Increased digitalis-like immunoreactive substances in patients with hypertrophic cardiomyopathy

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Aims Although increased digitalis-like immunoreactive substances have been found in cases of hypertension and heart failure, no information is available about digitalis-like immunoreactive substances in patients with hypertrophic cardiomyopathy. We investigated digitalis-like immunoreactive substances in the plasma and biopsied specimens of patients with hypertrophic cardiomyopathy.

Methods and Results In 40 patients with hypertrophic cardiomyopathy (27 with the non-obstructive type and 13 with the obstructive type), the plasma concentration of digitalis-like immunoreactive substances was studied by fluorescence polarization immunoassay. Right ventricular endomyocardial biopsy specimens were analysed immunohistochemically, using a monoclonal antibody against digoxin. An increase in digitalis-like immunoreactive substances of more than 0.2 ng ml⁻¹ in plasma was found in six of 27 patients with non-obstructive hypertrophic cardiomyopathy (22.2%) and five of 13 with obstructive hypertrophic cardiomyopathy (38.4%). Under light microscopy, positive staining against the antibody was observed heterogeneously on some cardiocytes. In non-obstructive hypertrophic cardiomyopathy, digitalis-like immunoreactive substances in the plasma correlated with the left atrial dimension and inversely with the cardiac index. In obstructive hypertrophic cardiomyopathy, plasma and myocardial digitalis-like immunoreactive substances were positively correlated; they also correlated with left ventricular end-diastolic pressures. Under electron microscopy, digitalis-like immunoreactive substances were detected at the sarcolemma in the free wall, T-tubules, intercalated discs and Z-bands of cardiocytes.

Conclusions Increased digitalis-like immunoreactive substances in plasma and cardiocytes, which may have been caused by pressure and/or volume overload, were found in patients with hypertrophic cardiomyopathy. Digitalis-like immunoreactive substances may act on the sarcolemma of cardiocytes and be transported into the cytoplasm.

Key Words: Digitalis-like immunoreactive substances, hypertrophic cardiomyopathy, immunohistochemistry, ultrastructure.

Introduction

In 1994, Hamlyn reported the existence of endogeneous ouabain, corresponding to digitalis-like factors, which are Na⁺-K⁺-ATPase inhibitors and may participate in the volume regulation of cells and tissues of mammals¹. However, the digitalis-like immunoreactive substances were found to be heterogeneous and may be involved in food ingestion. To date, increased concentrations of digitalis-like immunoreactive substances have been found under conditions of hypertension, cardiac failure, renal insufficiency and hepatic disorder, and also in the blood of pregnant women, fetuses and newborns⁵⁻⁷.

Hypertrophic cardiomyopathy is characterized by abnormal stiffness of the left ventricle with resultant impaired ventricular filling. The most characteristic anatomical finding is inappropriate myocardial hypertrophy that occurs without clear cause, such as aortic stenosis or hypertension. In addition, progression of hypertrophic cardiomyopathy to left ventricular dilatation and dysfunction without a gradient may occur in 10 to 15% of patients⁸⁻⁹.

No information is currently available regarding the existence or the role of digitalis-like immunoreactive substances in patients with hypertrophic cardiomyopathy. We hypothesized that digitalis-like immunoreactive substances increase in hypertrophic cardiomyopathy,
The left ventricle at rest [9].

Phy combined with Doppler recordings [8,9] and under-
with hypertrophic cardiomyopathy by echocardiogra-
controls. Tissue was removed from areas of normal
taken from the right ventricle, served as additional

Five patients (age 55–33) years, who showed no
obstructive type of hypertrophic cardiomyopathy (age
75, 10 females and 30 males), who were diagnosed
obstructive hypertrophic cardiomyopathy (age
35) and 13 the obstructive type (age 56–33) undergoing
cardiac catheterization; 27 patients had the non-
ystolic pressure; LVedp=left ventricular end-diastolic pressure; C.I.=cardiac index. Note that
Digitalis-like immunoreactive substance in the plasma
digitalis or spironolactone, which was confirmed by careful history taking and
from clinical charts.

