

Capecitabine Provides Low Toxicity Antitumor Chemotherapy During Perioperative Period Of Colectomy: Experimental Study In Rats

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Citation

H Konstantinidis, T Pissanidou, A Sioga, L Economou, C Demertzidis, N Pissanidis. *Capecitabine Provides Low Toxicity Antitumor Chemotherapy During Perioperative Period Of Colectomy: Experimental Study In Rats*. The Internet Journal of Oncology. 2006 Volume 4 Number 2.

Abstract

Background: Early induction of chemotherapy in patients operated for colorectal carcinomas, may cause a significant delay in wound healing and multiply the risk of septic complications. Capecitabine is a new fluoropyrimidine carbamate with antineoplastic activity, indicated for the therapy of colorectal cancer. The impact of capecitabine administration during the perioperative period after colectomy was studied.

Methods: Rats, which underwent colectomy and hand sutured colonic anastomosis, were pretreated with capecitabine. Sixty Wistar rats were randomized in two groups of 30 rats each. In the study group capecitabine was administered at therapeutic dose, from 1 week prior the operation throughout the study. Control group received placebo. Rats were sacrificed in groups of 10 animals on the 3rd, 7th and 14th postoperative days, in both experimental and control groups.

Results: All animals of the experimental group gained weight postoperatively. No negative impact on the healing of experimental animal's colonic anastomoses was reported. The median bursting pressure was found to be significantly higher and histological findings showed less necrotic effects in experimental animals sacrificed on the 3rd day, in comparison to controls.

Conclusions: Administration of capecitabine during colectomy does not have a negative impact on the recovery of preoperatively treated animals, neither on the healing of colonic anastomoses.

INTRODUCTION

Operative resection remains the most effective treatment in carcinomas of the colon and rectum. However, almost one third of the patients undergoing curative resection will develop local recurrence or metastatic disease, owing to operative manipulation of the tumor.¹ Adjuvant therapy with fluoropyrimidine-based regimens has been proposed to reduce these consequences, but on the other hand downregulates the healing process and multiplies the risk of anastomotic failure, resulting in increased morbidity and mortality rates.²

Capecitabine is an oral precursor of 5-fluorouracil (5-FU) with enhanced tumor selectivity, widely used in colorectal, breast and other malignancies.^{3,4} Capecitabine activation

follows a pathway with three enzymatic steps to form 5-FU.⁵ The final step requires thymidine phosphorylase, an enzyme that is significantly more active in tumor than in normal tissues.⁶ Our hypothesis is that this tumor selective attribute may provide an adjuvant antineoplastic therapy with fewer negative consequences on the postoperative recovery. This study investigates the impact of capecitabine administration at therapeutic doses, during the perioperative period of large intestine dissection with creation of a colonic anastomosis in rats.

MATERIALS AND METHODS

ANIMALS

Sixty Wistar rats, 10-12 weeks old, with mean body weight 224gr (157-243) were used. The animals were maintained on

a standard laboratory pellet diet, had free access to food and tap water throughout the study, and were kept in a stable environment of 20°C with natural light. The animals were randomly divided in two groups of 30. The experimental group received oral capecitabine and the control group normal saline orally. These two groups were further subdivided into three groups, each consisting of 10 animals. One group of 10 animals each, from both experimental and control groups, was sacrificed on the 3rd, 7th and 14th postoperative day, in order to examine the phases of the healing process. All experiments were conducted in conformity with the national and European Community laws. All animals were weighed before the operation and before sacrifice. Furthermore, five more animals (specifier group) were killed without any previous surgical or pharmaceutical intervention, in order to standardize the experimental conditions.

CHEMICALS

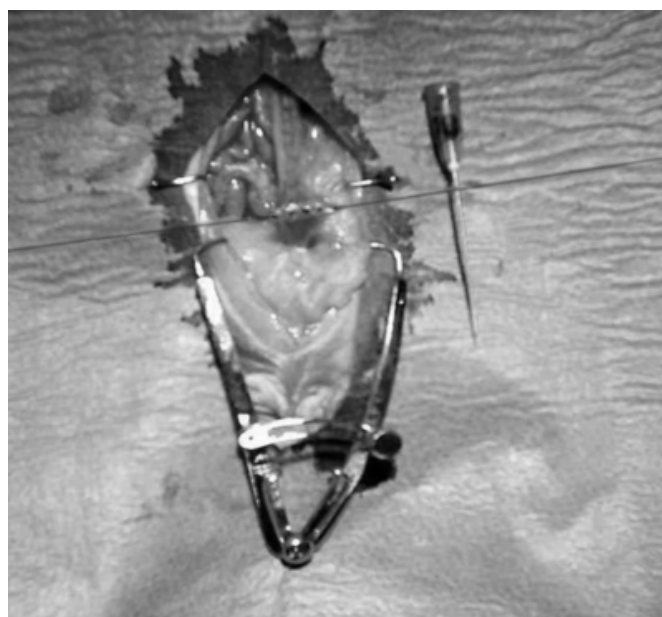
Capecitabine (Xeloda®, F. Hoffman La -Roche, Basle, Switzerland), in the form of white powder, was dissolved in 40mM citrate buffer (PH 6,0) containing 5% Arabic gum as the vehicle and then administered orally to rats.⁷ A therapeutic dose of 359 mg/kg (2/3 of the mean toxic dose₈) was administered starting 1 week prior to the surgical anastomosis and continued throughout the study. In the control group normal saline was administered during the same period.

