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Transient reduction of human left ventricular mass in carnitine depletion induced by antibiotics containing pivalic acid

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Abstract

***Objective*—To study the effect of induced carnitine depletion on myocardial structure and function.**

***Subjects and Design*—7 healthy adult volunteers given 1200 mg pivmecillinam per day for 7–8 weeks were studied by echocardiography before and after 7–8 weeks of treatment and a 15 months follow up after the treatment period.**

***Setting*—Teaching hospital.**

***Main outcome measures*—Carnitine concentration in serum, urine, and muscle and echocardiographic measurements.**

***Results*—After 7–8 weeks of treatment the median free serum carnitine concentration was reduced to 7% and the median total muscle carnitine concentration to 46% of the pretreatment levels. The median diastolic interventricular septum thickness decreased by 14% (mean 26%, P = 0·028) and the median left ventricular mass by 10% (mean 20%, P = 0·018). Fifteen months later these dimensions had increased but not completely returned to pretreatment values.**

***Conclusions*—Extended treatment with pivalic acid containing antibiotics causes carnitine depletion which may lead to changes in cardiac structure.**

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Keywords: carnitine depletion; left ventricular mass; antibiotics; pivalic acid

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Carnitine is essential for fatty acid transport through the inner mitochondrial membrane and thus for energy production in skeletal and cardiac muscle, which are dependent on fatty acid oxidation.[1] Most carnitine in the body is found in muscle (92–97%).[2] Strictly vegetarian adults have a total carnitine concentration in urine of about 25% of those on a mixed diet.[3] This indicates that the daily turnover represents both endogenous synthesis of carnitine and carnitine originating from meat in the diet. It is not known whether an increased demand for carnitine causes increased endogenous synthesis, but previous studies on the rate of replenishment indicate that there is no significant compensatory increase.[4]

Depletion of the body carnitine stores can be induced by treatment with pivalic acid containing drugs such as pivampicillin and pivmecillinam.[4–6] Pivalic acid forms an ester with carnitine, pivaloylcarnitine, which is excreted in the urine. After one to two days of treatment with pivalic acid containing drugs the concentration of free serum carnitine in adults was reduced to about 50%, and after 7 days to about 25% of the initial values.[6] Depletion of the muscle carnitine stores takes considerably longer.[2] With a body pool of 100 mmol in a 70 kg human adult[7] it would take about 50 days to reduce the muscle carnitine concentration to 50% of the initial value. Long term treatment with low doses of drugs containing pivalic acid to prevent urinary tract infection in children resulted in severe depletion of the body carnitine stores, measured as a very low carnitine concentration in serum and skeletal muscle.[4] Some of these children showed impaired ketone body production during fasting.

When given to rats, pivalic acid containing drugs caused reduction in the serum levels of carnitine.[8,9] Although this reduction was not as marked as in humans, a concomitant decrease of the carnitine concentration in the rat myocardium was observed. To study the effect of carnitine depletion on the human heart, we analysed the myocardial structure and function of healthy volunteers by echocardiography before and after administration of pivmecillinam.

Methods

SUBJECTS

Seven healthy volunteers, five women and two men, aged 17–54 years, were studied. To be accepted for the study, normal findings were required for medical history, physical examination, and electrocardiography. Normal values were found also for blood haemoglobin and sedimentation rate. No albuminuria, glycosuria, or haematuria was present.

No hypertensive subjects were included in the study. Subjects had a normal heart rate at rest which did not change during the study period.

Because of the season, after four weeks of the study period, subject No 7 travelled by bus instead of by bicycle to get to work (10 km per day). None of the others changed their physical activity.

Subject No 6 was a 54 year old woman and the oldest of the volunteers. She travelled by bicycle to her work on five days a week throughout the study period (14 km per day). Otherwise she was not active in any sport.

The subjects were told to keep to a varied diet but to avoid meals very rich in fat or carbohydrates. All food and beverages were

recorded for three days before the test procedures. No alcohol or physical exercise was allowed during 24 h before the procedures. Urine samples were frozen every other day to check compliance with taking the pivmecillinam. No other medication was permitted. The study was approved by the local ethics committee.

INVESTIGATIVE PROCEDURE

After 4 h of fasting, blood and muscle samples were obtained for analysis of carnitine. Electrocardiography and echocardiography were done. Thereafter, daily oral administration of 1200 mg pivmecillinam (2·7 mmol) was started and continued for 7–8 weeks (mean 54 days, range 49–56 days).

