MORPHOLOGICAL CHANGES IN JUXTAGLOMERULAR CELLS BY LONG-TERM TREATMENT WITH AN ANGIOTENSIN-CONVERTING ENZYME INHIBITOR IN BEIGE RATS (CHEDIAK-HIGASHI SYNDROME)

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Abstract: To determine the effect of long-term treatment with an angiotensin-converting enzyme (ACE) inhibitor on the morphological change in juxtaglomerular (JG) cells, and the influence of plasma angiotensin II (PAG II) in renin synthesis and secretion in beige rats, an animal model of Chediak-Higashi syndrome, show gradually enlarged and irregularly-shaped JG granules (renin in the granules) according to their degree of maturity. Beige rats were administrated trandolapril, an ACE inhibitor, orally for 4 weeks. Morphological changes in JG cells and renin-angiotensin system function were determined. These rats were examined histologically and ultrastructurally, focusing on morphological changes in JG cells along with blood pressure, PAG II, and plasma renin activity. Trandolapril reduced systolic blood pressure and decreased PRA remarkably, but PAG II was highly variable. The most conspicuous histological changes were hypertrophy and hyperplasia of JG cells. Ultrastructurally, JG cells contained an increased number of JG granules of varying morphology. Most JG granules decreased in size and contained smooth materials, but some showed a granular matrix. These results demonstrate that chronic ACE inhibition accelerates synthesis of JG granules including renin and inhibits secretion of renin, but it is not confirmed whether renin synthesis and secretion are affected by PAG II. (J Toxicol Pathol 9: 351~358, 1996)

Key words: Angiotensin-converting enzyme inhibitor, Beige rats, Juxtaglomerular cells

Introduction

ACE inhibitors block the activity of ACE (which converts angiotensin I to angiotensin II) resulting in a reduction in blood pressure¹. It is clear that long-term treatment of rats with ACE inhibitor induces hyperplasia and hypertrophy of JG cells. However, there is still no agreement on changes in fine ultrastructural morphology such as the shape of JG granules and cell organelles²-⁶, changes may be considered to be caused by the extinction of negative feedback with decreased angiotensin II levels⁷-⁹. Recent speculation that negative feedback control of angiotensin II does not occur is based on some reports in which the PAG II level either increased or was uninfluenced by long-term treatment with ACE inhibitors⁷-⁹. However, since these reports have not described of morphological changes in JG cells, the exact mechanism regulating PAG II level and JG granules is still disputed.

JG granules of mature rats are small and vary only slightly in size, with protogranules being rare and inconspicuous¹⁰. This morphological character of uniformity is one obstacle in establishing the degree of maturity of JG granules. Beige rats, which are an animal model of Chediak-Higashi syndrome, were recently reported to have abnormally giant granules in various cells¹¹. The mechanism for the formation of these giant granules is thought to be continuous fusion and this allows the degree of maturity of JG granules to be identified by size¹². Immunohistochemical and electron microscopical studies have shown renin in JG granules¹³. These giant granules of beige rats also contained renin. Giant JG granules of beige rats do not affect renin release and blood pressure at 2 months of age¹². Thus this model is suitable for this study.
The purpose of the present study is to examine the effect long-term treatment with an ACE inhibitor (trandolapril) on morphological changes in JG cells, and to determine the influence of PAG II in renin synthesis and secretion by using beige rats.

**Methods**

**Animals**

Beige rats originated from a mutant male among DA rats from the Australian National Institute of Genetics as an inbred strain. Since the time this rat was obtained at the Institute of Experimental Animals, Hamamatsu University School of Medicine, the mutant line has been maintained by brother and sister mating under conventional conditions. The rats in the present study were housed in an air-conditioned animal room where they were fed the standard laboratory diet and supplied water *ad libitum*. Fifteen male beige rats 2 months of age were used in this study.

**Experimental protocol**

Trandolapril (a gift from Nippon Roussel Co., Ltd.), one of a group of potent and long-acting ACE inhibitor from Roussel-Uclaf, was administrated by gavage for 28 days as a suspension in 0.5% methyl cellulose and at a dose volume of 5 ml/kg. Rats were divided into 3 groups in terms of dosage: 6 controls (no dose), 6 rats given 100 mg/kg, and another 3 given 200 mg/kg.

**Measurement of systolic blood pressure**

Before and following the administration period, individual rats were restrained in a chamber. The systolic blood pressure of conscious animals was taken by the tail-cuff method. Three blood pressure readings were performed for each rat.

**Measurement of PRA and PAG II**

Rats were anesthetized with ether, and blood from the abdominal aorta was collected in tubes containing EDTA2Na. The blood was centrifuged, and PRA and PAG II were assayed using a radio immunoassay.

