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# The effects of peroxisome proliferator-activated receptor gamma agonists and fluorouracil on Colon 38 cancer growth *in vitro*

Wpływ agonistów receptorów aktywowanych proliferatorami peroksysomów typu gamma i fluorouracylu na wzrost komórek raka linii Colon 38 *in vitro*

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## Abstract

**Introduction:** Colon cancer is a very serious medical problem in Europe and also in Poland. For many years fluorouracil has been used as a main agent in chemotherapy, but its effectiveness is not sufficient. Recently several modern drugs were introduced in the treatment of colon cancer and also various of therapeutic options have been tested but the survival time of patients with cancer has not been extended significantly. For that reason more efficient drugs have still been looked for. One of the potential anticancer drugs are thiazolidinediones (TZDs) – agonists of peroxisome proliferator-activated receptors gamma (PPARγ) which in the last years were used in diabetes treatment. Therefore, we decided to examine the effect of two TZDs – pioglitazone (PIO) and rosiglitazone (ROS) on the growth of murine colon cancer and to compare their action with the efficacy of routinely used fluorouracil (FU). Furthermore, we evaluated the PPARγ expression in colon cancer cells by using the immunocytochemical method.

**Material and methods:** Cell line of murine cancer – Colon 38 was used in our experiment. The growth of cancer cells was assessed by using EZ4Y kit based on the modified colorimetric Mosmann method. In 24 and 48 h cell culture the effects of ROS and PIO at concentrations $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ M and FU at concentrations 4 x $10^{-6}$ and $10^{-6}$ M were examined. Immunochistochemistry was performed with the use of murine specific polyclonal antibodies anti-PPARγ1,2 and the streptavidin-biotin-peroxidase method.

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**Results:** The immunopositive reaction for PPARγ was shown in the nuclei of Colon 38 cells. Both, the examined TZDs and fluorouracil significantly decreased the growth of colon cancer and their efficacy was dependent on the concentration of examined compounds and also the incubation time. Rosiglitazone, in all used concentrations, acted more strongly than PIO. Fluorouracil showed anti-cancer activity only in 48 h culture and its inhibitory effect was weaker than both TZDs at the highest concentration ($10^{-4}$ M).
**Conclusions:** Our data indicate that PPARγ agonists, especially rosiglitazone, inhibit the growth of Colon 38 cancer. However, the potential usefulness of rosiglitazone and pioglitazone for the chemoprevention and treatment of colon cancer requires further studies.

**Key words:** PPARγ agonists, pioglitazone, rosiglitazone, fluorouracil, colon cancer.

## Streszczenie

**Wstęp:** Rak jelita grubego stanowi poważny problem medyczny zarówno w Polsce, jak i w całej Europie. Przez wiele lat jako główny chemioterapeutyk stosowano fluorouracyl, niestety jego skuteczność okazała się niewystarczająca. Zastosowanie w terapii raka jelita grubego nowych leków i różnych opcji terapeutycznych nie wydłużyło istotnie czasu przeżycia pacjentów. Z tego powodu nadal poszukiwane są bardziej skuteczne formy leczenia. Związkami o potencjalnym działaniu przeciwnowotworowym są tiazolidinediony (TZDs) – agoniści receptorów gamma aktywowanych proliferatorami peroksysomów (PPARγ), które w ostatnich latach wykorzystywano w leczeniu cukrzycy. W związku z tym, w pracy oceniono wpływ dwóch TZDs – pioglitazonu (PIO) i roziglitazonu (ROS) na wzrost mysiego raka jelita grubego, a ich działanie porównano ze skutecznością fluorouracylu (FU). Ponadto w komórkach raka jelita grubego metodą immunohistochemiczną oceniono ekspresję PPARγ.
**Materiał i metody:** Badanie przeprowadzono na linii komórkowej mysiego raka jelita grubego – Colon 38. Wzrost komórek nowotworowych mierzono za pomocą zestawu EZ4Y opartego na metodzie kolorymetrycznej Mosmann'a. W hodowli 24- i 48-godzinnej oceniono wpływ ROS i PIO w stężeniach $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ M, a działanie fluorouracylu w stężeniach 4 x $10^{-6}$ i $10^{-6}$ M. W badaniu immunohistochemicznym wykorzystano mysie poliklonalne przeciwciała anty-PPARγ1,2 i streptawidynowo-biotynowo-peroksydazową metodę wizualizacji.
**Wyniki:** W komórkach raka Colon 38 wykazano immunopozytywny jądrowy odczyn dla PPARγ. Badane TZDs i fluorouracyl znacząco zahamowały wzrost raka jelita grubego, a ich skuteczność zależna była od stężenia i czasu inkubacji.

