Journal of Animal and Veterinary Advances 11 (23): 4472-4475, 2012
ISSN: 1680-5593

Immunohistochemical Study of the Effect of Resveratrol on the Expression of β-Catenin Protein in Experimental Colonic Carcinoma of Rat

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Abstract: Colorectal cancer is a major health problem worldwide. Resveratrol is a stilbenoid, a type of natural phenol and a phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi. The main objective of present study was to immunohistochemical assessment of the effect of resveratrol on the expression of β-catenin protein in experimental colonic carcinoma of rat. The 25 male Wistar rats aged 3-4 months old weighting 250-350 g were selected by chance. The rats of group 1 were received standard food and water without any changes in their nutritional condition. Rats of group 2 were received EDTA at the dose of 40 mg kg^{-1} as promotor of DMH twice a week for 2 weeks. Rats of group 3 were received DMH at the dose of 40 mg kg^{-1} twice a week for 2 weeks for induction the cancer. The rats of groups 4 and 5 after induction of cancer were received resveratrol at the dose of 10 and 20 mg/kg/day for 10 weeks orally, respectively. The 12 weeks after treatment with resveratrol, animals were constrained and anesthetized by xylazine and ketamine intraperitoneally. Then, segments of colon were sampled for histopathological assessments. Immunohistochemical evaluations showed that rate of the expression of β-catenin proteins in treatment groups was less than control group and there is a statistical significance among groups ($p<0.01$).

Key words: β-catenin proteins, colon carcinoma, resveratrol, rat, ketamine

INTRODUCTION

Beta-catenin (or β-catenin) is a protein that in humans is encoded by the *CTNNB1* gene (Kraus *et al*., 1994; MacDonald *et al*., 2009). In Drosophila, the homologous protein is called armadillo. β-catenin is a subunit of the cadherin protein complex and acts as an intracellular signal transducer in the Wnt signaling pathway (Peifer *et al*., 1991, 1994; Noordermeer *et al*., 1994). When β-catenin was sequenced it was found to be a member of the armadillo family of proteins. These proteins have multiple copies of the so-called armadillo repeat domain which is specialized for protein-protein binding. When β-catenin is not associated with cadherins and alpha-catenin it can interact with other proteins such as ICAT and APC. β-catenin is part of a complex of proteins that constitute Adherens Junctions (AJs). AJs are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. β-catenin also anchors the actin cytoskeleton and may be responsible for transmitting the contact inhibition signal that causes cells to stop dividing once the epithelial sheet is complete (Thompson and Monga, 2007). Recent evidence suggests that β-catenin plays an important role in various aspects of liver biology including liver development (both embryonic and postnatal), liver regeneration following partial hepatectomy, HGF-induced hepatomegaly, liver zonation and pathogenesis of liver cancer (Thompson and Monga, 2007). The gene that codes for β-catenin can function as an oncogene (Wang *et al*., 2008). An increase in β-catenin production has been noted in those people with basal cell carcinoma and leads to the increase in proliferation of related tumors (Saldanha *et al*., 2004). Mutations in this gene are a cause of Colorectal Cancer (CRC), Pilomatrixoma (PTR), Medulloblastoma (MDB) and ovarian cancer. Also, β-catenin binds to the product of the *APC* gene which is mutated in adenomatous polyposis of the colon.

Colorectal cancer, commonly known as colon cancer or bowel cancer is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine) or in the appendix. Genetic analysis shows that colon and rectal tumours are essentially genetically the same cancer (Cancer Genome Atlas Network, 2012). Symptoms of colorectal cancer typically include rectal bleeding and anemia which are sometimes associated with weight loss and changes in bowel habits.

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Most colorectal cancer occurs due to lifestyle and increasing age with only a minority of cases associated with underlying genetic disorders. It typically starts in the lining of the bowel and if left untreated can grow into the muscle layers underneath and then through the bowel wall. Screening is effective at decreasing the chance of dying from colorectal cancer and is recommended starting at the age of 50 and continuing until a person is 75 years old. Localized bowel cancer is usually diagnosed through sigmoidoscopy or colonoscopy.

Cancers that are confined within the wall of the colon are often curable with surgery while cancer that has spread widely around the body is usually not curable and management then focuses on extending the person's life via chemotherapy and improving quality of life. Colorectal cancer is the third most commonly diagnosed cancer in the world but it is more common in developed countries. Around 60% of cases were diagnosed in the developed world. It is estimated that worldwide in 2008, 1.23 million new cases of colorectal cancer were clinically diagnosed and that it killed 608,000 people (Ferlay *et al*., 2010). The main objective of present study was to Immunohistochemical assessment of the effect of resveratrol on the expression of β-catenin protein in experimental colonic carcinoma of rat.

