, 3-deoxy-2-C-(hydroxymethyl)tetronic acid (Nos. 17 and 22), 3-deoxyerythropentonic acid (Nos. 23 and 24) and 3-deoxy-2-C-(hydroxymethyl)erythropentonic acid (Nos. 26 and 28), were detected as large peaks, as shown in Fig. 4, compared with those of Fig. 3.

The peak of palmitic acid was detected as a very large peak because of contamination by an unknown compound which appeared at the same retention time as palmitic acid, as shown in Fig. 4 [7]. But the peak of palmitic acid (in Fig. 3) was detected as a relatively large peak, and peaks of stearic acids (shown in Figs. 3 and 4) were detected at almost the same height. It is considered that the content of fatty acids in HCM heart was almost the same as that of CCM heart.

Table I shows the relative peak height ratios of the major peaks to the internal standard among three groups — HCM, CCM and control. The ratios of almost all peaks of the CCM group were higher than those of the control group, and those of the HCM group were the lowest.

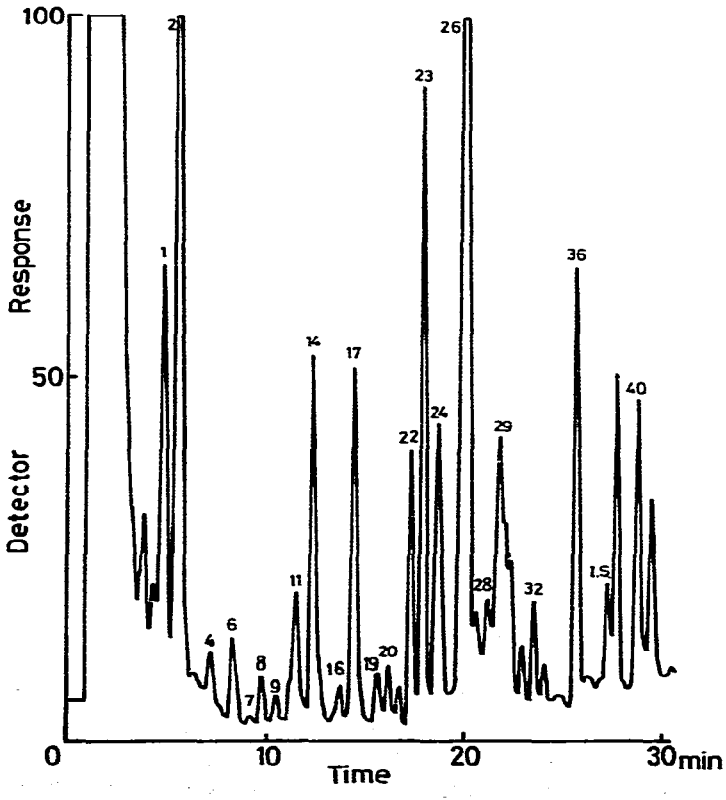

Fig. 3. Gas chromatogram of TMS derivatives of organic acids in heart biopsy from a patient with hypertrophic cardiomyopathy. The above peak numbers correspond to those in Fig. 1.

TABLE I

RELATIVE PEAK HEIGHT RATIOS (±S.D.) OF MAJOR COMPOUNDS TO THE INTERNAL STANDARD IN THE THREE GROUPS — HCM, CCM AND CONTROL

No.*	Compound	HCM (n=8)	CCM (n=3)	Control (n=9)
1	Lactate	0.418 (±0.372)	0.539 (±0.084)	0.587 (±0.415)
2	Glycolate	1.094 (±0.939)	2.339 (±1.327)	1.720 (±1.275)
4	3-Hydroxypropionate	0.309 (±0.158)	1.905 (±0.850)	0.875 (±0.809)
16	2-Deoxytetronate	0.270 (±0.343)	1.778 (±1.814)	0.870 (±1.023)
17 + 22	3-Deoxy-2-C-(hydroxymethyl)tetronate	0.157 (±0.184)	0.262 (±0.244)	0.195 (±0.159)
20	2,3-Dideoxypentonate	0.064 (±0.130)	0.946 (±0.764)	0.381 (±0.417)
23 + 24	3-Deoxyerthyropentonate	0.422 (±0.348)	3.267 (±2.488)	0.582 (±0.694)
26 + 28	3-Deoxy-2-C-(hydroxymethyl)erythropentonate	0.428 (±0.206)	0.946 (±0.764)	0.579 (±0.549)
36	Palmitate	0.883 (±0.511)	2.359 (±1.593)	1.075 (±0.622)
40	Stearate	0.906 (±0.455)	1.018 (±0.555)	1.112 (±0.726)

*The numbers refer to the numbered peaks in Fig. 1.

DISCUSSION

Profiling of organic acids in various tissues and fluids (rat heart and brain, human amniotic fluid and cerebrospinal fluid) have been reported using GC—MS [10, 11]. It is considered that the use of GC—MS is well adapted to this kind of study, because of its capabilities for simultaneous analysis and identification of compounds.

