Jpn. J. Cancer Res. (Gann)
79, 160-164; February, 1988

**IMMOBILIZED *DATURA STRAMONIUM*
AGGLUTININ COLUMN CHROMATOGRAPHY, A NOVEL METHOD TO
DISCRIMINATE THE URINARY hCGs OF
PATIENTS WITH INVASIVE MOLE AND
CHORIOCARCINOMA FROM THOSE OF
NORMAL PREGNANT WOMEN AND
PATIENTS WITH HYDATIDIFORM MOLE**

Tamao ENDO,^{*1} Koichi IINO,^{*2}
Shiro NOZAWA,^{*2} Rihachi IIZUKA^{*2} and
Akira KOBATA^{*1}

^{*1}Department of Biochemistry, the Institute of Medical Science, the University of Tokyo, Minato-ku, Tokyo 108 and ^{*2}Department of Obstetrics and Gynecology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160

Datura stramonium agglutinin (DSA) binds the asparagine-linked sugar chains commonly found in the human chorionic gonadotropin (hCG) purified from urine of patients with invasive mole or choriocarcinoma, but not in that of normal pregnant women or patients with hydatidiform mole. With the use of an immobilized DSA column, a novel method to discriminate urinary hCGs from various trophoblastic diseases was developed.

Key words: Human chorionic gonadotropin — *Datura stramonium* agglutinin — Trophoblastic disease — Affinity chromatography — Choriocarcinoma

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by trophoblasts of placenta. High levels of hCG in the blood and urine are also detected in patients with various trophoblastic diseases such as hydatidiform mole and choriocarcinoma.¹⁾

The abbreviations used are: hCG, human chorionic gonadotropin; DSA, *Datura stramonium* agglutinin; NeuAc, *N*-acetylneuraminic acid; Gal, galactose, GlcNAc, *N*-acetylglucosamine; Man, mannose; Fuc, fucose. All sugars mentioned in this paper were of D-configuration except for fucose, which was of L-configuration.

Therefore, urinary and serum hCGs have been measured as useful markers for the diagnosis and prognosis of trophoblastic diseases as well as normal pregnancy.

Many sensitive methods to determine the level of hCG in biological materials have been developed.^{2,3)} Although these methods have solved the problem of eliminating interfering materials which coexist in the samples, none of them provided a way to discriminate hCGs from various trophoblastic diseases. Differential diagnosis of trophoblastic diseases is important because it is essential for correct treatment of these diseases. Hydatidiform mole is considered to be essentially a benign lesion, although the rate of incidence of choriocarcinoma in patients with this disease is much higher than in normal pregnancy. Some hydatidiform moles show apparently more malignant characteristics than others, such as invasion into the surrounding tissues and metastasis, and are discriminated from typical moles by naming them invasive moles. Although prophylactic chemotherapy is effective to reduce the development of persistent gestational trophoblastic diseases such as invasive mole and choriocarcinoma, the use of chemotherapy at the time of molar evacuation is controversial because of the drug toxicity. Therefore, any method to discriminate invasive mole from hydatidiform mole would be useful to avoid indiscriminate prophylactic chemotherapy.

We have investigated the structures of the carbohydrate moieties of hCGs purified from the urine of patients with various trophoblastic diseases.^{4,7)} Although all hCGs contain four asparagine-linked sugar chains in one molecule, their structures are different depending on the disease. The asparagine-linked sugar chains of urinary hCGs from normal pregnant women and patients with hydatidiform mole contain oligosaccharides C, D and H (Fig. 1) in a molar ratio of 1:2:1.^{5,7)} In contrast, urinary hCGs from patients with invasive mole contain oligosaccharides A, B, C, D, G and H.⁶⁾ Urinary hCGs from

Fig. 1. The asparagine-linked sugar chains found in hCGs isolated from urine of patients with choriocarcinoma.

patients with choriocarcinoma contain all eight oligosaccharides shown in Fig. 1.^{4,5)} Accordingly, a method that specifically detects the hCGs containing the $\pm$ NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 4($\pm$ NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 2)Man group in their sugar moieties could be used to discriminate patients with invasive mole or choriocarcinoma from pregnant women or patients with hydatidiform mole.

By investigating the behavior of various complex-type oligosaccharides in several im-

mobilized lectin columns, we found that a *Datura stramonium* agglutinin (DSA)-Sephacrose column fractionates the oligosaccharides into three groups.⁸⁾ All oligosaccharides with either the Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 6(Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 2)Man group or the Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ group in non-substituted form are bound to the column and eluted with buffer containing β -N-acetylglucosamine oligomers. Oligosaccharides with the non-substituted Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 4(Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 2)Man

group are retarded in the column and eluted with buffer only. Oligosaccharides which contain none of the groups described above or the groups in either sialylated or fucosylated form pass through the column without interaction. Since the binding specificity of the DSA-Sepharose column was expected to be useful in distinguishing malignant hCGs from the normal counterpart, we have performed basic studies to establish a new diagnostic method for trophoblastic diseases with the use of the lectin affinity column.

