［課程－２］

審査の結果の要旨

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In this study, a simple method for evaluating rat serum oxidized albumin using HPLC has been established. This method is rapid and has an advantage over conventional methods and may be useful for future studies of animal models of oxidative stress-related diseases. The results are as follows.

1. The optimal condition for measurement of rat oxidized and reduced albumin are as follows: 25 mM phosphoric buffer with 60 mM sodium sulfate plus 1.5% ethanol (solution I, pH 5.3), and 1000 mM magnesium chloride (solution II). After balancing the column, the flow rate was set to 1 ml/min. The oven temperature was set to 40 ºC, and the samples volume was 3 microliters. The whole process of the serum analysis lasted for 12 minutes, and the linear gradient was 0-65% from solution I to solution II. Thus, only a total of 16 minutes was needed to analyze one sample (including the column balancing and sample analysis times).

2. The CV values of reproducibility for inter-day and intra-day reproducibility were 0.77% and 0.81%, respectively. Several samples were diluted for several times, the minimal oxidized albumin concentration for detection by was 6.4 mg/ml.

3. Incubation for 20 minutes at room temperature caused a significant increase in the percentage of oxidized albumin in a sample, and that the oxidation of albumin is time-dependent. This process was named “auto-oxidation”. Serum albumin could be auto-oxidized at room temperature after 20 minutes. The gradual increase in oxidized albumin in the intra-day reproducibility analysis may resulted from auto-oxidation. An “interference evaluation” study was conducted. In brief, commercially available oxidized albumin standards were used to calculate the percentage of oxidized albumin, and demonstrated a positive correlation between standard albumin/total albumin and oxidized albumin.

4. High salt-loading resulted in significantly higher systolic blood pressure in rats than normal salt loading ones. The urinary protein levels in the high salt diet group was also significantly higher than that of the normal salt diet group.

Urinary sodium level was significantly increased by high salt loading for 4 weeks

than normal salt loading group, the same effect cannot be seen in urinary potassium level.

Left ventricular weight to body weight (HW/BW) ratio was significantly increased in high salt loading rats than normal salt loading ones. Kidney weight to body weight (KW/BW) ratio was also significantly higher than in normal salt diet group.

At 4 weeks of treatment, Tempol significantly lowered systolic blood pressure compared with rats loading with high salt. Urinary protein level was significantly reduced in the presence of Tempol than that of the high salt diet loading group. These data suggested that Tempol can protect kidney function and have blood pressure lowering effect.

There were no significant changes in urinary sodium and potassium level at 4 weeks of treatment with Tempol. There was also no significant difference between left ventricular weight to body weight (HW/BW) ratio and kidney weight to body weight (KW/BW) ratio in rats treated with Tempol.

Oxidized albumin levels are higher in the high salt group compared to the normal salt group. and Tempol significantly reversed the effect of high salt. These results suggested that oxidative stress may play an important role in hypertension and proteinuria, and that oxidized albumin maybe a useful marker in a rat model of hypertension and proteinuria.

After 4 weeks of high salt loading, the 8-isoprostane level in the high salt diet group was significantly higher than that in the normal salt diet group, and Tempol significantly reversed the effect of high salt. The data suggested that 4 weeks high salt loading may be a reason for oxidative stress and Tempol can reversed this effect.

5. Correlations among urinary protein, 8-isoprostane and oxidized albumin

There is a positive relationship between oxidized albumin and urinary protein.

The areas under the curve (AUCs) were 0.643 for urinary 8-isoprostane and 0.917 for oxidized albumin, indicating that oxidized albumin was a superior marker to urinary 8-isoprostane ($p<0.01$) for oxidative stress-induced organ damage.

The above result shows a simple and rapid method for measuring oxidative stress levels with oxidized albumin was established and validated by using an established rat model of proteinuria and hypertension. This method has an advantage over conventional methods but not to discover the underlying molecular mechanism and progression of inflammation, maybe it is useful for future studies of animal models of oxidative stress.

よって本論文は博士（医学）の学位請求論文として合格と認められる。