**Plasma concentration of digitalis-like immunoactive substances**

Plasma from patients obtained on the day or a few days
before cardiac catheterization was analysed by fluores-
cence polarization immunoassay (Abbott TDX®,
Abbott Laboratories, Chicago, U.S.A.) [6,8,9], to deter-
the concentration of digitalis-like immunoactive substances. If there was more than 0·2 ng. ml⁻¹ of
digitalis-like immunoactive substance in the plasma
this was considered to be positive since the detection
limit by fluorescence polarization immunoassay was 0·2.

**Histology**

Specimens were obtained via endomyocardial biopsy
from the right ventricle from the 40 patients with hyper-

Ten patients with idiopathic arrhythmia, ranging in
age from 44 to 72 (56·4±6·33) years, who showed no
grounds of organic cardiac dysfunction at echocardiogra-
and who underwent cardiac catheterization includ-
ing right ventricular biopsy, constituted the control

Five patients (age 55·2±3·83) undergoing cor-
ony bypass surgery, in whom needle biopsies were
taken from the right ventricle, served as additional
controls. Tissue was removed from areas of normal
contractility and a normal blood supply, which is as
close as possible to normal human myocardium [10,11].

None of the subjects enrolled in this study had hyper-
tension or had been prescribed digitalis or spironolactone,
which was confirmed by careful history taking and
from clinical charts.

**Methods**

**Patients**

The subjects were 40 patients, aged 55·3±9·1 (range
35–75, 10 females and 30 males), who were diagnosed
with hypertrophic cardiomyopathy by echocardiogra-
phy combined with Doppler recordings [8,9] and under-
went cardiac catheterization; 27 patients had the non-

HNCM=non-obstructive type; HOCM=obstructive type; DLIS=plasma concentration of
digitalis-like substances; LAD=left atrial dimension; IVSd=diastolic ventricular septal thickness;
PWd=diastolic posterior wall thickness; Dd=diastolic left ventricular dimension; Ds=systolic left
ventricular dimension; FS=fractional shortening; Ao=aortic pressure; LVsys=left ventricular
systolic pressure; LVEDP=left ventricular end-diastolic pressure; C.I.=cardiac index. Note that
Disarray, Fibrosis, and Score were evaluated semiquantitatively (see text).

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### Table 1  Summary of data from patients with hypertrophic cardiomyopathy

<table>
<thead>
<tr>
<th></th>
<th>HNCM</th>
<th>HOCM</th>
<th>z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27</td>
<td>13</td>
<td>56·76</td>
<td>9·91</td>
</tr>
<tr>
<td>DLIS (ng. ml⁻¹)</td>
<td>27</td>
<td>13</td>
<td>0·33</td>
<td>0·43</td>
</tr>
<tr>
<td>LAD (cm)</td>
<td>27</td>
<td>13</td>
<td>4·60</td>
<td>0·82</td>
</tr>
<tr>
<td>IVSd (cm)</td>
<td>27</td>
<td>13</td>
<td>1·74</td>
<td>0·68</td>
</tr>
<tr>
<td>PWd (cm)</td>
<td>27</td>
<td>13</td>
<td>1·08</td>
<td>0·17</td>
</tr>
<tr>
<td>Dd (cm)</td>
<td>27</td>
<td>13</td>
<td>4·32</td>
<td>0·46</td>
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<td>Ds (cm)</td>
<td>27</td>
<td>13</td>
<td>2·47</td>
<td>0·33</td>
</tr>
<tr>
<td>FS (%)</td>
<td>27</td>
<td>13</td>
<td>40·15</td>
<td>7·47</td>
</tr>
<tr>
<td>Ao (mmHg)</td>
<td>26</td>
<td>13</td>
<td>124·96</td>
<td>18·86</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>26</td>
<td>13</td>
<td>124·85</td>
<td>18·34</td>
</tr>
<tr>
<td>C.I. (l. min⁻¹. mm⁻²)</td>
<td>25</td>
<td>13</td>
<td>12·26</td>
<td>6·91</td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>27</td>
<td>13</td>
<td>15·60</td>
<td>1·72</td>
</tr>
<tr>
<td>Disarray</td>
<td>26</td>
<td>13</td>
<td>2·00</td>
<td>0·91</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>27</td>
<td>13</td>
<td>2·08</td>
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<tr>
<td>Score</td>
<td>20</td>
<td>11</td>
<td>1·89</td>
<td>0·92</td>
</tr>
</tbody>
</table>

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when myocardial hypertrophy and cardiac failure
(diastolic failure with/without systolic failure) are
observed. We investigated digitalis-like immunoactive
substances in the plasma and biopsied specimens
of patients with hypertrophic cardiomyopathy, and
compared them with the haemodynamic data.
diameter of cardiocytes and semiquantitative analyses of myofibre disarrangement and collagen proliferation were conducted following methods previously published\(^{14}\). Briefly, each histological change was graded 0 to 4+ under light microscopy, according to the severity and the extent of the findings: grade (0), no apparent significant change; (1+), minimal degree; (2+), moderate degree; (3+), marked degree; (4+), excessively marked degree.