OPERATIVE PROCEDURES

Anesthesia was induced with a combination of light ether and intraperitoneal injection Ketamine Hydrochloride (Ketalar®, Warner Lambert Co, USA). Through a midline incision, a 2 cm segment of the distal colon was dissected. Continuity was restored by the creation of a single layer seromuscular end-to-end anastomosis (Figure 1), with ten interrupted polypropylene 6.0 sutures (Prolene®, Ethicon Ltd, Edinburgh, UK). Skin and fascia were closed in two layers with continuous sutures of 4.0 polypropylene (Prolene®, Ethicon Ltd, Edinburgh, UK).

Figure 1

Figure 1: Intraoperative picture, showing the creation of the colonic anastomosis and its size, comparably to a classic 21G needle.



Rats were re-operated in groups of 10 animals on postoperative days 3, 7, and 14. The peritoneal cavity was carefully inspected for adhesions. Any abscess or anastomotic leakage was recorded. The anastomosis site was gently dissected en block, as the mid-portion of a 5cm colonic segment. Blood samples were taken from the inferior vena cava for laboratory tests and then rats were sacrificed with a lethal dose of pentobarbital.

BURSTING PRESSURE

Anastomotic segments were preliminary washed with saline. The proximal end was ligated and a plastic catheter was secured to the distal end, connecting the portion to an infusion pump, while a manometer was interfaced by a side line. The bursting pressure was defined as the maximal intraluminal pressure the segment resisted, expressed in mm of Hg column. The breaking point was noted and the anastomotic tissue was sent for further laboratory assessment.

HISTOPATHOLOGY

After measuring the bursting pressure, the anastomotic site was divided in two parts, which were prepared following the standard procedures, one for light microscopy and the other for electron microscopy. For histopathological studies, serial longitudinal sections were evaluated at x20 to x200 magnifications. For electron microscopic assessment, semi-

thin and thin sections were observed in a Jeol TEM 2000 FX II at 80 KV. Findings from both electron and light microscopy are presented together.

BIOCHEMICAL ANALYSIS AND CYTOKINE DETERMINATION

Blood samples were centrifuged at 3,000 rpm for ten minutes and serum aliquots were collected. Routine biochemical tests (electrolyte and protein serum concentrations, renal and hepatic function tests) were performed in the Olympus (AU 640) biochemical analyzer. Determinations of interleukins 2 and 6 (IL-2 & IL-6), and tumor necrosis factor (TNF-a) were performed in an automatic Eliza analyzer (Mango Plus), using the Quantikine/Elisa kits (R&D Systems, Minneapolis, MN).

STATISTICAL ANALYSIS

All variables followed a skewed distribution and consequently all the results were expressed as median-interquartile range. Statistical analysis was performed using the nonparametric Mann-Whitney U test. P values of <0.05 were considered significant.

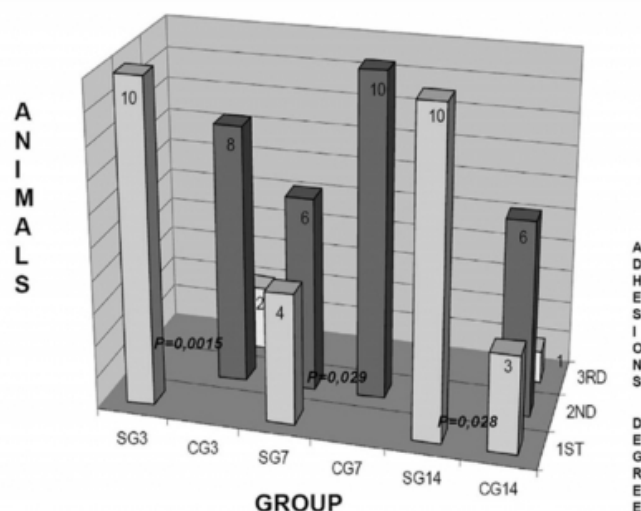
RESULTS

SURVIVAL, SEPTIC COMPLICATIONS AND ADHESION FORMATION

No deaths were reported during the experiment. No cases of anastomotic leakage or septic complications were observed. During sacrifice, inspection of the abdominal cavity did not reveal anomalies which could lead to late complications or death. Adhesion formation was graded according to the Van Der Hams four-degree scale., More prominent intra-abdominal adhesion formation was observed in the control than experimental animals and statistical significance was eventually confirmed between the two groups (Diagram 1).

