Can peritoneal carcinomatosis of colorectal origin be treated with oral everolimus alone or in combination with capecitabine? Preliminary results

Mehmet E. Yuksel¹, Osman Yuksel²

Abstract: Background: We wanted to evaluate the effectiveness of oral everolimus alone and in combination with capecitabine treatment on colorectal cancer-induced peritoneal carcinomatosis animal model.

Methods: Caco-2 colon adenocarcinoma cells were injected intraperitoneally to BALB/cOlaHsd-Foxn1nu mice to establish peritoneal carcinomatosis. Mice were divided into four groups (everolimus, everolimus plus capecitabine, capecitabine, control group) with 2 mice in each group. Treatment was initiated 5 days after inoculation of Caco-2 cells. 7.5 mg/kg everolimus was administered orally every 2 days. 2.1 mmol/kg capecitabine was administered orally every 5 days a week. 22 days after inoculation of Caco-2 cells, mice were sacrificed.

Results: The mean weight of the tumor was 25 ± 7.07 mg in the control group (n=2); 0.7 ± 0.7 mg in the everolimus group (n=2); 0.28 ± 0.36 mg in the capecitabine group; 0.48 ± 0.07 mg in the everolimus plus capecitabine group.

Conclusion: Tumor samples excised from BALB/cOlaHsd-Foxn1nu mice revealed that everolimus alone, and in combination with capecitabine suppressed tumor growth. However, a comparison of tumor weights revealed no statistically significant difference within the four groups (p=0.227). To reach statistically significant data, the sample size should be increased.

Keywords: colon cancer, everolimus, peritoneal carcinomatosis

INTRODUCTION

Peritoneal carcinomatosis secondary to colorectal cancer progression is characterized by the formation of solid tumor deposits on peritoneal surfaces [1]. Even today, chemotherapy protocols plus cytoreductive surgery and hyperthermic intraperitoneal chemotherapy applied for the treatment of metastatic colorectal cancer have been able to increase the median survival up to between 20-62 months at most [2-4]. Moreover, chemotherapy protocols used for the treatment of advanced colorectal cancer contain intravenous (iv) interventions and hours of chemotherapeutic agent infusions, which reduce both patient compliance and comfort.

In search of novel treatment options for peritoneal carcinomatosis of colorectal origin, an article published by Wagner et al. in 2009 drew our attention, in which they claimed that effective treatment of advanced colorectal cancer by rapamycin and 5-fluorouracil (5-FU)/oxaliplatin was achieved [5]. Rapamycin is both an antibacterial macrolide group antibiotic and a mammalian target of rapamycin (mTOR) inhibitor [6, 7]. Phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR signaling pathway is a central regulator in cell proliferation, cell growth, and angiogenesis [8-10]. Inhibition of mTOR by rapamycin prevents tumor proliferation and growth [7, 11]. Everolimus is the derivative of rapamycin that can be used orally and it has a higher

¹ Yıldırım Beyazıt University School of Medicine, Intensive Care Unit, Ankara, Turkey. ORCID: 0000-0002-7110-0
² Gazi University School of Medicine, Department of General Surgery, Ankara, Turkey. ORCID: 0000-0003-4784-908X

Corresponding author: Mehmet Eren Yuksel
doctormehmeteren@yahoo.com
bioavailability than rapamycin [12, 13]. Moreover, oral capecitabine treatment in metastatic colorectal cancer is equivalent to iv 5-FU plus leucovorin treatment has given the time to progression of the disease and overall survival [14]. Wagner et al. revealed that when oral rapamycin was administered in combination with iv 5-FU, colorectal cancer-induced peritoneal carcinomatosis was suppressed in a mouse model. However, 5-FU can only be used intravenously. Therefore, we thought that the treatment of peritoneal carcinomatosis with chemotherapeutic agents that could be used orally would both facilitate the compliance of the patient with the treatment and increase the patient’s comfort. Thus, this study aimed to administer everolimus and/or capecitabine orally to BALB/cOlaHsd-Foxn1nu mice with colon cancer-induced peritoneal carcinomatosis to examine whether tumor regression was achieved.

MATERIALS AND METHODS

Local ethics committee approval was obtained before the study (Approval number: 2013/60).

Cell Lines and Chemical Substances

Caco-2 human epithelial colorectal adenocarcinoma cells were purchased from American Type Culture Collection (ATCC®, Rockville, MD, USA). Dulbecco’s Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), Dulbecco’s Phosphate Buffered Saline (D-PBS), 0.05% trypsin-EDTA, and trypan blue stain 0.4% (dilution factor 20, 10 μl pellet in 200 μl mixture) were purchased. Everolimus (Certican 0.75 mg, Novartis) and capecitabine (Xeloda 500 mg, Roche) were supplied from the market.