For the immunohistochemical light microscopic study, additional sections were obtained from the paraffin block from 31 patients with hypertrophic cardiomyopathy (20 cases were of the non-obstructive type and 11 of the obstructive type), ranging in age from 40 to 73 (54.6 ± 12.2) years. In seven cases of non-obstructive hypertrophic cardiomyopathy and two cases of obstructive hypertrophic cardiomyopathy, the biopsied specimens were too small to obtain additional sections. The sections were placed on silanized glass slides (No. S3003, DAKO Japan, Kyoto, Japan), and the slides were dried, dewaxed in xylene, and rehydrated in graded concentrations of ethanol. For quenching endogenous peroxidase activity, the slides were incubated in 3% peroxide for 10 min. After incubation with normal blocking serum, the sections were incubated overnight at 4°C with monoclonal anti-digoxin antibody (MAB515, Chemicon International, Temecula, CA and #5111, Transformation Research, Framingham, MA, U.S.A.). After serial washing with phosphate-buffer solution, the slides were incubated with biotinylated secondary antibody for 45 min at room temperature. The sections were then allowed to react with Vectastain Elite ABC reagent (Vector Laboratories, Burlingame, CA, U.S.A.) for 30 min. After incubation in the peroxidase substrate solution (Vectastain 33′-diaminobenzidine substrate kit, Vector Laboratories, Burlingame, CA, U.S.A.), the slides were counterstained with Mayer–Hematoxylin, cleaned, and mounted for light microscopy\(^{15}\).

For the electron microscopic study, the specimens were fixed in 4% paraformaldehyde containing 0.25% glutaraldehyde and 4.5% sucrose, and embedded in LR White (London Resin Company, Hampshire, U.K.). Ultrathin sections were mounted on formvar/carbon support film grids (200 Ni/50, Electron Microscopy Sciences, Washington, PA, U.S.A.), and incubated in normal blocking serum. The sections were incubated overnight at 4°C with 10 nm gold conjugated monoclonal anti-digoxin antibody (GM-38-10, EY laboratories, San Meteo, CA, U.S.A.). After double staining with uranyl acetate and lead citrate, the sections were examined with a Hitachi H-7000 electron microscope. Close accumulation of five or more gold particles, based on a comparison with the control, was considered to be a positive reaction for digoxin. Positive reactions were also confirmed when a similar accumulation of particles was seen at the same site in serial sections\(^{16}\).

Histological specificity was checked by absorption tests using digoxin and bovine serum albumin. Specimens obtained from patients with dilated cardiomyopathy, who had been chronically treated with oral digoxin, were used as the positive control. Their data were reported elsewhere\(^{17}\).

As negative controls for the immunohistochemical procedures, substitution of an identical concentration of non-immune IgG for the primary antibody and direct incubation in colloidal gold without primary antibody were adopted.

**Semi-quantitative analysis by immunohistochemistry**

Following immunohistochemical analysis, the staining grade of digitalis-like immunoreactive substances was evaluated from 0 to 3+ under light microscopy in all specimens by three observers, who were blind towards the other available data. (Fig. 1). The average of the graded scores and the data of the plasma digitalis-like immunoreactive substance concentrations were compared with the haemodynamic, echocardiographic and histological parameters. All results were expressed as mean ± standard deviation (SD).

**Statistical analysis**

Statistical analysis was performed by the Fisher and the Wilcoxon methods, and the relationship between the scores of digitalis-like immunoreactive substances and each parameter was examined with linear regression analysis by the least squares method\(^{17}\). The results were considered statistically significant when \(P<0.05\).

