Warrington, PA, USA) in PBS at 0°C for 15 minutes and postfixed with 80% ethanol for at least 2 hours at –20°C. The fixed cells were washed twice in PBS and suspended in a 1% (w/v) solution of bovine serum albumin (Sigma-Aldrich) in PBS, to suppress nonspecific antibody binding. The cells were then incubated in 100 μL of 1% bovine serum albumin containing 1:100 diluted antiphosphohistone H2AX (Ser-139) monoclonal antibody (Upstate, Lake Placid, NY, USA) for 2 hours at room temperature, washed twice with PBS, and resuspended in 100 μL of 1:20 diluted fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of goat antimouse immunoglobulin (Dako, Glostrup, Denmark) for 30 minutes at room temperature in the dark. The cells were then counterstained with 5 μg/mL propidium iodide (Sigma-Aldrich) in the presence of 100 μL of ribonuclease A (Sigma-Aldrich) for 30 minutes.

Fluorescence measurements by flow cytometry

The fluorescein isothiocyanate (green) and propidium iodide (red) fluorescences of individual cells in suspension induced by excitation with a 488 nm argon ion laser were measured using a FACScan flow cytometer (BD). The green and red fluorescences from each cell were separated and quantified using standard optics and CellQuest software (BD). Ten thousand cells were measured per sample. All experiments were repeated at least three times.

After γH2AX and DNA staining, the DNA content and γH2AX content determined by flow cytometry were represented on the *x* and *y* axes, respectively. The γH2AX content in each cell cycle was determined, so as to allow examination of the relationships between cell kinetics and the DNA damage induced by antitumor agents.

Results

GEM

In the OVISE cells, treatment with 5 ng/mL or more of GEM mainly caused DNA damage in cells of the early S-phase. After exposure to 100 ng/mL or more of GEM, the S-phase cells showing DNA damage underwent apoptosis. Similarly, in the RMG-I cells, DNA damage was primarily seen in the early S-phase cells following exposure to 5 ng/mL or more of GEM. Treatment with 100 ng/mL or 1 μg/mL GEM induced DNA damage not only in S-phase cells but also in G_2/M-phase cells. These cells, however, did not undergo apoptosis (Figure 2A). To investigate the time course of the changes, both cell lines were treated with GEM at the minimum concentration causing DNA damage (5 ng/mL) for different periods of time. In the OVISE cells, DNA damage was mainly confined to S-phase cells after exposure to GEM for 24 hours or more. However, after exposure for 48 hours or more, DNA damage also extended to the cells of the G_2/M phase. The S-phase cells with DNA damage underwent apoptosis after exposure to GEM for 48 hours or more, while the number of cells in the G_1 phase gradually decreased and there was S-phase arrest. Moreover, G_2/M-phase cells showing DNA damage remained viable without undergoing apoptosis. In RMG-I cells, marked DNA damage was observed in the S-phase cells after 24 hours of exposure to GEM, although the cells underwent apoptosis after 72 hours' exposure to GEM. Similar to the OVISE cells, the gradual decreases in the number of cells in the G_1 phase and S-phase arrest were also noted in RMG-I cells. G_2/M-phase cells showing DNA damage remained viable without apoptosis even after 120 hours of exposure to GEM, and G_2/M-phase arrest was induced (Figure 2B).

CBDCA

DNA damage in the S phase was seen gradually after exposure to CBDCA for 24 hours in the OVISE and RMG-I lines at 1 μg/mL and 10 μg/mL, respectively (Figure 3A). Subsequently, cells with damaged DNA underwent apoptosis. Gradually, both cell lines showed DNA damage in the G_2/M phase and underwent apoptosis. OVISE showed S- and G_2/M-phase arrest, while RMG-I showed G_2/M-phase arrest (Figure 3B).[9]

Discussion

Numerous distinct dots of γH2AX are usually observed when cells are pretreated with antitumor agents and immunohistochemically stained using γH2AX antibodies. Each of these dots is considered to correspond to a site of DNA damage.[6] In apoptotic cells, because of DNA fragmentation, nuclear fragments showing strong staining of γH2AX are commonly observed. Thus, DNA damage and apoptosis can be visualized using γH2AX as an indicator. In a cell, all chromosomal DNA is replicated and the amount of DNA doubles during the S phase; then cell division occurs during the M phase to produce two daughter cells that initiate a new cell cycle. After immunofluorescence staining of γH2AX and counterstaining of DNA, histograms were constructed by plotting the amount of DNA and amount of γH2AX in the cells, determined by flow cytometry, on the *x* and *y* axes, respectively, to detect the amount of γH2AX formed in each cell-cycle phase; this allowed a visual estimation of

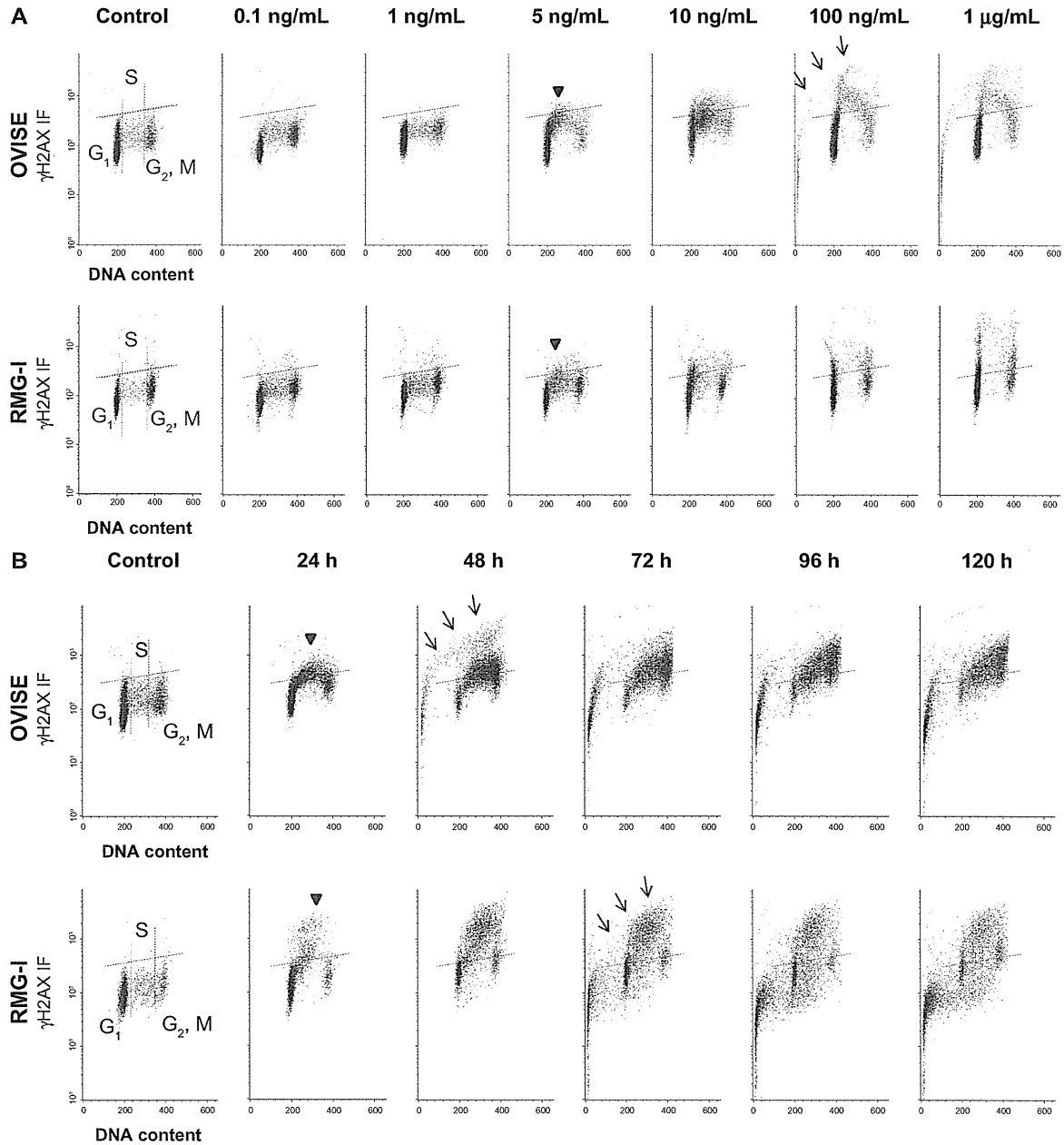

Figure 2 (**A** and **B**) Bivariate distributions (DNA content vs γH2AX) in the clear-cell carcinoma cell lines OVISE and RMG-I, treated with gemcitabine. The dotted lines indicate the upper level of γH2AX immunofluorescence in 95% of cells in the untreated (control) culture. Arrowheads indicate elevation in the immunofluorescence intensity of γH2AX, suggestive of DNA damage. Arrows indicate apoptotic cell populations with marked increase in the amount of γH2AX and gradual decrease in DNA. (**A**) Both cell lines were treated with various concentrations of gemcitabine for 24 hours. Both cell lines exhibited DNA damage in S-phase cells at the minimum concentration of 5 ng/mL. In the OVISE line, apoptosis was induced at concentrations of 100 ng/mL or higher. RMG-I cells remained with DNA damage. (**B**) Both cell lines were treated with 5 ng/mL, calculated as the minimum concentration inducing DNA damage, for various reaction times. S-phase cells of OVISE showing DNA damage underwent apoptosis after 48 hours. In addition, S- and G_2/M-phase arrest was observed. DNA damage was induced in the S-phase cells of RMG-I after 24 hours, and the cells showing DNA damage underwent apoptosis after 72 hours. Furthermore, cell-cycle arrest occurred in the S- and G_2/M phases.
Abbreviations: DNA, deoxyribonucleic acid; S, synthesis phase; G_1, Gap 1 phase; G_2, Gap 2 phase; M, mitotic phase; h, hours.

the extent of DNA damage caused by antitumor agents and examination of changes in the cellular kinetics. In this immunohistochemical γH2AX-detection method, DNA damage can be detected with high sensitivity at much lower concentrations of the necessary agents than in the comet assay, and the extent of DNA damage can be correlated with the phase of the cell cycle.[10]

Combination chemotherapy with PTX and CBDCA is established as the gold standard for ovarian cancer. This therapy, however, is not sufficiently effective for CCC, and it

