SENILE CARDIAC AMYLOIDOSIS IN SENESCENCE ACCELERATED MOUSE (SAM)

HIROMICHI OGAWA, M.D.

The characteristics of the senescence accelerated mouse (SAM), a new murine model for accelerated senescence, are early senescence and a high incidence of senile amyloidosis. This study was performed to clarify histopathologically the details of senile cardiac amyloidosis in SAM, and the incidence of amyloidosis in the heart of SAM (−P) was 46.0% (1+: 22.0; 2+: 16.0; 3+: 8.0%). Amyloid infiltrated the ventricular walls, interventricular septum, atrial walls and interatrial septum. Amyloid deposition was prominent around the myocardial fibers and in the vascular walls. Amyloid involvement was greater in the veins than in the arteries. Senile cardiac amyloidosis of SAM was mild or moderate and not severe, in general. The age dependency of amyloidosis incidence of the heart was confirmed. The heart/body weight ratio tended to parallel the grade of cardiac amyloidosis. SAM often had complications such as abscess, lymphoma, skin ulcer, etc. The incidence of amyloidosis was higher in SAM with these complications than in SAM without them. The complications seemed to promote the progress of cardiac amyloidosis and to superimpose secondary amyloidosis. In SAM senile cardiac amyloidosis is less frequent than renal amyloidosis (64.4%) or hepatic amyloidosis (63.3%).

SENESCENCE accelerated mouse (senescence prone series; SAM-P), a murine model for accelerated senility, was developed by Takeda, through sister-brother breeding from AKR mouse.1,2

A senescence resistant series of mice (aging normally) also resulted from this inbreeding and was named SAM-R; it serves as a control in experiments on SAM-P. As reported previously, the degree of senescence (grading score) in these mice is high.1,3 This high grading score in SAM-P is ascribed to the early onset of loss of reactivity, loss of glossiness and increased coarseness of the skin, hair loss, periorphthalmic lesions, increased lordokyphosis of the spine and a more marked increase in their severity with aging as compared to SAM-R. The earlier onset and prominent advance of senescence and the high frequency of senile amyloidosis are the characteristics of SAM-P.

This study was done to clarify the pattern of distribution and the progress of senile cardiac amyloidosis in SAM and the relationship between cardiac amyloidosis and frequent complications such as abscesses of various organs, nonthymic and thymic lymphoma, skin ulcer and/or gangrene and tumors.

MATERIALS AND METHODS

Hearts from 100 SAM-P (with sublines P1, P2 and P3) and SAM-R (with sublines R1 and R2) which were found dead during routine inspections of SAM (three times a week) and were

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Key words:
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Murine amyloidosis
Amyloidosis

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TABLE I

<table>
<thead>
<tr>
<th>1. Materials</th>
<th>Mean Lifespan</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-P</td>
<td>100 (P1 43, P2 31, P3 26)</td>
</tr>
<tr>
<td>SAM-R</td>
<td>78 (R1 52, R2 26)</td>
</tr>
</tbody>
</table>

2. Grade and Frequency of Amyloid Deposition (%)

<table>
<thead>
<tr>
<th></th>
<th>−</th>
<th>+</th>
<th>2+</th>
<th>3+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-P</td>
<td>54.0</td>
<td>22.0</td>
<td>16.0</td>
<td>8.0</td>
<td>46.0 (46/100)</td>
</tr>
<tr>
<td>SAM-R</td>
<td>79.5</td>
<td>16.7</td>
<td>3.8</td>
<td>0</td>
<td>20.5 (16/78)</td>
</tr>
</tbody>
</table>

3. Frequency of Amyloidosis in sublines of SAM-P (%)

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>65.1</td>
<td>35.5</td>
<td>26.9</td>
<td>46.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>72.1</td>
<td>58.1</td>
<td>69.2</td>
<td>64.4</td>
</tr>
<tr>
<td>Liver</td>
<td>71.4</td>
<td>53.3</td>
<td>61.2</td>
<td>63.3</td>
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</tbody>
</table>

4. Heart/Body Weight Ratio (%)

<table>
<thead>
<tr>
<th></th>
<th>−</th>
<th>+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-P</td>
<td>0.82</td>
<td>0.82</td>
<td>0.88</td>
<td>1.09</td>
</tr>
<tr>
<td>SAM-R</td>
<td>0.76</td>
<td>0.85</td>
<td>0.98</td>
<td>1</td>
</tr>
</tbody>
</table>

5. Cardiac Amyloidosis and Complications (%)

<table>
<thead>
<tr>
<th></th>
<th>No Complication</th>
<th>with Complications</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-P</td>
<td>18.0 (18/100)</td>
<td>28.0 (28/100)</td>
<td>46.0 (46/100)</td>
</tr>
<tr>
<td>SAM-R</td>
<td>7.7  (6/78)</td>
<td>12.8 (10/78)</td>
<td>20.5 (16/78)</td>
</tr>
</tbody>
</table>