Figure 2

Diagram 1: Intra-abdominal adhesions found after sacrifice. Number of animals with certain adhesion degree for every subdivision is shown. values between equivalent groups is also indicated. (SG: Experimental Group, CG: Control Group, 3-7-14: Sacrifice Day).

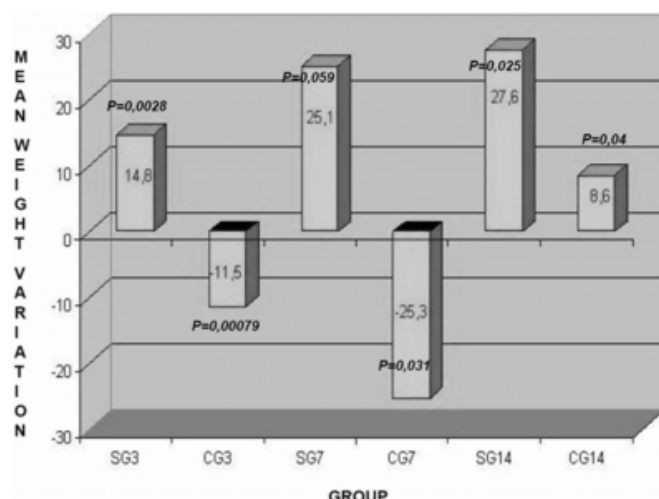


BODY WEIGHT CHANGES

Body weight changes during the experiment, for both study and control groups, are shown in diagram 2. There was a significant decrease of body weight in the control group animals up to the seventh postoperative day. Afterward, the animals regain their body weight. On the contrary, animals of the experimental group treated with Capecitabine showed a significant and progressive increase of body weight throughout the study.

Figure 3

Diagram 2: Median body weight variations per groups. Differential values expressed in grams and statistical significances for every group are indicated. (SG: Experimental Group, CG: Control Group, 3-7-14: Day of sacrifice).

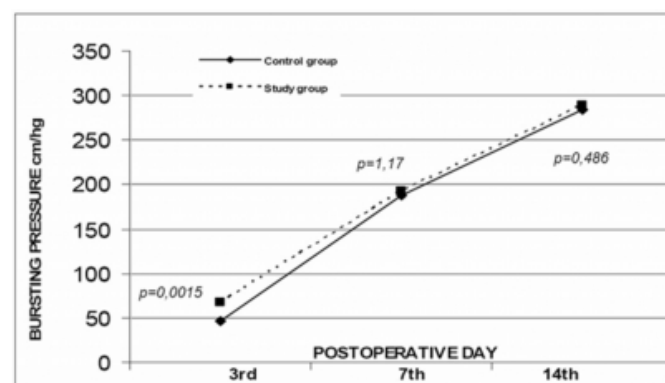


BURSTING PRESSURE

The mechanical strength of the anastomosis was assessed by measurements of the bursting pressure. The median bursting pressure was found to be statistically significant higher ($P = 0.0015$) in animals of the experimental group killed on the 3rd postoperative day (68 mmHg), in comparison to respective controls (46 mmHg). All ruptures occurred at the anastomosis site. The median bursting pressure was not found to be significantly different in animals killed on later postoperative days (Diagram 3).

Figure 4

Diagram 3: Variation of median bursting pressure for both experimental and control groups. values are indicated for equivalent subdivisions.



LABORATORY FINDINGS

Routine biochemical measurements did not reveal any

significant differences between control, experimental, and specifier groups. Nevertheless, differences were noticed in some cytokine serum levels. Control group animals of the second sacrifice had significantly higher serum concentrations of IL-6, than specifier group animals ($P = 0.03$). Furthermore, serum concentrations of TNF- α in controls ($P = 0.038$) and IL-6 in experimental group animals ($P = 0.038$), were found to be significantly higher during the third sacrifice, comparatively to specifier group values.

