Changes in CA602 and CA546 concentrations in patients during in-vitro fertilization and embryo transfer, and ovarian hyperstimulation syndrome

Kiyoshi Takamatsu1,2, Michiko Kasuga1, Masao Nakano2, Yasuhiro Udagawa1, Yasunori Yoshimura1 and Shiro Nozawa1

1Department of Obstetrics and Gynecology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan and 2Department of Obstetrics and Gynecology, Saiseikai Kanagawaken Hospital, 6-6 Tomiyacho, Kanagawa-ku, Yokohama-city, Kanagawa 221, Japan

© European Society for Human Reproduction and Embryology 441

Introduction

Tumour markers are potentially useful in the diagnosis of malignant diseases. Recently we established two new tumour markers for ovarian cancers, CA602 (Nozawa et al., 1991) and CA546 (Nozawa et al., 1989). CA602, a protein antigen, showed high positive rates in patients with ovarian cancer, especially in those with serous cystadenocarcinoma. This marker exhibits characteristics similar to those of CA125 (Bast et al., 1983) and shows a relatively high false-positive rate in the presence of endometriosis (Suzuki et al., 1990; Nozawa et al., 1991). CA546, a glycosidic chain antigen, shows high positive rates in patients with mucinous cystadenocarcinoma and low false-positive rates in patients with benign diseases including endometriotic cysts (Nozawa et al., 1992a). Thus, these two markers have complementary potential for establishing the diagnosis of ovarian cancer (Nozawa et al., 1992b).

Tumour markers are also used in fields other than oncology. Like CA125, CA602 appears in relatively high concentrations in patients with non-neoplastic conditions, such as endometriosis, ascites and pelvic inflammatory disease (Nozawa et al., 1991). Both CA602 and CA125 can be used as markers for the progression of endometriosis or the effect of its treatment (Barbieri et al., 1986; Suzuki et al., 1990).

The in-vitro fertilization (IVF) and embryo transfer procedure has become a common assisted reproductive technique. A serious complication of this procedure is ovarian hyperstimulation syndrome (OHSS), which is caused by the over-induction of ovulation by pharmacological means (Bettendorf and Lindner, 1987). Though the mechanism of OHSS is not clarified, the support of luteal function with injections of human chorionic gonadotrophin (HCG) is known to be one of the causes of OHSS. The discovery of indicators that could reveal the onset and the degree of severity of this syndrome would be therefore of the utmost clinical importance. A few attempts have been made to evaluate the usefulness of tumour markers in the diagnosis of OHSS, but only some studies have followed the changes in tumour markers from the beginning of ovarian stimulation (Jaeger et al., 1987; Eiermann and Collins, 1989; Lanzone et al., 1990; Zweers et al., 1990). Accordingly, in this study we measured the changes in serum concentrations of CA602 during IVF and embryo transfer to evaluate its use as a marker for the diagnosis of OHSS. Serum concentrations of CA546 were also monitored.

Materials and methods

Subjects

A total of 17 Japanese women were enrolled in this study with informed consent. They were being treated for infertility, especially bilateral tubal occlusion, in the outpatient clinic of Saiseikai Kanagawaken Hospital (Yokohama-city, Kanagawa, Japan). All patients desired the IVF and embryo transfer procedure. Five of the 17 patients were excluded because of endometriosis. Finally, 12 patients aged 26–40 years were registered.

A hormonal examination, including a gonadotrophin-releasing hormone (GnRH) and a thyrotrophin-releasing hormone test, before the IVF and embryo transfer procedure showed no abnormal hormonal conditions.

IVF and embryo transfer procedure

The IVF and embryo transfer procedure was performed as described previously (Konishi et al., 1993). Individualized ovarian stimulation was performed with pure follicle stimulating hormone (FSH)–human menopausal gonadotrophin (HMG)–HCG in combination with GnRH analogue. Briefly, while endogenous gonadotrophins were being suppressed with GnRH analogue (Suprecure; Hoechst, Frankfurt,
Figure 1. CA602 concentration during in-vitro fertilization and embryo transfer. Points of blood sample collection were: (i) during the previous cycle (not in the menstrual period); (ii) when the leading follicle grew to > 15 mm in diameter; (iii) at oocyte retrieval; (iv) at embryo transfer; and (v) 10 days after embryo transfer. (●) Cases with a positive pregnancy test 14 days after oocyte retrieval. n = number of patients; OHSS = ovarian hyperstimulation syndrome.