**Results**

**Digitalis-like immunoreactive substances in plasma**

None of the control patients was positive for digitalis-like immunoreactive substances in plasma. The plasma concentration of patients with hypertrophic cardiomyopathy ranged from 0 to 140 ng . ml\(^{-1}\), with an average of 0.14 ± 0.17 in the non-obstructive types and 0.33–± 0.43 in the obstructive types (Table 1). An increase in digitalis-like immunoreactive substances of more than 0.2 ng . ml\(^{-1}\) was found in six of 27 patients with non-obstructive hypertrophic cardiomyopathy (22.2% and five of 13 with obstructive hypertrophic cardiomyopathy (38.4%). The incidence of positive digitalis-like immunoreactive substances in plasma was slightly higher in obstructive type patients than in non-obstructive type patients.

Although there was no significant difference in left ventricular systolic and end-diastolic pressures between those with and those without digitalis-like immunoreactive substances, the left ventricular pressures tended to be elevated in plasma-positive patients. The left atrial dimension in those with digitalis-like immunoreactive substances was increased in patients with dilated cardiomyopathy, with a marked increase in patients with obstructive hypertrophic cardiomyopathy.
substances (4.7 ± 0.6 cm) was significantly higher than in those without digitalis-like immunoreactive substances (4.1 ± 0.5 cm \( P=0.01 \)).

**Histology**

The diameter of cardiocytes and the semiquantitative data found by ordinary light microscopic study are shown in Table 1. There was no significant difference between non-obstructive and obstructive hypertrophic cardiomyopathy.

**Immunohistochemistry**

In controls, no 3′3′-diaminobenzidine products or gold accumulation was observed (Fig. 2). Under light microscopy, positive staining against the antibody was observed heterogeneously on some cardiocytes, both in non-obstructive and obstructive hypertrophic cardiomyopathy (Fig. 2). The number of cardiocytes with positive staining tended to be greater in patients with obstructive hypertrophic cardiomyopathy than in those with the non-obstructive type. Some patients who were negative for digitalis-like immunoreactive substances in the plasma showed positive staining immunohistochemically in the myocardium. Under electron microscopy, digitalis-like immunoreactive substances were detected at the sarcolemma in the free wall, T-tubules and intercalated discs. Digitalis-like immunoreactive substances were also observed in the cytoplasm adjacent to the intercalated discs, nuclei and Z-bands of cardiocytes (Fig. 3). The cell membranes of the endothelial cells of capillary vessels also presented positive digitalis-like immunoreactive substances.

**Semiquantitative analysis by immunohistochemistry**

Following immunohistochemistry, the staining grade of digitalis-like immunoreactive substances was evaluated (Fig. 1), and the average score is shown in Table 1. There was no significant difference in the score between non-obstructive and obstructive hypertrophic cardiomyopathy. Nine of 31 patients with hypertrophic cardiomyopathy, whose plasma and myocardium were studied...
simultaneously, had increased digitalis-like immuno-reactive substances in the myocardium (average score more than 2·0); the number of patients with digitalis-like immunoreactive substances in plasma only was three (Table 2).

The correlation coefficients and $P$ values of the plasma concentrations of digitalis-like immunoreactive substances, the graded scores, and all the other parameters for patients with non-obstructive and obstructive hypertrophic cardiomyopathy are shown in Tables 3 and 4, respectively. The coefficients are listed above the diagonal and the $P$ values are below.

In non-obstructive hypertrophic cardiomyopathy, there was no correlation between the plasma concentration of digitalis-like immunoreactive substances and the graded score in biopsied specimens. However,
plasma digitalis-like immunoreactive substances correlated with the left atrial dimension and were inversely correlated with the cardiac index (Table 3).

In obstructive hypertrophic cardiomyopathy, on the other hand, plasma digitalis-like immunoreactive substances correlated with the graded score, left atrial dimension and left ventricular systolic and end-diastolic pressures; the graded score also correlated with left ventricular end-diastolic pressures (Table 4).

A significant difference was also recognized as follows: the degree of myofibre disarrangement (Disarray) correlated with collagen proliferation (Fibrosis), Disarray was inversely correlated with fractional shortening in non-obstructive hypertrophic cardiomyopathy (Table 3) and correlated with left ventricular end-diastolic pressures. Fibrosis was inversely correlated with fractional shortening in obstructive hypertrophic cardiomyopathy (Table 4).

