Peculiar Mitral Valve and Papillary Muscle Lesions Induced by Vagus Manipulations in Rabbits
An Experimental Model for Nonrheumatic Mitral Regurgitation

Kouji Imataka, M.D., Kazuhide Yamaoki, M.D., Akira Seki, M.D., Yoshiaki Takayama, M.D., and Jun Fujii, M.D.

SUMMARY
Peculiar lesions of mitral valves and papillary muscles developed in 50 to 80% of rabbits after clipping (N=38), crushing (N=15) or electrical stimulation (N=44) of their cervical vagi. Both right and left vagus manipulations induced similar cardiac lesions. The earliest manifestation was bleeding of the mitral leaflets, which was followed by swelling and fibrosis of the papillary muscles. Ventricular arrhythmias and systolic murmurs were frequently associated with the vagus manipulations. During or just after electrical stimulation of the vagus, ventricular premature contractions (VPCs) were observed in 30 of 44 animals. Twenty-nine of these 30 animals with VPCs (96.7%) were found to have mitral valve and/or papillary muscle lesions at autopsy, whereas only 5 of 14 animals without VPCs (35.7%) had the cardiac lesions. These results indicate that the occurrence of these ventricular arrhythmias during vagus manipulations was closely related with the mitral valve and papillary muscle lesions.

Additional Indexing Words:
Mitral valve prolapse Mitral valve bleeding Systolic murmurs Ventricular premature beats

SINCE the development of echocardiography, mitral valve and papillary muscle diseases have drawn increasing attention. However, the cause and mechanisms of these diseases are poorly understood. Some investigators have proposed that abnormal autonomic neural function is involved in the pathogenesis of mitral valve prolapse syndrome.1,2) We have recently found
that simple manipulations of the cervical vagus produce peculiar lesions con-
fined to the mitral valves and papillary muscles in rabbits. Mid- to late-
systolic murmurs can be auscultated in some of the animals. These lesions
are quite different in their distribution from those produced by electrical
stimulation of the stellate ganglion or by catecholamine infusions. The char-
acteristics and pathogenesis of the lesions have not been examined in detail.
This paper (1) compares cardiac lesions after right or left vagus manipulation,
(2) compares effects of different manipulations of the vagus, (3) documents
the time course of development of the lesions and (4) investigates the relation-
ship between ventricular arrhythmias occurring during the vagus manipula-
tions and the development of cardiac lesions.

MATERIALS AND METHODS

Cardiac lesions were examined 3 hours to 7 days after manipulation of
the cervical vagus in male rabbits weighing about 2 Kg. Under pentobar-
bital anesthesia (30 mg/Kg, i.v.), the right or the left cervical vagus was ex-
posed and either clipped loosely with a silver clip 0.7 mm in diameter, crushed
gently by forceps, or stimulated electrically. The electrical stimuli were 50
Hz square wave pulses, 1 msec in duration (0.3 to 0.8 V) given for 10 sec.
The voltage was adjusted to evoke marked sinus bradycardia. The stimula-
tion was repeated at 30-sec intervals for 10 to 30 min. Intact or sham-operated
animals served as controls. After sacrifice, hearts were excised and the endo-
cardial surfaces were exposed by a longitudinal incision. Special attention
was directed to the mitral valves, papillary muscles and chordae tendinae
during gross examination. Lesions were easily detected by visible deposits
of pretreated colloidal carbon, injected intravenously just before or 3 hours
after the vagus manipulations and once a day for the next 2 days. Injected
colloidal carbon was cleared from the blood by the reticuloendothelial system
in about 2 hours. Carbon particles were deposited into the damaged tissues
where the endothelial permeability was increased.