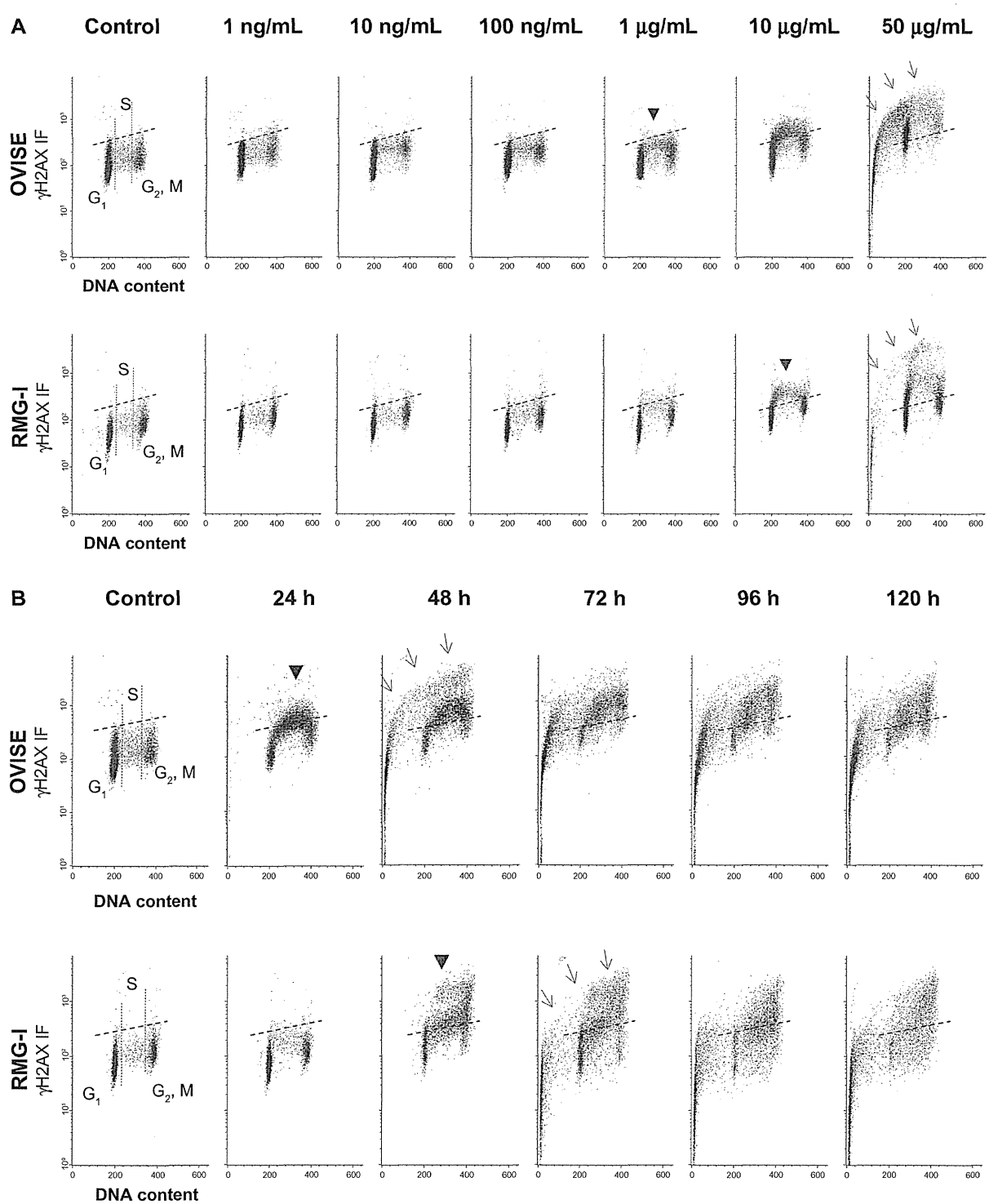

Figure 3 (**A** and **B**) Bivariate distributions (DNA content vs γH2AX) of OVISE and RMG-I cell lines, treated with carboplatin. The dotted lines indicate control. Arrowheads and arrows indicate DNA damage and apoptosis, respectively. (**A**) Both cell lines were treated with various concentrations of carboplatin for 24 hours. DNA damage was observed in the S-phase cells at 1 µg/mL and 10 µg/mL concentrations in OVISE and RMG-1, respectively. DNA damage was found in both cell lines at every cell cycle as the concentration increased, and apoptosis occurred at a concentration of 50 µg/mL. More cells remained free of DNA damage in RMG-1 than in OVISE. (**B**) Both cell lines were treated with 1 µg/mL, the minimum concentration inducing DNA damage in either cell line, for various reaction times. In OVISE, S-phase cells with DNA damage progressed to apoptosis after 48 hours. DNA damage was also found in G_2/M-phase cells after 48 hours, but most did not progress to apoptosis. S- and G_2/M-phase arrests were observed. DNA damage was found in S- and G_2/M-phase cells after 48 hours in RMG-1. The S-phase cells with DNA damage progressed to apoptosis 72 hours later, but G_2/M-phase cells showing DNA damage remained. S- and G_2/M-phase arrests were observed.

Abbreviations: DNA, deoxyribonucleic acid; S, synthesis phase; G_1, Gap 1 phase; G_2, Gap 2 phase; M, mitotic phase; h, hours.

has been pointed out that individualization of chemotherapy based on the histological subtypes is needed for this type of cancer.[4] GEM has proved to be effective in patients with ovarian cancer,[11,12] and a publication-based application of GEM was submitted in September 2010 in Japan. In this study, we attempted to demonstrate the efficacy of GEM and CBDCA, and examine the possibility of expanding the treatment options for patients with CCC, for whom the current treatment options are limited.

GEM is an antimetabolite used to treat recurrent ovarian cancer that is known to exert its antitumor activity via becoming incorporated into the cellular DNA. In both the CCC cell lines used in this study, GEM induced marked DNA damage and cell-cycle arrest in the S phase, probably as a result of the GEM-induced stalled replication forks. However, many cells with DNA damage remained viable even after exposure to GEM for 120 hours, indicating that GEM had only a weak cytocidal effect on the CCC cells.

The results of the study showed that GEM exerted a weaker antitumor effect on the RMG-I cells than on the OVISE cells. Possible reasons for this include the higher percentage of G_0/G_1 cells and lower percentage of S-phase cells in the RMG-I cell line. A relatively low proportion of cells in the S phase is generally observed in CCCs, and may account for the insufficient antitumor effect of GEM monotherapy in patients with this type of ovarian cancer. Unlike in human myelogenous leukemia cell lines,[13] GEM caused cell-cycle arrest not only in the S phase but also in the G_2/M phase in the CCC cell lines. In addition, cells arrested in the G_2/M phase of the cell cycle also showed DNA damage. Taking into account the GEM concentrations and the time course of the cellular changes, this may be attributable to the cell-cycle arrest induced at the G_2/M checkpoint after progression of the S-phase cells showing DNA damage to the G_2 phase. Although the factors involved in the phosphorylation of ataxia telangiectasia and Rad3-related protein after recognition of DNA damage are not yet clearly defined, it is considered that BRCA1, a human tumor-suppressor gene product, may play a role in the process and is responsible for G_2/M checkpoint regulation in response to DNA damage. A recent study has shown *BRCA1* mutations involved in CCC.[14] Thus, *BRCA1* gene mutations in CCC cell lines may be involved in the cell-cycle arrest at the G_2/M phase observed in this study.

After CBDCA administration, DNA damage was seen in the S and G_2/M phases in both cell lines. OVISE contained a remarkable cell population rescued from apoptosis and surviving with DNA damage. On the other hand, most RMG-I cells with DNA damage underwent apoptosis. These results suggest that cell lines respond differently to platinum agents, ie, RMG-I was cisplatin-resistant but responded to CBDCA.

This study infers that for residual cells in which the cell cycle remains arrested due to DNA damage caused by GEM, effective cytocidal action can theoretically be obtained by additionally or concomitantly administering CBDCA, which exerts effects on any cells in cell-cycle arrest (Table 1). These mechanisms of action for both drugs have already been elucidated in many types of carcinomas other than ovarian CCC. In this paper, the mechanisms of action in CCC are reported. The results obtained suggest that combination therapy with GEM plus CBDCA might be useful in the treatment of CCC. This conclusion was derived from our own study method using flow cytometry with γH2AX as a marker. In order to establish GEM-plus-CBDCA therapy, which is currently being administered in clinical trials, we considered it to be essential to demonstrate its usefulness not only for other types of carcinomas but also for CCC in basic studies. Moreover, another study is currently being conducted to assess whether there are synergistic effects of GEM plus CBDCA.

Currently, a randomized clinical trial (iPLAS) is ongoing as an intergroup study in Japan to compare the efficacy and safety of GEM plus CBDCA with those of polyethylene glycolated liposomal doxorubicin plus CBDCA in patients with platinum-sensitive, recurrent ovarian cancer. Molecular-targeted agents have come to be increasingly used in chemotherapy for ovarian cancer around the world. However, it is still impossible to use such drugs in clinical settings other than physician-initiated clinical trials in Japan. Therefore, effective treatment of CCC needs to be developed using antitumor drugs covered by health insurance. In this study, we used γH2AX as an indicator to examine the antitumor effects of GEM and CBDCA on OVISE and RMG-I cells, and the results suggested that combination chemotherapy with GEM plus CBDCA may be effective for CCC. The method employed in this study is convenient and very

Table 1 Cell kinetics of CCC cells treated with anticancer drugs

	DNA damage	Apoptosis	Cell cycle arrest
GEM			
OVISE	S	+	S, G_2/M
RMG-I	S, G_2/M	+	S, G_2/M
CBDCA			
OVISE	S, G_2/M	+	S, G_2/M
RMG-I	S, G_2/M	+	G_2/M

Abbreviations: CCC, clear cell carcinoma; CBDCA, carboplatin; GEM, gemcitabine; S, synthesis phase; G_2, Gap 2 phase; M, mitotic phase.

useful to examine the antitumor effects of anticancer drugs, as it takes only a short time for the effects of the agents to be assessed. In this study, we presented the data regarding single-agent administration of GEM and CBDCA. We are currently conducting a study on the combination, and will report the results in a future paper.

This report provides valid findings that would contribute to improvement of the prognosis of patients with CCC. We anticipate that our findings would also promote the development of further in vitro studies.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Sugiyama T, Kamura T, Kigawa J, et al. Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. *Cancer*. 2000;88:2584–2589.
2. McGuire WP, Hoskins WJ, Brady MF, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med*. 1996;334:1–6.
3. Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol*. 2003;21:3194–3200.
4. Sugiyama T, Fujiwara K. Clear cell carcinoma of the ovary. In: Govindan R, editor. *American Society of Clinical Oncology 2007 Educational Book*. Alexandria (VA); 2007:318–322.
5. Dickey JS, Redon CE, Nakamura AJ, Baird BJ, Sedelnikova OA, Bonner WM. H2AX:functional roles and potential applications. *Chromosoma*. 2009;118:683–692.
6. Bonner WM, Redon CE, Dickey JS, et al. GammaH2AX and cancer. *Nat Rev Cancer*. 2008;8:957–967.
7. Tanaka T, Kurose A, Halicka HD, Huang X, Traganos F, Darzynkiewicz Z. Nitrogen oxide-releasing aspirin induces histone H2AX phosphorylation, ATM activation and apoptosis preferentially in S-phase cells: involvement of reactive oxygen species. *Cell Cycle*. 2006;5:1669–1674.
8. Itamochi H, Kigawa J, Sultana H, et al. Sensitivity to anticancer agents and resistance mechanisms in clear cell carcinoma of the ovary. *Jpn J Cancer Res*. 2002;93:723–728.
9. Takatori E, Shoji T, Kumagai S, Sawai T, Kurose A, Sugiyama T. Are platinum agents, paclitaxel and irinotecan effective for clear cell carcinoma of the ovary? DNA damage detected with γH2AX induced by anticancer agents. *J Ovarian Res*. 2012;12:16.
10. Watters GP, Smart DJ, Harvey JS, Austin CA. H2AX phosphorylation as a genotoxicity endpoint. *Mutat Res*. 2009;679:50–58.
11. Pfisterer J, Plante M, Vergote I, et al. Gemcitabine plus carboplatin compared with carboplatin in patients with platinum-sensitive recurrent ovarian cancer: an intergroup trial of the AGO-OVAR, the NCIC CTG, and the EORTC GCG. *J Clin Oncol*. 2006;24:4699–4707.
12. Watanabe Y, Koike E, Nakai H, Etoh T, Hoshiai H. Phase II study of single-agent gemcitabine in heavily pretreated Japanese patients with recurrent ovarian cancer. *Int J Clin Oncol*. 2008;13:345–348.
13. Ewald B, Sampath D, Plunkett W. H2AX phosphorylation marks gemcitabine-induced stalled replication forks and their collapse upon S-phase checkpoint abrogation. *Mol Cancer Ther*. 2007;6:1239–1248.
14. Wu JY, Vlastos AT, Pelte MF, et al. Aberrant expression of BARD1 in breast and ovarian cancers with poor prognosis. *Int J Cancer*. 2006;118:1215–1226.