Działanie ROS było silniejsze niż PIO we wszystkich użytych stężeniach. Przeciwnowotworowe działanie fluorouracylu stwierdzono po 48 godzinach inkubacji, jednakże jego efekt był słabszy niż wpływ TZDs w najwyższym badanym stężeniu ($10^{-4}$ M).
**Wnioski:** Uzyskane wyniki wskazują, że agoniści receptorów PPARγ, a w szczególności roziglitazon, hamują wzrost raka Colon 38. Jednakże wykorzystanie roziglitazonu i pioglitazonu w leczeniu raka jelita grubego wymaga dalszych badań.

**Słowa kluczowe**: agoniści PPARγ, pioglitazon, roziglitazon, fluorouracyl, rak jelita grubego.

## Introduction

Colon cancer is a very serious medical problem in Europe and also in Poland. Although some progress has been made in the prevention and treatment of colon cancer, this disease still remains one of the common malignancies in the world [1]. According to Cancer Statistics 2009, it is the third cause of cancer death for both genders [2]. Moreover, the human population is ageing and the morbidity of colon cancer increases with age, so the health problem related to colon cancer is still growing. The possibility of the cure depends mainly on the stage of the disease at the diagnosis moment. However, most patients with colon cancer are diagnosed in the advanced stage of the disease, when the total surgical removal of the tumour is ineffective or impossible. Therefore, additional therapy is required after the operation. Until 1995 only one chemotherapeutic drug – fluorouracil (FU) was approved for the adjuvant treatment of colon cancer. For the last 15 years the real improvement of the pharmacological therapy has been made because some new agents such as: irinotecan, capecitabine - the oral form of FU, oxaliplatin, the biological compounds like bevacizumab - a monoclonal antibody against vascular endothelial growth factor and two anti-epidermal growth factor receptor inhibitors - cetuximab and panitumumab were used in chemotherapy [3-7]. Despite the introduction of several modern drugs and testing various therapeutic

options, statistical data indicate that the efficacy of colon cancer treatment is not satisfactory and the survival time of patients has not been extended significantly. Therefore, more efficient drugs are still looked for. One of the investigated compounds are thiazolidinediones (TZDs) named also glitazones which decrease insulin resistance and in the last years have been used in the treatment of diabetes mellitus. The most known of them are troglitazone, pioglitazone (PIO) and rosiglitazone (ROS). The majority of biological effects of TZDs like lipid and glucose metabolism control and influence on adipocytes differentiation depend on the binding with peroxisome proliferator-activated receptors gamma (PPARγ) [8-10]. Peroxisome proliferator-activated receptors gamma are expressed in adipocytes, hepatocytes, immune cells and also in gastrointestinal epithelial cells. Moreover, the high level of PPARγ has been detected in human malignancies including liposarcoma, breast cancer, prostate cancer and also colon adenocarcinoma [11]. The last research has shown that TZDs can inhibit the growth of various cancers *in vitro* and *in vivo* conditions [12-14]. The oncostatic action of TZDs is complex and includes some mechanisms like antiproliferative and proapoptotic effects, redifferentiation activity and inhibition of angiogenesis [11, 15, 16]. Some data indicated that PPARγ receptors play an essential role in physiological and pathological processes of intestinal epithelium and agonists of PPARγ such as TZDs inhibit colon carcinogenesis and the growth of the tumour [17]. Most experiments with TZDs concerned troglitazone, which indeed has colon anticancer activity but due to its hepatoxicity it was retired from medical use [18, 19, 20]. However, the studies assessing the influence of other TZDs on the growth of colon cancer are not numerous [21-24].

Therefore, we decided to examine the effect of PIO and ROS on the growth of murine colon adenocarcinoma and to compare their action with the efficacy of routinely used FU. Furthermore, in the present study, we have evaluated the expression of PPARγ in colon cancer cells by using the immunocytochemical method.