The test procedure was repeated at the end of the treatment period. Fifteen months after the treatment period, echocardiography was carried out again in five of the seven subjects (two subjects were then living abroad).

METHODS

The muscle biopsies were done with a concotome. A local anaesthetic, carbocain 1%, was injected subcutaneously and down to the fascia but not into the muscle. Samples with a weight of 50–80 mg were obtained from the lateral part of musculus vastus lateralis and immediately frozen in liquid nitrogen and stored at – 70°C.

Carnitine in serum, urine, and muscle was determined by the method described by Cederblad and Lindstedt,[10] with modifications.[6]

M mode echocardiographic measurements were obtained with an Acuson 128 XP/10 sector scanner using a 3·5 or 2·0 MHz transducer. Left atrial (LA) and aortic (Ao) root diameters, as well as left ventricular internal dimension, left ventricular posterior wall thickness, and interventricular septal thickness in systole and diastole, were measured as recommended by the committee on M mode standardisation of the American Society of Echocardiography.[11] Thus the LA/Ao ratio was measured in a parasternal long axis section of the heart in systole. The aortic root diameter was measured from the leading edge of the anterior aortic wall echo to the leading edge of the posterior aortic wall echo. The left atrial diameter was measured from the leading edge of the posterior aortic wall echo to the leading edge of the posterior atrial wall echo. The left ventricular dimensions (LVIDd), as well as the interventricular septal and posterior wall diameters (IVSd and LVPWd), were also measured in a parasternal long axis section of the heart at the level of the tip of the mitral valve, using leading edge technology as described for LA/Ao ratio.

Each variable was taken as the mean of three consecutive measurements. The left atrial/aortic root ratio and the left ventricular shortening fraction were calculated from the M mode measurements. In addition, the left ventricular ejection fraction, stroke volume and left ventricular output were calculated according to Teichholtz *et al.*[12] The left ventricular mass (LVM) was calculated using the formula LVM (g) = 1·04 [(LVIDd + PWd + IVSd)^3 – (LVIDd)^3] – 13·6.[13]

The coefficient of variation for repeated measurements of echocardiographic variables was determined in seven subjects with six measurements for each subject. The coefficient of variation was 12·5% for interventricular septal thickness in diastole (IVSd), 3·7% for left ventricular inner diameter in diastole (LVIDd), and 10·7% for left ventricular posterior wall thickness in diastole (LVPWd).

Statistical evaluation was performed using a non-parametric method, the Wilcoxon signed rank test for paired samples. Approximate 95% confidence intervals based on Hodge-Lehmans estimate[14] were calculated conservatively (exact 95·3% confidence intervals) for left ventricular mass and the three variables included in the formula given above.

Table 1 Carnitine levels before and after 7–8 weeks of treatment with pivmecillinam in seven subjects. Values are medians (range)

	Before treatment	*After treatment*	*Difference P value**
Serum total carnitine (μmol/l)	42·0 (24·0–50·0)	12·0 (6·3–17·0)	0·018
Serum acyl carnitine (μmol/l)	3·6 (0·9–5·4)	7·8 (4·3–10·0)	0·018
Serum free carnitine (μmol/l)	38·4 (22·6–46·6)	2·7 (2·0–7·0)	0·018
Muscle total carnitine (μmol/g protein)	17·0 (9·8–19·0)	7·8 (6·7–10·0)	0·028
Muscle acyl carnitine (μmol/g protein)	6·9 (1·2–9·6)	3·9 (3·3–4·9)	ns
Muscle free carnitine (μmol/g protein)	10·8 (2·9–13·8)	3·9 (2·1–6·5)	0·042
Urine total carnitine (mmol/mol creatinine)	18·0 (11·0–41·0)	91·0 (82·0–114·0)	0·018
Urine acyl carnitine (mmol/mol creatinine)	11·0 (9·5–19·0)	91·0 (82·0–114·0)	0·018
Urine free carnitine (mmol/mol creatinine)	7·0 (1·4–25·0)	0·0 (0·0–1·0)	0·018

*Wilcoxon non-parametric signed rank test for paired samples.