Fig.1. Slight amyloid infiltration surrounding myocardial fibers and in epicardium. (Yellow gold fluorescence in specimen stained with thioflavin T, × 50)

Fig.2. Moderate amyloid deposition around myocardial fibers and in superficial coronary artery and the pericardium. (H & E, × 30)

suitable for necropsy (not decayed) were fixed in 10% neutralized formalin and embedded in paraffin. H & E (4 μm sections) and alkaline Congo red stain (6 μm sections) were used for light microscopy. Amyloid was identified in the heart tissue stained with alkaline Congo red with

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a light microscope and a polarizing microscope (Olympus) by its green birefringence and in specimens stained with thioflavin T (4 μm sections) with a fluorescence microscope (Nikon) by its yellow gold fluorescence.

In half of the heart specimens used in this series (SAM-P and -R), the affinity of Congo red after incubation with potassium permanganate was examined by the method of Wright et al.5

RESULTS

In the present series the mean lifespan of SAM-P and SAM-R was 9.0 (± 4.4) months and 12.4 (± 6.7) months, respectively (Table I). In general, amyloid deposition was not discernible from the gross appearance of the heart. Suppurative pericardomyocarditis following cardiac abscess was observed in 2 hearts of SAM-P.

Microscopic examination showed amyloid in biventricular walls, the interventricular septum, both atrial walls and the interatrial septum. Amyloid was deposited around the myocardial fibers and in the blood vessel walls. Vascular deposits were observed in the superficial coronary arteries and in the veins of the interstitium, but many arteries in the interstitium were free of amyloid. As perifiber amyloid deposition increased, myocardial fibers were compressed and atrophied (Figs. 1, 2 and 3). Cardiac amyloidosis was classified into 3 grades:

Grade 1 (1+): Slight localized amyloid deposition.
Grade 2 (2+): Moderate patchy amyloid deposition.
Grade 3 (3+): High grade extensive amyloid deposition.

The incidence of amyloidosis of the heart was 46.0% in SAM-P and 20.5% in SAM-R (Table I). The grade and the incidence of cardiac amyloidosis in SAM-P are summarized in Table I: 1+ 22.0%, 2+ 16.0%, 3+ 8.0%.

Amyloid infiltrated the pericardium and epicardium in some SAM, and mitral and aortic valves and the root of the aorta in a few of these. Amyloid deposition was more prominent in the outer and medial layers of the left ventricle wall, the outer layer of the right ventricle wall and the right side of the interventricular septum.

The grade and the incidence of amyloidosis of the heart increased with age, as shown in Fig. 4.

The heart/body weight ratio tended to parallel the grade of cardiac amyloidosis in SAM-P (Table I). SAM often had various complications, such as abscesses of many organs (lung, liver, kidney, heart, skin and orbit), nonthymic and thymic lymphoma, skin ulcer and/or gangrene and tumors, (SAM-P 54.0%, SAM-R 55.1%). The incidence of amyloid deposition in SAM-P with these complications was 28.0%, and in SAM-P without them it was 18.0% (Table I).