## Material and methods

### Compounds

The following substances were examined in this study: rosiglitazone (Alexis Biochemicals, USA), pioglitazone (Alexis Biochemicals, USA) and 5-fluorouracil (Roche, Switzerland). Both thiazolidinediones were dissolved in DMSO - dimethylsulfoxide (DMSO, Sigma, USA).

### Cell culture

In the experiment we used the murine Colon 38 cancer cells kindly obtained from Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wroclaw. The Colon 38 is transplantable adenocarcinoma originally induced in the colon of C57BL/6 strain mouse by 1,2-dimethylyhydrazine [25]. The adaptation of Colon 38 cells to *in vitro* growth was made by Pajtasz-Piasecka and co-workers [26].

The continuous culture of the cells was maintained in a culture flask (Nunc Easy flask 25 $cm^2$, NUNC) in the presence of RPMI 1640 medium (Sigma), supplemented with 25 mM Hepes buffer, 4 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin solution (Sigma), 2 g/L sodium bicarbonate and 5% foetal calf serum (FBS, Biochrom). The cells were cultured in a humidified incubator at 37ºC and 5% carbon dioxide. Trypsynisation process was made every 4 days. The medium was removed from the culture flask and the cells were treated with preheated (37ºC) trypsin for 5 minutes (trypsin-EDTA, Sigma). Thereafter the cells were collected, rinsed three times in the culture medium, centrifuged and seeded in a culture flask ($2 \times 10^5$ cells/5 mL medium) for subsequent days.

### Immunocytochemistry

The immunocytochemical evaluation of PPARγ expression in monolayer of Colon 38 cells was performed. The cells were cultured on eight-well chamber plastic slides (in a number of approximately 3 x $10^4$ cells per well). Then the cancer cells were fixed in 4% p-formaldehyde for 30 min and incubated with 10% sheep serum. Afterwards, incubation with specific antibody (diluted 1:2000 in PBS; pH 7.4) was carried out overnight at 4°C. After that murine specific polyclonal antibodies anti–PPARγ1,2 (Calbiochem, Germany) were added. The cells were subsequently washed and incubated for 30 min at room temperature with the biotin-coupled secondary antibodies. Then the immunostaining was visualized by using the streptavidin-biotin-peroxidase method (Strept ABC Complex/ HRP kit, DakoCytomation, Denmark) and 3,3'-diaminobenzidine as the chromogenic substrate for peroxidase. The immunostained cells were assessed in light microscope. The wells with cells into which the first antibody was omitted were admitted as control.

### *In vitro* study

After one of the subsequent trypsinization the Colon 38 cells were suspended in the complete medium at a concentration 4 x $10^5$ cells/mL. Next into each well of the culture plate (Cell Culture Cluster Dish, Costar; Nunclon MicroWell Plater, NUNC), we seeded 50 μL of the suspension containing 2 x $10^4$ cells for 24 h culture and 1.5 x $10^4$ cells for 48 h culture. After 24 h of preincubation period the solutions of the investigated substances were added to the culture:

- rosiglitazone at the final concentration $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ M
- pioglitazone at the final concentration $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ M
- fluorouracil at the final concentrations 4 x $10^{-6}$ and $10^{-6}$ M.

The control groups for both thiazolidinediones were incubated in the presence of DMSO and for FU in complete medium only. The cancer cells viability was assessed after 24 and 48 h of incubation using the EZ4Y kit (Easy for You, the 4th Generation Non Radioactive Cell Proliferation & Cytotoxity

Assay, Biomedica Gruppe, Bellco Biomedica Poland). This kit involved the modified colorimetric Mosmann method which is based on the transformation of tetrazolium salt into colored formazan *via* mitochondrial enzymes. The extinction, called also optical density (OD), of each sample was measured at 450 nm wave length by using ELISA microplate reader. The optical density in the control groups was assumed to be 100% and OD of the examined drugs was presented as a percentage of the control group.

### Statistical analysis

The data were statistically analysed by Statistica 9 (StatSoft, Tulsa USA), using a one-way analysis of variance (ANOVA). Statistical differences between tested values were determined using the Least Significant Difference (LSD) test. The data were presented as the means±SEM. Differences were considered significant if $p < 0.05$.