Congenital Anomalies
THE JAPANESE TERATOLOGY SOCIETY
doi:10.1111/j.1741-4520.2012.00373.x
Congenital Anomalies 2013; **53**, 95–97

CASE REPORT

Monochorionic twin fetus with VACTERL association after intracytoplasmic sperm injection

Tomonobu Kanasugi[1], Akihiko Kikuchi[1], Atsushi Matsumoto[2], Miyuki Terata[1], Chizuko Isurugi[1], Rie Oyama[1], Akimune Fukushima[1], and Toru Sugiyama[1]

Departments of [1]Obstetrics and Gynecology and [2]Neonatology, Iwate Medical University School of Medicine, Morioka, Japan

ABSTRACT **We report a rare case of a monochorionic twin gestation after intracytoplasmic sperm injection (ICSI) in which one of the fetuses had VACTERL association. A 27-year-old woman became pregnant by ICSI and was found to have monochorionic twin fetuses. One fetus was noted to have the following anomalies: a multicystic, dysplastic left kidney with a hydroureter, and a dilated colon. A normal-sized stomach and normal amount of amniotic fluid were observed during the prenatal period with no other anomalies. The postnatal examination revealed hypospadias, and anal, esophageal, and duodenal atresia; thus, a diagnosis of VACTERL association was established. Although the prenatal diagnosis of this disorder is a challenge, even in a singleton, some of the characteristic features observed during antepartum ultrasonography may be a clue to the diagnosis, especially in a twin pregnancy after ICSI.**

Key Words: fetal ultrasound, intracytoplasmic sperm injection, monochorionic twin, prenatal diagnosis, VACTERL association

INTRODUCTION

VACTERL association (V, vertebral defects; A, anal atresia; C, cardiac anomaly; TE, tracheal-esophageal fistula with esophageal atresia; R, renal defects; and L, radial limb dysplasia) is a spectrum of fetal anomalies with an incidence of 1–9/100 000 infants (Botto et al. 1997; Solomon 2011). Patients are considered to have VACTERL association if three or more organ systems are involved. This disorder generally occurs sporadically in an otherwise normal healthy family. The etiology of VACTERL remains unknown. A familial pattern of VACTERL association also occurs in 8–10% of patients (Solomon et al. 2010). Although antepartum ultrasonography can demonstrate some of the characteristic findings, the prenatal diagnosis of VACTERL association is not always possible because it is very difficult to detect all of the symptoms associated with this disorder during the prenatal period. However, patients with VACTERL association require considerable postnatal surgical treatment and care, and thus the perinatal management of an affected infant is essential for obstetricians and neonatologists although the prenatal diagnosis of this disorder is a challenge.

Reports on fetuses with VACTERL association in twin pregnancies have been limited and there is a paucity of data in the literature regarding the incidence of this disorder in twin fetuses (Camacho et al. 2008; Athwal et al. 2010). We herein report a rare case of a monochorionic twin fetus with VACTERL association conceived by intracytoplasmic sperm injection (ICSI).

Correspondence: Akihiko Kikuchi, MD, Department of Obstetrics and Gynecology, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka, Iwate 020-8505, Japan. Email: dr-kiku@yj8.so-net.ne.jp

Received October 7, 2011; revised and accepted April 3, 2012.

CLINICAL REPORT

A 27-year-old Japanese nulligravida underwent ICSI and frozen embryo transfer as treatment for idiopathic spousal oligospermia and achieved a pregnancy. She was found to have monochorionic diamniotic twin fetuses in the first trimester and was referred to our hospital for perinatal management of this condition. An ultrasound at 20 weeks of gestation revealed the following in fetus A: a multicystic, dysplastic left kidney (Fig. 1); normal-sized stomach; no abdominal cysts; no cardiac anomalies; and no limb abnormalities. No congenital anomalies were detected in fetus B. At 31 weeks of gestation, fetus A developed a left hydroureter (Fig. 2). Moreover, dilatation of the colon progressed in fetus A (Fig. 3). Both of the fetuses grew normally with a normal amniotic fluid volume.

Preterm rupture of membranes occurred at 36 weeks of gestation and a cesarean section was performed. Infant A was a male weighing 2024 g and infant B was a male weighing 2208 g. Histologic examination of the placenta confirmed a monochorionic, diamniotic twin pregnancy. Infant B was healthy and no cardiac, renal, or congenital anomalies were demonstrated on ultrasound and physical examination. Infant A had hypospadias, and anal, esophageal (gross C type), and duodenal atresia. An X-ray did not reveal vertebral abnormalities except six lumber vertebrae which might merely reflect lumbarization of S1 (Fig. 4). No limb deficiencies were evident on X-ray. Postnatal ultrasound confirmed a left ureterocele, hydroureter, and a multicystic, dysplastic kidney without cardiac anomalies. The chromosomal analysis showed a normal karyotype (46, XY). Therefore, a postnatal diagnosis of VACTERL association was established in infant A. Repairs of the esophageal and duodenal atresia, and a colostomy for the anal atresia were successfully performed.

DISCUSSION

VATER association has been defined as the presence of at least three of the five classic VATER anomalies (vertebral defects, anal atresia, tracheoesophageal fistula, renal defects, and radial ray limb deficiency). Additional defects associated with VATER association include genital defects, cardiovascular anomalies, and small intestinal atresia, as well as rotary malpositioning of the hand, clinodactyly, or syndactyly. The expanded VATER association, including cardiac and limb defects, is referred to as VACTERL association (Temtamy and Miller 1974; Khoury et al. 1983; Czeizel and Ludanyi 1985). Herein we report monozygotic twins, one fetus of which had VATERL association based on the presence of anal atresia, tracheoesophageal fistula (gross C type esophageal atresia),

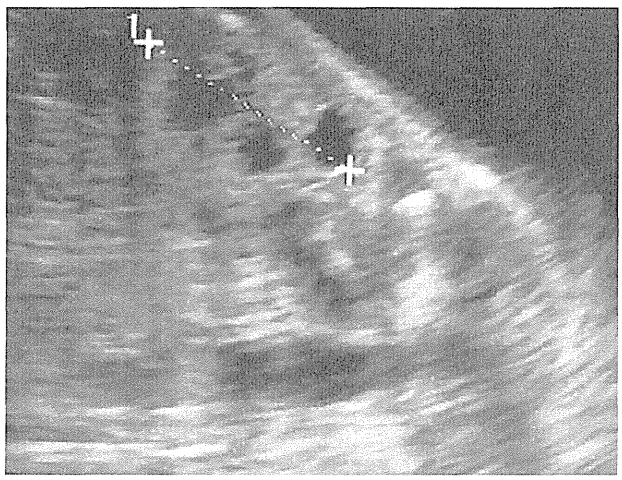

Fig. 1 Ultrasound at 20 weeks of gestation revealed a left multicystic, dysplastic kidney in fetus A.

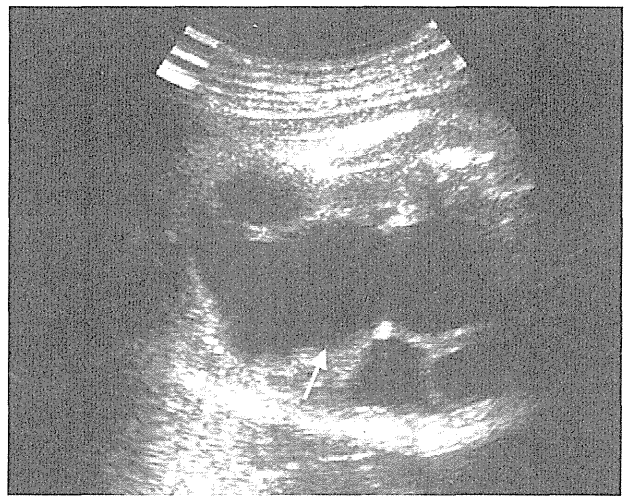

Fig. 3 Ultrasound sagittal view of the abdomen in fetus A revealed a remarkable dilatation of the colon.

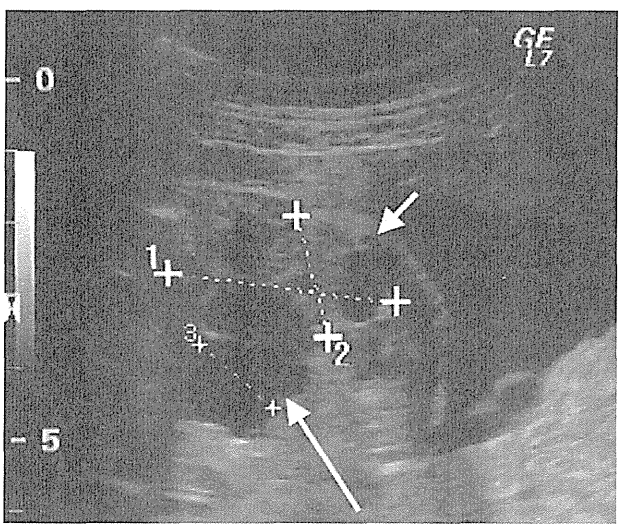

Fig. 2 Ultrasound at 31 weeks of gestation revealed a left hydroureter (long arrow) and a multicystic, dysplastic kidney (short arrow) in fetus A.

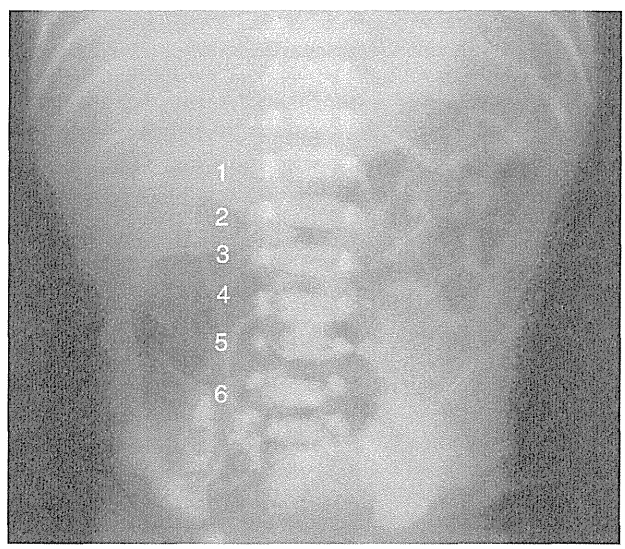

Fig. 4 Abdominal X-ray of infant A revealed six lumbar vertebrae.

a left ureterocele, hydroureter, and a multicystic, dysplastic kidney. The presence of these three defects of the six categories supports the diagnosis.

The differential diagnosis of VACTERL association is broad, and includes a number of conditions for which genetic testing is available. The presence of other features not typically observed in VACTERL association may suggest other disorders, such as pigmentary abnormalities in Fanconi anemia or hypocalcemia in deletion 22q11.2 syndrome. Ruling out these disorders includes evaluation for features that are not typical of VACTERL association, such as brain malformations, ophthalmologic anomalies, and hearing deficits (Solomon 2011). Infant A had duodenal atresia, which is a feature not typically present in VACTERL association; however, no other findings were noted in our case that would affect the differential diagnosis, including a chromosomal abnormality.

The embryology of VACTERL association is unknown. VACTERL association may result from a single early defect in fetal blastogenesis or mesoderm formation. It has been shown that the defects in VACTERL association are all due to defects in blastogenesis. Martinez-Frias et al. (1998) suggested that the entire embryo represents the developmental field during blastogenesis. Such a single 'hit' during blastogenesis may produce an association of anomalies, while later in gestation a similar hit would produce only a single anomaly.

The majority of studies comparing the prevalence of congenital anomalies at birth in singleton and plural births have observed an increased risk in the latter. Among twins, a higher prevalence of

congenital anomalies occurs in monochorionic twins compared to dichorionic twins (Glinianaia et al. 2008). The incidence of congenital anomalies in a twin pregnancy is approximately 1.5 times that of a singleton fetus, and the incidence of congenital anomalies in a monochorionic twin is approximately 1.5 times that of a dichorionic twin (Layde et al. 1980).