**Histopathology**

Rats were anesthetized with ether and underwent vascular perfusion for 5 min with physiologic saline containing heparin. The saline wash was followed by 60 min of perfusion fixation with 2% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.4). The kidneys were fixed in 10% phosphate buffered formalin (pH 7.4), dehydrated in a graded series of ethanols, and embedded in paraffin after which 4-μm sections were stained with hematoxylin and eosin, and Bowie's technique. The activity of JG apparatus was determined by microscopic examination of the Bowie-stained section. To evaluate the activity of JG apparatus, the number of JG apparatus containing Bowie-stained JG cells per 200 glomeruli was counted.

**Electron microscopy**

Tissue fragments from the kidneys were fixed in 2% glutaraldehyde solution (pH 7.4) overnight, post-fixed in 1% osmium tetroxide solution (pH 7.4) for 2 hours, dehydrated in graded-series of ethanols, and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined by electron microscope (JEM 1200EX, JEOL).

For morphometry of the size and number of the granules, nine randomly chosen electron micrographs of portions of the cytoplasm of JG cells were taken at an original magnification of ×4,000 from each thin section and enlarged to ×24,000. The area of granules per unit cytoplasmic area (36 μm²) was evaluated using the point-counting method. The number of granules per unit cytoplasmic area was counted. The area of a granule was calculated by dividing the area of granules per unit cytoplasmic area by the number of granules per unit cytoplasmic area.

**Statistical analysis**

All data were expressed as mean±S.D.. Differences were assessed by analysis of variance and Student's *t*-test.
Table 1. Mean Systolic Blood Pressure (mmHg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
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<tr>
<td>Pre-treatment</td>
<td>141.5±18.7</td>
<td>134.6±12.9</td>
<td>144.7±16.6</td>
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<tr>
<td>Post-treatment</td>
<td>123.9±20.9</td>
<td>76.1±12.9 ab</td>
<td>78.2±11.5 ac</td>
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Mean±S.D.

* $p<0.01$ compared with control group at post-treatment

b $p<0.01$ compared with 100 mg/kg group at pre-treatment
c $p<0.01$ compared with 200 mg/kg group at pre-treatment

Table 2. Plasma Renin Activity (PRA) and Angiotensin II Concentration (PAG II)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
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<tbody>
<tr>
<td>PRA (ng/ml/h)</td>
<td>88.3±15.5</td>
<td>14.6±6.1 a</td>
<td>9.6±2.0 a</td>
</tr>
<tr>
<td>PAG II (pg/ml)</td>
<td>183.0±190.1</td>
<td>76.2±50.4</td>
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</table>

Mean±S.D.

* $p<0.01$ compared with control group
/ not examined

Results

Systolic blood pressure, plasma renin activity, and angiotensin II

After the 4-weeks treatment, the systolic blood pressure and PRA of both trandolapril-treated groups were significantly lower than those of the control group (Tables 1 and 2). The PAG II level of the 100 mg/kg treated group also fell, but the difference was statistically insignificant (Table 2).

Histopathology

The JG apparatus of control beige rats consisted of some JG cells containing smaller number of heterogeneously-sized granules, some of which were of abnormal size, far larger than those in normal rats (Fig. 1a). JG apparatus of trandolapril-treated groups showed an increased number of hypertrophic JG cells (Fig. 1b) in which the JG granules were much smaller and more numerous. The number of Bowie-stained JG apparatus per 200 glomeruli of trandolapril-treated groups also increased compared to that of control animals (Table 3). Other histopathological changes included regeneration of renal tubules, thickening of the basement membrane of renal tubules, and lymphocytic infiltration in interstitium.

Electron microscopy

JG granules of control rats were less numerous, large and irregularly-shaped (Fig. 2a). Occasionally, two or more giant granules appeared to be fusing. These granules contained smooth material with moderate electron density. Small protogranules with
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Fig. 2. a) JG cells of control beige rat. JG cells included some granules of great size, much larger than normal. Most granules contain smooth material of moderate electron density. b) JG cells of 100 mg/kg. Hypertrophy and hyperplasia of JG cells show many kinds of JG granules and organelles. Hypogranular cells (HC) are also seen. AA: afferent arteriole. Uranyl acetate and lead citrate. ×4,000.

low electron density could not be recognized in these cells. These JG cell also had nucleus, small mitochondria, short rough endoplasmic reticulum, and small golgi apparatus.

JG apparatus of trandolapril-treated groups showed hypertrophic and hyperplastic changes in JG cells at the ultrastructural level. Hypertrophic JG cells contained increased numbers of JG granules of varying morphology and well-developed organelles (Fig. 2b). Among the granule rich JG cells, a small number of hypogranular cells were also seen (Fig. 2b). Most JG granules in the majority of JG cells decreased in size except for a small number of giant granules (Figs. 3 and 4). Almost all granules contained smooth materials with moderate electron density, but some granules had granular matrix with moderate to high electron density and myelin figure (Fig. 5). These granules resembled autophagic vacuoles. Well-developed rough endoplasmic reticulum and golgi apparatus were detected in hypo-granular cells. Cisternae of rough endoplasmic reticulum were dilated and filled with moderately electron dense materials. There was an accumulation of tubules emerging from rough endoplasmic reticulum. These contained high electron dense materials which were thought to be protogranular matrix (Fig. 6).