## Results

The positive reaction for PPARγ was shown in the nuclei of cells line Colon 38 (Fig. 1).

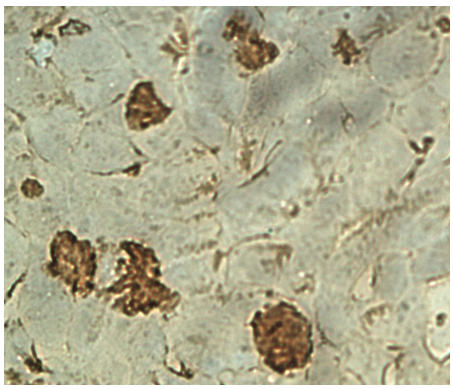

Fig. 1. PPARγ expression in Colon 38 cells. Magnification 600x.

Both examined TZDs and fluorouracil significantly decreased the viability of cancer cells and their efficacy was dependent on the concentration of the examined compounds and also the incubation time. Glitazones inhibited cancer growth in both checked time points, but their effectiveness was lower in 48 h cell culture than in 24 h cell culture (Fig. 2, 3). We noticed that ROS acts more strongly than PIO. Rosiglitazone decreased the viability of cancer cells at each examined concentration while PIO acted only at the two highest doses - $10^{-4}$ and $10^{-5}$ M.

The chemotherapeutic drug - FU showed antiproliferative activity only in 48 h culture – at concentration 4 x $10^{-6}$ M decreased OD value at about 40% and at almost 20% at lower concentration - $10^{-6}$ M (Fig. 2). Comparing the effects of FU and both examined TZDs, we noted that PIO and ROS at the highest investigated concentration ($10^{-4}$ M) inhibited the growth of Colon 38 cells in a significantly stronger way than chemotherapeutic drug. However, ROS applied at the concentration $10^{-6}$ M corresponding with the dose usually used in clinical study, was slightly weaker than FU, while PIO at the same concentration was ineffective.

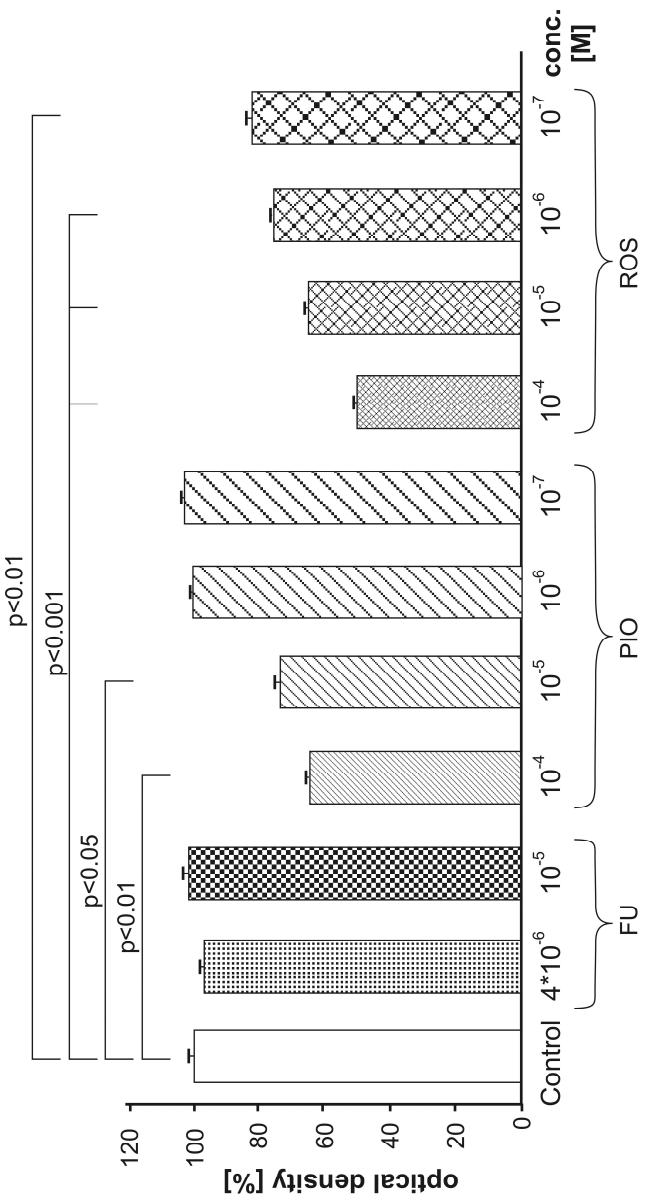


Fig. 2. The effects of pioglitazone (PIO), rosiglitazone (ROS) and fluorouracil (FU) on the growth of Colon 38 cancer cells in 24 h culture.