Machin (1996) reported that most monozygotic twin pairs are not identical. Indeed, there may be major differences in birth weight, genetic diseases, and congenital anomalies in monozygotic twin pairs. These facts indicate that post-zygotic events may lead to the formation of two or more cell clones in the inner cell mass and the early embryo that actually stimulates the monozygotic twinning event. There is also evidence that there may be an unequal allocation of the number of cells to the monozygotic twin; this may have widespread implications for the cascade of developmental events during embryogenesis, formation, and vascularization of the placenta.

Many studies suggest that infants resulting from ICSI pregnancies have an increased risk of major congenital anomalies compared with infants born after spontaneous conception. ICSI is a more invasive procedure than routine *in vitro* fertilization. When a spermatozoon is injected through the oocyte membrane, problems involving the technique of ICSI itself, chromosomal aberrations of sperm, and hereditary abnormalities may be transmitted to the conceptus (Bonduelle et al. 2002); however, there have been no reports demonstrating a relationship between VACTERL association and ICSI. Sunagawa et al. (2007) reported the first and only case in which both dichorionic twin fetuses conceived after ICSI had VACTRL association. Although Sunagawa et al. (2007) suggested a possible relationship between VACTERL association and ICSI, they concluded that their case might be explained by the natural occurrence rate of this association in twins, not by the ICSI procedure. Therefore, we do not know the exact basis for the etiology of this disorder in our monochorionic twin. Only a few cases with VACTERL association in a monochorionic twin pregnancy have been reported and there is a paucity of data in the literature describing the incidence of this disorder in a twin (Camacho et al. 2008; Athwal et al. 2010). Although Botto et al. (1997) reviewed 286 VATER infants, there was no mention of twins. Czeizel and Ludanyi (1985) reviewed 43 infants that included two sets of twins; however, they were not monozygotic.

Reports on the prenatal diagnosis of VACTERL association are rare. Tongsong et al. (1999) mentioned that the existence of several sonographic markers of the VATER complex allows this disorder to be detected on prenatal sonography. Of note, antepartum ultrasonography only revealed a multicystic, dysplastic kidney, a hydroureter, and enteric expansion in our case. Although the prenatal diagnosis of VACTERL association is not always possible because it is very difficult to detect all of the symptoms associated with this disorder during the prenatal period, antenatal suspicion of this disorder should be helpful for early postnatal definitive diagnosis and care of the affected infant.

In conclusion, we managed a rare case of a monochorionic twin fetus with VACTERL association conceived by ICSI. Whether or not the VACTERL association is a specific congenital anomaly among twin pregnancies after ICSI is more frequent than other anomalies is not known. Although the prenatal diagnosis of this disorder is still a challenge, even in a singleton fetus some of the characteristic features observed with prenatal ultrasonography may be a clue to the diagnosis, especially in a twin pregnancy through ICSI.

REFERENCES

Athwal S, Millard K, Lakhoo K. 2010. Twin reversed arterial perfusion (TRAP) sequence in association with VACTERL association: a case report. J Med Case Reports 4:411.

Bonduelle M, Van Assche EV, Joris H et al. 2002. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. Hum Reprod 17:2600–2614.

Botto LD, Khoury MJ, Mastroiacovo P et al. 1997. The spectrum of congenital anomalies of the VATER association: an international study. Am J Med Genet 71:8–15.

Camacho AF, Schneider A, Dorsainville D et al. 2008. Monozygotic twins discordant for VACTERL association. Prenat Diagn 28:366–368.

Czeizel A, Ludanyi I. 1985. An aetiological study of the VACTERL association. Eur J Pediatr 144:331–337.

Glinianaia SV, Rankin J, Wright C. 2008. Congenital anomalies in twins: a register- based study. Hum Reprod 23:1306–1311.

Khoury MJ, Cordero JF, Greenberg F et al. 1983. A population study of the VACTERL association: evidence for its etiologic heterogeneity. Pediatrics 71:815–820.

Layde PM, Erickson JD, Falek A et al. 1980. Congenital malformations in twins. Am J Hum Genet 32:69–78.

Machin GA. 1996. Some causes of genotypic and phenotypic discordance in monozygotic twin pairs. Am J Med Genet 61:216–228.

Martinez-Frias ML, Frias JL, Opitz JM et al. 1998. Errors of morphogenesis: a developmental field theory. Am J Med Genet 76:291–296.

Solomon BD. 2011. VACTERL/VATER association. Orphanet J Rare Dis 6:56.

Solomon BD, Pineda-Alvarez DE, Raam MS, Cummings DA. 2010. Evidence for inheritance in patients with VACTERL association. Hum Genet 127:731–733.

Sunagawa S, Kikuchi A, Yoshida S et al. 2007. Dichorionic twin fetuses with VACTERL association. J Obstet Gynaecol Res 33:570–573.

Temtamy SA, Miller JD. 1974. Extending the scope of the VATER association: definition of the VATER syndrome. J Pediatr 85:345–349.

Tongsong T, Wanapirak C, Piyamongkol W et al. 1999. Prenatal sonographic diagnosis of VATER association. J Clinical Ultrasound 27:378–384.

Ultrasound Obstet Gynecol 2013; 42: 609–610
Published online 2 October 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/uog.12484

Picture of the Month

Towards improved ultrasound-based analysis and 3D visualization of the fetal brain using the 3D Slicer

R. OYAMA*, M. JAKAB†, A. KIKUCHI*, T. SUGIYAMA*, R. KIKINIS† and S. PUJOL†

**Iwate Medical University, Department of Obstetrics and Gynecology, Morioa, Japan; †Harvard Medical School, Brigham and Women's Hospital, Department of Radiology, Boston, MA, USA*

Magnetic resonance imaging (MRI) provides useful three-dimensional (3D) information; however, there are some restrictions on its use during pregnancy due to safety concerns. In addition, fetal movements can create artifacts on MR images, as image quality depends on position of the fetus and placenta. In the past decade, 3D ultrasound imaging has been used in clinical practice to investigate the formation and volumetric size of critical anatomical structures of the fetus. However, current techniques rely mainly on analysis of sections of interest that do not integrate anatomical information concerning the shape of these structures.

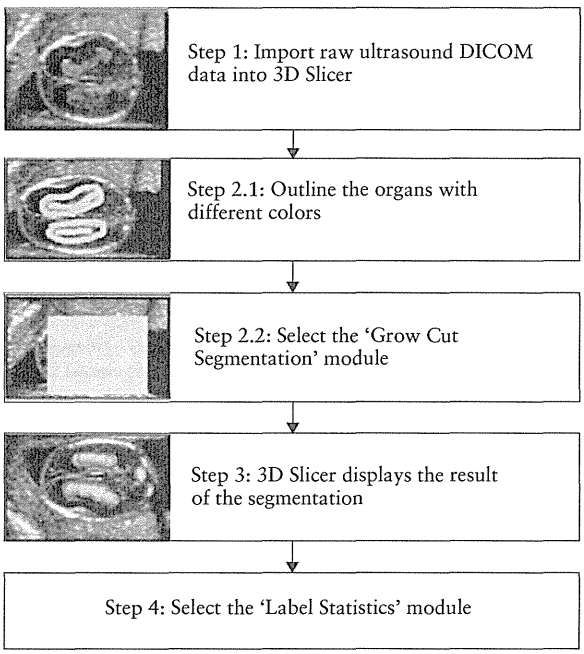

Figure 1 Flowchart describing the 3D Slicer workflow used in this study. DICOM, digital imaging and communications in medicine.

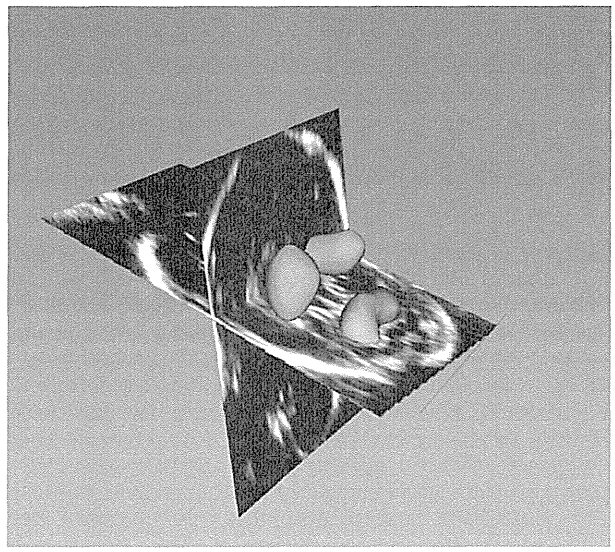

Figure 2 Result of 'Grow Cut Segmentation' of the fetal brain using the 3D Slicer. The blue structure represents the choroid plexus, and the yellow structure the cerebrum at 14 weeks of gestation (axial and coronal views).

We provide a brief description of a workflow for semi-automated segmentation and 3D visualization of fetal ultrasound volumes in the second trimester using the 3D Slicer open source software[1,2]. Our workflow allowed quantitative image analysis of the choroid plexus and cerebrum from 3D ultrasound images.

We acquired 3D ultrasound volumes from five healthy pregnant women at 12 ($n=2$), 14 ($n=2$) and 19 ($n=1$) weeks of gestation. Informed consent was obtained in each case. We used a Voluson E6 (GE Medical Systems, Zipf, Austria) ultrasound machine with a RAB4-8-D/OB D/4D 8-MHz transabdominal transducer. Our workflow consisted of four steps (Figure 1). Firstly, we imported DICOM (digital imaging and communications

Correspondence to: Dr R. Oyama, Iwate Medical University, Obstetrics and Gynecology, 19-1 Uchimaru Morioka 0208505, Japan (e-mail: oyamariegm@gmail.com)

in medicine) ultrasound volumes into the 3D Slicer. We then used the 'Grow Cut Segmentation' algorithm[3] of the interactive Editor module to extract critical structures from the ultrasound volumes. We reconstructed 3D surface models from segmented regions using the 'Marching Cubes' algorithm[4], and finally computed the volume of 3D anatomical models using the 'Label Statistics' module of the software.

Figure 2 shows a 3D surface model of the choroid plexus and cerebrum reconstructed from the original 3D ultrasound volumes. The corresponding volumes of these structures at 12, 14 and 19 weeks' gestation were, respectively: 431.1 mm^3, 698.9 mm^3 and 1203.3 mm^3 for the choroid plexus and 183.6 mm^3, 282.8 mm^3 and 469.8 mm^3 for the cerebrum.

Using the 3D Slicer, we were able to obtain patient-specific quantitative information and 3D visualization of anatomical structures within the fetal brain. We anticipate being able to perform segmentation that accurately matches the anatomy using different methods. We believe this method, combined with ultrasound or MRI data, will be helpful in monitoring fetal development and detecting anomalies of the brain as well as other anatomical structures.

References

1. Pieper S, Halle M, Kikinis R. 3D Slicer. Proceedings of IEEE International Symposium on Biomedical Imaging: Nano to Macro.2004; **1**: 632–635.
2. 3DSlicer, http://www.slicer.org. (April 25, 2013).
3. Vezhnevets V, Konouchine V. "Grow-Cut" Interactive Multi-Label N-D image Segmentation. *Proc Graphicon* 2005; 150–156.
4. Lorensen WE, Cline HE. Marching cubes: A high resolution 3D surface construction algorithm. *ACM Siggraph Computer Graphics* 1987; **21**:163–169.