HISTOLOGIC FINDINGS

Microscopic examination of the anastomoses revealed the preservation of the multilayered structure of the anastomotic site in both experimental and control groups killed on 3rd postoperative day. Nevertheless, the animals which received capecitabine had minor necrotic signs on the anastomosis, in comparison to those given placebo. An inflammatory reaction, with necrotic areas was found in the control group (Figure 2), but no significant differences were found in leukocyte population, fibroblastic activity, and collagen production. On the other hand, the experimental group had more constant intercellular junctions and better epithelial reconstruction (Figure 3). Microscopic examination did not reveal any substantial differences between the experimental and control groups killed on later postoperative days.

Figure 5

Figure 2: Lamina propria with collagen fibres (CF), capillary vessels (CV), destroyed cells with condense nucleus (CN). Epithelial cell nuclear (N). Electron microscopy, magnification $\times 6500$.

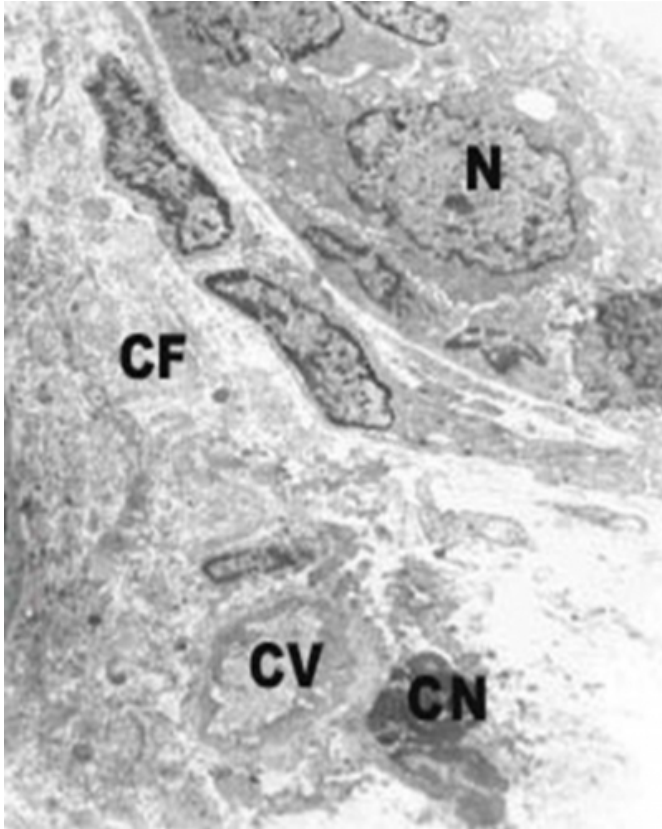
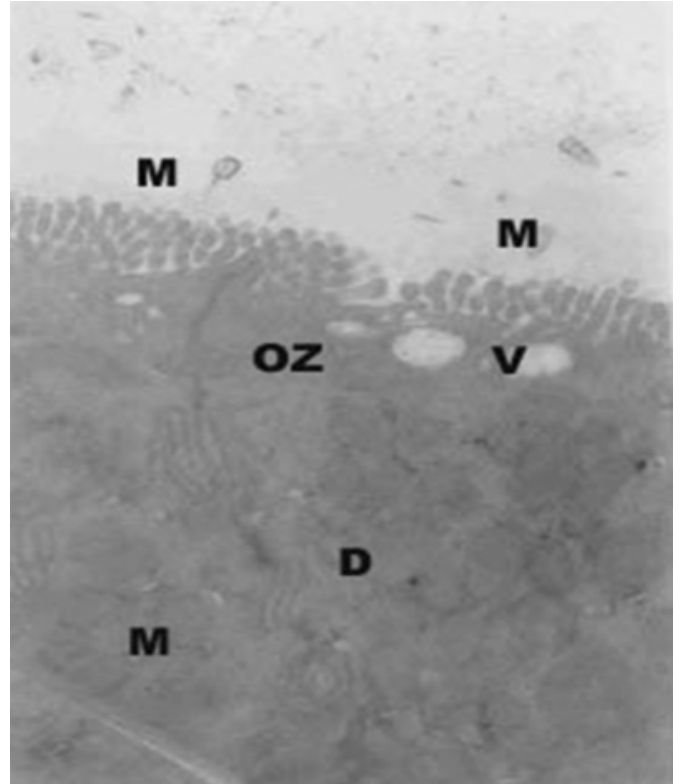


Figure 6

Figure 3: Epithelium with short microvilli (M). Cells are bonded together with firm intercellular junctions. Occludus zones (O?), desmosomes (D), mitochondria (?), vesicles (V). Electron microscopy, magnification $\times 15000$.