Figure 2. CA546 concentration during in-vitro fertilization and embryo transfer. Points of blood sample collection were as for Figure 1. (●) Cases with a positive pregnancy test 14 days after oocyte retrieval. See Figure 1 for abbreviations.

interassay coefficients of variation were < 4.4–6.0% for CA602 and < 2.5–7.5% for CA546.

The serum concentration of oestradiol was measured with semiautomatic equipment (SR-1; Biochem Immunosystems, Tokyo, Japan). The complete blood count and total protein concentration in serum were also measured.

To detect OHSS after embryo transfer, patients were checked using transvaginal ultrasonography and questioned about their condition. According to the classification of OHSS by the World Health Organization (WHO Scientific Group, 1973), patients with swelling of the ovaries to > 5 cm in diameter were categorized as having OHSS. Massive ascites and complaints of lower abdominal pain were also defined as OHSS.

Statistical analysis
Data are presented as the means ± SEM. A statistical analysis was performed using paired or non-paired Student’s t-tests. The post-hoc test was Scheffe’s F-test. Differences were considered to be statistically significant if the P value was < 0.05. All analyses were performed using standard computer software for statistical analysis (StatView-J, version 4.02; Abacus Concept Inc., Berkeley, CA, USA).

Results
Of the 12 women who underwent pharmacological induction of ovulation, five (41.6%) developed OHSS. All had mild or
CA602 and CA546 concentrations during IVF and embryo transfer

The OHSS group, the serum concentrations of CA602 were significantly increased 10 days after embryo transfer. At this point, the mean serum concentration of CA602 in the OHSS group was significantly higher than that in the non-OHSS group (Table I). The CA546 concentration did not change substantially during the course of IVF and embryo transfer (Figure 2). No difference in the CA546 concentration was found between the OHSS and the non-OHSS groups.

The concentrations of oestradiol before oocyte retrieval were 1358.4 ± 395.4 pg/ml in the OHSS group and 556.7 ± 111.3 pg/ml in the non-OHSS group; the difference was not statistically significant. However, 10 days after embryo transfer, the concentration of oestradiol in the OHSS group (3951.5 ± 706.0 pg/ml) was significantly higher than that in the non-OHSS group (773.5 ± 169.8 pg/ml) (Figure 3).

Discussion

CA602 and CA546 are newly established carbohydrate-related tumour markers in our institute, which are used for the diagnosis of ovarian cancers. To evaluate the possibility of tumour markers also acting as markers for OHSS, we measured the serum concentrations of CA602 and CA546 at five points during IVF and embryo transfer. Although no change in the CA602 concentration was observed until embryo transfer in the OHSS group, there was an immediate and sharp increase 10 days after embryo transfer. At the time of oocyte retrieval, a needle is inserted into the ovary; the bleeding that occurs could lead to stimulation of the peritoneum. However, in the non-OHSS group, there was no change in the course of the procedure of the study. In the OHSS group, three out of five patients became pregnant. This might suggest that the elevation of CA602 was due not to OHSS but to pregnancy. It has been reported that CA602 and CA125 concentrations do increase in the first trimester of pregnancy (Halila et al., 1986; Nozawa et al., 1991). However, there were two patients in the OHSS group who were not pregnant but who showed an increase in CA602, and one pregnant patient without OHSS but with no change in CA602. These results suggested that the elevation of CA602 in the OHSS group could indeed be explained by OHSS.

All cases of OHSS in this study were never more than at the grade II or moderate stage (Rabau et al., 1967; Schenker and Weinstein, 1978; Bettendorf and Lindner, 1987; Blankstein et al., 1987). Even at this early stage, the serum concentrations of CA602 were increased significantly compared with those in the non-OHSS group. Both differences were statistically significant (P < 0.01 and P = 0.01 respectively). At most, three fertilized oocytes were transferred. Four pregnancies occurred in the study subjects. The course of the three pregnancies in the OHSS group was uneventful. The one pregnancy in the non-OHSS group ended in a chemical abortion.

