Pathogenesis of Cerebral Hemorrhages in Experimental Hypertension in Rabbits with Particular Reference to Acute Vascular Lesions (Fibrinoid Necrosis) of Small Arteries and Arterioles

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ACUTE arterial lesions including fibrinoid necrosis are similar to essential features of the malignant phase of essential hypertension or malignant hypertension. The fibrinoid necrosis of cerebral small arteries and microaneurysm resulting from it are the primary vascular lesions producing cerebral hemorrhages in human hypertension.

The present report summarized the results from our laboratories a part of which has been published previously.?,9,10,12,17

The effect of salt, blood pressure and renal factors on the development of acute vascular lesions at the early and advanced stages was studied in rabbits through production of hypertension by means of unilateral or bilateral renal injury, with emphasis on pathogenesis of the fibrinoid necrosis of small arteries and arterioles in the brain as well as other tissues.

METHODS

Male rabbits weighting 2–2.5 kg were divided into 9 experimental groups as follows:
Group 1: Twenty one rabbits were maintained on a diet containing 0.1 g salt per day (Na: 7 mEq, K: 26 mEq per day). Hypertension was produced by constricting one renal artery applying a clip with 0.9 mm internal diameter and the other kidney was kept untouched. All animals were killed 7 days after renal manipulation.

Group 2: Twenty one rabbits were maintained on a diet containing 1.2 g of salt per day (Na: 28 mEq, K: 26 mEq per day) Other procedures were the same as in Group 1.

Group 3: Twenty-six rabbits were maintained on a diet containing 0.1 g salt per day. Hypertension was produced by constricting both renal arteries simultaneously applying clips with 0.9 mm internal diameter. Animals were killed 7 days after the renal manipulation.

Group 4: Sixteen rabbits were maintained on a diet containing 0.5 g salt per day. Other procedures were the same as in Group 3. Animals were killed 4 days after the renal manipulation.

Group 5: Twenty one rabbits were maintained on a diet containing 0.5 g salt per day. Bilateral nephrectomy was made simultaneously in these experiments. Twelve animals died or were killed within 4 days after bilateral nephrectomy. The remaining 9 animals were killed 4 days after the renal manipulation.

Group 6: Twenty rabbits were maintained on a diet containing 0.6 g salt per day. Hypertension was produced by constricting one renal artery applying a clip with 1.2 mm internal diameter several weeks after unilateral nephrectomy. Seven animals died by cerebral hemorrhages within 20 weeks. The remaining 13 animals were killed 20 weeks after the renal manipulation.

Group 7: Fifteen rabbits were maintained on a diet containing 0.6 g salt per day. Hypertension was produced by constricting one renal artery

Key Words:
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Cerebral hemorrhages in hypertension

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Acute Vascular Lesions of the Brain and Gastrointestinal Tract and Cerebral Hemorrhage in Rabbits with Various Renal Manipulation

<table>
<thead>
<tr>
<th>Group</th>
<th>Renal Manupulation</th>
<th>Salt Intake S/day</th>
<th>Survival Days</th>
<th>Average of B.P.(mmHg)</th>
<th>No of Cases</th>
<th>Incidence of A.V.L. Cerebral Hemorrhage</th>
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<td>(1)</td>
<td>Unilateral Clip</td>
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<td>10</td>
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<td>7</td>
<td>10</td>
<td>21</td>
<td>0 (6.28%)</td>
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All animals were killed 20 weeks after the renal manipulation.

Group 8: Ten rabbits were maintained on a diet containing 0.5 g salt per day. Hypertension was produced by one renal artery applying a clip with 0.9–1.0 mm internal diameter. Animals were killed from 16 to 36 months (24 months on the average) after the renal manipulation.

Group 9: Forty rabbits were maintained on a diet containing 0.5 g salt per day. Hypertension was produced by constricting one renal artery after another with interval of 2 or 3 weeks. A clip with 0.7–0.8 mm internal diameter was applied for the first manipulation and a clip with 0.9–1.0 mm internal diameter for the second one. These animals were observed until spontaneous death. Most animals died within 7 weeks. The longest survival period was 13 months.

