Contribution of hypoxia to the development of cardiomyopathy in hamsters

Yoshiyuki Watanabe, Hideo Kusuoka, Kazuki Fukuchi, Toshiyuki Fujiwara, Tsunehiko Nishimura

Division of Tracer Kinetics, Biomedical Research Center, Osaka University Medical School, 2-2 Yamadaoka Suitsa, Osaka 565, Japan

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Abstract

Objective: It has been hypothesized that microvascular spasms cause cardiomyopathy. To elucidate the contribution of hypoxia to the development of cardiomyopathy, the newly-developed hypoxia tracer, Tc-99m nitroimidazole, was applied to detect myocardial hypoxia in a hamster model. Methods: Tc-99m nitroimidazole (180 MBq) and I-125 iodoantipyrine (370 kBq) were injected into cardiomyopathic Syrian hamsters or control hamsters at age 10, 25, and 40 weeks (n = 6 in each group). The myocardial uptake of Tc-99m nitroimidazole was measured and dual tracer autoradiography was performed. Results: Histologic study revealed that the cardiomyopathic hamsters at age 10, 25 and 40 weeks were in the myocytolytic stage, the fibrotic and healing stage, and the hypertrophy and dilatation stage, respectively. Tc-99m nitroimidazole uptake was significantly greater in the cardiomyopathic hamsters than in the controls at age 25 weeks (cardiomyopathic hamsters, 33.3 ± 4.7% g dose/g; control, 25.2 ± 3.1), whereas there were no significant differences between both strains at age 10 and 40 weeks. The quantified concentration of I-125 iodoantipyrine in the cardiomyopathic hamster at age 40 weeks was significantly lower than that in the controls. When the Tc-99m nitroimidazole uptake was normalized by I-125 iodoantipyrine concentrations, the cardiomyopathic hamsters at age 25 and 40 weeks showed significantly greater uptake than the controls. Conclusion: The myocardium in cardiomyopathic hamsters was hypoxic at the fibrotic and healing stage and may be hypoxic at the hypertrophy and dilatation stage. This may contribute to the development of cardiomyopathy.

Keywords: Cardiomyopathy; Nitroimidazole; Hypoxia; Microcirculation; Hamster; Syrian hamster

1. Introduction

The cardiomyopathic Syrian hamster is a natural model of congestive heart failure due to cardiomyopathy and muscular dystrophy [1]. The pathogenesis of cardiomyopathy is still unknown, but many reports have demonstrated abnormalities of the microvascular circulation, calcium metabolism [2], and energy supply [3,4] in this model. Focal and transient microvascular spasms cause focal myocytolysis in cardiomyopathic hamsters [5]. Moreover, ischemia and repeated reperfusion cause extended myocytolytic lesions equivalent to those observed in cardiomyopathy [6]. Coupled with these observations, it has been hypothesized that the myocardium of cardiomyopathic hamsters is ischemic or hypoxic.

Recently, Tc-99m labeled nitroimidazole (BMS-181321) has been developed to image hypoxia [7,8]. The nitroimidazole compounds diffuse across the cell membrane and undergo reduction in the cytoplasm to form a radical [9–12]. When oxygen is abundant in the cell, it reacts with the radical anion and yields noncharged nitroimidazole that diffuses out of the cell. When intracellular hypoxia is present, the nitroimidazole radical anion is reduced further to form nitrous compounds that combine covalently with cytosolic macromolecules and are trapped intracellularly. Enhanced selective retention of BMS-181321 in hypoxic...
cells has been demonstrated in rats [13], swine [14], perfused hearts [15,16] and isolated myocytes [7,17]. To elucidate the degree of myocardial hypoxia in cardiomyopathic hamsters, we compared the uptake of BMS-181321 with that of the blood flow tracer, I-125 iodoantipyrine, in the myocardium of these animals.

2. Methods

Male cardiomyopathic Syrian hamsters (Bio14.6) at age 10, 25, and 40 weeks, and age-matched control hamsters (F1B) were used in this study (Bio Breeders Inc., Fitchburg, MA). Six hamsters were used for the hypoxia/blood flow studies, and two hamsters were only used for histologic examination in each age group. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, 1985).