Investigation of the clinicopathological features of fallopian tube malignancy

YOSHIHITO YOKOYAMA[1], MASAYUKI FUTAGAMI[1], TOSHIO FUJIMOTO[2], YUKIHIRO TERADA[2], ERIKO TAKATORI[3], TORU SUGIYAMA[3], TAKEO OTSUKI[4], NOBUO YAEGASHI[4], TAKANOBU KOJIMAHARA[5], HIROHISA KURACHI[5], HIROSHI NISHIYAMA[6], KEIYA FUJIMORI[6], TORU TASE[7] and HIDEKI MIZUNUMA[1]

[1]Department of Obstetrics and Gynecology, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562; [2]Department of Obstetrics and Gynecology, Akita University School of Medicine, Akita 010-8543; [3]Department of Obstetrics and Gynecology, Iwate Medical University School of Medicine, Morioka 020-8505; [4]Department of Obstetrics and Gynecology, Tohoku University School of Medicine, Aoba-ku, Sendai 980-8574; [5]Department of Obstetrics and Gynecology, Yamagata University School of Medicine, Yamagata 990-9585; [6]Department of Obstetrics and Gynecology, School of Medicine, Fukushima Medical University, Fukushima 960-1295; [7]Department of Gynecology, Miyagi Cancer Center, Natori 981-1293, Japan

Received February 11, 2013; Accepted April 5, 2013

DOI: 10.3892/or.2013.2439

Abstract. The present study investigated the clinicopathological features of fallopian tube malignancy (FTM) and elucidated the biological behavior of this disorder. Data were compiled concerning FTM from 68 patients from 7 institutes. The patients included 60 cases with fallopian tube carcinoma and 8 cases with fallopian tube carcinosarcoma. The clinical stage was stage III or higher in 72% of the cases. A complete response or partial response was achieved in 56 and 10 of the 68 patients with FTM, respectively, indicating a response rate of 97.1%. The median observation period for FTM was 41 months (3 to 126 months). Three of the 19 patients with stage I/II disease (16%) and 31 of the 49 patients with stage III/IV disease (63%) experienced recurrence, with a median progression-free survival of 17.5 months, and a 3-year overall survival of 77.2%. Regarding the site of recurrence, local intraperitoneal recurrence (26.2%) and solitary recurrences in lymph nodes (19.0%) and in the liver (16.7%) were relatively frequent. Secondary debulking surgery (SDS) was performed in 15 patients (44%) out of the 34 recurrent FTMs. Conversely, recurrence was associated with ascites (carcinomatous peritonitis) in 4 of the 34 recurrent patients, but all 4 patients died. The median survival period after recurrence was 28 months: 7.5 and 30 months with and without ascites, respectively ($P<0.001$). A univariate analysis showed that prognosis was significantly correlated only with whether SDS could be performed. These results suggest that since FTM frequently results in solitary recurrence, aggressive recurrence treatment including SDS could improve prognosis.

Correspondence to: Dr Yosihihito Yokoyama, Department of Obstetrics and Gynecology, Hirosaki University Graduate School of Medicine, 5-Zaifu-cho, Hirosaki, Aomori 036-8562, Japan
E-mail: yokoyama@cc.hirosaki-u.ac.jp

Key words: primary carcinoma of the fallopian tube, primary carcinosarcoma of the fallopian tube, solitary recurrence, secondary debulking surgery, prognosis

Introduction

Fallopian tube malignancy (FTM) is a rare disease that comprises only 0.14 to 1.8% of female genital malignancies (1). The incidence rate in the United States is estimated to be on average 3.6/million/year (2). Although rare, FTM is a disease that has increased 4.5-fold over the past 40 years (3). Even though the staging and therapeutic strategy for fallopian tube carcinoma are based on those of ovarian carcinoma, it is difficult to conduct a large-scale clinical study exclusively on fallopian tube carcinoma because of its rarity, and the pattern of metastasis as well as time to recurrence are not clear. Wethington *et al* (4) compared the outcome of fallopian tube carcinoma and ovarian carcinoma based on the Surveillance, Epidemiology, and End Results (SEER) database of the National Cancer Institute, and showed that fallopian tube carcinoma was associated with a more favorable long-term outcome than ovarian carcinoma. However, the biological behaviors of fallopian tube carcinoma such as location of recurrence and time to recurrence remain unknown (4).

This study was a retrospective, multicenter study that aimed to investigate the clinicopathological features of FTM and to elucidate the biological behaviors of this disease.

Materials and methods

All patients with FTM treated between January 2001 and December 2011 were eligible for this study. Institutional review board approval was obtained at each of the 7 participating academic centers prior to data acquisition, and informed

consent was obtained from the patients or the guardians. Cases were defined as FTM using diagnostic criteria as documented by Sedlis (5). Briefly, these criteria were as follows: i) the main tumor arises from the endosalpinx; ii) the histological pattern reproduces the endothelium of the tube mucosa; iii) transition from benign to malignant tubal epithelium is demonstrable; iv) the ovaries or endometrium are either normal or contain a tumor smaller than the tumor in the tube. In addition, we also adopted the definition of fallopian tube carcinoma of WHO classification which states that there is a primary lesion in the lumen or fimbriae of the fallopian tube and a lesion does not exist in the ovary and the uterine or it is different from the fallopian tube clearly even if it exists (6). All slides were reviewed by expert pathologists from each institution. A common database form was designed to be utilized by all participating centers. The medical records included information regarding age, gravidity and parity, clinical presentation, disease stage, type of surgery performed, treatment rendered, histological type, progression-free survival (PFS), overall survival (OS), and sites of recurrence. Patients of all stages were eligible. Staging was based on the International Federation for Obstetrics and Gynecology (FIGO) criteria. Procedures included in the standard surgery consisted of bilateral salpingo-oophorectomy, hysterectomy and greater omentectomy with or without cytoreduction. Staging laparotomy included retroperitoneal (pelvic, para-aortic) lymph node dissection (or biopsy) and intraperitoneal biopsies in addition to standard surgery. The 1-cm cutoff was used as a threshold for optimal cytoreduction (7). When adjuvant chemotherapy was indicated, patients generally received platinum/taxan-based combination chemotherapy. Cases that received neoadjuvant chemotherapy also were included in this retrospective study.

Clinical response was assessed in the enrolled patients with lesions that could be measured according to the revised RECIST guideline (version 1.1) (8). A complete response (CR) was defined as the complete disappearance of all measurable lesions. A partial response (PR) was defined as a 30% or greater decrease in the sum of the measurable lesions. Stable disease was defined as a steady state of response less than PR or an increase in <20% in the sum of the measurable lesions. Progressive disease was defined as an increase of 20% or more in the sum of the measurable lesions or the appearance of new lesions.

PFS was calculated from the date of the start of treatment to the date of recurrence or progression. OS was calculated from the date of the start of treatment to the date of death or last follow-up. The cumulative survival curve and median PFS time were estimated by use of the Kaplan-Meier method. Comparison between survival curves was carried out using the log-rank test. Difference in response rate between predictive variables was analyzed using the Chi-square test. Statistical significance was set at $P<0.05$.

Results

Patient characteristics. Patient characteristics are shown in Table I. Sixty-eight patients with FTM were enrolled for this retrospective analysis. The 68 patients with this disease included 60 cases with fallopian tube carcinoma and 8 cases with fallopian tube carcinosarcoma. The median age of the patients

Table I. Patient characteristics.

Clinical factors	No. of patients
Fallopian tube malignancy	
Carcinoma of the fallopian tube	60
Carcinosarcoma of the fallopian tube	8
Median age (range), in years	60 (38-85)
History of delivery	
Yes	64
No	4
Menstruation	
Premenopause	14
Postmenopause	54
Chief complaint	
Lower abdominal pain or distension	27
Atypical genital bleeding	21
Feeling of abdominal mass	5
Bloody bowel discharge	2
Asymptomatic	10
Others	3
Serum CA-125, median (range), U/ml	338.5 (4-16,000)
Preoperative diagnosis	
Ovarian carcinoma	45
Fallopian tube carcinoma	9
Endometrial carcinoma	9
Colon carcinoma	2
Others	3
Neoadjuvant chemotherapy	
Yes	10
No	58
FIGO stage	
I	11
II	8
III	40
IV	9
Histological type	
Serous	50
Endometrioid	6
Undifferentiated	2
Clear cell	1
Transitional	1
Carcinosarcoma	8
Debulking surgery	
Complete or optimal	44
Suboptimal	24
Type of surgery	
With staging	34
Without staging	34
Chemotherapy	
Paclitaxel + carboplatin	57
Docetaxel + carboplatin	3
Ifosfamide + epirubicin + cisplatin	4
Paclitaxel alone	1
None	3

Table II. Patient outcome.

Outcome	No. of patients
Response evaluation	
Complete response	56
Partial response	10
Stable disease	1
Progressive disease	1
Recurrence	
Yes	34
No	34
Secondary debulking surgery	
Yes	15 (44.1%)
No	19
Progression-free survival (median, months)	17.5
Three-year survival rate	77.2%

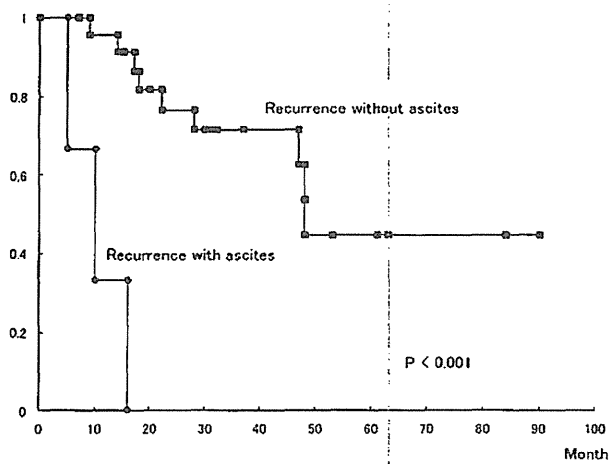

Figure 1. Survival time after recurrence. The median survival time after recurrence was 28 months: 7.5 and 30 months with and without ascites, respectively (P<0.001).

was 60 years (range, 38-85 years); initial symptoms included so-called Latzko's triad of lower abdominal pain (including bloating) in 27 patients (39.7%), atypical genital bleeding in 21 patients (30.9%), and the sensation of an abdominal mass in 5 patients (7.4%). Melena due to infiltration of the bowel was the initial symptom in 2 patients. Preoperative diagnoses included a suspected ovarian carcinoma in 45 patients (66.2%); fallopian tube carcinoma was suspected before surgery in just 9 patients (13.2%). Endometrial carcinoma was suspected and treatment for this condition was started in 9 patients (13.2%). Histological types were serous adenocarcinoma in 50 patients, endometrioid adenocarcinoma in 6 patients, and other types in 4 patients. All of the 8 carcinosarcomas were heterologous. The clinical stages were: stage I in 11 cases, stage II in 8 cases, stage III in 40 cases, and stage IV in 9 cases, indicating that

Table III. Sites of recurrence.

Recurrent site[a]	No. of patients (%)
Intraperitoneal (with ascites)	4 (9.5)
Intrapertitoneal (without ascites)	8 (19.0)
Pelvic cavity	11 (26.2)
Lymph nodes	8 (19.0)
Liver	7 (16.7)
Lung	3 (7.1)
Increase in CA-125 alone	1 (2.5)

[a]Included are 30 recurrent carcinomas and 4 recurrent carcinosarcomas of the fallopian tube. Several relapsed patients had one or more recurrent sites.

stage III or more comprised 72% of the cases. All of the 68 patients underwent standard surgery, and additionally staging surgery was performed in 34 patients. Optimal surgery was achieved in 44 patients (64.7%) (including 10 patients who underwent associated intestinal resection) and suboptimal surgery was achieved in 24 patients. The median number of chemotherapy cycles was 6 (range, 1-12). Postoperative chemotherapy included platinum-based combination treatment in 64 patients (94%).

Patient outcome. Patient outcome is summarized in Table II. The initial therapeutic effect in 68 patients with this disease was CR in 56 patients and PR in 10 patients, indicating that a response rate as high as 97.1% was achieved. The median observation period for FTM was 41 months (8-126 months). Three of 19 patients with stage I/II disease experienced recurrence and one patient died. The patient who died was a patient who received incomplete chemotherapy due to the presence of chronic renal failure. Recurrence occurred in 31 of 49 stage II/IV patients (63%), with a median PFS of 17.5 months, and a 3-year OS of 77.2%.