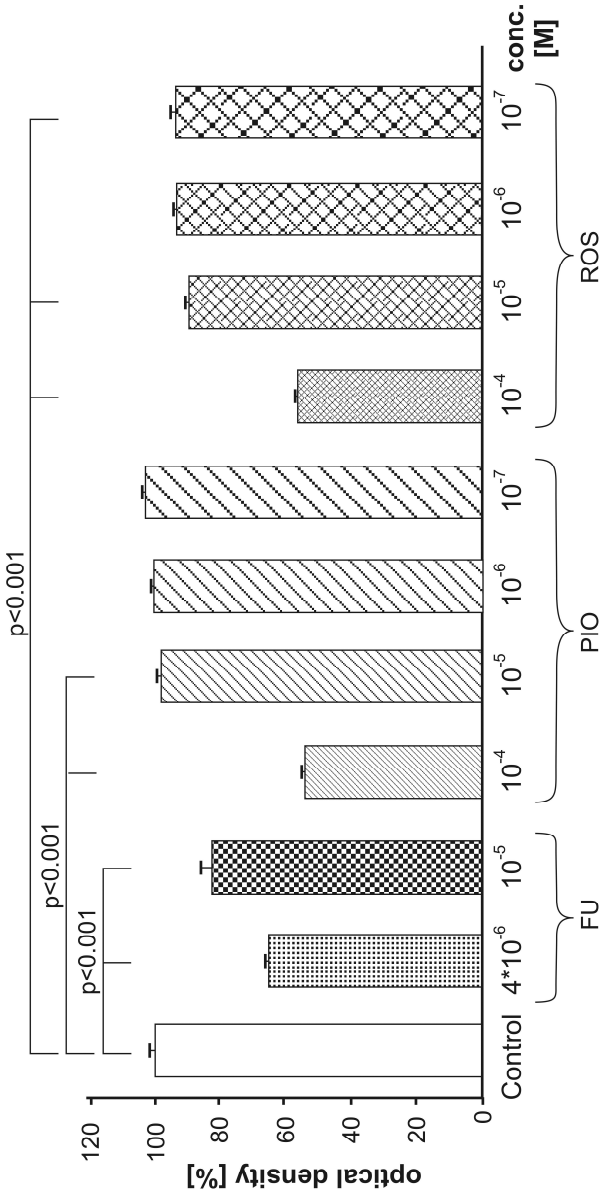


Fig. 3. The effects of pioglitazone (PIO), rosiglitazone (ROS) and fluorouracil (FU) on the growth of Colon 38 cancer cells in 48 h culture.

## Discussion

The obtained data demonstrate that PIO and ROS, which last time were applied in diabetes mellitus treatment, may inhibit the growth of colon adenocarcinoma cells. Both examined glitazones belong to PPARγ ligands. Several studies have shown that PPARγ are expressed at the high levels in normal colon mucosa and colorectal tissue as well as in colon cancer cells [17, 27]. In most experiments, conducted *in vitro* conditions, the human colon cancer line HT-29 has been used, whose cells possess a large number of PPARγ receptors [23, 28-30]. Although Colon 38 cancer cells are derived from murine colon adenocarcinoma [25], our earlier studies have shown that these cancer cell line is a good model for the assessment of oncostatic effect of various compounds [31-34]. Moreover, by using the immunohistochemistry method, we have found the expression of PPARγ in Colon 38 cancer cells (Fig. 1). The previous studies suggested that ROS suppresses proliferation of cancer cells in a dose- and a time-dependent manner [23, 35]. It was shown that ROS inhibits the growth of cancer at the concentrations higher than $10^{-5}$ M but the lower concentrations of ROS are ineffective. The study conducted on human adreno-cortical cell line H295 indicated that ROS is ineffective at the concentrations below $5 \times 10^{-6}$ M. The similar results were obtained by Zhang et al. [23], who used colon cancer line HT-29 and didn't notice antiproliferative and proapoptotic effects of ROS at concentrations below $3 \times 10^{-5}$ M. Our experiment demonstrated that ROS decreases the viability of cancer cells also at the low concentrations ($10^{-6}$ and $10^{-7}$ M) but its action is significantly weaker. Because the efficacy of this glitazone has been stronger in 24 h than in 48 h culture, we suppose that *in vitro* condition the oncostatic action of ROS decreases with the extension of the incubation time and the reduction of the used dose. Our results are compatible with the data obtained by other group which has shown that ROS could inhibit the cell growth of human colorectal cancer through inducing apoptosis and suppressing the cell cycle in a dose- and a time-dependent way [28]. Besides, it was proved that ROS at low

