Human chorionic gonadotropin like substance and cancer

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SUMMARY

1) HCGLS, present in the urine of the patients with carcinoma, was lacking in some antigenic properties of HCG and had a low biological activity compared with HCG.

2) The incidence of HCGLS is high in various carcinoma, especially those of urogenital origin, although clarification by the anti-HCGLS and HCGLS system were not obtained.

INTRODUCTION

A human chorionic gonadotropin like substance (refered as HCGLS) was observed in the urine of patient with gastric carcinoma. By double immunodiffusion (Ouchterlony's method) it was found that this substance was partially fused with human chorionic gonadotropin (HCG) and was lacking in some antigenic properties of HCG[1].

To determine the biological activity, Freedman's reaction was carried out and the results were negative by the same immunological amount of HCGLS where HCG produced positive reactions. Even ten fold amounts of HCGLS failed to bring the values to positive, but some effects on ovary were noted.

This paper deals with data concerning the radioimmunoassay of HCG in various sera with malignant diseases and the difference of biological activity and antigenic difference were discussed.

MATERIALS AND METHODS

Radioimmunoassay:

On 322 cases including various diseases and normal individuals, the radioimmunoassay of HCG was carried out by a commercial available kit (CEA) which is theoretically a double antibody technique. The standard curve was obtained from 1.0 ng/ml to 50 ng/ml.

Purification of HCGLS:

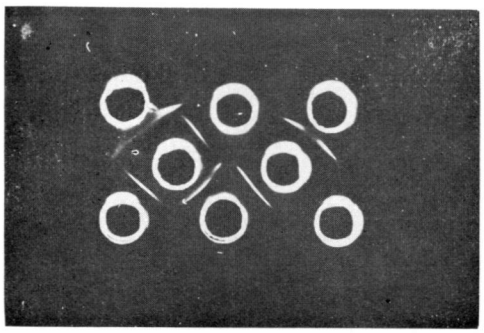
The urine samples of the patient, positive for RIA and which were positive by immunopregnancy test were extracted by Butt's method[2]. The extracted HCGLS was twice applied to DEAE cellulose column, followed by gel-filtration. Finally the highly purified HCGLS was applied to the anti-NHS coupled sepharose column.

Comparison between HCGLS and HCG:

Highly purified HCGLS and HCG were compared with each other by the Ouchterlony method using anti-HCG. HCGLS was also compared with the purified LH. These comparison were confirmed with the absorption test of anti-HCG by HCGLS.

Biological test:

The biological activity was studied by a quantitative mouse uterine assay[3]. The control mice were administered with 0.1 (0.5), 0.2 (1.0), 0.4 (2.0), 0.6 (3.0) immunological unit (biological unit) of HCG standard (pregnyl. Organon, Co. Ltd.). The standard curve was established by the mean values of each six mice. And several cases of the HCGLS from 0.36 to 3.0 immunological units were adjusted by the anti-HCG-HCG system which were administered to the groups consisting of six female mice. The activity was examined by the standard curve.



COMPARISON OF H.C.G.L.S. AND H.C.G.
BY OUCHTERLONY'S METHOD.

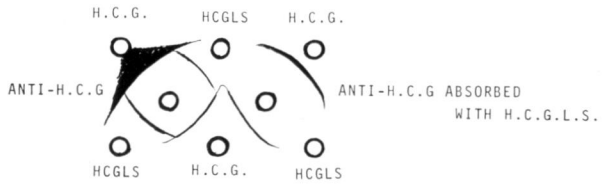


Fig. 1. Distribution of anti-HCG reacting substance in various diseases. ⊠···5 cases ●···1 case

RESULTS

Distribution of the substance reacting with anti-HCG in various diseases:

As shown in Fig. 1, the distribution of anti-HCG reacting substance in patients with cancer showed a relatively wide distribution compared to normal and benign diseases. In choriocarcinoma and epithelioma, seminoma and teratocarcinoma which are known to produce HCG extremely high levels of HCG were noted.

