Improvement in Fatty Acid Utilization in Relation to a Change in Left Ventricular Hypertrophy in Spontaneously Hypertensive Rats

Tomohide Ono, MD; Tetsuro Kohya, MD; Eriko Tsukamoto, MD*; Takaumi Mochizuki, MD*; Kazuo Itoh, MD*; Yoshinori Itoh, MD; Fumish Tomita, MD; Nagara Tamaki, MD*; Akira Kitabatake, MD

Although fatty acid metabolism is reportedly impaired in myocardial hypertrophy, it is unclear whether the antihypertensive drugs are associated with improved fatty acid metabolism. In order to evaluate the effects of antihypertensive drugs on fatty acid metabolism and myocardial perfusion, the simultaneous uptake of iodine-125(I) and 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP) and thallium-201 (TI) were measured in 3 groups of rats: (1) spontaneously hypertensive rats (SHR) without treatment (SHR-N), (2) SHR chronically treated with captopril (SHR-C), and (3) SHR chronically treated with hydralazine (SHR-H). Captopril and hydralazine were administered to their respective groups for 3 weeks from 12 weeks of age. The hearts were removed 10 min after simultaneous intravenous injections of BMIPP and TI and the 125I and 201TI counts were measured to calculate the uptake ratio. The systolic blood pressure (SBP) in SHR-N was 272 ± 10 mmHg, whereas the SHR-C and SHR-H groups showed significant SBP reduction (156 ± 11, and 158 ± 10 mmHg, respectively) (p < 0.01 each). The heart/bodyweight ratio was significantly lower in SHR-C (2.48 ± 0.09) than in SHR-N (2.74 ± 0.11) (p < 0.05). However, there was no significant difference in the heart/bodyweight ratio between SHR-N and SHR-H (2.62 ± 0.09). The ratio of BMIPP uptake to TI uptake (BMIPP/TI) was significantly higher in SHR-C (0.71 ± 0.13) than in SHR-N (0.50 ± 0.09) (p < 0.05). However, BMIPP/TI in SHR-H (0.53 ± 0.09) was similar to that in SHR-N. These results suggest that captopril improves fatty acid metabolism in the hypertrophied ventricle in SHR. The metabolic alterations may improve with left ventricular hypertrophy regression but are not affected by the reduction of blood pressure only. (Jpn Circ J 2000; 64: 117–120)

Key Words: Angiotensin converting enzyme inhibitor; BMIPP; Myocardial hypertrophy; Regression of hypertrophy; Thallium

Radioiodinated 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP) has recently been developed for myocardial fatty acid metabolic imaging. BMIPP is a methyl branched-chain fatty acid, which is trapped in the myocardial cells without further metabolism by β-oxidation. Thus, it provides a means of measuring regional myocardial fatty acid utilization in vivo. Our recent study demonstrated a decreased BMIPP uptake relative to the thallium-201 (TI) distribution in the hypertrophied myocardium in spontaneously hypertensive rats (SHR) in comparison with Wister-Kyoto rats (WKY)9. Other studies utilizing different fatty acid tracers have shown that the myocardial fatty acid distribution is uneven in severely hypertensive rats.10 However, it is not clearly understood whether the changes in myocardial fatty acid metabolism are caused by elevated blood pressure, left ventricular hypertrophy (LVH) or both.

It has been reported that hypertensive LVH regresses after long-term treatment with antihypertensive agents.11–13 Angiotensin-converting enzyme (ACE) inhibitors cause rapid regression of LVH by inhibiting conversion to angiotensin II, which directly promotes LVH.14–16 The purpose of this study was to evaluate the effect of antihypertensive drugs on LVH and its regression, on fatty acid metabolism and on myocardial perfusion.

Methods

Animals

Twelve-week-old male SHR, which show LVH7 were chronically treated with antihypertensive agents for 3 weeks. These 15-week-old rats were then used for the experiments. Experimental animals were allocated to 3 groups of 7 rats each: (1) SHR without treatment (SHR-N), (2) SHR chronically treated with captopril, an ACE inhibitor, (SHR-C), and (3) SHR chronically treated with the vasodilator hydralazine (SHR-H). The care of the animals strictly conformed with humane standards.18

Antihypertensive Treatment

Appropriate antihypertensive agents were added to the water supply for 3 weeks beginning at 12 weeks of age. The captopril group received 100 mg kg⁻¹ day⁻¹ and the hydralazine group received 12 mg kg⁻¹ day⁻¹, based upon previous reports concerning the antihypertensive effects of these dose rates in SHR.13,15,16

Japanese Circulation Journal Vol.64, February 2000
Measurement of Blood Pressure

At 12 weeks of age (prior to treatment), and at 15 weeks of age (during treatment), blood pressures and pulse rates were measured at the same time of day using the tail-cuff method.