In experiment 1, the incidence and distribution of cardiac lesions were
compared between animals with the right vagus (N=20) or left vagus (N=
24) electrical stimulation. All animals were killed after 7 days. In experi-
ment 2, the cardiac lesions induced by three different manipulations [right or
left vagus clip (N=35), left vagus crush (N=15) and the electrical stimu-
lation of the right or the left vagus (N=44)] were also compared. Thirty-
three animals served as controls. In experiment 3, the time course of the
cardiac lesions was examined in animals at various intervals between the vagus
manipulation and sacrifice. Twenty animals were killed 1 day, 17 animals
2 days and 15 animals 7 days after crush of the right or the left vagus. Eleven animals were killed 3 hours and 44 animals were killed 7 days after electrical stimulation of the right or the left vagus. In experiment 4, relationships between ventricular arrhythmias, heart murmurs and cardiac lesions were examined in 44 animals that were killed 7 days after electrical stimulation of the vagus. In these animals the electro- and phonocardiograms were monitored during electrical stimulation. Chi-square analysis was used to assess statistical significance.

Results

1. Comparison of cardiac lesions after right and left vagus manipulation

Table I shows the incidence of cardiac lesions in 20 animals after right vagus and 24 animals after left vagus electrical stimulation. The characteristic lesions were identified by visible carbon deposits found in the mitral valves and papillary muscles. The papillary muscles were swollen and stiff. Both mitral valve and papillary muscle lesions were found in 80.0% of animals after right vagus, and in 66.7% after left vagus stimulation. The differences were not statistically significant. Compared with the mitral valve complex, other parts of the heart (e.g., left ventricular free wall and interventricular septum) were relatively free of lesions.

<table>
<thead>
<tr>
<th>Electrical stimulation</th>
<th>Cardiac lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mitral valve</td>
</tr>
<tr>
<td>Right vagus (N=20)</td>
<td>16 (80.0%)</td>
</tr>
<tr>
<td>Left vagus (N=24)</td>
<td>16 (66.7%)</td>
</tr>
</tbody>
</table>

Table II. Comparison of Cardiac Lesions after Different Manipulations of the Vagus

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Cardiac lesions after 7 days</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mitral valve</td>
</tr>
<tr>
<td>Vagus clip (N=35)</td>
<td>18 (51.4%)**</td>
</tr>
<tr>
<td>Vagus crush (N=15)</td>
<td>11 (75.3%)**</td>
</tr>
<tr>
<td>Electrical stimulation of vagus (N=44)</td>
<td>32 (72.7%)**</td>
</tr>
<tr>
<td>Control</td>
<td>4 (12.1%)</td>
</tr>
</tbody>
</table>

Compared to control: ** p<0.01.
2. Comparison of cardiac lesions after different manipulations of the vagus

Table II shows the incidence of cardiac lesions in animals after three different vagal manipulations. The lesions of mitral valves, papillary muscles and other parts of left ventricle developed in 51.4%, 60.0% and 20.0% of animals with a vagus clip (N=35), respectively; in 73.3%, 80.0% and 0% of animals after vagus crush (N=15) and in 72.7%, 72.7% and 15.9% of animals after electrical stimulation (N=44). There were no significant differences in the incidence of mitral valve and papillary muscle lesions among the three vagus manipulations. The incidence of mitral valve and papillary muscle lesions induced by the vagus manipulations was significantly greater than in the control animals.

These results indicate that mitral valve and papillary muscle lesions are not directly related to either the laterality or the nature of the vagus manipulations. Fig. 1 shows typical lesions of the mitral valves and papillary muscles in a rabbit killed 7 days after left vagus crush.

3. Time course of cardiac lesions

To investigate the time course of cardiac lesions, animals were killed at various intervals after the vagus manipulations. Table III shows the incidence of mitral valve and papillary muscle lesions in 20 animals 1 day, 17 animals 2 days and 15 animals 7 days after left vagus crush, and in 11 animals 3 hours and 44 animals 7 days after electrical stimulation of the right or left vagus. As shown in Table III, the mitral valve lesions developed earlier

Fig. 1. Typical mitral valve and papillary muscle lesions in a rabbit killed 7 days after crushing the left vagus. Carbon deposits on mitral valves and papillary muscles are visible. Papillary muscle swelling and increased stiffness are also apparent.
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Table III. Incidence of Cardiac Lesions in Different Stages

<table>
<thead>
<tr>
<th></th>
<th>Mitral valve</th>
<th>Papillary muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagus crush</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day (N = 20)</td>
<td>12 (60.0%)</td>
<td>3 (15.0%)</td>
</tr>
<tr>
<td>2 days (N = 17)</td>
<td>11 (64.7%)</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>7 days (N = 15)</td>
<td>11 (73.3%)</td>
<td>12 (80.0%)</td>
</tr>
<tr>
<td>Electrical stimulation</td>
<td>3 hours (N = 11)</td>
<td>9 (81.8%)</td>
</tr>
<tr>
<td></td>
<td>7 days (N = 44)</td>
<td>32 (72.7%)</td>
</tr>
</tbody>
</table>

* p<0.05,  ** p<0.01.