While the most frequent site of recurrence was intraperitoneal dissemination, local pelvic peritoneal recurrence (26.2%) and solitary recurrences in the lymph nodes (19.0%) and the liver (16.7%) were relatively frequent (Table III). Secondary debulking surgery (SDS) such as resection of the relapsed abdominal/pelvic mass or lymph nodes, or partial hepatectomy was performed in as many as 44% of patients with recurrence (Table II). Conversely, recurrence was associated with ascites (carcinomatous peritonitis) in just 4 of the relapsed 34 patients, but all 4 patients died. The median survival time after recurrence was 28 months: 7.5 and 30 months with and without ascites, respectively (P<0.001) (Fig. 1).

Determination of factors predicting poor outcome in fallopian tube malignancies. Univariate analysis by histological type, FIGO stage, age, CA-125 level, operative procedure, completion rate, recurrent site, presence or absence of SDS performance, and time to recurrence was performed to assess predictive factors related to the prognosis of FTM. Prognosis was significantly correlated solely with whether SDS could be performed (Table IV).

Table IV. Determination of factors predicting poor outcome in fallopian tube malignancy.

Factors	No. of patients	Death events from this disease	P-value
Histology			
Carcinoma of the fallopian tube	60	12	
Carcinosarcoma of the fallopian tube	8	2	ns
Stage			
I/II	19	2	
III/IV	49	12	ns
Age, years			
≥60	33	8	
<60	35	6	ns
CA-125 value			
≥330	33	7	
<330	35	7	ns
Completion of surgery			
Complete or optimal	44	10	
Suboptimal	24	4	ns
Staging laparotomy			
Yes	34	7	
No	34	7	ns
Recurrent sites			
Intraperitoneal	12	6	
Others	30	8	ns
Ascites in recurrence			
Yes	4	4	
No	30	10	ns
Secondary debulking surgery			
Yes	15	1	
No	19	13	<0.01
Time to recurrence			
≥6 months	30	13	
<6 months	2	1	ns

ns, not significant.

Discussion

This study revealed that, although intraperitoneal recurrence occurred in fallopian tube carcinoma and carcinosarcoma, recurrence presented as carcinomatous peritonitis was noted in only 4 of the 34 patients with relapsed disease; isolated metastases to the abdominal/pelvic cavity or lymph nodes and sole metastasis to the liver were relatively frequent. Therefore, it appears that post-recurrence survival was prolonged since in many patients SDS such as excision of the relapsed abdominal/pelvic mass or the relapsed lymph node or partial hepatic resection was indicated. This fact was also supported by the univariate analysis which revealed that prognosis was significantly correlated solely with whether SDS was performed. Previous studies have shown that FTM easily infiltrates surrounding organs and progresses aggressively (1), and that lymph node metastasis occurs more frequently than that in ovarian carcinoma (9). The present study, however, showed that only 4 of the 34 patients with recurrent disease developed carcinomatous peritonitis, demonstrating for the first time that carcinomatous peritonitis is uncommon. It is already known in cases of ovarian carcinoma that the prognosis of a solitary recurrence in the liver is significantly improved by partial hepatic resection (10). Thus, the results of the present study suggest that SDS should be positively introduced to recurrent FTMs unless patients develop carcinomatous peritonitis.

With regard to the initial site of recurrence, FTM shows contrasting findings with those for ovarian carcinoma (Table III). While the present results demonstrated a high frequency of lymph nodes recurrence (19.0%) and solitary liver metastasis (16.7%), Ushijima reported that lymph node metastases (7.1%) and solitary liver metastases (6.3%) occur

infrequently in ovarian carcinoma (11). FTM is richly permeated with lymphatic channels that drain into the para-aortic lymph nodes through infundibulopelvic lymphatics (12), and indeed a more frequent rate of lymph node metastasis than ovarian carcinoma has been reported (9,11). In addition, Ajithkumar *et al* (13) found that metastasis to the retroperitoneal lymph nodes was observed at the initial visit in ~50% of patients, irrespective of the stage of disease advancement. In this study as well, metastasis to the retroperitoneal lymph nodes was observed in 16 of the 34 patients (47.1%) on whom staging surgery was conducted initially (data not shown).

It has been reported that platinum-based chemotherapy for advanced or recurrent fallopian tube carcinoma resulted in CR in 75% of patients (14) and that an 82.2% response rate was obtained in progressive fallopian tube carcinoma (9). In this study, the combination therapy with platinum and a taxane was chosen as a postoperative chemotherapeutic regimen for most patients, and CR to postoperative chemotherapy was 82% in the present patients, although suboptimal surgery accounted for ~35% of all patients. There was no difference in the number of deaths between patients who received optimal surgery and those who received suboptimal surgery, suggesting that aggressive postoperative chemotherapy was mandatory and the combination of platinum and a taxane was effective, even in patients undergoing suboptimal surgery. The results of the present study were compatible with those of Baekelandt *et al* (15) and Gemignani *et al* (16), in which an 87.5% response rate and significant prolongation of PFS were observed. These reports and this study suggest that combination therapy with platinum and a taxane is effective against FTM. Given that the response rate to chemotherapy in epithelial ovarian carcinoma was found to be 56% (17) and that the rate for ovarian carcinosarcoma was 20 to 68% (18,19), the favorable effect of initial therapy in FTM is noteworthy. This favorable therapeutic effect may be attributed to the histological type of fallopian tube carcinoma, which is largely composed of serous and endometrioid adenocarcinoma.

Within Latzko's triad, atypical genital bleeding was observed in ~30% of patients in this study. If an adnexal tumor is detected in patients with atypical genital bleeding or a watery vaginal discharge, FTM should be considered as a differential diagnosis. Nevertheless, a definitive preoperative diagnosis of FTM is difficult in many patients. It has been reported that FTM is diagnosed preoperatively in 0 to 10% of patients (20-22). Moreover, Meng *et al* (23) reported that in 50% of patients, even intraoperative findings fail to identify that the primary site is the fallopian tube. In this study, preoperative diagnosis was feasible in 13.2% of patients, probably by virtue of advances in imaging diagnosis. Transvaginal ultrasonography has been reported to be more effective than abdominal ultrasonography (24), and the presence of a sausage-like mass and/or multilobular mass with a 'cog-and-wheel' appearance is considered to be the basis for suspecting a FTM (25-27). Diagnostic ability has improved with the development of color Doppler and three-dimensional Doppler transvaginal ultrasonography (28). However, findings that clearly discriminate FTM have not been identified, even with full use of CT and MRI. Although this provides no clinical specificity, the CA-125 level is increased in 80% or more of patients with fallopian tube carcinoma (13,29). Although it has been reported occasionally that the preoperative CA-125 level is an independent predictive factor for the prognosis of fallopian tube carcinoma (13,30) and that this level correlates with responsiveness to chemotherapy (31,32), such a correlation with the prognosis and therapeutic effect was not observed in this study. More cases need to be accumulated to establish the clinical significance of the CA-125 level in fallopian tube carcinoma.

A retrospective multicenter study was conducted recently to clarify the prognosis of fallopian tube carcinoma. Moore *et al* (33) used matched, case-controlled methods to compare the prognosis of fallopian tube carcinoma in 96 patients and epithelial ovarian carcinoma in 189 patients. Comparison of stage I/II with a median observation period of 57 months for fallopian tube carcinoma and 42 months for ovarian carcinoma showed a 5-year survival rate of 95% for fallopian tube carcinoma and 76% for ovarian carcinoma (P=0.02); a similar comparison in stage III/IV disease with a median observation period of 33 months for fallopian tube carcinoma and 35 months for ovarian carcinoma, showed a 3-year survival rate that was comparable (59%) for both diseases. Accordingly, Moore *et al* (33) re-evaluated the concept that therapy for fallopian tube carcinoma could be similar to that for ovarian carcinoma. Conversely, Pectasides *et al* (34) analyzed 64 patients with fallopian tube carcinoma and reported that advanced stage and residual tumor diameter correlated significantly with prognosis. In stage III/IV fallopian tube carcinoma, while the 5-year survival in patients whose residual tumors could be debulked to less than 1 cm was 55%, the 5-year survival of patients with larger residual tumor deposits was 21% (9). Thus, optimal debulking and subsequent platinum/taxan therapy were concluded to confer a favorable prognosis. In addition, a large population-based tumor registry study in which 416 patients with fallopian tube carcinoma were included showed that the 5-year survival rate was 95% for stage I, 75% for stage II, 69% for stage III, and 45% for stage IV, respectively, indicating that the prognosis was better than the 5-year survival rate for each stage of ovarian carcinoma evaluated at around the same time (35). More recently, using SEER database, the outcome of 1,576 women with fallopian tube carcinoma and 54,249 with epithelial ovarian carcinoma was compared (4). Five-year cancer-specific survival was 54 and 36% in women with stage III and IV fallopian tube carcinoma, respectively, vs. only 30 and 14% in those with the same stage epithelial ovarian cancer, respectively (4). However, initially relapsed locations and time to recurrence were not clarified in the SEER database (4). The present results suggest that there are many isolated recurrences and few recurrences of carcinomatous peritonitis for FTMs. Thus, because there are many cases of FTMs for which recurrent tumors can be extracted by surgery, even if the disease relapsed, prognosis would be improved. Although a hypothesis for the origin of ovarian and peritoneal carcinoma has been proposed, based on the concept of transport and implantation of malignant cells from the fallopian tube to the ovary and peritoneum (36), the results of this study suggest that FTM has a different biological behavior when compared to ovarian carcinoma.

The present study suggests that FTM frequently results in solitary recurrence, and that aggressive recurrence treatment including SDS would improve the prognosis of this disease.

However, several limitations must be acknowledged in this study. While differentiation of fallopian tube and ovarian carcinoma for women with advanced stage disease is sometimes difficult (37), a central pathology review was not performed as a retrospective nature. Although clinical features of fallopian tube carcinoma are not distinct from those of ovarian carcinoma as reported using case-control analysis (38), this study did not adopt a case-control method. Further study to elucidate the molecular mechanisms involved in the development of FTM and to improve decision-making for disease treatment is warranted.