Experimental Protocol

In the 2 treatment groups, the antihypertensive agents were withdrawn 24 h before the experiments in order to eliminate acute pharmacological effects. After 12 h of fasting, each rat was weighed and anesthetized with an intraperitoneal injection of pentobarbital. All rats were injected intravenously with 370kBq of $^{125I}$-BMIPP and 920kBq of $^{201Tl}$ simultaneously. The heart was removed 10 min after injection and separated into the right and left ventricles. These were weighed, and the radioactivity of $^{201Tl}$ in the left ventricle (LV) was measured using an auto well counter (ARC-380, Aloka, Tokyo, Japan) with the energy window range set at 60–80keV. The $^{125I}$-BMIPP activity was counted using the same well counter with the energy window at 20–40keV. The radioactivity associated with each radionuclide in other energy windows was determined by using reference standards and the resultant crossover corrections were applied to tissue samples containing both tracers. This was achieved by evaluating aliquots of the pure radionuclides individually and then combined to evaluate the count rate in each counting window to determine the crossover contribution of each radionuclide.

Calculation of Parameters

The left ventricle per body weight ratio (LVW/BW, mg/g) was calculated as an index of myocardial hypertrophy. The percent uptake of $^{125I}$-BMIPP and that of $^{201Tl}$ in the left ventricular myocardium were calculated by 2 methods:

\[
\text{% injected dose} = \left(\frac{\text{LV counts of tracer}}{\text{injected dose counts}}\right) \times 100
\]

\[
\text{% injected dose/g tissue} = \left(\frac{\text{LV counts of tracer}}{\text{injected dose counts}}\right) \times 100 / \text{tissue weight}.
\]

In addition, we calculated the ratio of % injection dose/g tissue for $^{125I}$-BMIPP to that of $^{201Tl}$ (BMIPP/Tl) as an index of myocardial fatty acid metabolism relative to myocardial perfusion.

Data Analysis and Statistics

The data were expressed as mean and standard deviation (SD). Differences were examined by using one-way factorial ANOVA and Fisher's PLSD multiple comparison test, and p<0.05 was considered the level of significance.

| Table 1 | Comparison of Parameters in the 3 Groups of 15-Week-Old Spontaneously Hypertensive Rats (SHR) |
|--------|---------------------------------|---------------------------------|---------------------------------|
|        | SHR-N                           | SHR-C                           | SHR-H                           |
| SBP (mmHg) | 222±10                         | 156±11*                         | 158±10*                         |
| Pulse rate (beats/min) | 420±14                         | 425±3                           | 436±5                           |
| LVW/BW (mg/g) | 2.74±0.11                      | 2.48±0.09**                      | 2.65±0.09                       |
| TI uptake (%g) | 3.9±0.43                      | 3.35±0.33**                      | 4.1±0.62                        |
| BMIPP uptake (%g) | 1.99±0.15                    | 2.35±0.40                       | 2.33±0.25                       |
| BMIPP/Tl ratio | 0.93±0.09                     | 0.71±0.13**                      | 0.53±0.09                       |

SHR-N: no treatment; SHR-C: captopril 100 mg kg⁻¹ day⁻¹; SHR-H: hydralazine 12 mg kg⁻¹ day⁻¹; SBP: systolic blood pressure; LVW: left ventricular weight; BW: body weight; %g, % injection dose/g tissue. *p<0.05 vs SHR-N, **p<0.05 vs SHR-H.

Results

Blood Pressure and Pulse Rate (Table 1)

The systolic blood pressure (SBP) in SHR at 12 weeks of age was 210±8 mmHg. In the SHR-N group it increased to 222±10 mmHg, but the SHR-C and SHR-H groups both showed a significantly decreased SBP (156±11 mmHg, 158±10 mmHg, respectively) (p<0.05).

The pulse rate in the SHR-N, SHR-C and SHR-H groups was 432±14, 425±3, and 436±54 beats/min (bpm), respectively. There was no significant difference in the pulse rate among the groups.

Left Ventricular Hypertrophy (Table 1)

The LVW/BW in SHR-N was 2.74±0.11 mg/g, which did not differ significantly from the value at 12 weeks of age (2.66±0.13 mg/g). The LVW/BW were significantly lower in SHR-C (2.48±0.09 mg/g) than in SHR-N (p<0.05), but not in SHR-H (2.65±0.09 mg/g).

