D_4. Measurement E (In Vitro Assay, C-Peptide)

Fundamental Studies on the Development of C-Peptide Radioimmunoassay Kit

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We carried out the fundamental studies of C-peptide Radioimmunoassay that recently reported with an aim to materialize "C-peptide kit" which can be usable as one of the pancrease function tests.

The antiserum was prepared by immunizing synthetic human proinsulin connecting peptide to rabbit, and ^{125}I-C-peptide was obtained by labelling ^{125}I to tyrosilated connecting peptide by Hunter & Greenwood's modified method.

As assay system, the double antibody method was carried out mainly, and on B, F separation the polyethylene glycol method was studied as well.

The usable antiserum at final dilution of 1/10,000 was obtained, and it hardly showed any cross reactions against other peptide hormones.

On B, F separation, the double antibody method was more suitable than the polyethylene glycol method. First incubation of 4°C, 48 hrs. was best in the double antibody method.

In our method, the recovery rate was good; 98.4−107.3%, the standard coincided well with the endogenous C-peptide, the serum of 100−300 μl seemed usable for assay with this method.

The variations of intraassay and interassay in C-peptide values of sera were small. Any remarkable variations were not detected in the control serum values we measured for 7 weeks using the kit (Storage at 4°C).

In glucose tolerance test, the curve of C-peptide and that of insulin were parallel in the case of normal blood.

From the above results, this method seems usable enough for C-peptide measurement in blood.

Analysis of C-Peptide Radioimmunoassay (RIA) Kit

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Human C-peptide RIA kit was manufactured recently by Daiichi RI Laboratories Ltd.

The antiserum did not cross-react with human insulin. The mean recovery was 111.7%. Intraassay variability was less than 4.2% (C.V.).

0.5 ng/ml of C-peptide in the smallest amount

that can be detected with confidnece in assays.

Basal levels of plasma C-peptide in normal subjects, mild diabetics, moderate diabetics, and severe diabetics were 3.2±0.4 ng/ml (M±SE), 3.1±0.25, 3.4±0.3, 2.9±0.9 respectively. The plasma C-peptide rose gradually following oral glucose loading, with the mean peak level occurring 60 min. after glucose loading, which value was 9.8± 1.3 ng/ml. Plasma C-peptide response to glucose in diabetics was lower than in normal subjects, as well as insulin response. Measurment of C-peptide was unable and very low with insulin antibodies.

In all normal subjects intravenous glucose administration elicited a significant rise in plasma insulin and C-peptide within 3 min. . The plasma insulin level soonly returned to basal level, whereas plasma C-peptide level tended to maintain high concentration.

These results suggest that measurments of plasma C-peptide level is a useful tool to estimate beta cell function, and metabolic clearance rate of C-peptide may be more slowly than that of insulin.

Clinical Significance of Human C-Peptide Radioimmunoassay

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Serum c-peptide immunoreactivity (CPR) before and after carbohydrate loads (100 g OGTT and 2 hr postprandial) was measured in 65 insulin-treated diabetics, using C-Peptide RIA Kit (Daiichi Radioisotope Lab.). Standard curve, recovery, reproducibility and dilution curve were all satisfactory.

Insulin-requiring diabetes could be classified into two groups (Ia and Ib) according to the response of serum CPR to carbohydrate loads. Group Ia showed low, but significant response (fasting 2.11±1.23 ng/ml (M±SD) and at 90′ 4.36±0.81 max.), but group Ib no response (fasting 1.03± 0.93 and at 60′ 1.25±0.93 max.) in 100g OGTT. Postprandial CPR in normal controls, group Ia and Ib were 4.34±1.23, 4.60±1.60 and 1.13±0.72.

Urinary CPR was measured also. In 30: 1−60: 1 dilution of urine stable data could be obtained, suggesting the influence of urinary inhibitors on CPR assay could be minimized at these dilutions. Recovery of added human c-peptide (0.39−12.5 ng/ml) was 101.6±4.7% (M±SD) in 30: 1 dilution and 102.8±6.9% in 40: 1 dilution. Intraassay variation in 30: 1 and 40: 1 dilution was 10.3 and 3.5% respectively.

CPR excreted in 24 hr urine was 70.7 and 107.8 μg/day in two normal subjects. Twenty-four hr urinary CPR in two cases of Ia group was 60.5 and 109.4 μg/day, but undetectable in a case of Ib group.

It was suggested that measurements of CPR response after carbohydrate load and 24 hr urinary CPR were simple and very useful for clinical studies of insulin-requiring diabetes, because the classification of insulin-treated diabetes into two groups was possible according to presence or absence of endogenous secretion of insulin.