concentrations enhances significantly the anticancer activity of fluorouracil by increasing the apoptotic rate in HT-29 cell line [23]. On the other hand, Chinthallapali and co-workers reported that ROS at concentrations $10^{-6}$ M, 5 x $10^{-6}$ M and $10^{-5}$ M decreases the viability of HT-29 cancer after six days of incubation if glitazone had been added to the culture every 48 h [21].

Pioglitazone in our both cultures acted only at high concentrations ($10^{-4}$ and $10^{-5}$ M) but its activity was weaker than ROS. Similar results were obtained by Feruzzi et al., who assessed the influence of both glitazones on adrenocortical cancer cells [35]. They also observed that PIO at concentrations below $10^{-5}$ M is ineffective. The data related with the effect of PIO on colon cancer are not numerous. The study with SW480 and LS174T colon cancer cell lines proved that PIO inhibits the proliferation of cancer cells in a dose-dependent manner [24]. The next examination showed that PIO inhibits not only colon cancer proliferation but also restrains liver metastasis [29]. Other group *in vitro* study demonstrated that pioglitazone inhibits proliferation and enhances apoptosis in colon cancer cell lines SNU-C4 and SNU-C2A [22]. Moreover, in mice PIO significantly reduces number of aberrant crypt foci (APC) which are precancerous lesions of the colon and in this way suppresses colon carcinogenesis [19]. The opposed results were published by Choi et al. [36]. In their study PIO was applied to APC gene-mutated HT-29 cell line. The high concentrations of PIO ($10^{-5}$ and $10^{-4}$ M) suppressed the growth of colon tumours, while low concentrations ($10^{-8}$ - $10^{-6}$ M) promoted the growth of the cancer.

The oncostatic action of fluorouracil in the treatment of colon cancer has been confirmed by several investigations, but in the present study this drug has not caused significant changes in Colon 38 cells viability in 24 h culture. The oncostatic activity of fluorouracil was noted only after 48 h of incubation – OD value was decreased at about 40% at the concentration 4 x $10^{-6}$ M and at almost 20% at the concentration $10^{-6}$ M. Our observations are in accordance with the results obtained by others, who documented the increase of anticancer effect of FU on Colon 38 cells with the extension of the incubation time [33, 37].

Recently, the growing evidence from clinical study have shown that glitazones, especially ROS, increase the rate of cardiovascular events including edema and heart failure in patients with type 2 diabetes [38-40]. For this reason ROS was withdrawn from European market and the close monitoring therapy of ROS was recommended by Food and Drug Administration in the United States [41, 42]. On the other hand, it is well documented that most anticancer drugs induce the undesirable side effects, for example, myelotoxicity and hepatotoxicity produced by fluorouracil. However, the side effects of chemotherapeutic agents usually do not exclude these drugs from cancer therapy.

Summing up, our data and results of other investigators indicate that thiazolidinediones, especially rosiglitazone, suppress the growth of colon cancer. However, the potential usefulness and therapy safety of PPAR ligands for the chemoprevention and treatment of colon cancer require further studies.