TI Uptake in the Left Ventricle (Table 1)

The TI uptake (% injection dose/g tissue) in SHR-N was 3.9±0.43%, which was not different from the value at 12 weeks of age (3.6±0.51%). The values in SHR-C and SHR-H were 3.35±0.33, and 4.1±0.62%, respectively, with the TI uptake significantly lower in SHR-C than in SHR-N (p<0.05).

BMIPP Uptake in the Left Ventricle (Table 1)

The BMIPP uptake (% injection dose/g tissue) in SHR-N, SHR-C, SHR-H was 1.95±0.15, 2.35±0.40, and 2.13±0.23%, respectively. The uptake in SHR-C was slightly but not significantly higher than that of SHR-N and SHR-H.

BMIPP/Tl Ratio (Table 1)

The BMIPP/Tl was calculated as an index of fatty acid metabolism relative to perfusion in the SHR-N, SHR-C, and SHR-H it was 0.50±0.09, 0.71±0.13, and 0.53±0.09, respectively. Compared with SHR-N, the BMIPP/Tl in SHR-C was significantly higher (p<0.05), whereas that of SHR-H was not significantly different.

Discussion

In the present study we found that changes in fatty acid metabolism were present in LVH and that these changes were reversed with the regression of LVH induced by captopril, but not with the blood pressure reduction alone induced by hydralazine.

SBP was already elevated in SHR at 12 weeks of age (prior to the treatments) and thereafter increased until 15 weeks of age (SHR-N). In both SHR-C and SHR-H rats...
following 3 weeks of antihypertensive treatment the SBP dropped to the levels comparable to normotensive rats. Therefore, we consider that the dosages of the antihypertensive agents were appropriate.

The LVW/BW in SHR-C was significantly lower than that in SHR-N, indicating LVH regression due to captopril treatment. Conversely, the LVW/BW in SHR-H did not decrease significantly despite lowered blood pressure. Hydralazine is thought to increase reflexive sympathetic tone, and therefore cannot induce regression of LVH.5

In SHR-H, the TI uptake by the left ventricle did not significantly differ from SHR-N. In SHR-C, a decrease in TI uptake was observed with regression of LVH, which indicates a decrease in total blood flow into the left ventricle, in association with the regression of LVH due to captopril.

In the present study we used BMIPP/TI (% injection dose/g tissue for 131-I-BMIPP to the % injection dose/g tissue for TI) as an index on fatty acid metabolism. Because BMIPP uptake is dependent upon myocardial perfusion as well as fatty acid metabolism, we used the ratio of BMIPP to TI uptake in order to eliminate the effect of myocardial perfusion.6,7,9,10 Myocardial perfusion is increased in hypertrophied hearts and the energy demand increases accordingly. Although myocardial perfusion was increased because of an increase in myocardial weight, BMIPP uptake tended to be lower in the hypertrophied heart (SHR-N and SHR-H) than in the hearts with regression of LVH (SHR-C).

Our previous study using combinations of TI and BMIPP demonstrated changes in fatty acid metabolism with increased LVH. However, the reason why changes in metabolism occur with hypertrophy remains unclear. A study using murine cultured cells reported that the accumulation of BMIPP as a fatty acid tracer parallels the intracellular ATP concentration. Decreased intracellular ATP concentration is frequently observed during hypertrophy with myocardial ischemia,20,21 so changes in fatty acid metabolism mediated by changes in ATP cannot be excluded. A positron emission tomography (PET) study using a similar model of hypertension showed an increase in fluorodeoxyglucose (FDG) accumulation at myocardial sites corresponding to decreased accumulation of a fatty acid tracer. Therefore, there is also the possibility that a switch from fatty acid metabolism to glucose metabolism causes changes in BMIPP accumulation in SHR.22,23

In the present study, we found that the changes in fatty acid metabolism of LVH were reversed by LVH regression. SHR at 12–15 weeks of age are reported to show complete LVH without marked irreversible changes such as fibrosis.24–27 Therefore, regression appears to have resulted in an improved intracellular ATP concentration and a change of the energy substrate demand from glucose metabolism to fatty acid. Precise correlation of the changes in perfusion and BMIPP uptake with the biochemical and histological findings in this model is required. In addition, further studies are needed to determine whether a change in fatty acid metabolism precedes LVH regression.

We conclude that an alteration of fatty acid metabolism in LVH may be reversed in association with the regression of LVH induced by captopril, but not with blood pressure reduction alone that is induced by hydralazine. Myocardial hypertrophy itself is an important factor in changes in fatty acid metabolism.

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