Throughout the observed period systolic blood pressure was measured every day in experiments of Group 1, 2, 3, 4 and 5, and every week in experiments of Group 6, 7, 8 and 9, on the central artery of the ear by an indirect method. Terminally, plasma renin activity was measured in experiments of Group 1, 2 and 3. PRA was measured every week through 20 weeks in Group 6 and 7. Plasma renin activity was measured by the method of Pickens et al. with a minor modification. BUN was measured at the terminal stage in experiments of Group 4, 5 and 9.

All animals were killed at the estimated 4 days (Group 4 and 5), 7 days (Group 1, 2 and 3), 20 weeks (Group 6 and 7) and a longer period (Group 8) after the renal manipulation excluding those died spontaneously. The specimen of brain, kidneys, stomach, small intestine, lung, aorta were fixed in formal saline. Paraffin sections were stained with Hematoxylin and Eosin, Masson Trichrom, Elastica Masson and examined microscopically.

**RESULTS**

1. Blood Pressure

The blood pressure of normal rabbits was ranged between 80 and 90 mmHg. Average value of the maximal grade of blood pressure at each terminal stage of experiments was in the Fig.1.

The influence of salt on the development of hypertension after unilateral constriction of the renal artery was shown in experiments of Group 1 and 2. Blood pressure gradually rose daily in both groups either the low or high salt regimen. The difference of the terminal blood pressure at 7th day after renal manipulation between two groups was statistically significant (p<0.01).

The animals which were observed for longer term showed sustained high blood pressure about 2 weeks after the renal manipulation and tended to elevate gradually thereafter. The average value of the maximal grade of blood pressure at each terminal period of these experiments on a diet with the same amount of salt showed no remarkable difference between Group 6, Group 7 and 8, despite the different renal procedures.

2. Acute Vascular Lesions

Acute vascular lesions consisted of the fibrinoid necrosis of small arteries and arterioles, proliferation of cells in the intima and media, perivascular infiltration of cells and glomerular changes.

Among acute arterial lesions, the most prominent lesion consisted of the fibrinoid necrosis of small arteries and arterioles with a wide distribution. However, as far as the fibrinoid necrosis was concerned, while acute arterial lesions showed a wide spread distribution, small arteries of gastrointestinal tract especially those of stomach were
Terminal Maximum Blood Pressure and Term from Renal Manipulation at Cerebral Hemorrhage

<table>
<thead>
<tr>
<th>B.P.</th>
<th>No of Cases</th>
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<tr>
<td>above 160</td>
<td>9</td>
</tr>
<tr>
<td>160 ~ 131</td>
<td>22</td>
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<td>130 ~ 101</td>
<td>6</td>
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Fig.2.

predominantly vulnerable. Acute lesions of small arteries and arterioles in the brain were less frequent than those in the gastro-intestinal tract. The lesions were less frequent in small arteries, arterioles and glomeruli even in nonischemic kidney and were rare in the vessels of other tissues examined.

Incidence of the arterial lesion similar to periarteritis nodosa was low in rabbits. It usually occurred with fibrinoid necrosis elsewhere. Medium size and small arteries in the heart often showed proliferative changes in the intima with or without fibrinoid necrosis in other tissues.

1) Height of blood pressure and incidence of fibrinoid necrosis

The fibrinoid necrosis of small arteries and arterioles was induced in animals with blood pressure above 120 mmHg among experiments of Group 1 and 2 with one ischemic kidney. The incidence of fibrinoid necrosis was 59% (10/17) in animals with blood pressure above 130 mmHg, whereas the incidence in those with blood pressure less than 130 mmHg was 40% (10/25). The incidence of fibrinoid necrosis did not seem to be related to the grade of high blood pressure. (the relation was not statistically significant) although the grade of fibrinoid necrosis was weak.

In experiments of Group 3 with bilateral ischemic kidneys, however, the fibrinoid necrosis of small arteries and arterioles was induced in animals with blood pressure above 95 mmHg. The incidence of fibrinoid necrosis in the intestine and brain was 50% (8/16) and 31% (5/16) respectively in animals with blood pressure less than 120 mmHg.

2) Amount of salt intake and incidence of fibrinoid necrosis

The fibrinoid necrosis of small arteries and arterioles in gastrointestinal tract was found in 6 (28%) of 21 low salt animals (Group 1) and 14 (67%) of 21 high salt animals (Group 2). This difference was statistically significant (p<0.05). No fibrinoid necrosis of small arteries and arterioles was found in the brain of either group.

