Phase II study of medroxyprogesterone acetate plus metformin as a fertility-sparing treatment for atypical endometrial hyperplasia and endometrial cancer†

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Background: Metformin, widely used in the treatment of type 2 diabetes mellitus, reduces the risk of cancer and relapse after treatment. Fertility-sparing treatment for endometrial cancer (EC) with progesterin is associated with a high chance of disease regression, and the high relapse rate continues to be a problem. We assessed the efficacy of metformin in preventing recurrence after medroxyprogesterone acetate (MPA) as fertility-sparing treatment for atypical endometrial hyperplasia (AEH) and EC.

Patients and methods: This phase II study enrolled 17 patients with AEH and 19 patients with EC limited to the endometrium (age, 20–40 years). MPA (400 mg/day) and metformin (750–2250 mg/day) were administered for 24–36 weeks to achieve a complete response (CR). Metformin was administered until conception, even after MPA discontinuation. The primary end point was relapse-free survival (RFS) after remission. We analyzed all efficacy end points in the full analysis set.

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Results: The body mass index was ≥25 kg/m² in 27 patients (mean, 31 kg/m²; range, 19–51 kg/m²), and the homeostasis model assessment for insulin resistance index was ≥2.5 in 24 patients (mean, 4.7; range, 0.7–21). Two patients showed progression at 12 weeks [95% confidence interval (CI) 2–18]. At 36 weeks, 29 (81%; 95% CI 65–90) patients achieved CR, and 5 (14%; 95% CI 6–29) patients achieved partial response. During a median follow-up of 38 months (range, 9–66 months) after remission, relapse was confirmed in three of the patients who had achieved CR (relapse rate, 10%). The 3-year estimated RFS rate was 89%. No patients experienced severe toxicity.

Conclusions: Metformin inhibited disease relapse after MPA therapy. The combination of metformin and MPA in EC treatment should be studied further.

Trial registration number: UMIN00002210.

Key words: endometrial cancer, metformin, medroxyprogesterone acetate, insulin resistance, fertility-sparing treatment

introduction

Progestin therapy is one of the most popular treatment options for fertility preservation in patients with endometrial cancer (EC) or atypical endometrial hyperplasia (AEH). Progestin therapy can achieve a high response rate, but patients also show a high recurrence rate [1, 2]. Accordingly, a novel strategy is needed to prevent recurrence after progestin therapy.

EC is often associated with obesity and/or diabetes mellitus, indicating that insulin resistance is a risk factor for EC [3–5]. Hyperinsulinemia caused by insulin resistance and its related signaling pathways, the PI3K/Akt and mitogen-activated protein kinase (MAPK) pathways, play an important role in carcinogenesis [6, 7]. Epidemiological and basic research studies support this notion; therefore, insulin resistance may be a promising preventive and therapeutic target of EC [8–10].

Metformin is a biguanide that is widely prescribed for the treatment of type 2 diabetes mellitus. Metformin stimulates adenosine monophosphate-dependent kinase (AMPK), which reduces gluconeogenesis while enhancing glucose uptake in skeletal muscles, therefore reducing insulin levels and modulating cancer growth [11]. Population-based studies suggested that metformin use decreased the incidence of cancer and cancer-related mortality in diabetic patients [12, 13]. Moreover, metformin inhibited the growth of breast, ovarian, endometrial, and prostate cancers, probably through suppression of MAPK, cyclin D1, and mammalian target of rapamycin (mTOR) activity [8–10]. The protective effect of metformin in terms of EC development as well as prevention of recurrence has been retrospectively shown [14, 15]. In addition, the results of our preliminary study supported the notion that metformin use reduces the levels of growth markers of EC cells, probably through suppression of MAPK and mTOR activity by lowering some humoral factors, such as insulin-like growth factor 1, insulin, and leptin [16].