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## References

1. Kulbacka J, Saczko J, Chwiłkowska A. Rak jelita grubego – charakterystyka i oporność na leczenie. Onkol Prak Klin. 2008; 4: 135–140.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009; 59: 225-249.
3. Schrag D. The price tag on progress – chemotherapy for colorectal cancer. N Engl J Med. 2004; 351: 317-319.
4. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004; 350: 2335-2342.
5. Iqbal S, Lenz H-J. Integration of novel agents in the treatment of colorectal cancer. Cancer Chemother Pharmacol. 2004; 54: S32-39.

6. Saltz L. Current role of bevacizumab in colorectal cancer. Clin Adv Hematol Oncol. 2009; 7: 375-376
7. Marshall JL, Gramont A, Köhne CH. Accomplishments in 2008 in the adjuvant treatment of colon cancer. Gastrointest Cancer Res. 2009; 3: 2–7.
8. Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta. 1996; 1302: 93-109.
9. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolizm. Endocr Rev. 1999; 20: 649-688.
10. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature. 2000; 405: 421-425.
11. Koeffer HP. Peroxisome proliferator-activated receptor γ and cancers. Clin Cancer Res. 2003; 9: 1-9.
12. Winczyk K. Receptory aktywowane proliferatorami peroksysomów gamma - ich znaczenie w karcinogenezie i terapii nowotworów. Fol Med Lodz. 2007; 34: 117-140.
13. Winczyk K. The role of peroxisome proliferators-activated receptors (PPARgamma) in neoplasms of endocrine glands. Pol J Endocrinol. 2008; 59:156-166.
14. Blanquicett C, Roman J, Hart M. Thiazolidinediones as anti-cancer agents. Cancer Ther. 2008; 6: 25–34.
15. Wang YL, Miao Q. To live or to die: prosurvival activity of PPAR γ on cancers. PPAR Res. doi:10.1155/2008/209629.
16. Hatton JL, Yee LD. Clinical Use of PPARgamma Ligands in Cancer. PPAR Res. 2008:159415. Dec 18.
17. Thompson EA. PPAR γ physiology and pathology in gastrointestinal ephithelial cells. Mol Cells. 2007; 24: 167-176.
18. ShimadaT, Kojima K, Yoshiura K, Hiraishi H, Terano A. Characteristics of the peroxisome proliferators-activated receptor gamma ligand induced apoptosis in colon cancer cells. Gut. 2002; 50: 658-664.
19. Osawa E, Nakajima A, Wada K, Ishimine S, Fujisawa N, Kawamori T et al. Peroxisome proliferators-activated receptor gamma ligands suppress colon carcinogenesis induced by azoxymethane in mice. Gastroenterology. 2003; 124: 361-367.

20. Yasui Y, Hosokawa M, Kohno H, Tanaka T, Miyashita K. Troglitazone and 9cis, 11trans, 13trans-conjugated linolenic acid: comparison of their antiproliferative and apoptosis-inducing effects on different colon cancer cell lines. Chemotherapy. 2006; 52: 220-225.
21. Chintharlapalli S, Smith R, Samudio I, Zhang W, Safe S. 1, 1-bis(3'-indolyl)-1-(psubstitutedphenyl) methanes induce peroxisome proliferators – activated receptor γ–mediated growth inhibition, transactivation, and differentiation markers in colon cancer cells. Canc Res. 2004; 64: 5994-6001.
22. Lee CJ, Han JS, Seo CY, Park TH, Kwon HC, Jeong JS et al. Pioglitazone, a synthetic ligand for PPARgamma, induces apoptosis in RB-deficient human colorectal cancer cells. Apoptosis. 2006; 11: 401-411.
23. Zhang YQ, Tang XQ, Sun L, Dong L, Qin Y, Liu HQ et al. Rosiglitazone enhances fluorouracil - induced apoptosis of HT-29 cells by activating peroxisome proliferator - activated receptor gamma. World J Gastroenterol. 2007; 13: 1534-1540.
24. Shen D, Deng C, Zhang M. Peroxisome proliferator-activated receptor gamma agonists inhibit the proliferation and invasion of human colon cancer cells. Postgrad Med J. 2007; 83: 414-419.
25. Corbett TH, Griswold DP, Roberts BJ, Peckham JC, Schabel FM. Tumor induction relationships in development of transplantable cancers of the colon in mice for chemotherapy assays, with a note on carcinogen structure. Cancer Res. 1975; 35: 2434-2439.
26. Pajtasz-Piasecka E, Glazman-Kuśnierczyk, H, Salwa J, Konarski L, Radzikowski C. Growth inhibition of transplantable tumors in mice by mIL-2-secreting murine plasmocytoma cells used alone or in combination with a cytostatic agent. Arch Immunol Ther Exp. 1995; 43: 281-292.
27. Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB et al. Differentiation and reversal of malignant changes in colon cancer through PPARgamma. Nat Med. 1998; 4: 1046–1052.
28. Lin MS, Chen WC, Bai X, Wang YD. Activation of peroxisome proliferator-activated receptor gamma inhibits cell growth via apoptosis and arrest of the cell cycle in human colorectal cancer. J Dig Dis. 2007; 8: 82-88.
29. Takano S, Kubota T, Nishibori H, Hasegawa H, Ishii Y, Nitori N et al. Pioglitazone, a ligand for peroxisome proliferator-activated receptor-gamma acts