By morphometrical analysis, the average number of JG granules of trandrapril-treated groups were significantly greater than in the control (Table 4). The average area of a JG granule of trandrapril-treated groups was significantly smaller than in the control (Table 4).

Discussion

Several investigations have studied chronic ACE inhibition in rats. By histological examination, JG apparatus hypertrophy and hyperplasia were apparent. Electron microscopy revealed such changes to be associated with an increase in the number of JG granules, a decrease in size and increased pleomorphism, or a rise in the number of JG granules without pleomorphism. In this study, in which the rats with abnormal giant granules were used, hypertrophy and hyperplasia of JG cells were consistently observed, and JG granules increased in number and decreased in size. These results were essentially similar to previous reports, whereas the finding specifically observed in beige rats was an increased number of JG granules of smaller size, which is considered as an expression of accelerated synthesis of the granules, taking a progressively fusing nature of JG granules. These changes may be attributable to an increased synthesis of renin. However, the real answer is not clear and would require more dynamic and kinetic studies using labeling.

Many authors have speculated about the relationship between the functional state of JG cells and ultrastructural changes in JG granules. Regarding the pleomorphism of renin granules, they found the following: partial focal vesicular to membranous
inclusions in granules were considered matrical alterations in exocytosis\textsuperscript{15,16}; altered granules with high electron dense matrix and discontinuous limiting membranes were thought to be renin secretion occurring through leak either from a stretch or from the whole granule outline\textsuperscript{17}; and granule matrix alternation was considered an autophagy of granules\textsuperscript{18}. Under chronic ACE inhibition, granule matrix changes indicated an autophagy of granules\textsuperscript{5}. Many altered granules in the present study were similar in appearance to autophagic granules. Furthermore, taking the finding of the present study, i.e. a decrease in PRA, renin secretion from the JG cells appeared to be inhibited in contrast to increased JG granule synthesis including renin; i.e., it is likely that acceleration of JG granule synthesis and inhibition of renin secretion occurred simultaneously in a cell, and that an excess in JG granules might result from hypersynthesis and hyposecretion. Generally, crinophagy has been considered a regulatory process designed to suppress an excess of secretory granules and to degrade the quantities of granules in endocrine cells\textsuperscript{19}. In the present case, an excess of JG granules might be degraded by autophagy in a similar manner.
Therefore, pleomorphism of granules might have resulted from autophagy of JG granules.

PRA under chronic ACE inhibition has been reported as increased\(^7,9,20\), unchanged\(^21\), and decreased\(^22\). The reasons for these discrepancies are not completely clear, but they may be due in part to the concentration of ACE inhibitors. Hartmann et al\(^20\) reported that higher doses of ACE inhibitor resulted in a decline in PRA. Our study is consistent with that finding. A possible reason for this may be some non-specific effect or the activation of a mechanism of renin suppression\(^20\). A deeper understanding of such a mechanism awaits further study.

Various reports have show the PAG II level to be increasing\(^7\), unchanged\(^9\), and decreasing\(^20\) by chronic ACE inhibition. AG II is produced by ACE or an alternate pathway\(^22\). Increased or unchanged PAG II were explained by the activation of the alternate pathway of AG II\(^7,9\). However, PAG II in this study did not reach significance and was
Table 4. Average Area of a JG Granule and Number of JG Granules per Unit Cytoplasmic Area (36 μm²) of JG Cell

<table>
<thead>
<tr>
<th>Group</th>
<th>Control 100 mg/kg</th>
<th>200 mg/kg</th>
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<tbody>
<tr>
<td>Average area of a JG granule</td>
<td>1.77 ± 0.96</td>
<td>0.45 ± 0.20*</td>
</tr>
<tr>
<td>Number of JG granules per unit cytoplasmic area (36 μm²)</td>
<td>26.0 ± 11.6*</td>
<td>30.1 ± 12.3*</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>12.2 ± 3.5</td>
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*p < 0.01 compared with control group

highly variable. Thus the question of feedback of PAG II levels on renin release and/or synthesis can not be answered from this study.

In summary, the present study demonstrates that, after chronic treatment of beige rats with relatively higher doses of ACE inhibitor, JG granules synthesis including renin is accelerated as suggested by histological findings, and renin secretion is inhibited as suggested by both histological findings and PRA determination. However, it remained unclear whether PAG II affect synthesis and secretion of renin.

References