DISCUSSION

Colorectal cancer is one of the most common malignancies today.¹⁰ Neoadjuvant chemotherapy has been proposed in order to improve the five-year survival and local recurrence rate. Currently, after the surgical removal of the tumor, the initiation of adjuvant therapy with anticancer agents is usually delayed for several weeks. The main reason for this delay is the fear of anastomotic rupture potentially caused by certain chemotherapeutic agents.¹¹ The early phase of colonic healing sequence is characterized by a transient loss of strength in the anastomotic segment. Further reduction of wound healing during this period, such as induced by perioperative chemotherapy, may compromise anastomotic integrity and increase the risk of dehiscence.¹²

The strong inhibitory effect of most antineoplastic agents on anastomosis healing, including 5-FU, has been well documented.¹³ Capecitabine is an oral, tumor-activated, prodrug of 5-FU.^{14,15} Capecitabine has similar activity against metastatic colorectal cancer than conventionally administered intravenous 5-FU,¹⁶ with significantly less

toxicity, improved quality of life, and lesser cost.^{17,18,19}

Capecitabine is selectively metabolized to 5-FU by tumor cells. It is initially converted to 5'-DFCR by the carboxylesterase reaction in the liver, then to 5'-DFUR by the cytidine deaminase, an enzyme highly expressed in the liver and tumor cells, and finally to 5-FU by the thymidine phosphorylase, reaction that is preferentially located in various types of cancer cells.²⁰ As a result of the unique localization of these enzymes, capecitabine is expected to deliver the active 5-FU selectively to tumor tissues and to have a better safety profiles and fewer side effects.²¹ Our data supports the non toxic effect of capecitabine.

Capecitabine not only does not impair mechanical strength of large bowel anastomoses, but it increases the rate and effectiveness of healing process during the first postoperative days (Diagram 3), but not during the mid and late postoperative days. Even though wound healing is a complex and dynamic process, during the first three days the main pathophysiologic reaction is an acute inflammatory phenomenon, described as the inflammatory stage.^{22,23} During this stage, which is an essential step of intestinal healing, macrophage and polymorphonuclears migrate from the circulation to the wound. Local ischemia and necrosis are present, simultaneous with cell regeneration.^{24,25} This phase is followed by fibroblastic proliferation, synthesis of collagen, remodeling of connective tissue and its parenchymal component, and the acquisition of wound strength.²⁶ The data obtained in this experiment shows that experimental animals sacrificed on the 3rd postoperative day, when compared to respective controls, had better recovery of the bowel wall layers, less necrotic effects and better regeneration of the epithelium. Intestinal glands and absorptive cells presented more reconstructed at these animals, as well as basic membrane cells were found to have more profound polyribosomes, evidence which indicate aggravated metabolism.

Higher bursting pressure during the early postoperative days has been reported in other studies, in which anti-inflammatory factors, vitamins, antibiotics and low doses of immunosuppressant drugs were used.^{27,28} The proposed mechanism of action for these factors, in order to explain the positive effect on wound healing, is the partial inhibition of the inflammatory phase, so that a positive balance is accomplished between regeneration and necrotic effects on the anastomosis line.^{29,30} Our experiments, in line with that interpretation, also revealed diminished cell destruction in

capecitabine treated animals.

The hypothesis of partial inhibition of the undesirable effects of the inflammatory phase on wound healing may provide an explanation for the statistically significant lower grade of adhesion formation observed in the experimental group. Increased collagenolysis and cell necrosis, arising from high cytokine concentrations, leads to anastomotic rupture.^{31,32} Our experiments did not reveal any significant increase in IL-2, IL-6, and TNF- α levels in capecitabine-treated animals during the early and more vulnerable days of intestinal healing.

In a mouse model of cachexia caused by implantation of murine colon 26 adenocarcinoma cells (colon 26 adenocarcinoma model),³³ 5'-DFUR and its precursor, capecitabine, could reverse progressive weight loss and improve hypoglycemia, hyperglycorticisism, and hepatic malfunctions.³⁴ Similarly, despite surgical injury and stress, all experimental animals gained weight during the early and late postoperative period. Based on the results of routine biochemical tests, there was no evidence that this weight gain was a result of low protein edema, neither renal nor liver malfunction. Capecitabine, by improving the healing process, offered experimental animals a quicker recovery, coinciding with better nutrition and weight gain.

In conclusion, early induction of Capecitabine administration, simultaneously or even before colonic surgical excision, does not impair the recovery of animals which underwent colonic resection and anastomosis. Further clinical investigations are needed to confirm the outcome observed in rats, and possibly to change standard practice in colorectal patients.

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