Animal Model

Eight BALB/cOlaHsd-Foxn1nu mice, 14-18 week-old female, weighing 14.1-24.2 g were used within this study. BALB/cOlaHsd-Foxn1nu mice were purchased from Harlan (Israel). BALB/cOlaHsd-Foxn1nu mice were housed in individually ventilated cages at 12/12 hours light/dark cycle with a midline incision. Saline solution was injected into the intraabdominal cavity and aspirated. Intraabdominal
peritoneal washing fluid samples were reserved for cytological examination (Figure 1). Afterward, the peritoneum was excised without damaging other organs and the tumor developed at the intraperitoneal injection site was excised. The dimensions of the total tumor tissue removed were measured and the tumor was weighed. Tumor tissue formed in the peritoneum, mesenteric lymph nodes, liver, spleen, kidney, small intestine, colon, stomach, and intraperitoneal injection site were placed in formaldehyde solution for pathological examination.

| Table 1 | Treatment protocol |
|---------|--------------------|
|         | Everolimus         | Everolimus + Capecitabine | Capecitabine | Control Group |
| Number of Mice | 2             | 2                         | 2            | 2            |
| Dose     | 7.5 mg/kg          | 7.5 mg/kg + 2.1 mmol/kg/day | 2.1 mmol/kg/day | PBS* |

*PBS: Phosphate-buffered saline

Figure 1: (A) 0.9% NaCl was given into the abdominal cavity intraperitoneally and aspirated. Cytological examination of the aspirated fluid was investigated for malignant cells. (B) Excision of the peritoneum.

![Figure 1](image1.png)

Statistical Analysis

Statistical analysis was performed using SPSS version 15.0. Kruskal-Wallis analysis of variance was used to evaluate the differences between continuous variables. P<0.05 was considered statistically significant.

RESULTS

Control Group

In the first mouse within the control group, a 1.5 cm lesion was observed where Caco-2 cells were injected in the left lower quadrant of the abdomen. The tumor which was located intraperitoneally was excised. The tumor size was 2x1.5x1.5 cm weighing 20 mg (Figure 2). No macroscopic tumor was found in other intraabdominal organs. Poorly differentiated adenocarcinoma was detected in the pathological examination of the tumoral lesion excised from BALB/cOlaHsd-Foxn1nu mouse.

In the second mouse within the control group, the size of the lesion was 2.5x1.8x1.5 cm weighing 30 mg. The pathological examination of the excised tumor revealed poorly differentiated adenocarcinoma. No tumor, metastasis, or infiltration was detected in the peritoneum, mesenteric lymph nodes, liver, spleen, kidney, small intestine, colon, and stomach. No tumor cell was detected in the cytological examination of the peritoneal washing fluid.

Everolimus Group

During exploration, a tumoral lesion 0.2x0.4x0.4 cm in size,
weighing 0.2 mg was detected in the first mouse. The cytological examination of intraabdominal fluid revealed no tumor cells. Tumor size was 1x0.6x0.4 cm weighing 1.2 mg in the second mouse. In addition, metastatic mesenteric, peripancreatic, and perisplenic lymph nodes were detected in the pathological examination. At the same time, two tumor deposits were detected on the small intestine surface. However, no tumor cell was detected in the cytological examination of the peritoneal washing fluid.

**Capecitabine Group**

In the first mouse given capecitabine, the tumor was associated with peritoneal tissue in exploration. Tumor size was 0.2x0.1x0.3 cm (Figure 3). No other macroscopic tumor tissue was detected. In the second mouse treated with capecitabine, a tumor 0.4x0.5x0.6 cm in size was detected. Apart from the other mice, poorly differentiated adenocarcinoma infiltrating diaphragm striated muscle was detected.

**Figure 3:** (A) Tumoral lesion detected on the peritoneum. (B) Poorly differentiated adenocarcinoma metastasis within the mesenteric lymph node (Hematoxylin&eosin; x40 magnification).

**Everolimus plus Capecitabine Group**

Tumor size was 1.2x1x0.8 cm in the first mouse which was treated with both everolimus and capecitabine. However, pathological examination revealed poorly differentiated adenocarcinoma metastases in mesenteric lymph nodes. Tumor size within the second mouse was 0.5x0.6x0.4 cm (Figure 4). No malignant cells were detected in other intraabdominal organs, peritoneum, and peritoneal lavage fluid.

**Comparison of Tumor Weights**

In the control group, tumor weights were 20 mg and 30 mg. In the group treated with everolimus tumor weights were 0.2 mg and 1.2 mg. Within the group treated with capecitabine, tumor weights were 0.03 mg and 0.54 mg. The tumor weights were 0.43 mg and 0.54 mg within the group treated with both everolimus and capecitabine (Figure 5).