Urine samples were collected from five normal pregnant women, from six patients with hydatidiform mole, from three with invasive mole and from five with choriocarcinoma. The samples were either concentrated or diluted with distilled water to adjust the concentration of hCG to the range of 5,000 to 10,000 mIU/ml.

The Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 4(Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 2)Man group in the sugar chains of invasive mole hCG and choriocarcinoma hCG may or may not be sialylated. Since the sialylated pentasaccharide residues do not interact with a DSA-Sepharose column,⁸⁾ the behavior of hCGs in the urinary samples must be investigated before and after removal of sialic acid residues. In order to remove sialic acid residues from hCG, each urine sample (50 μ l) was adjusted to pH 5.0 by adding 0.5 M acetate buffer, pH 3.5, and incubated with 200 mU of *Arthrobacter ureafaciens* sialidase (Nakarai Chemicals Ltd., Kyoto) at 37° for 16 hr. It was confirmed by the thiobarbituric acid method⁹⁾ that all protein-bound sialic acids in the urine were released as free sialic acid by this treatment (data not shown). The reaction mixture, thus obtained, and the untreated urine samples were adjusted to pH 7.0 by adding 1M Tris, and subjected to DSA-Sepharose column chromatography.

DSA-Sepharose, prepared as described in the previous paper,⁸⁾ was packed to make a column of 1 cm diameter and 6 cm height. Urine samples were loaded on the DSA column and left at 4° for 30 min. Then, elution was started with 15 ml of 10mM Tris-buffered saline, pH 7.4, followed by 10 ml of 10mM Tris-buffered saline, pH 7.4, containing 1% of a mixture of equal amounts of *N,N'*-diacetylchitobiose, *N,N',N''*-triacetylchitotriose and *N,N',N'',N'''*-tetraacetylchitotetraose, and

finally with 10 ml of 0.1N acetic acid. Chromatography was performed at 4° at a flow rate of approximately 5 ml/hr. Fractions (1 ml) were collected and the amount of hCG in an aliquot (50 μ l) of each fraction was determined by enzyme immunoassay with the use of the HCG-CTP test (Takeda Chemical Co., Osaka).

Elution profiles of two representative choriocarcinoma hCGs and a normal hCG are shown in Fig. 2. Almost all hCGs in the urine of a pregnant woman passed through the column (Fig. 2A, solid line). None of them bound to the column after sialidase digestion (Fig. 2A, dotted line). In contrast, only a part of hCG in the urine of a choriocarcinoma patient passed through the column. The remainder was not eluted even with buffer containing *N*-acetylglucosamine oligomers, but was completely recovered by elution with 0.1N acetic acid (Fig. 2B, solid line). This unexpectedly strong binding might be produced because the hCG molecule contains

Fig. 2. Chromatography of hCG in urine samples on a DSA-Sepharose column. Samples were applied to a DSA-Sepharose column and fractions were collected as described in the text. The amounts of hCG (urine samples, —; desialylated urine samples, - - -) in each fraction was determined. Arrows 1 and 2 indicate the points where elution buffer was changed to a 1% solution of a mixture of equal amounts of *N,N'*-diacetylchitobiose, *N,N',N''*-triacetylchitotriose and *N,N',N'',N'''*-tetraacetylchitotetraose, and to 0.1M acetic acid, respectively. A, Urine from a normal pregnant woman; B and C, urine from choriocarcinoma patients.

at least two oligosaccharides with the pentasaccharide group interacting with DSA. Therefore, the elution step with 1% chito-oligosaccharide solution was omitted to obtain the data in Fig. 3. As expected, the portion of hCG bound to the DSA-Sepharose column increased after sialidase digestion (Fig. 2B, dotted line). Most other choriocarcinoma hCGs behaved as shown in Fig. 2B. However, one choriocarcinoma hCG bound completely to the DSA column without sialidase treatment (Fig. 2C, solid line).

After the affinity chromatography, the percentage of the bound hCG fraction was calculated for each urine sample and the data are summarized in Fig. 3. The values of normal pregnant women and of patients with hydatidiform mole were less than 15%, which did not increase after sialidase digestion (Fig. 3A and B). The values of three patients with invasive mole were also within this limit. However, they became much higher after sialidase treatment (Fig. 3C). These results are in good accord with the evidence revealed

by structural studies of asparagine-linked sugar chains of hCGs purified from urine of patients with trophoblastic diseases.⁴⁻⁶ Although invasive mole hCGs contain sugars with the $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4(\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 2)\text{Man}$ group, these sugars are almost completely sialylated.