References

1. Pectasides D, Pectasides E and Economopoulos T: Fallopian tube carcinoma: a review. Oncologist 11: 902-912, 2006.
2. Rosenblatt KA, Weiss NS and Schwartz SM: Incidence of malignant fallopian tube tumors. Gynecol Oncol 35: 236-239, 1989.
3. Riska A, Leminen A and Pukkala E: Sociodemographic determinations of incidence of primary fallopian tube carcinoma, Finland 1953-97. Int J Cancer 104: 643-645, 2003.
4. Wethington SL, Herzog TJ, Seshan VE, *et al*: Improved survival for fallopian tube cancer: a comparison of clinical characteristics and outcome for primary fallopian tube and ovarian cancer. Cancer 113: 3298-3306, 2008.
5. Sedlis A: Primary carcinoma of the fallopian tube. Obstet Gynecol Surv 16: 209-226, 1961.
6. World Health Organization Classification of Tumours: Pathology & Genetics. Tumours of the Breast and Female Genital Organs. IARC Press, Lyon, 2003.
7. Hoskins WJ, McGuire WP, Brady MF, *et al*: The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. Am J Obstet Gynecol 170: 974-979, 1994.
8. Eisenhauer EA, Therasse P, Bogaerts J, *et al*: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
9. Gadducci A, Landoni F, Sartori E, *et al*: Analysis of treatment failures and survival of patients with fallopian tube carcinoma: a Cooperation Task Force (CTF) study. Gynecol Oncol 81: 150-159, 2001.
10. Roh HJ, Kim DY, Joo WD, *et al*: Hepatic resection as part of secondary cytoreductive surgery for recurrent ovarian cancer involving the liver. Arch Gynecol Obstet 284: 1223-1229, 2011.
11. Ushijima K: Treatment of recurrent ovarian cancer - at first relapse. J Oncol 2010: 497429, 2010.
12. Klein M, Rosen AC, Lahousen M, *et al*: Lymphadenectomy in primary carcinoma of the fallopian tube. Cancer Lett 147: 63-66, 1999.
13. Ajithkumar TV, Minimole AL, John MM and Ashokkumar OS: Primary fallopian tube carcinoma. Obstet Gynecol Surv 60: 247-252, 2005.
14. Peter WA III, Andersen WA, Hopkins MP, *et al*: Prognostic features of carcinoma of the fallopian tube. Obstet Gynecol 71: 757-762, 1988.
15. Baekelandt M, Joruun Nesbakken A, Kristensen GB, *et al*: Carcinoma of the fallopian tube. Cancer 89: 2076-2084, 2000.
16. Gemignani ML, Hensley ML, Cohen R, *et al*: Paclitaxel-based chemotherapy in carcinoma of the fallopian tube. Gynecol Oncol 80: 16-20, 2001.
17. Katsumata N, Yasuda M, Takahashi F, *et al*: Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial. Lancet 374: 1331-1338, 2009.
18. Anderson B, Turner DA and Benda J: Ovarian sarcoma. Gynecol Oncol 26: 183-192, 1987.
19. Tate Thigpen J, Blessing JA, DeGeest K, *et al*: Cisplatin as initial chemotherapy in ovarian carcinosarcima: a Gynecologic Oncology Group study. Gynecol Oncol 93: 336-339, 2004.
20. Eddy GL, Copeland LJ and Gershenson DM: Second-look laparotomy in fallopian tube carcinoma. Gynecol Oncol 19: 182-186, 1984.
21. Podratz KC, Podczaski ES, Gaffey TA, *et al*: Primary carcinoma of the fallopian tube. Am J Obstet Gynecol 154: 1319-1326, 1986.
22. Huber-Buchholz MM, Buchholz NP and Staehelin J: Analysis of 23 cases of primary carcinoma of the fallopian tube over 50 years. J Obstet Gynaecol Res 22: 193-199, 1996.
23. Meng ML, Gan-Gao, Scheng-Sun, *et al*: Diagnosis of primary adenocarcinoma of the fallopian tube. J Cancer Res Clin Oncol 110: 136-140, 1985.
24. Timor-Tritsch IE and Rottem S: Transvaginal ultrasonographic study of the fallopian tube. Obstet Gynecol 70: 424-428, 1987.
25. Subramanyam BR, Raghavendra BN, Whalen CA, *et al*: Ultrasonic features of fallopian tube carcinoma. J Ultrasound Med 3: 391-393, 1984.
26. Kol S, Gal D, Friedman M and Paldi E: Preoperative diagnosis of fallopian tube carcinoma by transvaginal sonography and CA-125. Gynecol Oncol 37: 129-131, 1990.
27. Ajjimakorn S, Bhamarapravati Y and Israngura N: Ultrasound appearance of fallopian tube carcinoma. J Clin Ultrasound 16: 516-518, 1988.
28. Kurjak A, Kupesic S, Sparac V and Kosuta D: Three-dimensional ultrasonographic and power Doppler characterization of ovarian lesions. Ultrasound Obstet Gynecol 16: 365-371, 2000.
29. McMurray EH, Jacobs AJ, Perez CA, *et al*: Carcinoma of the fallopian tube. Management and sites of failure. Cancer 58: 2070-2075, 1986.
30. Rosen AC, Klein M, Hafner E, *et al*: Management and prognosis of primary fallopian tube carcinoma. Austrian Cooperative Study Group for Fallopian Tube Carcinoma. Gynecol Obstet Invest 47: 45-51, 1999.
31. Tokunaga T, Miyazaki K, Matsuyama S, *et al*: Serial measurement of CA 125 in patients with primary carcinoma of the fallopian tube. Gynecol Oncol 36: 335-337, 1990.
32. Hefler LA, Rosen AC, Graf AH, *et al*: The clinical value of serum concentrations of cancer antigen 125 in patients with primary fallopian tube carcinoma: a multicenter study. Cancer 89: 1555-1560, 2000.
33. Moore KN, Moxley KM, Fader AN, *et al*: Serous fallopian tube carcinoma: a retrospective, multi-institutional case-control comparison to serous adenocarcinoma of the ovary. Gynecol Oncol 107: 398-403, 2007.
34. Pectasides D, Pectasides E, Papaxoinis G, *et al*: Primary fallopian tube carcinoma: results of a retrospective analysis of 64 patients. Gynecol Oncol 115: 97-101, 2009.
35. Kosary C and Trimble EL: Treatment and survival for women with fallopian tube carcinoma: a population-based study. Gynecol Oncol 86: 190-191, 2002.
36. Kurman RJ and Shih IeM: Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer - shifting the paradigm. Hum Pathol 42: 918-931, 2011.
37. Berek JS, Crum C and Friedlander M: Cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet 119: S118-S129, 2012.
38. Dunn MS, Manahan KJ and Geisler JP: Primary carcinoma of the fallopian tube and epithelial ovarian carcinoma: a case-control analysis. J Reprod Med 53: 691-694, 2008.

Int J Clin Oncol
DOI 10.1007/s10147-013-0539-4

ORIGINAL ARTICLE

A pilot study of oxaliplatin with oral S-1 as second-line chemotherapy for patients with recurrent adenocarcimona of the uterine cervix

Eriko Takatori · Tadahiro Shoji · Yasuko Suga · Hanae Niinuma · Yuki Miura · Yoshitaka Kaido · Anna Takada · Masahiro Kagabu · Satoshi Takeuchi · Toru Sugiyama

Received: 2 December 2012 / Accepted: 12 February 2013

Abstract
Background The efficacy and safety of S-1/oxaliplatin (SOX) therapy in patients with recurrent adenocarcinoma of the uterine cervix were examined in a pilot study.
Patients and methods S-1 was orally administered for 14 days at a dose of 80–120 mg/body/day to 7 patients with recurrent adenocarcinoma of the uterine cervix, with oxaliplatin being administered intravenously at a dose of 100 mg/m^2 on day 1. Each therapy cycle was 21 days, and the patients received 6 cycles at most. The antitumor effect, adverse events, progression-free survival (PFS), and overall survival (OS) were investigated.
Results The median age of the patients was 49 years. The antitumor effect was rated as a complete response in 2 patients, partial response in 2, and stable disease in 3. The overall response rate was 57.1 %, and the disease control rate was 100 %. Regarding hematological toxicities of grade 3 or more, leukopenia, neutropenia and thrombocytopenia occurred in 42.9, 28.6 and 14.3 %, respectively; regarding non-hematological toxicities, grade 3 rectovaginal fistula occurred in 14.3 %, as well as grade 2 fatigue in 14.3 % of the patients. The median PFS and OS were 5 months (range 3–9 months) and 7 months (range 4–43 months), respectively.
Conclusions These results suggest that SOX therapy is useful for the treatment of recurrent adenocarcinoma of the uterine cervix, having a promising antitumor effect and minimal adverse effects. It was also suggested that SOX therapy may contribute to improving the prognosis for patients with adenocarcinoma of the uterine cervix.

Keywords Oxaliplatin · S-1 · SOX · Cervical adenocarcinoma · Chemotherapy

E. Takatori · T. Shoji (✉) · Y. Suga · H. Niinuma · Y. Miura · Y. Kaido · A. Takada · M. Kagabu · S. Takeuchi · T. Sugiyama
Department of Obstetrics and Gynecology, Iwate Medical University School of Medicine, Uchimaru 19-1, Morioka, Iwate 020-8505, Japan
e-mail: tshoji@iwate-med.ac.jp

Introduction

Currently, adenocarcinoma of the uterine cervix accounts for about 20–25 % of all uterine cervix cancers, and its frequency is rising [1]. It has been reported previously that adenocarcinoma of the uterine cervix carries a poorer prognosis than squamous cell carcinoma [2–4]. The reasons cited for this poorer prognosis include the occurrence of lymph node metastasis at a relatively early stage [5] and low sensitivity to radiation therapy [6]. Therefore, chemotherapy is expected to be more effective for improving the prognosis than radiation or chemoradiotherapy. Although new therapeutic strategies should be tried, few studies have focused on adenocarcinoma, and thus no high-level evidence has yet been obtained.

Katsumata et al. used S-1 therapy (80–120 mg/day) in 37 patients with advanced or recurrent carcinoma of the uterine cervix; each cycle of S-1 therapy consisted of 4-weeks' continuous administration followed by a 2-week rest period. They reported that the response rate was 30.6 %, the median time to progression was 5.2 months, and the median survival time was 15.4 months. Although adverse events of grade 3 or more, including diarrhea, anemia and anorexia, occurred in 22, 16 and 16 % of patients, respectively, the severity of these symptoms was within an acceptable range [7]. At present, a phase-III clinical study using S-1 (cisplatin 50 mg/m^2 on day 1 + S-1 80–120 mg/day on days 1–14 vs. cisplatin 50 mg/m^2 on day

Published online: 14 March 2013

1) in patients with advanced or recurrent carcinoma of the uterine cervix is underway on a global scale. S-1 is considered to be a promising drug that may serve as a new therapeutic strategy for carcinoma of the uterine cervix. In addition, with regard to chemotherapy for colorectal carcinoma, it was reported that 39.5 % of patients with inoperable advanced or metastatic colorectal carcinoma responded to S-1 [8]. The results of phase-I and phase-II studies of S-1 in combination with L-OHP (oxaliplatin) (S-1 + oxaliplatin; SOX therapy) showed a high response rate of 56 % [9], attracting considerable attention to this combination regimen as an alternative to FOLFOX therapy. In Japan, an intergroup phase-II clinical study of the efficacy and safety of SOX therapy for mucinous adenocarcinoma of the ovary has been underway since 2008, and the results are currently awaited.

We conducted a pilot study of the SOX regimen in patients with adenocarcinoma of the uterine cervix. Here, we describe the efficacy and safety of this therapy as evaluated in the present study.

Subjects and methods

The subjects were 7 patients, diagnosed with recurrent adenocarcinoma of the uterine cervix between April 2007 and September 2012, who gave informed consent to participate in this study. They had evaluable lesions that were histologically confirmed to be adenocarcinoma of the uterine cervix. All 7 patients had a history of chemotherapy, and ranged in age from 20 to 75 years, had a performance status (PS) of 0–2, and had well-maintained function of their major organs. Patients who were judged to be unsuitable for safe implementation of this treatment, such as those with intestinal paralysis, intestinal obstruction, diarrhea, interstitial pneumonia, pulmonary fibrosis, and/or uncontrolled diabetes mellitus, were excluded from the study.

S-1 was orally administered twice daily for 14 consecutive days (from the evening of day 1 until the morning of day 15). Oxaliplatin was administered on day 1. The S-1 dosing regimen was 80–120 mg/body/day, per os. More specifically, the S-1 dose was 80 mg/body/day for patients whose body surface area (BSA) was less than 1.25 m^2, 100 mg/body/day for those who had a BSA of 1.25 to less than 1.5 m^2, and 120 mg/body/day for those with a BSA of 1.5 m^2 or more. Oxaliplatin, 100 mg/m^2 dissolved in 250 mL of 5 % glucose, was administered intravenously by drip infusion over 120 min. Each cycle of combination therapy consisted of 21 days, and patients received 6 cycles at most (Fig. 1).