Fig. 2. Representative mitral valve and papillary muscle bleeding 3 hours after electrical stimulation of the left vagus. The bleeding is massive in posterior mitral leaflet.

than papillary muscle lesions. The mitral valve lesions were found in 12 of 20 animals (60.0%) killed 1 day after vagus crush, but papillary muscle lesions were found in only 3 of these animals (15.0%) at that time. The incidence of papillary muscle lesions increased with time and reached 12 of 15 animals (80.0%) after 7 days. There was a significant difference in the incidence of papillary muscle lesions between animals killed 1 day and 7 days after the vagus crush (p<0.01). A similar time course was observed in animals after electrical stimulation of the vagus. Papillary muscle lesions were detected in only 4 of 11 animals (36.4%) examined 3 hours after electrical stimulation and were found in 32 of 44 animals (72.7%) examined after 7 days. The difference between the 2 groups was statistically significant (p<0.05).

Bleeding into the mitral valve was the most prominent lesion in the initial stage. Among 11 animals killed 3 hours after electrical stimulation, 9 had bleeding into the posterior mitral leaflet and the lesion extended into the
anterior leaflet in 6 of these animals. Fig. 2 shows massive bleeding into the mitral valve, especially in the posterior leaflet, in an animal killed 3 hours after electrical stimulation of the left vagus. The hematomas disappeared with time and were completely absorbed in the animals killed after 7 days, and only carbon deposits remained in the mitral valves. The papillary muscle lesions were obscure in the early stages but became obvious with time. After 7 days, they were characterized by swelling and increased stiffness with carbon deposits.

4. Relation of ventricular arrhythmias and systolic murmurs to the cardiac lesions

Transient ventricular arrhythmias were observed during the vagus manipulations on an electrocardiographic monitor. Almost all of the arrhythmias were ventricular premature contractions (VPCs), which frequently occurred in a bigeminal or trigeminal pattern. These VPCs had such long coupling intervals that they usually superimposed the next P waves (R-on-P phenomenon). Fig. 3 shows frequent VPCs with long coupling intervals on a tracing recorded during the electrical stimulation of the left vagus. The transient VPCs appeared during the vagus manipulations and lasted for several minutes. Mid- to late-systolic murmurs (with or without systolic clicks) were auscultated during or immediately after the appearance of VPCs. They increased in intensity in a few minutes, lasted 3 to 7 days and virtually disappeared after 7 days. Fig. 4 shows phonocardiograms recorded before and after electrical stimulation in an animal.

Table IV shows the relation of VPCs and systolic murmurs to the cardiac lesions in 44 animals in which electrocardiograms were monitored during electrical stimulation of the right or the left vagus. All animals were killed after 7 days. VPCs were observed during electrical stimulation in 30 of 44
Table IV. Relation of VPCs and Systolic Murmurs to the Cardiac Lesions in Animals after Electrical Stimulation of the Vagus

<table>
<thead>
<tr>
<th>VPCs</th>
<th>Systolic murmurs</th>
<th>Cardiac lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+) 30</td>
<td>(+) 19 (63.3%)</td>
<td>(+) 19 (100%)</td>
</tr>
<tr>
<td></td>
<td>(−) 11 (36.7%)</td>
<td>(−) 0 (0%)</td>
</tr>
<tr>
<td>(−) 14</td>
<td>(+) 2 (14.3%)</td>
<td>(+) 2 (100%)</td>
</tr>
<tr>
<td></td>
<td>(−) 12 (85.7%)</td>
<td>(−) 9 (75.0%)</td>
</tr>
</tbody>
</table>

Animals (68.2%) and 29 of these 30 animals (96.7%) were found to have cardiac lesions at autopsy. On the other hand, cardiac lesions were found in 5 of the 14 animals (35.7%) that did not display VPCs, a significantly smaller proportion than in animals with VPCs (p<0.01). Systolic murmurs were auscultated in 21 of 44 animals, and all 21 animals with murmurs had cardiac lesions. These results indicate that the cardiac lesions are closely related to the VPCs and the systolic murmurs.