2.1. Measurement of myocardial BMS-181321 uptake

Hamsters were anesthetized with pentobarbital (0.01 g/100 g i.p.), and catheterized at femoral vein. Tc-99m propylene amine oxime-1,2-nitromidazole (BMS-181321; 185 MBq, Bristol-Myers Squibb Co., Princeton, NJ) was injected first, and I-125 iodoantipyrine (ANT; 370 kBq) was injected 55 min later. The hamsters were sacrificed by injection of saturated KCl 5 min after injection of ANT. The hearts were excised, blotted on filter paper, and weighed. Tc-99m activity was measured by a single channel analyzer with a 2" × 2" NaI(Tl) scintillator (Ohyo Koken Kogyo, Tokyo, Japan). The myocardial uptake of BMS-181321 (BMS uptake) was calculated as follows [18]:

\[
\text{BMS uptake} = \frac{\text{myocardial count/heart weight}}{\text{total injection dose/heart weight}} \times 100 \left( \% \text{g dose/g} \right).
\]

2.2. Dual tracer autoradiography

After counting myocardial radioactivity, the hearts were frozen in powdered dry ice, embedded in a compound of polyvinyl alcohol and polyethylene glycol (Miles Inc., Elkhart, IN), and sectioned with a cryomicrotome in the direction perpendicular to the longitudinal axis of the left ventricle. Myocardial sections of 20 μm thickness were cut and mounted onto poly-L-lysine coated slides. The slides were placed against a phosphor imaging plate for exposure. The first exposure was carried out for 1 h to detect the BMS-181321 distribution. The second exposure was initiated 7 days later, following the decay of Tc-99m activity, and carried out for 8 h to image the distribution of ANT. The image data were analyzed using a computerized imaging analysis system (Fujix Bio-Imaging Analyzer BAS2000, Fuji Photo Film, Tokyo, Japan) [19]. The left ventricular dimension was measured from autoradiograms of BMS-181321. The left ventricular dimension was expressed as the average of lengths of the major axis and the minor axis.

Autoradiographic I-125 microscales (Amersham, Tokyo, Japan) of known radioactivity were placed on the imaging plate during ANT exposure. The quantified concentration (kBq/g tissue) of ANT was determined by using a calibration curve obtained from the graded I-125 microscales, and the concentration was corrected for the body weight of each hamster. To distinguish hypoperfused myocardium from the normally-perfused one with fibrosis, the BMS uptake was divided by quantified ANT concentrations (i.e., BMS/ANT).

To quantitate the myocardial distribution of BMS-181321 or ANT, each myocardial section was divided into 4 regions: the endocardial and epicardial regions of the left ventricular free wall, the septum, and the right ventricular wall. In each region, the relative uptake of tracer was defined as the ratio of the regional radioactivity to that of the septum.

2.3. Histologic examination

Myocardial sections of 10 μm thickness in those adjacent to the autoradiographic studies were stained with hematoxylin–eosin solution. In separate experiments, hearts were fixed with 10% buffered formaldehyde, embedded in paraffin, and cut into sections 5 μm thick. The specimens were stained with hematoxylin–eosin and Masson's trichrome solution.

The severity of myocyte lysis or fibrosis was evaluated by two independent observers, and scored semiquantitatively by an arbitrary scale of 0 to 4: 0 designates no lesion; grade 1, mild lesion; grades 2 and 3, moderate lesion; and grade 4, severe lesion [20]. The transnuclear width of the myocytes was measured from longitudinally-oriented myocytes in the left free wall muscle bundles with a calibrated microscope eyepiece reticule on randomly-selected fields (×400).

2.4. Radiopharmaceuticals

BMS-181321 was prepared from provided kits according to a method previously reported [7]. The radiochemical purity of Tc-99m complex was determined by paper chromatography using a German solvent saturation pad developed in diethyl ether. The average radiochemical purity was 93.1%. BMS-181321 was injected within 30 min of preparation.

I-125 4-iodoantipyrine (ANT) was prepared as described previously [21]. In brief, a solution (0.5 ml) containing Na[I-125] (185 MBq, 2.3 nmo) was added to the
4-bromoantipyrine solution, heated for 15 min at 100°C, and cooled to room temperature. The solution was purified by reversed-phase HPLC (eluent, 45% methanol; flow rate, 5 ml/min; retention time, 72 min). The radiochemical purity of the I-125 iodoantipyrine was more than 99% with a TLC plate (acetone/toluene = 1:1).

2.5. Statistical analysis

Data are presented as mean ± s.d. Statistical analysis was performed using the unpaired t-test or Mann-Whitney’s U-test to compare between the cardiomyopathic and control hamsters where appropriate. We used two-way analysis of variance (ANOVA) with Scheffe’s method or the Kruskal-Wallis test for multiple comparison among ages or myocardial regions. Probability of the null hypothesis less than 0.05 was considered as significant.