**DISCUSSION**

The mTOR (mammalian target of rapamycin) is a serine/threonine-protein kinase [17]. There are two mTOR
complexes, mTORC1 and mTORC2 [18, 19]. mTORC1 includes mTOR, Raptor (regulatory-associated protein of mTOR), and mLST8. mTORC2 contains mTOR, Rictor (rapamycin-insensitive companion of mTOR), mLST8, and mSIN1 (mammalian stress-activated protein kinase interacting protein). The mTORC1 eukaryotic translation initiation factor controls protein synthesis through the ED (eIF4E) binding protein (4E-BP1) and p70S6 ribosomal kinase (S6K) [20]. mTORC2 is involved in cell growth. Rapamycin combines with FK506 binding protein (FKBP) 12 to block mTOR activity. The mTORC1 is sensitive to rapamycin, while mTORC2 becomes susceptible to rapamycin only if it is exposed to the rapamycin effect for a long time [21]. Rapamycin-like drugs such as everolimus, temsirolimus, and ridaforolimus which are called rapalogs, have been produced after the antitumoral properties of rapamycin were discovered [22].

Nowadays, everolimus is used because of its tumor suppression feature in patients with subependymal large cell astrocytoma, advanced breast cancer, progressive pancreatic neuroendocrine tumor, subependymal giant cell astrocytoma, and advanced renal cell carcinoma [22]. In addition, capecitabine is used for the treatment of colorectal cancer and metastatic breast cancer [12, 23-25]. The side effects of both everolimus and capecitabine are very well known in detail because they are included within the current treatment protocols [26-28].

The positive effect of everolimus on peritoneal carcinomatosis has been demonstrated in several experimental models. Taguchi et al. used 58As1 human stomach cancer cell series, which were prone to peritoneal metastasis, to demonstrate the effectiveness of everolimus in advanced gastric cancers with peritoneal spread, and showed that everolimus significantly reduced peritoneal spread [29]. Chu et al. tested everolimus on a human colorectal carcinoma cell line with KRAS mutation in a mouse model and revealed that everolimus alone had significant antitumor activity [30]. Mabuchi et al. reported that everolimus delayed tumor onset and progression in the transgenic mouse model in ovarian cancer [31]. Moreover, studies on humans investigated the effects of everolimus on tumor growth when used as a single agent. Okamoto et al. published the results of phase 1 studies on daily everolimus treatment for patients with advanced solid tumors and reported significant tumor shrinkage in patients with esophageal and gastric cancer with 10 mg/day everolimus treatment [32]. However, Yoon et al. stated that 10 mg/day of everolimus treatment had only a limited impact on advanced gastric cancer [33]. There have been also phase studies on human beings with everolimus and capecitabine.

Deenen et al. published the results of the phase I study of oral capecitabine and everolimus treatment in patients with advanced solid cancers [34]. In this study, 18 patients with the primary pancreas, major duodenal papilla, esophageal, gallbladder, brain, osteosarcoma, and hepatocellular cancer were treated with capecitabine 1.000 mg/m² bid and everolimus 10 mg/day every 3 weeks for 14 days, and prolonged clinical efficacy was observed in 39% of patients with advanced solid malignancies. Lim et al. published the results of a phase I study of capecitabine and everolimus treatment for patients with previously treated metastatic gastric cancer [35]. Fifteen patients with metastatic stomach cancer who had progression after chemotherapy were included in the study. With 650 mg/m² capecitabine twice daily and 5 mg everolimus twice daily, the median time without progression of the disease was 1.8 months (range: 0.8–2.8 months). At the same time, the reduction in the longest diameter of the tumor was 28.7%.

In our study, tumors formed in mice within the control group were approximately x20 times larger than tumors excised from other groups, however, tumor growth in the control group was limited only to the inoculation site of Caco-2 cells. Everolimus and capecitabine treatment suppressed primary tumor growth in the region where Caco-2 adenocarcinoma cells were injected. No liver toxicity or mortality were observed during treatment with everolimus and/or capecitabine.

In light of these findings, the mTOR inhibitor everolimus suppressed tumor growth in the mouse model of colon cancer-induced peritoneal carcinomatosis when administered orally or in combination with capecitabine. However, the limitation of this study was the small sample size. Our study is unique because it has investigated whether peritoneal carcinomatosis could be treated with oral chemotherapeutic agents. However, to reach a definitive conclusion, it is necessary to work with a larger experimental group.

Acknowledgments
I would like to thank Prof. Emin Umit Bagriacik, Assoc. Prof. Ozgur Ekinci, Dr. Emine Avci, Serhat Ozgermen, and Basak Ozgermen for their valuable contribution.

This study was presented at 49. Congress of the European Society for Surgical Research as an oral presentation on 21-24th May 2014, Budapest, Hungary.

Funding
No funding was received.

Conflict of interests
It is not declared by the authors.
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