Table 2 Results of echocardiographic examination before and after 7–8 weeks treatment with pivmecillinam in seven subjects. Values are medians (range)

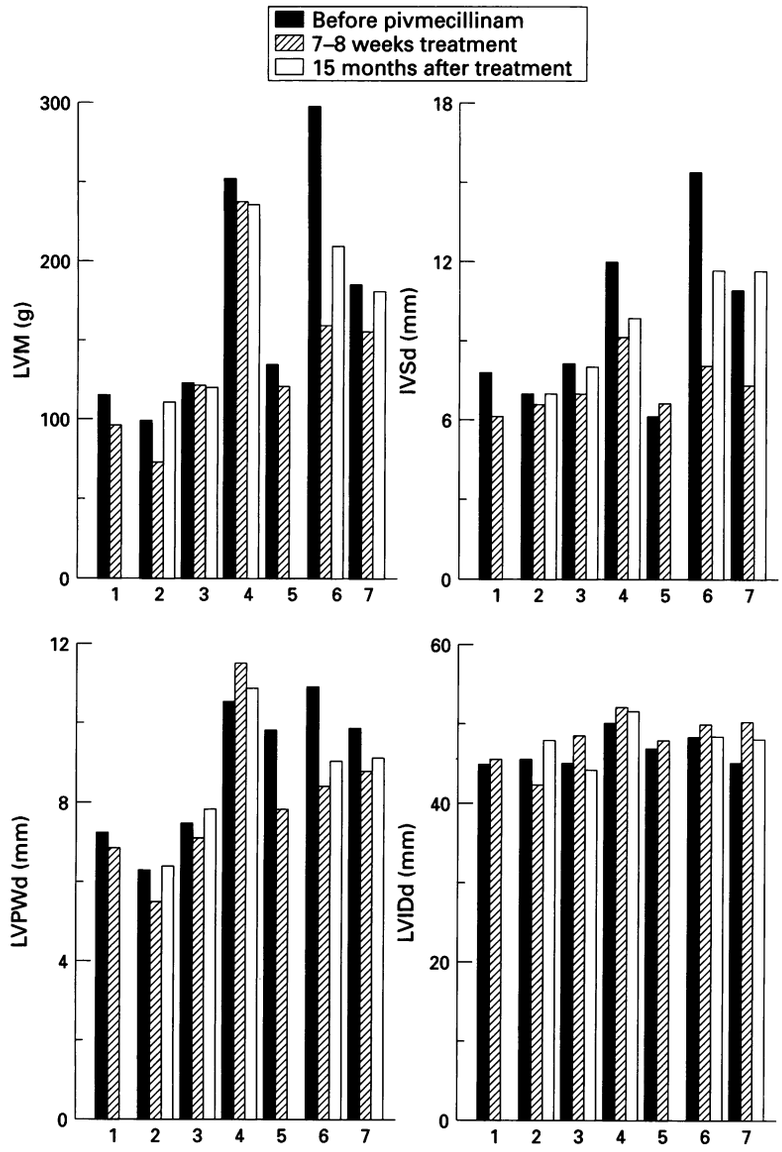
	Before treatment	*After treatment*	*Difference P value**	*Confidence interval*
LA/Ao	1·22 (0·96–1·47)	1·04 (0·94–1·44)	ns	
LVIDd (mm)	45·3 (44·8–49·9)	48·3 (42·1–51·9)	ns	– 1·15 to 3·55
LVSF (%)	33·0 (29·0–44·0)	37·3 (28·3–47·3)	ns	
EF (%)	61·7 (55·3–75·0)	67·0 (54·0–78·3)	ns	
SV (ml/stroke)	62·8 (55·5–68·7)	70·2 (45·9–92·8)	ns	
LVO (l/min)	3·58 (3·21–3·98)	4·20 (2·99–5·48)	ns	
LVPWd (mm)	9·8 (6·3–10·9)	7·8 (5·5–11·5)	ns	– 0·30 to 2·0
IVSd (mm)	8·1 (6·1–15·3)	7·0 (6·1–9·1)	0·028	0·35 to 5·10
LVM (g)	135·1 (99·0–297·2)	121·9 (73·5–237·3)	0·018	7·95 to 82·1

EF, ejection fraction (%); IVSd, interventricular septal thickness in diastole; LA/Ao, left atrial/aortic root ratio; LVIDd, left ventricular internal dimension in diastole; LVM, left ventricular mass; LVO, left ventricular output; LVPWd, left ventricular posterior wall thickness in diastole; LVSF, left ventricular shortening fraction (%); SV, stroke volume.
*Wilcoxon non-parametric signed rank test for paired samples.