In the entire group of 40 patients with hypertrophic cardiomyopathy, the plasma concentration of digitalis-like immunoreactive substances correlated with Fibrosis and the left atrial dimension ($P=0.023$ and 0.001, respectively), and the graded score correlated with Fibrosis and left ventricular end-diastolic pressures ($P=0.031$ and 0.026, respectively).

**Discussion**

**Identification of digitalis-like immunoreactive substances**

A number of antibodies to digoxin or ouabain cross-react with steroid hormones. This is partly due to structural similarities in the epitope of the antigen. In several studies using the immunohistochemical technique, with an anti-digoxin monoclonal antibody, the digitalis-like immunoreactive substances were localized in the hypothalamus or the adrenal medulla. We previously described a histochemical technique to localize the immunoreactive products of anti-digoxin monoclonal antibodies in patients who were chronically treated with digoxin.

Digitalis-like immunoreactive substances are known to inhibit the sodium pump of the mammalian cell membrane, and might be considered a new class of hormone participating in the volume regulation of cells and tissues of mammals. In general, in situ fixation of the steroid hormone is difficult. Assuming that digitalis-like immunoreactive substances function like new steroid hormones, digitalis-like immunoreactive substances could be identified immunohistochemically, from a specific binding globulin responsible for transport.

Thus, immuno-positive findings against the anti-digoxin monoclonal antibody observed in this study are considered to be due to digitalis-like immunoreactive substances.

**Digitalis-like immunoreactive substances in plasma**

By fluorescence polarization immunoassay, 22.2% of non-obstructive and 38.4% of obstructive hypertrophic cardiomyopathy patients were positive for plasma digitalis-like immunoreactive substances. The difference in the incidence might be due to the greater elevation of left ventricular systolic and end-diastolic pressures in obstructive hypertrophic cardiomyopathy. Statistical analysis revealed that plasma digitalis-like immunoreactive substances were correlated with the left atrial dimension both in non-obstructive and obstructive hypertrophic cardiomyopathy. Furthermore, in obstructive hypertrophic cardiomyopathy, plasma digitalis-like immunoreactive substances correlated with left ventricular systolic and end-diastolic pressures, suggesting that increased digitalis-like immunoreactive substances in plasma were a secondary phenomenon caused by pressure and/or volume overload.

The incidence of positive digitalis-like immunoreactive substances in plasma should be compared with other physiological and pathological conditions. Further investigation may be necessary.

**The role of digitalis-like immunoreactive substances in hypertrophic cardiomyopathy**

It is known that digitalis-like immunoreactive substances are Na$^+$-K$^+$-ATPase inhibitors and may

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**Table 2** Summary of the number of patients with hypertrophic cardiomyopathy whose plasma and myocardium were positive or negative for DLIS

<table>
<thead>
<tr>
<th></th>
<th>Plasma (−)</th>
<th>Plasma (+)</th>
<th>Plasma (−)</th>
<th>Plasma (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myocardium (−)</td>
<td>Myocardium (+)</td>
<td>Myocardium (−)</td>
<td>Myocardium (+)</td>
</tr>
<tr>
<td>HNCM (n=20)</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>HOCM (n=11)</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total (n=31)</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