From the distribution range in normal male and female, positive was designated as over 2.5 ng/ml in male and 3.0 ng/ml in female arbitrarily.

Incidence of the positive cases:

As summarized in Table 1, positive reaction was observed in various carcinoma. The incidence among carcinoma was 32.6% in male and 37.9% in

Table 1. *Incidence of positive reaction.*

Diagnosis	male (≧2.0 ng/ml)		female (≧2.5 ng/ml)	
	No. cases	positive	No. cases	positive
Ca. Stomach	69	12	32	13
Esph., rect.	17	1	14	6
Lung.	13	3	6	2
Mam.	*	*	7	2
Hepatoma	22	7	3	1
Ca. Kidney	5	4	1	1
Bladder	8	6	*	*
Prostate	13	10		
Uterus	*	*	9	2
Seminoma	7	5	*	*
Chorio.	1	1	1	1
Malig. terat.	2	1	1	0
Ca. Ovary	*	*	3	2
AML, RS. HD.	11	6	5	2
Other malig. dis.	8	1	5	1
Total	176	57	87	33
Livercirrhosis	8	0	4	0
Hepatitis	12	2	7	1
Total	20	2	11	1
Normal	14	0	15	0
Total	210	59	113	34

female. Except for chorioepithelioma, seminoma and teratocarcinoma, the incidence was 30.3% (50 cases out of 165) in male and 36.6% (30 cases out of 82) in female. In all carcinoma, it was striking fact that the incidence of urogenital carcinoma was quite high (77.9%) in male. And it was also high in malignant hematopoietic diseases and carcinoma of the alimentary tract. On the other hand carcinoma of the alimentary tract, especially esophagus cancer showed a high incidence.

In benign diseases, including livercirrhosis and hepatitis, this substance was positive in hepatitis, but the amount was only slightly over the normal limit.

Biological activity of the HCGLS by a quantitative mouse uterine assay:

The mouse administered with 1.4 immunological unit of HCGLS were negative. However as it increased to 3.0 immunological unit, it turned positive. On the other hand, the purified standard HCG (pregnyl) gave a positive reaction as low as 0.1 immunological unit.