Collectively, metformin use seems to be a promising therapy for controlling recurrence after progestin treatment. Here, we conducted a prospective observational study on the combined use of progestin and metformin as treatment for fertility preservation and evaluated the efficacy of metformin in preventing recurrence after medroxyprogesterone acetate (MPA) treatment.

methods

study design and participants

Patients were eligible if they were 20–40 years old, had histologically confirmed AEH or well-differentiated adenocarcinoma grade 1, had International Federation of Gynecology and Obstetrics stage IA disease, had EC limited to Federation of Gynecology and Obstetrics stage IA disease, had EC limited to the endometrium, and if they desired and consented to fertility-sparing therapy. Endometrial tissue sampling for diagnosis was carried out by dilatation and curettage. Detailed eligibility criteria are provided in supplementary material, available at Annals of Oncology online. All patients, except those already diagnosed as having diabetes mellitus, underwent a 75-g oral glucose tolerance test (OGTT) to assess insulin resistance. Data regarding age, gravidity, parity, infertility, obesity (defined as body mass index (BMI) ≥25 kg/m²), diabetes, and polycystic ovary syndrome (PCOS) were obtained. PCOS was diagnosed according to the Rotterdam 2003 criteria. The pathological diagnoses of the endometrial samples were reviewed by two independent pathologists. The Institutional Review Board of Chiba University approved the study protocol and all patients provided written informed consent before participation.

procedures

The patients received a daily oral dose of 400 mg MPA with 100 mg aspirin for 24 weeks, followed by a lower-dose monophasic agent (1 mg norethisterone and 0.035 mg ethinyl estradiol) for six cycles. Patients who achieved a partial response (PR) at 24 weeks were administered MPA for an additional 12 weeks. Metformin (initial dose, 750 mg/day; increased weekly by 750 mg up to 2250 mg/day if no adverse effects occurred) was administered concurrently with MPA from the initial point of treatment. Metformin therapy was continued after MPA administration until conception or disease recurrence. The treatment protocol was halted, and hysterectomy was recommended in the following situations: stable or progressive disease at 12 weeks, progressive disease at 24 weeks, or no complete response (CR) at 36 weeks. If a patient wanted to conceive immediately, fertility treatment was initiated after remission followed by six cycles of low-dose contraceptive administration. Otherwise, a low-dose contraceptive was prescribed until the patient wished to conceive.

outcomes

The primary end point was relapse-free survival (RFS), which was measured from the remission date until the date of recurrence. Secondary end points were as follows: the efficacy of MPA with metformin, including overall response; the safety of and degree of toxicity with this combination; the changes in metabolic status, including in insulin resistance and BMI; and the conception rate following treatment.

We determined the proportion of patients who achieved a response [95% confidence interval (CI)]. The efficacy and safety were evaluated in the final evaluation carried out at 24 months after completion of enrollment. We graded the adverse effects according to the National Cancer Institute Common Toxicity Criteria version 3.

statistical analysis

We calculated the sample size based on Fleming’s single-stage design of phase II studies. We set a 2-year RFS rate of 52% as a baseline survival rate.
and 20% as the high level of interest, with a power of 0.8 at a one-sided significance level of 0.05, requiring an accrual of 36 eligible patients.

All data were analyzed for the full analysis set. For the baseline variables, summary statistics were constructed employing frequencies and proportions for categorical data, and means and 95% CIs were calculated for continuous variables. Primary end point analysis was carried out using the Kaplan–Meier method to obtain estimates of RFS. Kaplan–Meier curves were generated to display event distributions over time. Comparisons between paired values were made using the Wilcoxon signed-rank test.

All comparisons were planned, and the tests were two-sided. A $P$ value of $<0.05$ was considered significant. All statistical analyses were carried out using SAS software version 9.3 (SAS Institute, Cary, NC) and SPSS software version 20 (IBM-SPSS, Inc., Chicago, IL).

### results

**patient characteristics**

Between July 2009 and December 2012, 38 patients (17 with AEH and 21 with EC) were enrolled. Two patients with EC were deemed ineligible after enrollment because they were diagnosed as having grade 2 endometrioid adenocarcinoma. The patients’ characteristics, including their metabolic profiles, are shown in supplementary Table S1, available at *Annals of Oncology* online only. Of the total patients, 16 (44%) showed impaired glucose tolerance, and 24 (67%) had insulin resistance. The mean glucose level at 2 h after OGTT was 138.5 U/ml (95% CI 122.7–154.4). The mean insulin level at 2 h after OGTT was 154.3 U/ml (95% CI 105.0–203.5) (supplementary Figure S1, available at *Annals of Oncology* online only).