Among marked hypertensive animals with the terminal blood pressure above 130 mmHg in Group 1 and 2, the incidence of fibrinoid necrosis was higher (9/12) in the high salt group than in the low salt group (1/5). This difference was statistically significant (p<0.01).

3) Development of fibrinoid necrosis and renal involvement (Renal factors)

Comparing Group 1 with Group 3, the incidence of fibrinoid necrosis was predominant in Group 3 with both ischemic kidneys; nevertheless, both groups receiving the same amount of salt had almost the same blood pressure. The fibrinoid necrosis in the brain was induced in Group 3 with both ischemic kidneys even on the low salt regimen, but not in Group 2 with one ischemic kidney on the high salt regimen.

The incidence of the fibrinoid necrosis of small arteries and arterioles in the brain in Group 3, however, was lower than that in Group 6 and
9 in which animals were observed or survived longer.

The fibrinoid necrosis was not induced in the brain during either the early or advanced stage of experiments with one ischemic and the other intact kidneys (Group 1, 2, 7 and 8), although it was induced in the gastro-intestinal tract during the early stage (Group 1 and 2), not during the advanced stage (Group 7 and 8).

The fibrinoid necrosis of small arteries and arterioles was induced in the stomach in experiments of Group 4 with bilateral nephrectomy although the grade of fibrinoid necrosis was weak.

4) Plasma renin activity and fibrinoid necrosis

Plasma renin activity in animals with one ischemic kidney was 17.7 ng on the average (SE: 2.7) under high salt regimen (Group 1 and 13.9 ng on the average (SE: 2.7) under low salt regimen (Group 2). Plasma renin activity in Group 3 with simultaneously induced bilateral renal ischemia was almost within normal range except 3 experiments with slight or moderate rise. No correlation was found between plasma renin activity and incidence of fibrinoid necrosis. Plasma renin activities followed once a week in Group (6) were almost normal or rather decreased with exceptional increase and those in Group (7) were increased more than a half of experiments during the first several weeks and tended to decrease thereafter through 20 weeks. The incidence of the acute arterial lesion (fibrinoids necrosis), however, was higher in Group (6), while the acute arterial lesion was scarcely found in Group (7). According to these experiments, elevation of plasma renin activity did not seem to be responsible for the development of the acute arterial lesion.

5) Development of fibrinoid necrosis during the early and advanced stages after renal manipulation

The fibrinoid necrosis of small arteries and arterioles was induced during the early stage of hypertension after the renal manipulation. It was induced at 4th day in experiments with both ischemic kidney (Group 4) and 7th day in those with one ischemic kidney (Group 1 and 2) and those with both ischemic kidneys (Group 3). Comparing Group 3 with Group 4 (experiments with both ischemic kidneys), the incidence of fibrinoid necrosis was higher in Group 3 (7 day survival) than in Group 4 (4 day survival) in which no fibrinoid necrosis was found in small arteries in the brain.

The fibrinoid necrosis of small arteries and arterioles was not seen during the advanced stage of hypertension in experiments with one ischemic and the other intact kidneys (20 weeks in Group 7 and from 16 to 36 months in Group 8). It was induced, however, during the advanced stage of hypertension in experiments with both renal injuries (several months in Group 6 and 9).

3. Cerebral Hemorrhages

Massive cerebral hemorrhages were induced in Group 6 and 9 with both renal injury. These were never induced in experiments with one ischemic and the other intact kidneys during the early and even the advanced stages of hypertension after the renal manipulation.

Cerebral hemorrhages were induced from several days to 13 months, mostly with 6 weeks after the renal manipulation. (Fig. 2).

The maximal value of blood pressure during the terminal stage in the animals with cerebral hemorrhage showed under 100 mmHg in 2 cases, from 100 to 130 mmHg in 6 cases, from 131 to 160 mmHg in 22 cases and above 161 mmHg in 9 cases.

Development of cerebral hemorrhages as well as acute arterial lesions were not related with the height of plasma renin activity, as shown in Group (6) and (7). High plasma renin activity did not seem to be responsible for cerebral hemorrhages.