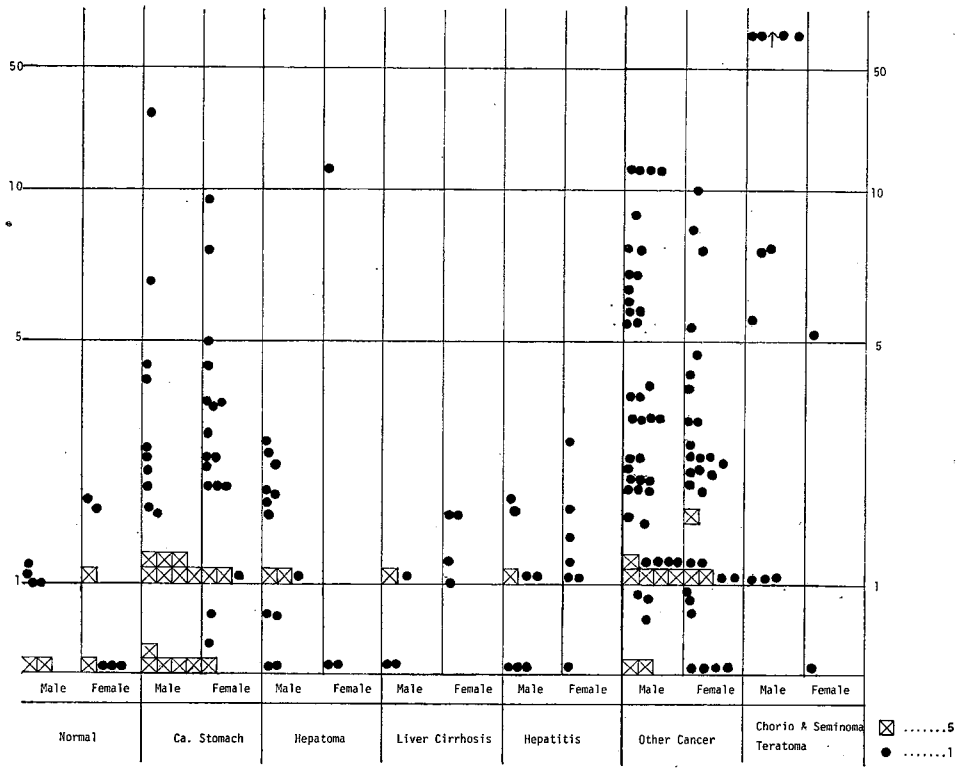


Fig. 2. Comparison of HCGLS and HCG by anti-HCG on Ouchterlony mehtod.

Comparison between HCGLS and HCG by the Ouchterlony method:

As shown in Fig. 2, the Ouchterlony method revealed that HCG and HCGLS showed a precipitation line to the anti-HCG. The precipitation lines were partially fused and a spur was observed against HCGLS. The absorbed anti-HCG could not produce a precipitation line against HCGLS, but developed a line to HCG. Comparison of HCGLS to LH revealed that HCGLS and LH had the same antigenic determinant, but the absorbed anti-HCG was still active to LH. The purified HCGLS samples, isolated from urine, showed the same reaction, namely lacking some antigenicity of HCG.

DISCUSSION

Relation between HCGLS and HCG

The HCGLS, lacking some antigenic properties of HCG, had a weak biological activity, although the Freedman reaction was negative. By a quantitative assay of the mouse uterine weight, it was noted that the activity was about 30 times less than HCG, comparing by the same immunological units. Since this substance was isolated from patients with gastric carcinoma, adenocarcinoma, mucocellular HCGLS was classified under the category of the carcinoplacental protein. The relation between HCGLS and HCG was not completely resolved. However the chromatographic resolution on Sephadex and DEAE cellulose column revealed that the molecular weight was similar to and the elution pattern was defferent from HCG.

As mentioned by others, the HCG from pregnant women and from choriocarcinoma were antigenically similar to each other, but the biological activity thereof were different. Especially the HCG with different biological activity was separated partially by DEAE cellulose column[4]. The HCGLS was similar to the HCG with a low biological activity and not to the "HCG analog". Recently Gustine mentioned that there were developmental changes in several proteins in mice which should mostly be attributed to the content of sialic acid[5]. On the other hand by the neuraminidase treatment, the biological activity was reduced coincidently with the grade of the desialylation. From these reports, the HCGLS can be considered as an "HCG analog" with an extremely low biological activity.

Incidence of HCGLS among carcinoma

For the titration of HCGLS, the anti-HCGLS-HCGLS system is indispensable. However the anti-HCG-HCG system was employed for the preliminary detection of HCGLS, since the system remains unclarified. Using

radioimmunoassay, positive sera were examined and the urine of positive sera was also examined for HCG reaction substance. Most cases were also positive, in urine, followed by the extraction according to Butt and purified by gel-filtration and DEAE. The identification of HCGLS among the anti-HCG reacting substance was performed by the characteristic of DEAE resolution and the double immunodiffusion method. In three patients selected, the anti-HCG reacting substances were identical to HCGLS and satisfied the definition of HCGLS[6]. From random sampling, three cases among anti-HCG reacting sera were positive HCGLS. The possibility of HCGLS among positive sera is high except in the HCG producing diseases.

As shown in the Table and Figures, it was striking that the incidence of positive cases in cancer of urogenital origin was extremely high and it reminds us that this clinic is related to the development of the organ. It is reasonable to compare the HCG microheterogeneity during development[7].

Recently Braunstein reported that the neoplasma produced human chorionic gonadotropin is of ectopic production and the biological activity and reactivity were the same as normal HCG[8]. However he did not compare the identity with each other by the Ouchterlony and the relative biological activity per immunological units. Since the reactivity of HCGLS to anti-HCG was almost the same as HCG and it was hardly distinguishable from HCG by its reactivity, it is difficult to conclude the ectopic production of HCG.

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