**relapse-free survival**

Of the 36 eligible patients who completed the MPA therapeutic protocol, 23 (64%) achieved CR within 6 months and 6 (17%) achieved CR within an additional 3 months. Thus, 29 (81%; 95% CI 65–90) patients who achieved CR within 36 weeks were determined as being in remission. Of these, 16 patients with AEH and 13 patients with EC were analyzed for RFS. Only 3 (10.3%) of the 29 patients who had achieved CR relapsed during a median follow-up period of 38 months (range, 9–66 months) after remission (Figure 1). In two of these, recurrence occurred during infertility treatment, at 6 and 9 months after MPA treatment. These patients were treated with additional MPA because of a strong desire to maintain fertility, and their lesions had regressed at the time of analysis. One patient showed recurrence 18 months after MPA treatment and opted to undergo hysterectomy. There was no significant difference in the 3-year RFS rate between patients undergoing infertility treatment and those not undergoing infertility treatment (88% versus 92%; hazard ratio, 1.7; 95% CI 0.15–19.2; $P = 0.65$).

**response to MPA treatment**

Five patients who achieved PR at 36 weeks continued with the MPA treatment. Three of these achieved CR within 3 months, and one achieved CR within 12 months; all four had no recurrence during the study period. Another EC patient underwent hysterectomy without remission at 12 months. Two patients with EC showed progression at 12 weeks after the initial treatment. In both patients, the pathological diagnosis changed from endometrioid adenocarcinoma grade 1 to undifferentiated adenocarcinoma, indicating dedifferentiation. In one patient, bone metastasis and lymphadenopathy were also detected, and the patient consequently received combination chemotherapy with carboplatin and paclitaxel. She died of the disease 14 months after the initial treatment. The other patient underwent hysterectomy 14 weeks after the initial treatment and remained alive without evidence of disease during the study period.

**adverse events**

No severe toxicities were observed (supplementary Table S2, available at *Annals of Oncology* online only). Abnormal blood test results, including liver function and coagulation tests, were not reported during MPA treatment. Three patients presented with liver dysfunction caused by fatty liver after MPA treatment. In one patient, nonalcoholic steatohepatitis was diagnosed, and metformin was interrupted at 23 weeks after remission because of grade 2 liver dysfunction. There were no cases of thrombosis or treatment-related death. Grade 2 diarrhea and nausea occurred at a metformin dose of 2250 mg/day in six patients, four of whom showed considerable improvement when the metformin dose was reduced to 1500 mg/day. Metformin was interrupted in two patients because of grade 2 nausea but was resumed after childbirth. No weight gain (grade ≥1) occurred in any patient when MPA was administered in combination with metformin.

**response for metabolic profiles**

Metformin improved the patients’ metabolic profiles (supplementary Table S3, available at *Annals of Oncology* online only). Metformin administration after completion of MPA therapy led to a decrease in the patients’ weight, and significant improvements in insulin resistance (supplementary Figure S2, available at *Annals of Oncology* online only).
pregnancy outcome

Sixteen patients among the 29 CR patients wanted to conceive immediately. The mean infertility period before enrollment was 4.4 years (range, 0–12 years). The mean age of the infertile patients was 35 years (range, 26–41 years). Eight of these 16 patients (50%) had 11 conceptions during the study period. All 11 pregnancies were due to fertility treatment using an in vitro fertilization and embryo transfer program. There were six live births during the follow-up period.

discussion

The present study demonstrated that metformin inhibited EC recurrence and prolonged RFS after MPA therapy. This is the first prospective evidence that an antidiabetic dose of metformin can suppress cancer development, which had been suggested previously in epidemiological studies but had not yet been proven.

We found that adjuvant metformin improved the hormonal therapy outcomes to a greater extent than expected. A recent review evaluating 408 EC patients and 151 AEH patients supports this conclusion [2]. This study showed that CR rates after progestin therapy in EC and AEH patients were 76.2% and 85.6%, respectively, whereas the recurrence rates were 40.6% and 26.0%, respectively [2]. The largest study reported previously, which was included in the review, was the most comparable with our study in terms of its prospective study design, subject number (n = 45), ethnic background (Japanese), AEH/EC ratio, MPA dose, and oral contraceptive use during the follow-up period [17]. In this previous study, the initial CR rate was 67%, and the recurrence rate during the 3-year follow-up period was 47% [17]. Our results exceeded these results regarding both the initial CR rate (81% at month 9) and the recurrence rate (3-year recurrence rate, 10%), although the median follow-up period of the current trial was shorter (38 versus 48 months). Nevertheless, our results are encouraging, because the follow-up period in our study almost covered the initial 2 years, within which most recurrences occurred in the control group. A review of eight independent studies (n = 60) showed that 68% of recurrences occurred within 2 years, in which the median recurrence period was 20 months (range, 4–81 months), suggesting that our follow-up period was sufficient to account for most incidences of relapse [17–23].

