VENTRICULAR TACHYARRHYTHMIA-RELATED BASAL CARDIOMYOPATHY IN RABBITS WITH VAGAL STIMULATION—A NOVEL EXPERIMENTAL MODEL FOR INVERTED TAKOTSUBO-LIKE CARDIOMYOPATHY

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Summary
Background: Electrical stimulation of the intact (unsectioned) cervical vagus in rabbits frequently provokes ventricular tachyarrhythmias that are often accompanied by mitral regurgitation. Unique pathological lesions often arise on the mitral valve, papillary muscles, and mitral annulus (mitral complex), the latter two of which become swollen and stiffened. These lesions are reversible in nature. Previous studies have essentially ignored the basal portion except for the mitral annulus. Therefore, the present study examined pathological lesions on the left ventricular basal portion in rabbits.

Methods: The intact right vagal nerves of 20 anesthetized rabbits were repeatedly electrically stimulated under electrocardiographic monitoring. Colloidal carbon (1 ml) was injected intravenously immediately after the end of the stimulation and all animals were killed 1 week later. Pathological lesions were identified as carbon deposits visible at gross examination.

Results: Ventricular bigeminy was induced after vagal stimulation in 15 (75%) of the 20 rabbits. Pathological lesions were evident on the basal portion in 16 (80%) and on the mitral valve and papillary muscles of 15 (75%) of the 20 rabbits. Ventricular bigeminy was closely associated with the development of the pathological lesions, which were rarely observed on the ventricular apex.

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Introduction

Interest in arrhythmia-induced cardiomyopathy has recently increased. Persistent supraventricular or ventricular tachycardias can induce reversible cardiac lesions known as tachycardia-induced cardiomyopathy and frequent ventricular premature contractions (VPCs) can induce reversible cardiac lesions. A series of experimental studies using rabbits at our laboratory has shown that repeated electrical stimulation of the intact vagus frequently provokes ventricular tachyarrhythmias that are often followed by transient mitral regurgitation and the development of unique pathological lesions on the mitral complex. The involved papillary muscles and mitral annulus become swollen and stiffened. These lesions are reversible in nature. The distribution of these lesions differs considerably from that of tachycardia-induced cardiomyopathy, which involves all cardiac chambers.

We previously observed lesions predominantly on the mitral complex and hardly examined those on the basal portion except for the mitral annulus. Therefore, the present study examines pathological lesions that develop on the basal portion of the left ventricles upon electrical stimulation of the intact vagus in rabbits.

Materials and Methods

Our institutional Animal Care Committee approved the study protocol. The experimental procedures have already been described elsewhere. In brief, 20 fasting female rabbits weighing about 2 kg were anesthetized using intravenous pentobarbital sodium (20 mg/kg). Electrical stimulations of 50 Hz and 1 ms duration with stepwise voltage increases from 0.1 to 1.0 volt were applied to the right cervical intact vagus of the rabbits under electrocardiographic monitoring. Each stimulation was maintained for 1–2 min with pauses for 2–3 min between each for a period of about 1 h. Mitral regurgitation before and after the procedures was examined using a stethoscope and color Doppler echocardiography. In rabbits, signals of mitral regurgitation were never observed in the late systolic period before vagal stimulation. Therefore, significant mitral regurgitation was defined as any signal of mitral regurgitation in the late systolic period on color Doppler images. We always compared the color Doppler echocardiogram after vagal stimulation with that before vagal stimulation. Systolic regurgitant murmur was frequently heard when mitral regurgitation was observed. Anesthesia was maintained at a level that was light enough to allow regular spontaneous respiration. Immediately after completing the vagal stimulation, 1 ml of colloidal carbon (Sho-eki, Kaimei, Saitama, Japan) was injected via a marginal ear vein to detect cardiac lesions later at autopsy.

Conclusion: Cardiomyopathic lesions involving the basal portion and mitral complex were frequently induced in rabbits by vagal stimulation. These lesions bear a close similarity in distribution and reversibility to inverted Takotsubo cardiomyopathy.
Figure 1  Typical electrocardiogram before (upper column) and during (middle and lower columns) continuous vagal stimulation. No arrhythmias are evident before stimulation. Ventricular bigeminy that developed at 6 s after starting stimulation was converted into non-sustained monomorphic ventricular tachycardia and then into ventricular bigeminy.