3. Results

The body weight of the cardiomyopathic hamsters was significantly lower than that of the controls at all ages (Table 1). The heart weight of the cardiomyopathic hamsters was significantly lower than that of the controls at age 10 weeks, but significantly higher at age 40 weeks. The left ventricle dilated in the cardiomyopathic hamsters at age 40 weeks (Table 1). However, the cardiomyopathic hamsters did not show any signs of congestive heart failure such as subcutaneous edema, ascites, hydrothorax, or hydroepicardium, even at age 40 weeks. These results indicate that the cardiac function of the cardiomyopathic hamsters at age 40 weeks was compensated.

3.1. Histologic examination

In the cardiomyopathic hamsters, the myocardium demonstrated progressive changes (Fig. 1), as observed previously [22]. At age 10 weeks, there was multifocal myocytolysis with surrounding inflammation and fibrosis (Fig. 1A,B). In the 25-week-old hamsters, myocytolytic lesions were still present, but fibrosis became more prominent (Fig. 1C,D). At age 40 weeks, there were few active myocytolytic lesions and compensatory myocardial hypertrophy was observed (Fig. 1E,F).

In the cardiomyopathic hamsters, the myocytolysis score (Table 2) was highest at age 10 weeks, and decreased gradually at age 25 and 40 weeks. The fibrosis score (Table 2) was higher at age 25 weeks than at age 10 and 40 weeks. In contrast, the control hearts showed some residual level of myocytolysis or fibrosis (Table 2). The myocyte width in the cardiomyopathic hamsters was sig-

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Table 1

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Heart weight (g)</th>
<th>LV dimension (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio14.6 F1B</td>
<td>Bio14.6 F1B</td>
</tr>
<tr>
<td>10 weeks</td>
<td>89.5 ± 3.0 b</td>
<td>114.4 ± 6.5</td>
</tr>
<tr>
<td>25 weeks</td>
<td>118.7 ± 9.1 a b</td>
<td>127.9 ± 3.9</td>
</tr>
<tr>
<td>40 weeks</td>
<td>116.8 ± 6.5 b</td>
<td>154.1 ± 4.2</td>
</tr>
</tbody>
</table>

Bio14.6 = cardiomyopathic hamsters; F1B = control hamsters.

a P < 0.05, b P < 0.005 vs. F1B by unpaired t-test.
Table 2
Histologic analysis in the 3 age groups of hamsters

<table>
<thead>
<tr>
<th></th>
<th>Score of myocytolysis</th>
<th>Score of fibrosis</th>
<th>Myocyte width (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio14.6</td>
<td>F1B</td>
<td>Bio14.6</td>
</tr>
<tr>
<td>10 weeks</td>
<td>2.29 ± 0.95 a</td>
<td>0.17 ± 0.41</td>
<td>0.71 ± 0.49 a</td>
</tr>
<tr>
<td>25 weeks</td>
<td>1.50 ± 0.55 b</td>
<td>0.17 ± 0.41</td>
<td>2.17 ± 1.47 c</td>
</tr>
<tr>
<td>40 weeks</td>
<td>0.67 ± 0.52 c</td>
<td>0.33 ± 0.51</td>
<td>1.83 ± 0.75 b</td>
</tr>
</tbody>
</table>

Myocyte width was measured in 10 cells. Bio14.6 = cardiomyopathic hamsters; F1B = control hamsters.

a P < 0.05 vs. F1B by Mann-Whitney’s U-test.

b P < 0.005 vs. F1B by unpaired t-test.

c P < 0.05 vs. 10-week-old hamsters by Kruskal-Wallis test.

d P < 0.05 vs. 10- and 25-week-old hamsters by ANOVA by Scheffé’s method.

Table 3
BMS uptake, qANT, and BMS/ANT in cardiomyopathic and control hamsters

<table>
<thead>
<tr>
<th></th>
<th>BMS uptake (%g dose/g)</th>
<th>qANT (kBq/g)</th>
<th>BMS/ANT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio14.6</td>
<td>F1B</td>
<td>Bio14.6</td>
</tr>
<tr>
<td>10 weeks</td>
<td>23.7 ± 5.0</td>
<td>22.3 ± 1.6</td>
<td>92.9 ± 26.2</td>
</tr>
<tr>
<td>25 weeks</td>
<td>33.3 ± 4.7 c</td>
<td>25.2 ± 3.1</td>
<td>63.0 ± 13.8</td>
</tr>
<tr>
<td>40 weeks</td>
<td>24.0 ± 2.7</td>
<td>23.1 ± 3.5</td>
<td>78.9 ± 11.1 a</td>
</tr>
</tbody>
</table>

Bio14.6 = cardiomyopathic hamsters; F1B = control hamsters; qANT = quantitative I-125 iodoantipyrine concentrations; BMS/ANT = BMS uptake normalized by qANT.

a P < 0.05, b P < 0.005 vs. F1B by unpaired t-test.

c P < 0.05 vs. 10- and 40-week-old hamsters by ANOVA with Scheffé’s method.