DLIS= digitalis-like immunoreactive substances; HNCM= non-obstructive type; HOCM= obstructive type; Plasma (+)= positive DLIS in plasma; Plasma (−)= negative DLIS in plasma; Myocardium (+)= positive DLIS in the myocardium; Myocardium (−)= negative DLIS in the myocardium.
<table>
<thead>
<tr>
<th></th>
<th>DLIS</th>
<th>LAD</th>
<th>LVsd</th>
<th>PWd</th>
<th>Dd</th>
<th>Ds</th>
<th>FS</th>
<th>Ao</th>
<th>LVsys</th>
<th>LVdp</th>
<th>C.I.</th>
<th>Diam</th>
<th>Disarray</th>
<th>Fib</th>
<th>Score</th>
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</thead>
<tbody>
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<td>DLIS</td>
<td>0.456</td>
<td>-0.005</td>
<td>-0.299</td>
<td>0.157</td>
<td>0.285</td>
<td>-0.270</td>
<td>-0.233</td>
<td>-0.294</td>
<td>0.029</td>
<td>-0.543</td>
<td>-0.184</td>
<td>0.223</td>
<td>0.389</td>
<td>0.110</td>
<td></td>
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<tr>
<td>LAD</td>
<td>0.033</td>
<td>-0.087</td>
<td>-0.167</td>
<td>0.327</td>
<td>0.221</td>
<td>0.071</td>
<td>0.006</td>
<td>0.057</td>
<td>-0.025</td>
<td>-0.269</td>
<td>0.375</td>
<td>0.079</td>
<td>0.155</td>
<td>-0.004</td>
<td></td>
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<tr>
<td>LVsd</td>
<td>0.984</td>
<td>-0.667</td>
<td>#</td>
<td>-0.422</td>
<td>-0.339</td>
<td>-0.126</td>
<td>-0.280</td>
<td>-0.163</td>
<td>-0.138</td>
<td>-0.017</td>
<td>0.238</td>
<td>0.027</td>
<td>0.250</td>
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<td>PWd</td>
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<td>-0.272</td>
<td>0.004</td>
<td>0.114</td>
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<td>0.082</td>
<td>-0.292</td>
<td>-0.169</td>
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<tr>
<td>Dd</td>
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<td>0.056</td>
<td>0.084</td>
<td>0.057</td>
<td>#</td>
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<td>0.011</td>
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<td>-0.237</td>
<td>-0.200</td>
<td>0.001</td>
<td>0.010</td>
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<td>0.000</td>
<td>#</td>
<td>-0.653</td>
<td>-0.313</td>
<td>-0.327</td>
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<td>0.000</td>
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<td>Ao</td>
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<td>0.975</td>
<td>0.428</td>
<td>0.580</td>
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<td>#</td>
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<td>0.145</td>
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<td>0.010</td>
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<td>0.005</td>
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<td>0.783</td>
<td>0.501</td>
<td>0.600</td>
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<td>0.000</td>
<td>#</td>
<td>0.142</td>
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<td>-0.292</td>
<td>0.096</td>
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<td>0.516</td>
<td>0.385</td>
<td>0.481</td>
<td>0.489</td>
<td>#</td>
<td>0.055</td>
<td>-0.046</td>
<td>-0.194</td>
<td>-0.052</td>
<td>0.244</td>
</tr>
<tr>
<td>CI</td>
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<td>0.193</td>
<td>0.251</td>
<td>0.343</td>
<td>0.987</td>
<td>0.103</td>
<td>0.039</td>
<td>0.113</td>
<td>0.311</td>
<td>0.792</td>
<td>#</td>
<td>0.036</td>
<td>0.111</td>
<td>0.199</td>
<td>-0.293</td>
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<td>0.412</td>
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<td>0.894</td>
<td>0.684</td>
<td>0.116</td>
<td>0.998</td>
<td>0.251</td>
<td>0.323</td>
<td>0.366</td>
<td>0.822</td>
<td>0.137</td>
<td>#</td>
<td>0.123</td>
<td>0.168</td>
<td>-0.418</td>
</tr>
<tr>
<td>Disarray</td>
<td>0.330</td>
<td>0.700</td>
<td>0.219</td>
<td>0.148</td>
<td>0.169</td>
<td>0.040</td>
<td>0.036</td>
<td>0.201</td>
<td>0.147</td>
<td>0.343</td>
<td>0.957</td>
<td>0.550</td>
<td>#</td>
<td>0.461</td>
<td>-0.007</td>
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<tr>
<td>Fibrosis</td>
<td>0.074</td>
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The correlation coefficients are listed above the diagonal and p values are below. DLIS = plasma concentration of digitalis-like substances; LAD = left atrial dimension; LVsd = diastolic ventricular septal thickness; PWd = diastolic posterior wall thickness; Dd = diastolic left ventricular dimension; Ds = systolic left ventricular dimension; FS = fractional shortening; Ao = aortic pressure; LVsys = left ventricular systolic pressure; LVdp = left ventricular end-diastolic pressure; CI = cardiac index; Diam = diameter; Fib = fibrosis. Note that Disarray, Fib, and Score were evaluated semiquantitatively (see text).
### Table 4  Statistical analysis in obstructive hypertrophic cardiomyopathy