Figure 2  Typical macroscopic findings of whole lesions in left ventricle. Pathological lesions identified as carbon deposits have developed on entire basal portion, mitral valve and papillary muscles of left ventricle but never on apical area at gross examination. Involved basal portion and papillary muscles are swollen and stiff. AO, aorta; PM, papillary muscle.

the mitral valve and hypeeosinophilia of the myocytes were also observed in the basal portion and involved papillary muscles, although they are not shown in Fig. 4. Table 1 shows the incidence of pathological lesions on each part of the left ventricle. The lesions frequently developed on the basal portion as well as on the mitral valve and papillary muscles. Ventricular bigeminy and the unique lesions were closely related. Typical lesions involved the basal portion, mitral valve, and papillary muscles in 12 (80%) of the 15 animals with ventricular bigeminy and in only 1 (20%) of 5 animals without ventricular bigeminy. The incidence of lesions was significantly greater in animals with ventricular bigeminy than in those without ventricular bigeminy (p < 0.04). How-

| Table 1  Incidence of peculiar cardiac lesion in 20 animals with vagal stimulation. |
|-----------------------------------|-------------------|
| Lesions                           | Incidence         |
| Ventricular basal portion         | 16 (80%)          |
| Whole lesions                     | 8 (40%)           |
| Partial lesions                   | 8 (40%)           |
| Mitral valve and papillary muscles | 15 (75%)         |
| Apical area                       | 0 (0%)            |
Figure 3  Typical macroscopic findings of partial lesions in left ventricle. Pathological lesions identified by carbon deposits on partial basal portion (subaortic lesion and part of mitral annulus). AO, aorta; PM, papillary muscle.

Figure 4  Microscopic examination shows focal myocardial cell necrosis, disseminated interstitial fibrosis and colloid carbon deposits (arrows) in involved basal portion and papillary muscles. Such pathological findings are scarce in apical area (Masson-trichrome stain).

However, we identified some discordance between ventricular bigeminy and pathological lesions in a few rabbits. The lesions were not found in two rabbits that developed ventricular bigeminy during vagal stimulation. On the other hand, these lesions developed only on the basal portion in four rabbits and only on the mitral complex in one that had developed sporadic VPCs without bigeminal rhythm.