DISCUSSION

The fibrinoid necrosis of cerebral small arteri- es and microaneurysm resulting from it are the primary vascular lesions producing cerebral hemorrhages in human hypertension and experimental hypertension as well. [Matsuoka, S., 1958; Kameyama M. 1964; Ooneda, G. 19641, 13, 14; Ikeda, M. 19649]

Several hypotheses have been advanced on the development of acute arterial lesions especially of the fibrinoid necrosis of small arteries. According to one, they are the consequence of high blood pressure itself2, 3, and the dilatation of arterial wall due to hypertension. Another one concerns renal factors, such as renin1, 5, 6 or non-pressor permeability factor of renal origin12, 15, 16, 17. Increase in permeability of the arterial wall by renal factors was suggested by several authors. Kolesky, S11 has suggested that high salt intake with or without steroid would enhance the development of the acute vascular lesion in rats.

Most studies on hypertensive vascular lesions have been made mainly in rats. A critical study

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of vascular lesions in rabbits has been made by Pickering et al (1939), Allison et al. (1967) and Campbell et al (1959, 1963). The acute fibrinoid arterial lesion of the brain as well as other tissues in rabbits is very similar to those of human hypertensives and stable to non-specific factors compared with those in rats, so that rabbits were used for this study.

The present study indicates that renal factors play a great role in producing acute arterial lesions of renal hypertension. A renal factor responsible for the development of the fibrinoid necrosis of small arteries and arterioles is considered by several investigators to be a vascular toxic or permeability accelerating substance. We have reported previously the concept on the role of non-pressor fractions of extracts of ischemic renal cortex in the production of acute arterial lesions probably as permeability factor. The ischemic effect for the kidney may differ in individual animal in spite of applying a clip with the same internal diameter for narrowing the renal artery. The amount of the renal factor could be influenced by the grade of renal ischemia. However, the evidence that the incidence of fibrinoid necrosis was higher in experiments deriving from both renal injury than in those from one ischemic and the other intact kidneys, suggests the following two possibilities. One of them is that the amount of the renal factor for the development of fibrinoid necrosis is increased more according to sever renal ischemia by both renal injury. Another one is that the intact kidney remained untouched may play a role to protect the development of acute arterial lesions. The results of experiments in Group 5 seem to support the idea that normal kidney may contain a protecting factor against the development of acute vascular lesions, although the exact mechanism is not clear yet.

Despite the possible role of the renin-angiotensin-aldosterone system (the concept that renin is the permeability factor in the production of acute arterial lesions) there was no correlations between plasma renin activity and the development of acute arterial lesions and cerebral hemorrhages in our experiments.

The grade of high blood pressure was not parallel to the incidence of fibrinoid necrosis although high blood pressure may contribute to the development of the lesions. The fibrinoid necrosis was induced in those with blood pressure above 120 mmHg among animals with one ischemic kidney (Group 1 and 2), whereas it was induced in those with blood pressure above and also below 120 mmHg among animals with bilateral ischemic kidneys. (Group 3) The results seem to indicate that renal factors were pre-dominant compared with mechanical factor of high blood pressure in the production of acute arterial lesions.

High and low sodium diet in our experiments were in physiological range. The low and high salt regimen in these experiments approximately correspond to the diet with 2 g and 25 g salt per day respectively in human being. Analysis of electrites of the diet showed that the low salt regimen contained 7.0 mmEq Na and 26 mmEq K per day and the high salt one contained 28 mmEq Na and 26 mmEq K per day. The high salt regimen may have the significant effect in the development and sustenance of high blood pressure and also that of rendering the vascular-ture more than normally susceptible to effects of the elevated pressure or to effects of renal permeability factors (possibly as a synergic action of sodium or sodium/potassium ratio and renal factors).

Cerebral vessels were more resistant and thus less involved than vessels of the gastro-intestinal tract. The vessels of the stomach were more susceptible than that of the intestine in rabbits. Acute fibrinoid lesions are essential to be induced in small arteries and arterioles in the brain for the development of cerebral hemorrhages according to experiments in Group 6 and 9.

Elevation of PRA is not essential for the production of fibrinoid necrosis of small arteries and arterioles and also for the development of cerebral hemorrhages as in Group 6 and 7. A renal factor other than renin is considered to be responsible for the development of acute vascular lesions and cerebral hemorrhages.

