Histopathological Changes in Spontaneously Hypertensive Rats after Feeding of Shiitake (*Lentinus edodes*) and Maitake (*Grifola frondosa*) Mushroom Diets

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Summary Histopathological changes in spontaneously hypertensive rats were studied after the animals had been fed Shiitake (*Lentinus edodes*) and Maitake (*Grifola frondosa*) mushroom powder diets, with 0.5% NaCl solution as drinking water for 9 weeks. Histological changes in control and Shiitake-fed rats were essentially the same, consisting of necrosis of the medial smooth muscle cells, vacuolization in the media of the aorta, fatty liver development, maceration in the myocardial muscle cells and vacuolization in the myocardium. In Maitake-fed animals large amounts of glycogen were observed in the liver, but the animals were otherwise normal in all respects. Dietary Maitake seems to play an important role in preventing the histological degenerative changes in SHR and thus may imply some benefits to be gained through blood pressure reduction and an improvement of lipid metabolism.

Key Words: mushrooms, spontaneously hypertensive rat (SHR), histopathology, blood pressure

Previously we reported [1, 2] that Shiitake and Maitake mushrooms prevented the increase of blood pressure in spontaneously hypertensive rats (SHR), but that only Maitake reduced the blood pressure. The favorable effect of a reduction of blood pressure on mortality caused by "pressure related" diseases such as cardiac failure or strokes has been well documented [3-5]. Hypertension plays some role in the formation of cardiovascular lesions [6-8], the reduction of which depend on the effectiveness of blood pressure control [4, 9-11]. The older the

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animal and the longer the duration of the hypertension, the more difficult it seems to be to induce regression of the hypertensive structural changes in the heart and vessels. This is true for both SHR [12] and renal hypertensive rats [13].

The present study was undertaken to investigate the histopathological changes in SHR that accompany high blood pressure, and its subsequent prevention by mushroom diets.

MATERIALS AND METHODS

All animals used in this experiment were the same strain as those described in the previous report [1]. Namely, male SHR, weighing about 116 g each, were fed 5% Shiitake and Maitake diets with 0.5% NaCl solution as drinking water for 9 weeks. The rats were kept under controlled experimental conditions and blood pressure was recorded as previously described [1].

The animals were sacrificed after a 9-week feeding period. Gross and histopathological observations were made of the abdominal aorta, liver, and heart. These organs were fixed in 10% buffered neutral formalin solution, dehydrated through a series of graded ethanol solutions, embedded in paraffin, and cut into thin sections (4 μ m). The sections were stained with hematoxylin-eosin (HE) and periodic acid-Schiff stain (PAS) for histological examination under the light microscope. The preparation and staining of the sections from the 3 groups (control, Shiitake-fed, Maitake-fed), each group consisting of six animals, were carried out simultaneously in a single batch under the same conditions.

The results obtained were analyzed by Student's *t*-test to determine significant differences between means.

RESULTS

Characteristics of the groups studied are shown in Table 1. The histological findings are given in Table 2.

Aorta

The changes in the aorta of control and Shiitake groups were essentially the same. In both groups, the histological changes occurred principally in the media and consisted primarily of the necrosis of smooth muscle cells (SMCs) along the internal elastic membranes of the upper layer of the media (Fig. 1a). Disorganization of the elastic membranes and smooth muscle cells was marked in these lesions. Vacuoles in the media were observed in all SHR irrespective of their dietary group. In Maitake-fed animals (Fig. 1b), necrosis of the smooth muscle cells was not observed and the vacuoles were fewer than those in the control and Shiitake groups. In general, the inner and middle layers of the media remained intact and appeared normal in microscopic appearance compared with those of the upper layer of the media.

Table 1. Characteristics of animal groups studied.

Diets	Body weight (g)		Final systolic blood pressure (mm Hg)	Heart wt. Body wt. $\times 10^4$
	Initial	Final		
Control	117.7 $\pm$ 3.9	324.8 $\pm$ 4.8	204.8 $\pm$ 5.1	41 $\pm$ 1
Shiitake	115.7 $\pm$ 3.1	301.8 $\pm$ 8.7*	191.2 $\pm$ 4.9**	43 $\pm$ 1
Maitake	113.8 $\pm$ 3.9	288.2 $\pm$ 6.2**	191.3 $\pm$ 3.6**	39 $\pm$ 2

Values are mean $\pm$ SE for 6 rats. Significantly different from control, * p <0.05; ** p <0.001.

Table 2. Histopathological findings in spontaneously hypertensive rats fed the experimental diets.

Organ	Pathologic condition	Diets		
		Control	Shiitake	Maitake
Aorta	Necrosis of the medial smooth muscle cells	+	+	—
	Vacuoles in media	++	++	+
Liver	Fatty liver	++	++	—
	Glycogen deposition	T	+	+++
Heart	Myocardial edema	+	—	—
	Vacuoles in myocardium	+	+	T
	Maceration in myocardium muscle cells	+	+	—

—, No change; T, trace; +, mild; ++, moderate; +++, severe.

Liver

The most outstanding histopathological finding in this organ was the fatty changes (Fig. 2a). The liver of almost all of the control and Shiitake-fed rats showed fatty changes and little glycogen deposition, with slightly greater changes in the Shiitake-fed rats. On the other hand, a larger amount of glycogen was found in the liver of the Maitake-fed animals (Fig. 2b), and almost no fatty changes were visible by light microscopy.

