博士論文概要

高脂肪食ラットのインスリン作用に対するアンジオテンシ II AT1 受容体拮抗薬と運動トレーニング併用の効果

大学院医学系研究科
健康社会医学専攻　健康増進医学講座　健康スポーツ医学分野
博士課程4 年　　王　　忠華
指導教員　　押田　芳治

[Introduction]

Overactivity in the renin-angiotensin system has been implicated in insulin resistance. ARBs and ACE inhibitors as well as exercise training have been shown to prevent the new onset of type 2 diabetes. Several evidences have shown that the combined treatment of ACE inhibition and exercise training improves insulin action compared with each treatment alone. However, the combined effect of ARB and exercise has not yet been proven. Recently, candesartan cilexetil, one type of ARB, has been shown to improve insulin resistance in hypertensive rats. Therefore, we investigated the effects of candesartan and exercise training, alone and in combination, on impaired glucose homeostasis in F344/NSlc rats fed with high-fat (HF) diet.

[Methods]

Male F344/NSlc rats were obtained at 7 weeks of age. After 1-week acclimation period, rats were randomly divided into 5 groups: (1) normal chow group (N, n=6) fed with standard chow diet; (2) HF diet group (HF, n=8) fed with high-fat diet; (3) HF diet with daily candesartan (AT1 receptor antagonist, 5 $mg \cdot kg^{-1} \cdot day^{-1}$ administered through oral gavage) treatment group (HF+C, n=10); (4) HF diet with exercise-trained group (HF+Ex, n=6) fed with HF diet and exercise (treadmill, 15 m/min, 6% grade, 1 h/day, 5 days/week for 4 weeks); (5) HF diet with daily candesartan and exercise-trained group (HF+C+Ex, n=6). At the sixth week after the treatment, all rats underwent surgery for cannulation. Exercise was resumed approximately 3 days after the surgery when body weights recovered to preoperative levels. Exercise and candesartan treatment were continued until the day before euglycemic-hyperinsulinemic clamp procedure was performed. The clamp under high-dose insulin infusion condition was performed to assess insulin action in peripheral tissues. Western blotting was performed for analysis of protein expression in soleus muscle. All data are presented as means ± SD. The significant difference between groups was assessed by one-way analysis of variance (ANOVA) with post hoc Dunnett's test. Differences were considered to be statistically significant when $p < 0.05$.

[Results]

The mean values for daily caloric intake in each group were 51.3 ± 2.5 kcal in N, 71.5 ± 2.1 kcal in HF, 72.5 ± 2.9 kcal in HF+C, 65.8 ± 3.4 kcal in HF+Ex, and 68.9 ± 4.2 kcal in HF+C+Ex. Both candesartan and exercise training significantly suppressed body weight gain and epididymal fat weight gain in HF diet groups. Systolic blood pressure (SBP) values in HF group, HF+Ex group and N group were similar. However, the SBP in both candesartan groups with (102 ± 2 mmHg; $p < 0.01$) and without exercise (101 ± 9 mmHg; $p < 0.01$) was significantly lower than that in HF group (126 ± 8 mmHg). Plasma levels of insulin in HF group were higher than those in other groups. Oral glucose tolerance test (OGTT) showed that HF-diet induced increase in glucose levels was significantly attenuated at 30, 60, and 120 min after treatment with candesartan or exercise. The difference between the combination and each treatment alone was significant at 60 min. Area under the curve (AUC) of the OGTT of N group was significantly smaller than that of HF group ($p < 0.01$), HF with candesartan group ($p < 0.05$), and HF with exercise group ($p < 0.05$), but there were no significant differences between other groups. Euglycemic-hyperinsulinemic clamp procedure showed that the glucose infusion rate (GIR) was decreased in the HF group compared with the N group. Candesartan treatment or exercise training significantly suppressed the decrease in the GIR, whereas their combination suppressed the decrease in the GIR more

compared with that after each treatment alone. The total contents of Glut-4 protein in the N, HF+Ex, and HF+C+Ex groups were significantly higher than those in the HF and HF+C groups. Finally, there were no significant differences in phosphorylation (Ser473) of AKT and protein expression of AMPKα and PI3K.

[Discussion]

Several lines of evidence have shown that inhibition of the renin-angiotensin system improves insulin action. However, the effects and inhibition mechanisms of ACE inhibitors and ARBs, and even those of different ARBs, may differ. In the present study, the increase in body weights and the amount of epididymal fat of rats fed on HF diet were suppressed with candesartan, suggesting that candesartan affects adipose tissue rearrangements. Glut-4 protein expression in the soleus muscle was recovered with exercise but not with candesartan treatment in rats on HF-diet. We further examined the phosphorylation of AKT, upstream molecule of Glut-4. Unexpectedly we could not find differences in phosphorylation levels of AKT after treatment with candesartan. Our results may suggest the involvement of AKT-independent pathways (e.g. AMPKα, aPKC). In addition, skeletal muscles other than soleus need to be investigated because signaling mechanisms may be varied among different types of muscles.

[Conclusions]

The combination of candesartan cilexetil and exercise prevented the worsening in oral glucose tolerance test results and GIR in F344/NSlc rats fed with HF diet compared to each treatment alone. Our findings may provide information regarding the effects of candesartan cilexetil and exercise training on insulin resistance in patients with metabolic syndrome or in those at the prediabetic stage.