This is the first report of analysis of organic acids in human heart using a packed column. A capillary column is able to separate more peaks clearly, but this is not suitable for profiling because of its poor reproducibility and its use is time-consuming. Consequently, the packed column was used for the current study.

It might be supposed that the profiling of organic acids in human heart is similar to that in rat heart muscle [4] even though different ventricles were used (right in human and left in rat), and no characteristic compound different to those found in rat heart was detected in human heart.

Let us consider two points as to why deoxyaldonic acids were accumulated more in patients with CCM than in those with HCM. First, the number of patients with CCM was small (three cases). One must be cautious in comparing the ratios of peaks to internal standard in CCM with those in HCM. However, as Table I demonstrates that means of these ratios in CCM are clearly larger than those in HCM, the difference in case numbers may not be of importance in interpreting the increase in deoxyaldonic acids in CCM. Secondly, the protein

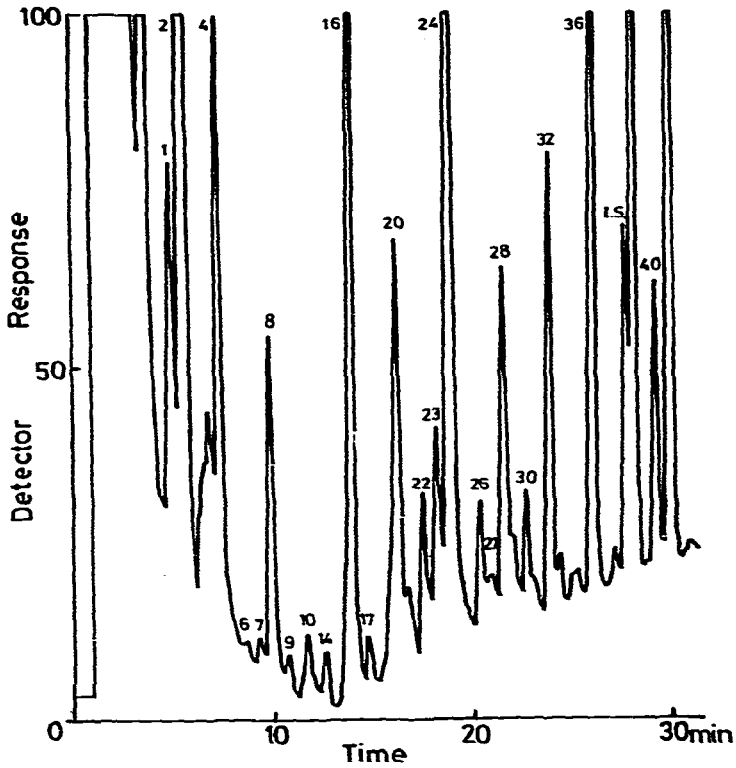

Fig. 4. Gas chromatogram of TMS derivatives of organic acids in heart biopsy from a patient with congestive cardiomyopathy. The peak numbers correspond to those in Fig. 1.

content in CCM appeared to be lower than that in HCM, the average values being 240 and 450 mg/g wet tissue, respectively. However, the ratio of protein content in CCM to that in HCM was only one half. And as the ratios of each peak in CCM were more than twice those in HCM, the increase in deoxyaldonic acids in CCM does not seem to be related to a difference of protein content in the hearts. It is concluded, therefore, that deoxyaldonic acid was actually accumulated in large amounts in CCM heart.

Haragachi et al. [8] reported that deoxyaldonic acids, which were accumulated in CCM heart, had been gradually accumulated during the time elapsed after decapitation in rat heart muscle. These results mean either that significant accumulation of deoxyaldonic acid might cause deterioration of the heart function, or that the accumulation might be induced by the heart damage. The relevance of these data to the present findings is unclear, because it is not known whether or not biochemical functions of the rat and human heart are completely identical. However, one possible interpretation is that the heart of CCM might be more dysfunctional or damaged than that of HCM, probably due to the accumulation of deoxyaldonic acids. In fact, in contrast to HCM patients, the prognosis of patients with CCM is generally bad and heart failure readily occurs.

As neither the function nor the metabolism of deoxyaldonic acids detected in heart muscle is clearly understood, the metabolic changes in CCM and HCM

have not been discussed biochemically. It is known, however, that nuclei of CCM heart muscle cells are morphologically damaged and the DNA content decreases. It may be possible to assume that 2-deoxyaldonic acids are derived from 2-deoxyribonucleic acid produced by decomposition of the nucleus. On the other hand, some researchers have reported that derivation of 2-deoxytetronic acid from carbohydrate was indirectly demonstrated [9, 12, 13]. It is presumed, therefore, that a change in carbohydrate metabolism, particularly of the pentose phosphate cycle which normally plays an important role in the heart [14], induced by unknown causes, might lead to increased deoxyaldonic acids in CCM and decreased acids in HCM.

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