There were no age-dependent differences in F1B.

3.2. Myocardial uptake of BMS-181321

At age 25 weeks, BMS uptake was significantly higher in the cardiomyopathic hamsters than in the controls (P < 0.05), whereas there was no significant difference between the two strains at the other ages (Table 3). Furthermore, BMS uptake in the cardiomyopathic hamsters was significantly higher at age 25 weeks than at age 10 or 40 weeks.

Fig. 2. Autoradiograms of BMS-181321. The myocardial distribution of BMS-181321 was homogeneous in both strains at all ages. Even in Bio14.6 at age 25 weeks, whose BMS uptake was significantly greater than F1B, the BMS-181321 distribution did not reveal regional differences. Scale bar = 2 mm.

Fig. 3. Autoradiograms of I-125 iodoantipyrine. The myocardial distribution of I-125 iodoantipyrine was homogeneous in both strains at all ages. Scale bar = 2 mm.
Table 4

Relative uptake of cardiomyopathic and control hamsters

<table>
<thead>
<tr>
<th></th>
<th>Endocardium</th>
<th>Epicardium</th>
<th>RV wall</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tc-99m nitroimidazole</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 weeks</td>
<td>1.00 ± 0.05</td>
<td>1.00 ± 0.04</td>
<td>1.03 ± 0.13</td>
</tr>
<tr>
<td>F1B</td>
<td>1.00 ± 0.03</td>
<td>1.00 ± 0.02</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>25 weeks</td>
<td>0.98 ± 0.05</td>
<td>0.98 ± 0.06</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>Bio14.6</td>
<td>0.99 ± 0.03</td>
<td>0.99 ± 0.03</td>
<td>1.03 ± 0.13</td>
</tr>
<tr>
<td>F1B</td>
<td>1.02 ± 0.04</td>
<td>1.02 ± 0.04</td>
<td>1.00 ± 0.07</td>
</tr>
<tr>
<td>40 weeks</td>
<td>1.02 ± 0.03</td>
<td>1.00 ± 0.02</td>
<td>0.99 ± 0.04</td>
</tr>
<tr>
<td>F1B</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*I-125 iodoantipyrine*

<table>
<thead>
<tr>
<th></th>
<th>Endocardium</th>
<th>Epicardium</th>
<th>RV wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 weeks</td>
<td>1.00 ± 0.02</td>
<td>1.02 ± 0.04</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>F1B</td>
<td>1.01 ± 0.09</td>
<td>1.04 ± 0.11</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>25 weeks</td>
<td>0.99 ± 0.02</td>
<td>1.02 ± 0.03</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>Bio14.6</td>
<td>0.99 ± 0.02</td>
<td>0.99 ± 0.04</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>F1B</td>
<td>1.02 ± 0.06</td>
<td>1.02 ± 0.06</td>
<td>0.97 ± 0.04</td>
</tr>
<tr>
<td>40 weeks</td>
<td>1.01 ± 0.03</td>
<td>1.02 ± 0.03</td>
<td>0.95 ± 0.04</td>
</tr>
<tr>
<td>F1B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bio14.6 = cardiomyopathic hamsters; F1B = control hamsters.

There are no regional differences among each myocardial regions by ANOVA.

(P < 0.05). In contrast, there were no age-dependent differences in the controls.

3.3. Autoradiography of BMS-181321 and I-125 iodoantipyrine

The myocardial distribution of BMS-181321 (Fig. 2) and of I-125 iodoantipyrine (ANT; Fig. 3) was homogeneous in both strains at all ages. Even in the cardiomyopathic hamsters at age 25 weeks, whose BMS uptake was significantly greater than the controls, the BMS-181321 distribution was homogeneous and did not reveal regional differences. The relative uptake of BMS-181321 and ANT showed no differences among the myocardial regions or between the cardiomyopathic and control hamsters (Table 4). The quantified concentration of ANT in the cardiomyopathic hamsters was significantly lower at age 40 weeks compared to the controls (Table 3). BMS/ANT was significantly higher in the cardiomyopathic hamsters at age 25 weeks (P < 0.005) and 40 weeks (P < 0.05) compared to the controls (Table 3).