Results

After treatment with pivmecillinam, the serum free carnitine concentration decreased from a median value of 38·4 μmol/l to 2·7 μmol/l. The median concentration of total skeletal muscle carnitine decreased from 17·0 μmol/g protein before to 7·8 μmol/g protein after treatment (table 1).

The echocardiographic results are shown in table 2. All subjects showed a decrease in median left ventricular mass by 10% (mean 20%) (figure). There was also a decrease in



Left ventricular mass (LVM), interventricular septal thickness in diastole (IVSd), left ventricular posterior wall thickness in diastole (LVPWd) and left ventricular internal dimension in diastole (LVIDd) before pivmecillinam, after 7–8 weeks treatment with pivmecillinam, and 15 months later.

median diastolic interventricular septal thickness by 14% (mean 26%) (figure). When subject No 6, who had a very marked decrease, was excluded from the statistical evaluation, the changes in diastolic interventricular thickness ($P = 0.046$) and the left ventricular mass ($P = 0.028$) were still significant. No other significant differences were found. However, of seven subjects, six had a decreased left ventricular posterior wall thickness in diastole as well as an increased left ventricular internal dimension in diastole.

In the five subjects who were reinvestigated 15 months after the treatment period, the echocardiographic findings had essentially returned to pretreatment values (figure). The median left ventricular mass had increased to 180·8 g and the median interventricular septal thickness in diastole to 9·8 mm (table 3).

Table 3 Results of echocardiographic examination in five subjects investigated before, after 7–8 weeks treatment with pivmecillinam, and 15 months later. Values are medians (range)

	Before	*After 7–8 weeks*	*15 months later*
IVSd (mm)	10·9 (7·0–15·3)	7·3 (6·5–9·1)	9·8 (7·0–11·6)
LVM (g)	185·1 (99·0–237·3)	156·2 (73·5–237·3)	180·8 (111·2–235·8)

IVSd, interventricular septal thickness in diastole; LVM, left ventricular mass.

Discussion

In primary carnitine deficiency both hypertrophic and dilated cardiomyopathy occur.[15–19] In several genetic disorders carnitine stores are depleted because of pathological accumulation of organic acids.[20] However, little is known about cardiac involvement in these disorders, although cardiomegaly is a common feature, for example in children with long chain 3-hydroxyacyl-CoA dehydrogenase deficiency.[21–24] In cases with fatty acid oxidation disorders it is not possible to decide whether the cardiomyopathy is caused by the primary fatty acid oxidation defect or, for example, by the secondary carnitine deficiency.

In our study, administration of a pivalic acid containing drug for seven to eight weeks caused a decrease of the median free serum carnitine concentration to 7% of the control levels and of median total muscle carnitine concentration to 46% of control levels. Therefore it is reasonable to suppose that there is also carnitine depletion in cardiac muscle tissue. Furthermore Diep *et al*[25] recently showed that rats treated with pivampicillin orally had the highest pivaloylcarnitine concentration in brown adipose tissue as well as in cardiac muscle. The concentration of pivaloylcarnitine as a percentage of free carnitine was six times higher in cardiac muscle than in skeletal muscle. As carnitine deficiency has been reported to be associated with cardiac hypertrophy we were surprised to find a significantly decreased left ventricular myocardial mass in our carnitine deficient subjects. It is possible, however, that the initial response of the myocardium to carnitine deficiency may be different from what is observed at later stages of the disease, when the cardiomyopathic state is established. A decrease of the median left ventricular mass by 10% (mean 20%) may seem small but its restoration after the end of the treatment period indicates that the phenomenon is related to the treatment.

Drugs containing pivalic acid are widely used against urinary tract infections. The manufacturers recommend a treatment period of 7–10 days. However, patients with complicated infections are sometimes treated for several weeks and those with recurrences may be given repeated courses, where the drug-free periods are too short to allow body carnitine stores to be restored. Our results suggest that such patients may be at risk of developing cardiac changes. Although the effect on the myocardial function was reversible over a period of about 15 months, a decrease of cardiac muscle mass may still be of significance to the patient, especially if myocardial function is already decreased secondary to other causes such as coronary insufficiency.

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