Factors influencing the lesions may be different between the early and advanced stages of hypertension. In the early stage of renal hypertension, renal factors in addition to high blood pressure and high salt intake seem to play a great role for the development of acute vascular lesions, but other unknown factors may not be ignored or excluded. In the advanced stage of renal hypertension, renal factors seem to play a role in the production of acute arterial lesions, although sustained high blood pressure may be one of the factor responsible for that. The factors enhancing development of lesions may differ in accordance with race, strain, age, sex of animals, and type and stage of hypertension as well. It is clear,
however, that the pathogenesis of acute fibrinoid lesions of small arteries and arterioles in hypertension is quite different from that of atherosclerosis of large arteries and the aorta. The higher the grade of high blood pressure, the higher is the risk of rupture of cerebral vessels with the fibrinoid lesion or microaneurysm deriving from it. However, further studies are necessary to determine how much these enhancing factors or inhibitory factors may be responsible for the production of fibrinoid necrosis during the early as well as the advanced stage in experimental hypertension and also in human essential hypertension, what kind of substances are these enhancing factors and inhibitory factors and their mechanism in producing or protecting acute vascular lesions and cerebral hemorrhages.

**SUMMARY**

The effect of salt, high blood pressure and renal factors on the development of acute vascular lesions of small arteries and arterioles and cerebral hemorrhages was studied in rabbits through production of hypertension by means of unilateral or bilateral renal injuries.

1. Massive cerebral hemorrhages were induced in animals with bilateral renal injuries and these were never induced in animals with one ischemic and the other intact kidneys even during advanced stage of hypertension.

2. Animals died due to cerebral hemorrhages usually accompanied with marked high blood pressure during the advanced stage. However, cerebral hemorrhages were induced in animals with blood pressure less than 120 mmHg as well as in those with higher blood pressure during the early stage after renal manipulation.

3. Among acute arterial lesions, the most prominent lesion consisted of the fibrinoid necrosis of small arteries and arterioles with a wide distribution. The vessels of gastro-intestinal tract especially those of stomach were predominantly vulnerable.

4. Fibrinoid necrosis of small arteries and arterioles in the brain was found all of the experiments with cerebral hemorrhages. The incidence of the acute arterial lesion in the brain and other tissues was higher in experimental groups in which cerebral hemorrhages developed. These results convince that the fibrinoid necrosis of small arteries and arterioles in the brain is the primary vascular lesion responsible for cerebral hemorrhages.

5. The incidence of the fibrinoid necrosis in small vessels was not related with the grade of high blood pressure especially during the early stage of hypertension. It was not clear, however, how much the high blood pressure itself contributed to the development of the acute arterial lesion during the advanced stage of hypertension.

6. Renal factors played a great role in producing the acute arterial lesion (fibrinoid necrosis). A renal factor other than renin seemed to be responsible for the acute arterial lesion and cerebral hemorrhages. The acute arterial lesion was induced in animals with one ischemic and the other intact kidneys during the early stage of hypertension. But, it was scarcely produced during the advanced stage of hypertension. Bilateral renal injuries (two ischemic kidneys or one ischemic kidney after one nephrectomy) enhanced the development of the acute arterial lesion.

7. High salt intake augmented the incidence of the acute arterial lesion.

**REFERENCES**


Discussion:

Chairman: NISHIMORI

The presentation was focused on the degree of participation of blood pressure in the pathogenesis of vascular fibrinoid necrosis that is seen in Goldblatt type renovascular hypertension of rabbits. Particularly, emphasis was placed on the possibility that the pathogenesis of fibrinoid necrosis is participated more strongly by renal factor than by blood pressure, since increase of blood pressure is preceded by vascular fibrinoid necrosis in early stage of experiment.

In comment on this presentation, Dr. Ebihara stated from his experience with various types of experimental hypertension that vascular fibrinoid necrosis is independent from renin but mostly dependent on blood pressure.

Further, Dr. Onoyama inquired about the degree of participation of blood pressure in brain hemorrhage in long-term experiment and about perivascular edema of the brain.

Dr. Ikeda replied that he would still place emphasis on renal factor though the participation of blood pressure in brain hemorrhage in long-term experiment cannot be denied.

Chairman Nishimori commented that he was in favor of the theory that renin and blood pressure are not directly related with the pathogenesis of vascular fibrinoid necrosis and emphasized the necessity of further study on the mechanism of vascular permeability including renal factor which has recently drawn attention in pathological studies of the blood vessel.