The requirements for therapy initiation were as follows: neutrophil count, 1500/mm^3 or more; platelet count, 75000/mm^3 or more; AST/ALT, less than 100 IU/L (less than 150 IU/L when there was liver metastasis); total bilirubin, less than 1.5 mg/dL; serum creatinine, less than 1.5 mg/dL; and no signs of diarrhea or infection. The requirements for initiation of a second cycle were: neutrophil count, 1500/mm^3 or more; platelet count, 75000/mm^3 or more; and the absence of non-hematological toxicities of grade 2 or more excluding nausea, vomiting, anorexia, fatigue, and hair loss.

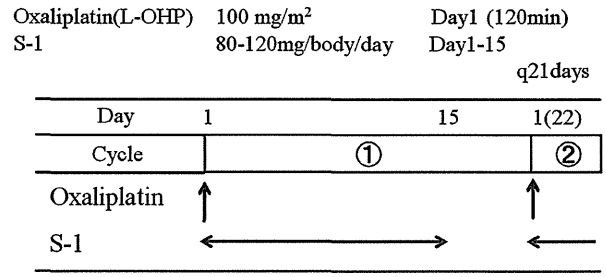

Fig. 1 Oxaliplatin, 100 mg/m^2 dissolved in 250 mL of 5 % glucose, was administered on day 1 by intravenous drip infusion over 120 min. S-1 was orally administered twice daily for 14 consecutive days (from the evening on day 1 until the morning on day 15). The dose of S-1 was 80 mg/body/day in patients with a body surface area (BSA) of less than 1.25 m^2, 100 mg/body/day in those with a BSA of 1.25 m^2 or more and less than 1.5 m^2, and 120 mg/body/day in those with a BSA of 1.5 m^2 or more. Each cycle of therapy consisted of 21 days

In patients with grade 4 thrombocytopenia in the previous cycle or whose platelet count did not reach 75,000/mm^3 on the day scheduled for initiation of the next cycle but who showed a restored count within 14 days, the dose of oxaliplatin was reduced from 100 to 75 mg/m^2. When the platelet count did not reach 75,000/mm^3 even after 14 days, the therapy was discontinued. When grade 4 neutropenia persisted for at least 5 days, or when febrile neutropenia of grade 3 or more occurred, the dose of oxaliplatin was reduced from 100 to 75 mg/m^2, and the dose of S-1 was reduced by one level (e.g., a previous dose of 120 mg/body/day was decreased to 100 mg/body/day; similarly, 100 was reduced to 80 mg/body/day and 80 to 50 mg/body/day). When grade 2 sensory nerve disorder occurred and did not improve to grade 1 by the day scheduled for initiation of the next cycle, the oxaliplatin dose was reduced from 100 to 75 mg/m^2. When the same symptoms developed a second time, the oxaliplatin dose was reduced from 75 to 50 mg/m^2. If the same symptoms were observed a third time, the therapy was discontinued. When sensory nerve disorder of grade 3 occurred but had improved to grade 1 by the day scheduled for initiation of the next cycle, the oxaliplatin dose was reduced as described above. If the symptoms did not improve to grade 1, the therapy was discontinued.

The antitumor effect, adverse events, progression-free survival (PFS), and overall survival (OS) were investigated. The antitumor effect was evaluated using the Response

Evaluation Criteria in Solid Tumor (RECIST). The National Cancer Institute Common Toxicity Criteria (NCI-CTCAE) version 3.0 was used for evaluation of adverse events. PFS and OS were calculated by the Kaplan–Meier method.

Results

Patient characteristics

The median age of the patients was 49 years (range 43–58 years), and PS was 0 in 5 patients, 1 in 1, and 2 in 1. The disease was clinical stage I in 1 patient, stage II in 2, stage III in 1, and stage IV in 3. The histological type was mucinous adenocarcinoma in all 7 patients. The median number of previous therapeutic regimens was 2 (range 1–4), and the treatment-free interval was less than 6 months in 6 patients and 6 months or more in 1. All patients had received taxane or platinum therapy. The median number of cycles of SOX therapy was 5 (range 4–6) (Table 1).

Antitumor response

The antitumor effect in terms of the response to therapy was rated as a complete response (CR) in 2 patients, partial response (PR) in 2, and stable disease (SD) in 3. The overall response rate was 57.1 %, and the disease control rate was 100 % (Table 2).

Table 1 Patient characteristics ($N = 7$)

Age, years		Treatment-free interval, months	
Median	49	<6	6 (85.7 %)
Range	43–58	≥6	1 (14.3 %)
Performance status (PS)		Cell type	
0	5 (71.4 %)	Mucinous	7 (100 %)
1	1 (14.3 %)	Site of metastasis	
2	1 (14.3 %)	Lymph nodes	4 (57.1 %)
No. of previous regimens		Vaginal stump	4 (57.1 %)
1	3 (42.9 %)	Bone	2 (28.6 %)
2–3	3 (42.9 %)	Others	3 (42.9 %)
≥4	1 (14.3 %)	No. of cycles	
FIGO stage		4	2 (28.6 %)
I	1 (14.3 %)	5	2 (28.6 %)
II	2 (28.6 %)	6	3 (42.9 %)
III	1 (14.3 %)		
IV	3 (42.9 %)		

Toxicity

Regarding hematological toxicities of grade 3 or more, leukopenia occurred in 42.9 %, and neutropenia in 28.6 %, of patients. Both patients with grade 4 neutropenia showed improvement before initiation of the next cycle, without administration of granulocyte-colony stimulating factor (G-CSF). Thrombocytopenia of grade 3 or more developed in 1 (14.3 %) patient during the 5th cycle of therapy. In this patient, the platelet count was restored to more than 75,000/mm^3 on day 28 without platelet transfusion, but the therapy was discontinued because of progressive disease (PD). Anemia of grade 3 or more occurred in 2 (28.6 %) patients during the 4th and 3rd cycles, respectively, and both received erythrocyte transfusion. As for non-hematological toxicities, grade 3 rectovaginal fistula occurred in 1 (14.3 %) patient, and therapy was discontinued after 5 cycles. This patient underwent a colostomy. Grade 2 fatigue was the only other non-hematological toxicity of grade 2 or more, and occurred in only 1 (14.3 %) patient. Sensory nerve disorder of grade 1 developed in all (100 %) patients (Table 3).

Table 2 Response

Response	No. of patients	(%)
CR	2	28.6
PR	2	28.6
SD	3	42.9
PD	0	0
Overall response rate	4	57.1
Disease control rate	7	100

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Table 3 Adverse events of SOX therapy ($n = 7$)

	Grade				
	1	2	3	4	≥3 (%)
Leukopenia	2	2	3	0	3 (42.9)
Neutropenia	3	2	0	2	2 (28.6)
Thrombocytopenia	3	2	1	0	1 (14.3)
Anemia	3	2	1	1	2 (28.6)
Nausea	5	0	0	0	0
Vomiting	0	0	0	0	0
Diarrhea	0	0	0	0	0
Sensory neuropathy	7	0	0	0	0
Mucositis	1	0	0	0	0
Fatigue	3	1	0	0	0
Vaginal fistula	0	0	1	0	1 (14.3)

Status of therapy

Three (42.9 %) of the 7 patients completed 6 cycles of therapy. PD occurred during therapy in 3 (42.9 %) of the 7 patients. In 2 of these 3, therapy was discontinued after 4 cycles and the regimen was altered. In the other patient with PD, the therapy was switched to a palliative regimen after 5 cycles. The aforementioned adverse events necessitated discontinuation of the therapy after 5 cycles in 1 (14.3 %) of the 7 patients.

In total, 36 cycles of this therapy were administered to the 7 patients. Thirteen (44.8 %) of 29 cycles were delayed by 1 week. The reason for the delay was unrestored neutrophil count for 1 cycle, and onset of peritonitis and pyelitis for 1 cycle each. Another cycle was delayed at the physicians discretion because there was a downward trend in the patient's platelet count although it remained over 75,000/mm^3. As for the other 9 cycles, the delay was at the patient's request because of fatigue, nausea, etc. Ultimately, only 1 (3.4 %) cycle was delayed by 1 week because of failure to satisfy the requirements for initiation of the next cycle of therapy. No dose reductions of oxaliplatin or S-1 occurred in any of the patients due to adverse events.

Survival

The median follow-up period was 7 months (range 4–43 months), the median PFS was 5 months (range 3–9 months), and the median OS was 7 months (range 4–43 months).

Discussion

Cisplatin (CDDP) has been used as monotherapy or in combination with other drugs as chemotherapy for adenocarcinoma of the uterine cervix [10, 11]. Chemotherapy with taxanes has been studied in recent years [12, 13]. In the GOG 204 study, which was a randomized controlled study involving 435 patients with stage-IVB or recurrent carcinoma of the uterine cervix including 54 with adenocarcinoma and 36 with adenosquamous carcinoma, vinorelbine/CDDP therapy, gemcitabine/CDDP therapy, and topotecan/CDDP therapy failed to show a therapeutic effect superior to that of paclitaxel (PTX)/CDDP therapy [14]. Therefore, at present, combination chemotherapy with taxane plus a platinum agent is generally considered to be the most effective regimen for the treatment of adenocarcinoma of the uterine cervix. However, there is no established second-line chemotherapy for patients who have recurrent disease after receiving first-line therapy with taxane plus a platinum agent. The histological type of adenocarcinoma of the uterine cervix is mucinous adenocarcinoma in a high proportion of patients with this disease, and it carries a poor prognosis in comparison with other histological types [15]. Uterus and ovary have the same embryological origin: the mullerian duct. Mucinous adenocarcinomas of both cervix and ovary show very similar histological characteristics. Moreover, cervical and ovarian mucinous adenocarcinomas consist of endocervical and intestinal types. We considered that both carcinomas had a similar chemosensitivity for the SOX regimen. Thus, we carried out a pilot study of the SOX regimen that is used for mucinous adenocarcinoma of the ovary in clinical study, and examined its efficacy and safety in patients with recurrent adenocarcinoma of the uterine cervix.

The important elements requiring serious attention when considering chemotherapy for recurrent cancer include: (1) minimal toxicity, (2) quality of life (QOL) maintenance, and (3) simplicity, in addition to a promising antitumor effect. Although the SOX therapy response rate was 57.1 % in this study, there were 2 (28.6 %) cases with CR (Fig. 2).

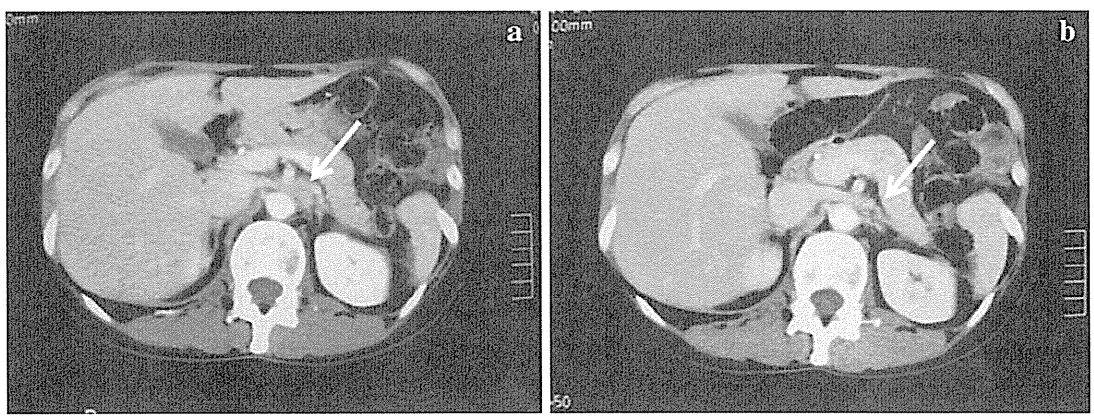

Fig. 2 **a** Before SOX therapy: para-aortic lymph nodes were enlarged to 1.5 cm × 1.5 cm, confirming lymph node involvement. **b** After 6 cycles of SOX therapy: para-aortic lymph node metastasis had disappeared