**DISCUSSION**

The present study showed that clipping, crushing and electrical stimula-
tion of the cervical vagus were equally likely to produce mitral valve and papillary muscle lesions in 50–80% of the animals. The laterality of vagal electrical stimulation did not affect the incidence of cardiac lesions. These results can be explained, not by direct vagus effects on any localized areas of the heart, but by a common pathway that exerts specific effects on the mitral valves and papillary muscles. The common pathway is probably related to the occurrence of the R-on-P type VPCs because the cardiac lesions developed in almost all animals in which the VPCs had been observed during the vagus manipulations. When VPCs occur on P waves, the atrium and ventricle contract simultaneously and left ventricular distorted contractions arise, both of which may produce abnormal tension on the mitral valves and papillary muscles. However, we did not determine whether crush or clip of the vagus induced acute premature contractions. Furthermore, the reason why 12% of control animals would have lesions of the mitral apparatus is unclear, but may be explained by the influences of pentobarbital anesthesia or a “false positive” rate associated with the colloidal carbon method. The fact that 5 of 14 animals with no VPCs exhibited mitral lesions may also be explained by anesthesia during operation or “false positives”, or by overlooking of VPCs which occurred after electrocardiographic monitoring. The detailed mechanism of these cardiac lesions should be further investigated.

Considering the time course of the cardiac lesions, mitral valve bleeding, especially to the posterior leaflet, is probably the earliest change, because mitral valves are more vulnerable to mechanical tension during R-on-P type VPCs than papillary muscles. These mitral valve infarcts may indicate mitral valve dysfunction, resulting in heart murmurs immediately after the vagus manipulations. Mid- to late-systolic heart murmurs were occasionally followed by a systolic click that resembled mitral valve prolapse syndrome in humans. Papillary muscle lesions characterized by swelling and increased stiffness may be secondary to the acute mitral valve regurgitation. Our pre-

![Graph](Fig. 5. Time courses of several parameters after vagus manipulation are illustrated. Vertical arrows indicate the time of carbon injection.)
Previous study has revealed that these papillary muscle changes correspond to myocardial degeneration and interstitial fibrosis on microscopic observation. The suspected time courses of the cardiac lesions are illustrated in Fig. 5.

The distribution of cardiac lesions produced by the vagus manipulations was substantially different from lesions after sympathetic stimulation, administration of catecholamines or stimulation of various brain structures. In contrast to sympathetic or catecholamine cardiomyopathy in which focal myocardial necroses were disseminated in the ventricular free wall, interventricular septum and papillary muscles, the cardiac lesions produced by the vagus manipulations were confined to the mitral valves and papillary muscles.

Production of cardiac lesions through vagus stimulation has been challenged by some investigators. Manning et al. carried out prolonged electrical stimulation of the vagus nerve in dogs to investigate the harmful effects of vagus stimulation on the myocardium; they observed non-specific myocardial damages including papillary muscle infarction and atrioventricular valve congestion and bleeding. They also observed systolic murmurs many times throughout the experiments without further comments. However, the vagus was stimulated for more than 24 hours and, in the longest case, for 120 hours, periods that were far beyond the realm of physiological limits. Their conclusion was that parasympathetic over-excitation resulted in myocardial damage, the role of ventricular arrhythmias in myocardial damage was not discussed. Groover and Stout electrically stimulated the baboon vagus nerve more gently and examined cardiac lesions 4 weeks later. They observed myocardial fibrosis in the anterior part of the interventricular septa, the anterior wall of the left ventricle and the anterior papillary muscle. In contrast to these experiments, we have successfully produced specific cardiac lesions confined to the mitral valve and papillary muscles in rabbits by simple manipulations of the vagus, and showed a close relationship between R-on-P type VPCs induced by vagus manipulations and development of cardiac lesions.

References

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