The incidence of cardiac amyloidosis of
<table>
<thead>
<tr>
<th>Author (et al.)</th>
<th>Year</th>
<th>Strain</th>
<th>Ventricles</th>
<th>Atrium</th>
<th>Aorta</th>
<th>Interstitium</th>
<th>Blood vessels</th>
<th>Involved organs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LV</td>
<td>RV</td>
<td>IVS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gorer, TB.</td>
<td>1940</td>
<td>C57 black</td>
<td>(+, Patchy areas of amyloid frequent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kidney, Adrenal</td>
</tr>
<tr>
<td>Dunn, TB.</td>
<td>1944</td>
<td>Hybric ABC</td>
<td>(+, Surrounding muscle fibers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kidney, Spleen, Liver, Duodenum, Stomach, Colon, Lung, Ovary, Testicle, Adrenal, Endometrial stroma, Skin</td>
</tr>
<tr>
<td>Thung, PJ.</td>
<td>1957</td>
<td></td>
<td>(+, Surrounding muscle fibers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kidney, Adrenal, Ovary, Testes, Spleen, Liver, Intestine, Pancreas, Thyroid, Salivary gland</td>
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<tr>
<td>Mühlbock, O.</td>
<td>1957</td>
<td></td>
<td>1. DBA\textsuperscript{f}</td>
<td>2. O\textsuperscript{30}</td>
<td>3. O\textsuperscript{30} xDBA\textsuperscript{f}</td>
<td></td>
<td></td>
<td>1. Adrenal, ovary, Kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Adrenal, Kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. Various organs</td>
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<tr>
<td>West, WT.</td>
<td>1965</td>
<td>A/Sn</td>
<td>+</td>
<td>+</td>
<td>Only slightly affected</td>
<td>Pericapillary</td>
<td></td>
<td>Lung, Tongue, Stomach, Cervical lymph node, Duodenum, Colon, Mesenteric lymph node, Liver, Testes, Spleen, Urinary bladder, Salivary gland</td>
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<tr>
<td>Zchiesche, W.</td>
<td>1972</td>
<td>AB\textsuperscript{J}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spleen Liver, Kidney,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/J Han Jena</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scheinberg, MA</td>
<td>1976</td>
<td>SJL/J</td>
<td>(+, moderate to large amounts of hyaline material in subepicardia &amp; interstitial area)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Liver, Kidney, Lymph node</td>
</tr>
<tr>
<td>Chai, SK.</td>
<td>1976</td>
<td>LLC</td>
<td>(+, trace ~ large localized areas)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spleen, Kidney, Liver, Adrenal, Tongue, Intestine, Pancreas, Lung, Thyroid, Lymph node</td>
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<tr>
<td>Næsø, P.</td>
<td>1977</td>
<td>obob</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adrenal, Liver, Spleen, Kidney, Pancreas, Adipose tissue</td>
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<tr>
<td>Eisenbud, L.E.</td>
<td>1981</td>
<td>LLC</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Spleen, Kidney</td>
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<tr>
<td>Present study</td>
<td>1985</td>
<td>SAM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Systemic</td>
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SAM-P with only lymphoma, only abscess, only skin ulcer and/or gangrene and mixed complications was 55.6%, 40.0%, 25.0% and 33.3%, respectively. Deposited amyloid was potassium permanganate resistant.

DISCUSSION

In this series the incidence of cardiac amyloidosis in SAM-P is higher than in SAM-R and the mean lifespan of SAM-P is shorter than that of SAM-R. However, the incidence of complications is higher in SAM-R than in SAM-P. It is supposed that SAM-P dies young from early senescence or senile amyloidosis, and/or has weak resistance to complications.

Spontaneous murine amyloidosis and human senile amyloidosis have been described by many investigators\cite{1-35} and the pattern of amyloid distribution is diverse (Table II). It has been noted that mice are susceptible to senile and experimental amyloidosis, which is more severe in mice than in other species\cite{8,36} However, none of the reports made an analysis of senescence or of the relationship between senility and murine amyloidosis. Deposited amyloid in some mice seems to be senile amyloid, but in others it is not so clear. The molecular weight of this unique

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senile amyloid of SAM (AS/SAM) is 5,200 daltons, as reported previously. The descendants of SAM have been maintained as a newly established animal model for accelerated senescence and a comprehensive study of senility and amyloidosis was first performed in SAM.

Amyloid deposition in the heart in SAM was often patchy, and in some cases it was more extensive. Severe diffuse cardiac amyloidosis was rare, and amyloid involvement of the heart did not seem to be the direct cause of death in most SAM, although involvement of the conduction system in the heart awaits further study. Peripheral amyloid infiltration and greater amyloid involvement of the veins than the arteries were the features of the cardiac amyloidosis of SAM.

This study confirmed that the incidence of cardiac amyloidosis is age dependent in SAM-P (r = 0.54), but not in SAM-R (r = 0.26). The incidence of cardiac amyloidosis is different among the 3 sublines of SAM-P but some older or very elderly SAM-P have no amyloid involvement (Fig. 4, Table I).

These findings suggest differences among sublines, a lower tendency to amyloidosis especially in the heart of SAM (Table I) or appearance of a new subline during inbreeding. In fact, various sublines (and series) of SAM have been readjusted during inbreeding.

The higher incidence of cardiac amyloidosis in SAM with complications than in SAM without suggests that amyloidosis maybe increased by further acceleration of senescence due to complications and that secondary amyloidosis is superimposed on senile amyloidosis because of such complications. Simultaneous deposition of AS/SAM and protein AA in the liver of SAM has been demonstrated immunohistochemically and it has also been observed in the heart.

Many authors, but not Pirkkänen et al, have noted that human senile amyloid deposition is confined to a few organs, especially the heart. In organs other than the heart, amyloid infiltration is infrequent, mild, and mostly confined to the blood vessels. Senile cardiac amyloidosis (SCA) isolated atrial amyloidosis (IAA) senile aortic amyloidosis (SAAO) and senile seminal vesicle amyloidosis (SSVA) have been reported in human senile amyloidosis.

Senile amyloidosis of SAM is systemic and involves almost all organs, as well as both the ventricular and the atrial walls in the heart. It is noteworthy that the incidence of amyloidosis of the heart in SAM-P (46.0%) is lower than that of the kidney (64.4%) or the liver (63.3%).

Amyloidosis in SAM is being studied further, and the species differences will also be investigated.

Acknowledgment
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