Metformin combined with MPA showed other benefits in addition to recurrence prevention. First, metformin improved insulin resistance in young EC patients. Many patients who are candidates for fertility-preserving treatment are obese and have insulin resistance; these patients are at risk of developing diabetes and metabolic syndrome. It is reasonable to use a combination of metformin and MPA in these patients because metformin is effective for diabetes prevention [24]. Second, metformin may prevent weight gain, a common adverse event of MPA treatment, and the present study confirmed this. In fact, patients’ weight decreased after completion of MPA. Finally, metformin enhanced ovulation in PCOS patients and improved pregnancy rates after MPA treatment [25].

The result that metformin inhibited EC recurrence after MPA therapy supports the findings of several case–control studies of diabetes, which showed that metformin prevented cancer development [9]. We have previously reported that preoperative metformin administration reduced Ki-67 expression in EC tissues via AMPK-dependent mTOR inhibition and extracellular signal-regulated kinase dephosphorylation [16], and this result was confirmed by two independent studies [26, 27]. In addition, ex vivo assays showed a reduction in growth-supporting potential of patients’ sera obtained after metformin treatment. This may be explained by metformin-induced changes in humoral factors such as insulin, insulin-like growth factor 1, and/or leptin. On the basis of these previous studies, we speculate that decreases in humoral factors after metformin treatment could prevent the development of new EC lesions after MPA treatment.

In the present study, the most common metformin-related adverse events were gastrointestinal symptoms, including nausea and diarrhea. Most of these adverse events improved because the dose was reduced to 1500 mg/day. In some patients, asymptomatic liver enzyme increases indicative of fatty liver were observed during MPA and metformin administration. The majority of patients who desired fertility-sparing treatment were obese, increasing the potential risk of fatty liver. Therefore, attention must be paid to the development of hyperlipidemia during this treatment. Nevertheless, no patient developed fatal adverse events such as lactate acidosis, and long-term metformin was generally well tolerated and safe for patients with EC, similar to in patients with diabetes.

The optimal dose of MPA for fertility-sparing treatment has not been established, although many case studies used 200–600 mg MPA. In Japan, the most frequently used dose of MPA is 600 mg, because that dose was recommended in a phase II study [17]. However, in our previous study [18], similar response rates were found in patients treated with 400 and 600 mg/day. Furthermore, the current study revealed that side-effects such as weight gain, thrombosis, and liver dysfunction were less frequent in patients who received 400 mg MPA compared with those who received 600 mg, which was also reported in a Japanese phase II study [17]. Whether this finding was the result of the combined treatment with metformin remains unclear; however, 400 mg MPA plus metformin was found to be a reasonable regimen.

Two single-arm phase II studies (NCT01968317, NCT02035787) and one randomized phase II study (NCT01686126: Femme trial) of metformin combined with progestin for fertility-sparing treatment have been ongoing since 2012. The primary end point of these studies is the pathological response rate. Patients’ metabolic status and relapse rate have not been mentioned even as a secondary end point in two of these studies. A combination of metformin and MPA could reduce disease recurrence after remission and improve patients’ metabolic status; however, it did not improve the remission rate in our study. In this regard, additional studies are required to confirm the inhibitory effect of metformin.

The primary limitation of the present study is that it was a single-center, nonrandomized, phase II study with a relatively small sample size. An additional limitation is that >80% of the patients were obese and had insulin resistance; therefore, it is unclear whether metformin is effective in nonobese, insulin-sensitive patients. For these reasons, the results need to be confirmed in larger cohorts including nonobese patients.

In summary, this study shows promising results in terms of metformin reducing the relapse rate after MPA therapy.
Furthermore, this is the first prospective study to demonstrate that an antidiabetic dose of metformin suppressed cancer development. A phase III study is needed to confirm the efficacy of metformin.

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disclosure
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references