The mean concentrations of CA602 in the OHSS group were higher than those in the non-OHSS group at all sampling points through to embryo transfer, but at no time was there a statistically significant difference. During stimulation of the ovaries and until embryo transfer, the CA602 concentrations in both the OHSS and the non-OHSS groups were steady, almost below the threshold value of 63 U/ml (Figure 1). In

CA602 and CA546 concentrations during IVF and embryo transfer
Table I. CA602 concentrations (U/ml) during in-vitro fertilization and embryo transfer at various sampling times

<table>
<thead>
<tr>
<th>Group</th>
<th>Sampling point#</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHSS</td>
<td></td>
<td>29.1 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.9 ± 8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.3 ± 7.7&lt;sup'id&lt;/sup&gt;</td>
<td>41.9 ± 8.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>155.6 ± 57.5</td>
</tr>
<tr>
<td>Non-OHSS</td>
<td></td>
<td>25.6 ± 2.9</td>
<td>29.2 ± 2.8</td>
<td>25.9 ± 2.8</td>
<td>30.8 ± 4.0</td>
<td>35.9 ± 3.6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

OHSS = ovarian hyperstimulation syndrome.

<sup>a</sup>Points of blood sample collection: (i) during the previous cycle (not in the menstrual period); (ii) when the leading follicle became >15 mm in diameter; (iii) at oocyte retrieval; (iv) at embryo transfer; and (v) 10 days after embryo transfer.

<sup>b</sup>-<sup>g</sup>P < 0.01 compared with point 5.

<sup>e</sup>P = 0.03 compared with the corresponding sampling point in the OHSS group.

be suitable predictors of OHSS, but may be indicators of OHSS. Compared with CA125, the great advantage of CA602 is higher sensitivity. The detection limits of both markers were almost the same since it was known that 258 CA602 units were equal to 108 CA125 units (Nozawa et al., 1991). The gradient of the standard curve (units–absorbance curve) for the CA602 kit is steeper than that for the CA125 kit. Thus, CA602 can detect a smaller change with an at least 2-fold greater sensitivity than CA125. Recently, a second-generation CA125 assay was introduced into clinical use. However, it was reported that the ability of this assay to differentiate between patients with and without endometriosis is small, especially in the lower range (Hornstein et al., 1995). From these points of view, CA602 is especially useful when small changes in a narrow range are considered, as in OHSS.

The origin of CA602 in OHSS remains to be clarified. One hypothesis is that the gonadotrophic stimulus leads to ovarian enlargement, which modifies the surface of the ovary and also increases steroid production, which in turn increases the thickness of the endometrium; the tumour marker then pours into the circulatory stream (Scarpellini and Scarpellini, 1992). Some investigators have concluded that the high CA125 concentration in OHSS patients could be related to a peritoneal contribution (OezakSit et al., 1993). On the other hand, CA125 concentrations in serum from ovarian cancer patients were not correlated with the size of the ovarian tumour (Fleuren et al., 1987). In animal models, isolation of both ovaries from the peritoneal cavity did not prevent ascites formation in the presence of OHSS (Yarali et al., 1993). With respect to peritoneal stimulation, repeated aspiration of ovarian follicles and early corpus luteum cysts has been found to reduce the risk of OHSS (Amit et al., 1993). In endometriosis, some investigators focused on the angiogenic factor released by the ovary (McLarend et al., 1996). This factor may also be released in OHSS and alter the permeability of the peritoneum and ovary, resulting in the increased concentrations of CA602.

Among current biochemical analyses, oestriadiol concentrations in serum or urine are only used for the evaluation of OHSS (Schenker and Weinstein, 1978). In this study, oestriadiol concentrations in the OHSS group were significantly higher than those in the non-OHSS group at 10 days after embryo transfer. The oestriadiol concentration is, however, thought to be associated with the number of follicles (MacDougall et al., 1993), and the serum oestriadiol concentrations before oocyte retrieval exhibited a relatively wide range compared with the concentrations of CA602. There was a case in our study, for example, in which the oestradiol concentration was high, ~2800 pg/ml, before oocyte retrieval, but the CA602 concentration was in the normal range, 52 U/ml. In the patients excluded from this study because of endometriosis, high oestradiol concentrations before oocyte retrieval were not related to CA602 concentrations (data not shown).

From the economic point of view, the analysis of tumour markers is a relatively high cost procedure. However, this is balanced by high sensitivity, and much progress has been made towards establishing simpler and cheaper kits for measuring CA602.

In conclusion, our preliminary results suggest that CA602 may serve as an indicator of OHSS. Further prospective investigations of large numbers of patients will reveal its usefulness.

Acknowledgements

The authors are indebted to Dr Shin-ichi Yoshimura, Dr Jun-ichi Kobayashi and Dr Yasuhiro Konishi for their support and suggestions, and to Ms Hiromi Enomoto and Ms Miho Murata for their excellent technical assistance. We also thank Mochida Co. Ltd for generously providing the kits for measuring CA602 and CA546.

References