The results in the case of choriocarcinoma hCGs showed more variation than in the case of invasive mole hCGs (Fig. 3D). Some of them behaved very similarly to invasive mole hCGs. A significant portion of a choriocarcinoma hCG could be bound to the column before sialidase digestion, although the value became much higher after desialylation. One of the choriocarcinoma hCGs bound completely to the column before sialidase digestion, as already documented in Fig. 2C. These data accord with the fact that all choriocarcinoma hCG contain the eight oligosaccharides shown in Fig. 1, which are sialylated to various extents.⁵

Therefore, this affinity chromatography can effectively be used to discriminate malignant hCGs from normal hCGs in urine samples. Desialylation is essential for this purpose. However, a comparative study of the behavior of hCGs on a DSA column before and after sialidase treatment should cast light on the problem of sialylation. We have previously observed an interesting case in which the asparagine-linked sugar chains of the hCG obtained from urine of a choriocarcinoma patient were completely sialylated, while a sample obtained from the same patient at a later stage contained non-sialylated sugars.⁶ This observation may indicate that the aberration of sialyltransferase might occur only at the advanced stage of choriocarcinoma. This possibility might be confirmed by analyzing more choriocarcinoma samples by means of this affinity chromatography.

This study was supported in part by a Grant-in-Aid for Special Project Research (Cancer-Bioscience) from the Ministry of Education, Science and Culture of Japan. The authors wish to thank Miss Yumiko Kimizuka for her expert secretarial assistance.

(Received Nov. 17, 1987/Accepted Jan. 9, 1988)

Fig. 3. Percent molar ratio of urinary hCGs bound to a DSA-Sepharose column before (○) and after (●) sialidase digestion. A, Urine samples from normal pregnant women; B, those from hydatidiform mole patients; C, those from invasive mole patients; D, those from choriocarcinoma patients.

REFERENCES

- 1) Pierce, J. G. and Parsons, T. F. Glycoprotein hormones: structure and function. *Ann. Rev. Biochem.*, **50**, 465-495 (1981).
- 2) Chen, H.-C., Hodgen, G. D., Matsuura, S., Lin, L. J., Gross, E., Reichert, L. E., Jr., Birken, S., Canfield, R. E. and Ross, G. T. Evidence for a gonadotropin from non-pregnant subjects that has physical, immunological, and biological similarities to human chorionic gonadotropin. *Proc. Natl. Acad. Sci. USA*, **73**, 2885-2889 (1976).
- 3) Stenman, U., Alfthan, H., Myllynen, L. and Seppälä, M. Ultrarapid and highly sensitive time-resolved fluoroimmunoassay for chorionic gonadotropin. *Lancet*, **2**, 647-649 (1983).
- 4) Mizuochi, T., Nishimura, R., Derappe, C., Taniguchi, T., Hamamoto, T., Mochizuki, M. and Kobata, A. Structures of the asparagine-linked sugar chains of human chorionic gonadotropin produced in choriocarcinoma: appearance of triantennary sugar chains and unique biantennary sugar chains. *J. Biol. Chem.*, **258**, 14126-14129 (1983).
- 5) Mizuochi, T., Nishimura, R., Taniguchi, T., Utsunomiya, T., Mochizuki, M., Derappe, C. and Kobata, A. Comparison of carbohydrate structure between human chorionic gonadotropin present in urine of patients with trophoblastic diseases and healthy individuals. *Jpn. J. Cancer Res. (Gann)*, **76**, 752-759 (1985).
- 6) Endo, T., Nishimura, R., Kawano, T., Mochizuki, M. and Kobata, A. Structural differences found in the asparagine-linked sugar chains of human chorionic gonadotropins purified from the urine of patients with invasive mole and with choriocarcinoma. *Cancer Res.*, **47**, 5242-5245 (1987).
- 7) Endo, Y., Yamashita, K., Tachibana, Y., Tojo, S. and Kobata, A. Structures of the asparagine-linked sugar chains of human chorionic gonadotropin. *J. Biochem.*, **85**, 669-679 (1979).
- 8) Yamashita, K., Totani, K., Ohkura, T., Takasaki, S., Goldstein, I. J. and Kobata, A. Carbohydrate binding properties of complex-type oligosaccharides on immobilized *Datura stramonium* lectin. *J. Biol. Chem.*, **262**, 1602-1607 (1987).
- 9) Warren, L. The thiobarbituric assay of sialic acids. *J. Biol. Chem.*, **234**, 1971-1975 (1959).