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The correlation coefficients are listed above the diagonal and p values are below. DLIS=plasma concentration of digitalis-like substances; LAD=left atrial dimension; IVSd=diastolic ventricular septal thickness; PWd=diastolic posterior wall thickness; Dd=diastolic left ventricular dimension; Ds=systolic left ventricular dimension; FS=fractional shortening; Ao=aortic pressure; LVsys=kft ventricular systolic pressure; LVedp=kft ventricular end-diastolic pressure; CI=cardiac index; Diam=diameter; Fib=fibrosis. Note that Disarray, Fib, and Score were evaluated semiquantitatively (see text).
participate in the volume regulation of cells and tissues in mammals\cite{1}. Delva et al. found a strong and significant correlation between plasma concentrations of digoxin-like immunoreactivity and haemodynamic variables which characterize the pulmonary circulation in patients with various cardiovascular diseases\cite{28}. In the study by Bagrov et al., plasma Na\(^+-\)K\(^-\)-ATPase inhibitory activity was inversely correlated with the cardiac index and cardiac output\cite{23}. Our study revealed that plasma digitalis-like immunoreactive substances and the cardiac index were inversely correlated in non-obstructive hypertrophic cardiomyopathy, whereas it did not exist in obstructive hypertrophic cardiomyopathy.

Under immunoelectron microscopy, digitalis-like immunoreactive substances were detected at the sarcolemma in the free wall, T-tubules and intercalated discs, indicating the localized site of Na\(^+-\)K\(^-\)-ATPase in situ. According to a previous study, in the hypertrophied heart induced by pressure overload, the number of Na\(^+-\)K\(^-\)-ATPase units was reduced, although the affinity of the enzyme was unchanged\cite{23}. The localization and expression of cardiac Na\(^+-\)K\(^-\)-ATPase in hypertrophic cardiomyopathy should be examined in detail.

Digitalis-like immunoreactive substances were also found in the cytoplasm of cardiocytes, such as the Z-bands near the site of intercalated discs. Cardiac glycosides may be sequestered or internalized, together with their binding sites, as a part of membrane turnover\cite{59}. Thus, the immunoreactivity observed in cardiocytes indicates that digitalis-like immunoreactive substances were transported into the cytoplasm.

Some patients with hypertrophic cardiomyopathy, who were negative for digitalis-like immunoreactive substances in the plasma, showed positive staining immunohistochemically in the myocardium (Table 2). No characterization was detected in available clinical data of these patients. Further investigations should be conducted to explain this unusual finding.

**Additional subjects**

The statistical analysis of the histochemical results showed that Fibrosis correlated with Disarray (P=0.018) and Disarray was inversely correlated with the fractional shortening (P=0.036) in non-obstructive hypertrophic cardiomyopathy, and that Fibrosis was inversely correlated with fractional shortening (P=0.017) in obstructive hypertrophic cardiomyopathy (Tables 3 and 4). The proliferation and subtypes of collagen may be important factors to understand the pathophysiology of hypertrophic cardiomyopathy\cite{11}.

**Limitations of the study**

In many cases, the biopsied specimens were too small to obtain additional sections for immunohistochemical analysis. We could not perform immunostaining in all cases of hypertrophic cardiomyopathy examined in this study.

We previously reported the incidence of plasma digitalis-like immunoreactive substances in patients with hypertensive heart disease, and the immunohistochemical study of the myocardium in hypertensive heart disease is also necessary\cite{57}. Currently, endomyocardial biopsy specimens from patients with hypertensive heart disease are not available, and animal models should be studied in future examinations.

In this study, distantly related tissue, such as skeletal muscle, was not examined. It might be important to study other tissue in patients who showed positive digitalis-like immunoreactive substances in plasma and/or in the myocardium.

**Conclusions**

Increased digitalis-like immunoreactive substances in plasma and cardiocytes, which are correlated with pressure and/or volume overload, were found in patients with hypertrophic cardiomyopathy. Digitalis-like immunoreactive substances may act on the sarcolemma of the cardiocytes and be transported into the cytoplasm.

We thank Dr Hisao Yamada, Department of Anatomy, Shiga University of Medical Science, and Dr Hakuo Takahashi, Department of Clinico-Pathology, Kansai Medical University, for their thoughtful suggestions. This study was supported in part by a Grant from the Vehicle Racing Commemorative Foundation.

**References**