4. Discussion

The hereditary cardiomyopathic strain of Syrian hamsters, Bio 14.6, is a natural model of congestive heart failure. Focal myocytolysis begins about 1 month of age, leading to congestive heart failure within 1 year [22]. The histologic progress of cardiomyopathy has been separated into 4 temporal phases: the myocytolytic stage, the fibrotic and healing stage, the hypertrophy and dilatation stage, and the continuing dilatation and terminal heart failure stage [20]. In recent years, it has become well known that disease severity in Bio14.6 is attenuated and that these histopathological changes progress slowly [23]. In our experiments, histologic examination revealed that our strains of Bio14.6 were in the myocytolytic stage at age 10 weeks, in the fibrotic and healing stage at age 25 weeks, and in the hypertrophy and dilatation stage at age 40 weeks.

There is much evidence to support the hypothesis that myocardial ischemia and/or hypoxia plays a significant role in the pathogenesis and development of cardiomyopathy. In cardiomyopathic hamsters, focal and transient spasms of small blood vessels occur before the development of myocytolysis, and that these spasms become more severe with the progress of cellular necrosis [5]. Moreover, embolization of the coronary microvasculature of dogs with 25 or 50 μm microspheres leads to focal myocytolysis very similar to the changes seen in cardiomyopathy [24], and intermittent brief periods of ischemia induced by inflating and deflating the balloon of an intracoronary catheter cause cumulative myocardial necrosis [6]. In human cardiomyopathy, coronary capillary and arteriolar spasms are also observed in patients with a congestive cardiomyopathy [25], and elevated glucose utilization and the decreased myocardial flow exist in hearts with hypertrophic cardiomyopathy [26].

Our study demonstrated that BMS uptake in 25-week-old cardiomyopathic hamsters in the fibrotic and healing stage was significantly greater than in the controls (Table 3) while ANT concentration did not differ. Although BMS uptake was not different at age 40 weeks, normalization by ANT concentration revealed that the BMS/ANT in the cardiomyopathic hamsters was significantly higher than that in the controls (Table 3). Thus, the myocardium of the cardiomyopathic hamsters appeared to be hypoxic at age 25 weeks and may be hypoxic at age 40 weeks.

Radiolabeled iodoantipyrine is used to evaluate myocardial blood flow because iodoantipyrine is inert and rapidly diffusible into intracellular water [27]. Thus, we assumed that ANT concentration reflected the number of myocytes. Although BMS uptake was not different at age 40 weeks on a per-unit-mass basis, normalization by ANT concentration (that is, by the number of viable cells) revealed that BMS/ANT in the cardiomyopathic hamsters was significantly higher than that in the controls (Table 3). This result suggests that the individual myocyte in the cardiomyopathic hamsters took up more BMS-181321 than that in the controls. Alternatively, if the ANT concentration reflects myocardial flow, these data would seem to show an abnormal low-flow state in the 40-week-old hamster’s heart, which was nonetheless normoxic. This could reflect adaptive changes in energy consumption. This also indicates that there were some abnormalities in the microvascular circulation. Even though there were different interpretations of the results obtained in 40-week-old hamsters, it was definitely shown that the myocardium of cardiomyopathic hamsters was hypoxic at age 25 weeks.

Microvascular abnormalities have been observed before the myocytolytic stage [5,28] (i.e., before 10 weeks in this
study). However, hypoxia was detected in the cardiomyopathic hamsters at least at age 25 weeks. The BMS-181321 distribution in the cardiomyopathic hamsters showed no regional differences at all ages (Table 4), whereas multifocal myocytolysis was observed on histologic examination. These results suggest that hypoxia contributes to the development of cardiomyopathy, and may not be associated with myocytolysis.

There are some possible mechanisms for hypoxia in 25-week-old cardiomyopathic hamsters and also for that in 40-week-old ones if hypoxia exists. The cardiomyopathic hamsters at age 25 weeks showed severe fibrotic changes; the fibrosis may impair diffusion and O\textsubscript{2} transport at the capillary [29]. At age 25 and 40 weeks, the cardiomyopathic heart showed a hypertrophy of the myocyte (Table 2). Consequently, the intercapillary distance increases, and this may impede the diffusible metabolic supply and myocardial metabolism [30]. These mechanisms could result in myocardial hypoxia.

In summary, the myocardium in cardiomyopathic hamsters was hypoxic at the fibrotic and healing stage and may be hypoxic at the hypertrophy and dilatation stage. This may contribute to the development of cardiomyopathy.

Acknowledgements

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References