Discussion

The present study demonstrated that ventricular tachyarrhythmia-related pathological lesions frequently developed on the basal portion as well as on the mitral valve and papillary muscles in rabbits after vagal stimulation. The unique distribution of these lesions involving
the basal portion but not the apical area implies that they can serve as a novel experimental model of basal cardiomyopathy. The cardiac lesions without involvement of the apical area in the rabbits were somewhat similar to inverted Takotsubo cardiomyopathy in humans [15–18] with respect to the following three points. The first is that the apical area remained free of lesions in the rabbits, as it does in human inverted Takotsubo cardiomyopathy. The second is the reversibility of the lesions: both inverted and regular Takotsubo cardiomyopathy are reversible [17,18]. The cardiac lesions in the present experimental animals were not progressive and were rather regressive. We previously reported that the hydroxyproline content of the left ventricular basal portion 1 week after vagal stimulation was significantly increased compared with that in control animals, and that the hydroxyproline content of the left ventricular basal portion 4 weeks after vagal stimulation was significantly decreased compared with that 1 week after stimulation [12]. Immunohistochemical stains also indicated that the increased type III collagen in the perianular tissue at 1 week after vagal stimulation clearly regressed in 4 weeks. We also reported that swelling and stiffness of mitral ring and papillary muscle improved 3 or 4 weeks after vagal stimulation using Doppler echocardiography and autopsy [13]. In the present study, we did not evaluate cardiac function, but we are now measuring cardiac function before, and 1, 2, 4, 6, and 8 weeks after vagal stimulation using tissue Doppler echocardiography and autopsy [13]. The third is involvement of neurogenic mechanisms in the pathogenesis of cardiac lesions. Stress and catecholamines might be pathogenetic factors involved in inverted Takotsubo cardiomyopathy [19,20]. One case report described a patient with subarachnoid hemorrhage complicated with inverted Takotsubo cardiomyopathy-like dysfunction and functional mitral regurgitation [21]. Of course, the cardiac lesions in the experimental animals developed based on a neurogenic mechanism initiated by vagal stimulation. In the present study, we stimulated the intact (both afferent and efferent) vagus. Stimulation of the intact vagus may cause reflex sympathetic nerve stimulation. We will present a study in the future that stimulation of only afferent vagus causes similar cardiac lesions as stimulation of the intact vagus. We previously reported that injection of adrenaline or noradrenaline in rabbit made similar cardiac basal lesion as vagal stimulation, but injection of isoproterenol did not [22]. We also reported that α-adrenergic blockade reduced the development of the adrenaline-induced cardiac basal lesion, but β-adrenergic blockade did not [23]. On the other hand, the two conditions differ somewhat. The first is the role of arrhythmias. Although the development of cardiac lesions in the rabbits was closely associated with ventricular tachyarrhythmias, inverted Takotsubo cardiomyopathy does not seem to involve arrhythmia. However, the possibility that arrhythmias of short duration develop under stress and excessive catecholamine release in patients with inverted Takotsubo cardiomyopathy cannot be ruled out. The second dissimilarity is complication with lesions of the mitral complex. This complication has been identified in patients with regular Takotsubo cardiomyopathy [24], but not in those with the inverted type, except for one case report [21]. The present study found that transient mitral regurgitation lasted for a few days in a third of the stimulated rabbits and that lesions of the mitral complex developed in 75% of them. Transient mid-ventricular akinesia has recently been described as a variant form of Takotsubo cardiomyopathy [25,26]. Inverted Takotsubo cardiomyopathy might also be a variant of Takotsubo cardiomyopathy that develops due to a common underlying mechanism.

The underlying mechanisms of the unique cardiac lesions have already been discussed [8,27]. In brief, we gained critical information at autopsy 1 h after vagal stimulation [27]. Widespread fresh punctuate bleeding was evident on leaflets of the mitral valve, papillary muscles, and mitral annulus almost exclusively in animals that had developed ventricular bigeminy during vagal stimulation. As the distribution of fresh bleeding was exactly the same as that of the unique cardiac lesions, we recognized fresh bleeding as one of the earliest precursors of these lesions. The fact that the fresh bleeding occurred exclusively in animals with ventricular bigeminy supports the notion that it is a result of abnormal mechanical stress induced by repeated VPCs of a type that can be characterized as being superimposed on subsequent P waves. When VPCs occur on P waves, distorted atrial and ventricular contraction can provide abnormal tension against the mitral portion, papillary muscles, and the basal portion.

Spontaneous VPCs occasionally develop in supine anesthetized rabbits, probably because this position is extremely unnatural for these animals. Unique cardiac lesions frequently occurred in rabbits with spontaneous VPCs as well as in those with VPCs induced by vagal stimulation [28]. However, VPCs do not always induce such lesions. The incidence of unique lesions profoundly differed among animals that develop VPCs induced by vagal stimulation, or by right and left ventricular pacing [28,29]. The highest rate of unique cardiac lesions was generated by VPCs induced by vagal stimulation followed by those induced by left and right ventricular pacing in that order. Two of fifteen animals with ventricular bigeminy did not develop these lesions in the present study. The development of these lesions might not depend on quantity but rather on the quality of VPCs. We have reported [28,29] that the incidence of unique cardiac lesions in animals with VPCs induced by left ventricular pacing was significantly greater than in animals with VPCs induced by right ventricular pacing. Thus, it seems that the origin of VPCs is important. We have used sham-operated controls in previous studies [19,29], but we did not use sham-operated controls in the present study. We will use controls in the future. This is an experimental limitation.

In conclusion, the present study demonstrates that cardiac lesions related to ventricular arrhythmias involving the basal portion, mitral valve, and papillary muscles but not the apex develop in rabbits with vagal stimulation. These lesions could serve as a novel experimental model of basal cardiomyopathy with similarity in distribution and reversibility to inverted Takotsubo cardiomyopathy.

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References