Heart

In all groups of animals, vacuoles were observed in the arterioles of the heart, with the control and Shiitake groups showing slightly higher numbers. Maceration in the myocardium muscle cells was also observed in control (Fig. 3a) and Shiitake-fed rats. In the control animals mild edema was observed in the outer layer of the heart, with evidence of atrophy in the myocardium (Fig. 3a). Edematous changes were observed in only a small number of animals in the control group and not at all in Shiitake or Maitake groups. Most of the animals fed Shiitake exhibited changes similar to those of the control. A similar incidence of vacuoles around the cardiac muscle fibers and myofibril atrophy was also observed in the heart of some of the control animals. The hearts of Maitake-fed rats were normal in all respects (Fig. 3b) compared with those of the control and Shiitake-fed rats.

DISCUSSION

The effect of mushroom diet on the histopathogenic changes in SHR were described in this study. Wrogemann, and Pena [14] suggested that the necrosis of muscle cells in various muscle diseases may be caused by an excessive influx of calcium ions influxed into cells which ions accumulate in the mitochondria, leading to a defect in oxidative phosphorylation. It has been also reported that the necrosis may be due to the impairment of energy utilization [15]. The results obtained here suggested the possibility of a similar mechanism in the pathogenesis of the necrosis in the medial smooth muscle cells of the aorta in the control and Shiitake-fed animals. These mechanisms might be involved metabolic disturbances in the arterial wall [16]. In the case of Maitake, reduction of blood pressure with the decrease of plasma total cholesterol and amelioration of lipid metabolism [1] might reverse these pathogenesis mechanisms.

In this study, the control and Shiitake-fed rats had fatty changes in livers, but not the Maitake-fed rats. Necrosis of the smooth muscle cells along the internal elastic membranes of the aorta might cause the fatty changes seen in the liver. A relationship between the destruction of the internal elastic lamina of the aorta and the increased permeability to plasma substances is described [17, 18]. Wexler *et al.* [19] showed that SHR displayed fatty livers despite their outward appearance of being lean. The livers of repeatedly inbred SHR show focal, but advanced, fatty infiltration of the liver [20]. The progressive development of a fatty liver is most likely related to the progressively worsening hyperlipidemia and hyperglycemia which are characteristics of SHR [21]. The plasma total cholesterol level in the Maitake-fed rat is lower than that of the control and Shiitake-fed rats, while the percentage of free cholesterol level is significantly higher [1]. Also the high density lipoprotein-cholesterol level is significantly higher in Maitake-fed rat than that of the Shiitake-fed rat. Although the effect of Shiitake on blood pressure is the same as that of Maitake, its effects on lipid metabolism are quite different from those of the Maitake [1]. The reduction of the blood pressure was probably not the only

Fig. 1. The abdominal aorta of spontaneously hypertensive rats. HE stain. $\times 400$. (a) Control rat: Note the vacuoles (V) and necrosis of smooth muscle cells (SMCs) in the media. Disorganization of the elastic membranes and SMCs was marked in these lesions. (b) Maitake-fed rat: No necrosis was observed and the vacuoles are few compared with those in the control.

Fig. 2. The liver of spontaneously hypertensive rats. PAS stain. $\times 100$. (a) Control rat: The vacuoles in the hepatocytes were significantly larger than those in the Maitake-fed rats, indicating fatty changes of the liver. (b) Maitake-fed rat: Larger amounts of glycogen without fatty changes were observed in the liver compared with those in the control. CV, Central vein.

Fig. 3. The heart of spontaneously hypertensive rats. HE stain. $\times 200$. (a) Control rat: Edema (E) and atrophy (A) in myocardium. Maceration in myocardium muscle cells was also present. (b) Maitake-fed rat: Normal in all respects.

factor for an improvement in the histopathological changes in SHR, because an amelioration of the histopathological features was observed only in Maitake-fed rats. The dietary *Ganoderma lucidum* mushroom significantly decreases the liver cholesterol level in SHR [22]. The liver of the Maitake-fed animals contained a

considerable amount of glycogen as a constituent, but almost no lipid was visible by light microscopic observation. The dietary Maitake seemed to affect the basic pathogenetic mechanism operative in SHR, since they prevent the excessive fatty accumulation in the liver as well as reduce the blood pressure. Relationship between blood pressure control by mushrooms and the lipid levels in SHR is not obvious [2]. The results here showed that the metabolism of lipid seemed to play an important role in the histological features in SHR.

Freis [23] reported that the myocardium of the control, but not that of antihypertensive drug-treated animals showed different degenerative changes in 6 months experimental period. During this 9 weeks experimental period, the myocardium of control rat showed various degenerative changes. On the other hand, Maitake-fed animals showed none of the advanced pathological changes seen in the hearts of the control and Shiitake groups (Table 2 and Fig. 3b). It is possible that Maitake feeding might initiate metabolic, neural or humoral alterations causing a primary fall in transmural pressure which then signals a secondary process leading to prevent the degenerative changes which normally accompany hypertension and acts to lower the blood pressure.

This study showed that after feeding Maitake mushroom to SHR, the aorta, the liver, and the coronary arterial system of these rats appeared to be normal.

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