as an inhibitor of colon cancer liver metastasis. Anticancer Res. 2008; 28: 3593-3599.

30. Fu JN, Li J, Tan Q, Yin HW, Xiong K, Wang TY et al. Thioredxin reductase inhibitor ethaselen increases the drug sensitivity of the colon cancer cell line LoVo towards cisplatin via regulation of G1 phase and reversal of G2/M phase arrest. Invest New Drugs. 2011; 29: 627-636.
31. Mełeń-Mucha G, Winczyk K, Pawlikowski M. Effects of somatostatin analogs octreotide and lanreotide on the proliferation and apoptosis in colon 38 tumor: interaction with 5-fluorouracil. Neuroendocrinol Lett. 2000; 21: 137-142.
32. Winczyk K, Pawlikowski M, Karasek M. Melatonin and RZR/ROR receptor ligand CGP 52608 induce apoptosis in the murine colon cancer. J Pineal Res. 2001; 31: 179-182.
33. Mełeń-Mucha G, Ławnicka H, Mucha S. Ten combine effects of progluminde and fluorouracil on the growth of murine Colon 38 cancer cells in vitro. Pol J Endocrinol. 2005; 6: 933-938.
34. Winczyk K, Fuss-Chmielewska J, Lawnicka H, Pawlikowski M, Karasek M. Luzindole but not 4-phenyl-2- propionamidotetralin (4P-PDOT) diminishes the inhibitory effect of melatonin on murine Colon 38 cancer growth in vitro. Neuro Endocrinol Lett. 2009; 30: 657-662.
35. Ferruzzi P, Ceni E, Tarocchi M, Grappone C, Milani S, Galli A et al. Thiazolidinediones inhibit growth and invasiveness of the human adrenocortical cancer cell line H295R. J Clin Endocrinol. 2005; 90: 1332-1339.
36. Choi IK, Kim YH, Kim JS, Seo JH. PPAR-gamma ligand promotes the growth of APC-mutated HT-29 human colon cancer cells in vitro and in vivo. Invest New Drugs. 2008; 26: 283-288.
37. Mełeń-Mucha G, Ławnicka H. Lepton promotes the growth of Colon 38 cancer cells and interferes with the cytotoxic effect of fluorouracil in vitro. Pol J Endocrinol. 2007; 1: 2-6.
38. Lago RM, Singh PP, Nesto RW. Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. Lancet. 2007; 29: 1129-1136.
39. Mannucci E, Monami M, Di Bari M, Lamanna C, Gori F, Gensini GF et al. Cardiac safety profile of rosiglitazone: a comprehensive meta-analysis of randomized clinical trials. Int J Cardiol. 2010; 143: 135-140.

40. Komajda M, McMurray JJ, Beck-Nielsen H, Gomis R, Hanefeld M, Pocock SJ et al. Heart failure events with rosiglitazone in type 2 diabetes: data from the RECORD clinical trial. Eur Heart J. 2010; 31: 824-831.
41. Woodcock J, Sharfstein JM, Hamburg M. Regulatory action on rosiglitazone by the U.S. Food and Drug Administration. N Engl J Med. 2010; 36: 1489-1491.
42. Blind E, Dunder K, de Graeff PA, Abadie E. Rosiglitazone: a European regulatory perspective. Diabetologia. 2011; 54: 213-218.