MATERIALS AND METHODS

Experimental plan: This experimental study was carried out in Islamic Azad University Research Center and all procedures and works on animals was conducted under Animal Rights Monitoring Committee of Islamic Azad University Research Center. Present study was an interventional type of researches.

Animals and procedure: About 25 male wistar rats aged 3-4 months old weighting 250-350 g were selected by chance. All animals were kept at room with 22-27°C temperature and 60% humanity and at a natural photoperiod (12/12 darkness/lightness) for 2 weeks before experiment execution in order to adaptation. A commercial balanced diet and tap water were provided *ad libitum*.

Animals were divided into the 5 groups by chance. The rats of group 1 were received standard food and water without any changes in their nutritional condition. Rats of group 2 were received EDTA at the dose of 40 mg kg^{-1} as promotor of DMH twice a week for 2 weeks. Rats of group 3 were received DMH at the dose of 40 mg kg^{-1} twice a week for 2 weeks for induction the cancer. The rats of groups 4 and 5, after induction of cancer were received resveratrol at the dose of 10 and 20 mg/kg/day for 10 weeks orally, respectively. The 12 weeks after treatment with resveratrol, animals were constrained and anesthetized by xylazine and ketamine intraperitoneally at the dose of 5 and 60 mg kg^{-1}, respectively. Then, segments of colon were sampled for histopathological assessments.

Statistical analysis: The statistical package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 13.0 was used for statistical analysis. All data are presented as mean±SEM. Before statistical analysis all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. The $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Immunohistochemical evaluations showed significant changes in the rate of β-catenin proteins in the rats treated with resveratrol than control group ($p<0.01$, Table 1). So that the aggregation of these cells in the mucosal layer in the control groups was more than treatment group (Fig. 1 and 2).

Colorectal cancer is the second most frequent malignancy and the second leading cause of death from cancer in Europe with 412,900 cases diagnosed and 207,400 deaths in 2006. By sex, it constitutes the second most frequent tumour in women after breast cancer and the third in men after lung and prostate tumours (Ferlay *et al*., 2007).

Table 1: Mean value of changes in β-catenin protein in colon tissue of control and treatment groups

β-catenin	Mean	SD	p-value
Treatment group	0.9643	0.1204	<0.01
Control group	2.3210	0.1460	

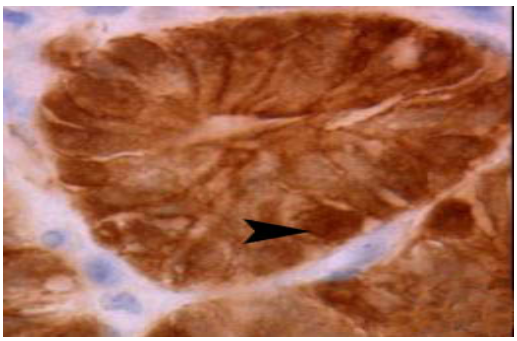

Fig. 1: Microscopic view from the neoplastic colon epithelium of rats of control group. Emerging of β-catenin proteins in the aberrant crypt foci as light brown color is obvious that have more extension (arrows). Immunohistochemistry, 100x

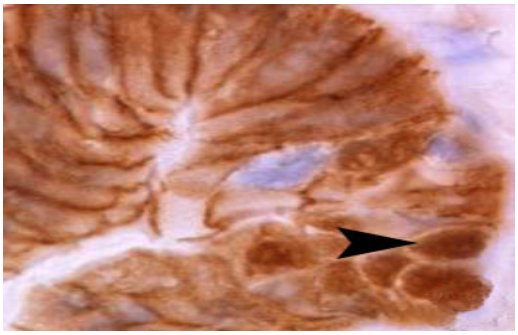

Fig. 2: Microscopic view from the neoplastic colon epithelium of rats of treatment group. Emerging of β-catenin proteins in the aberrant crypt foci as light brown color is obvious that have less extension than control group (arrows). Immunohistochemistry, 100x

The modern day approach to cancer management is multidisciplinary consisting of surgery, radiation therapy and chemotherapy with potential side effects. Several investigations are underway to improve the efficacy of these treatment modalities or to find new ways to treat or prevent cancer. Proteomics technology plays an important role in finding and validating biomarkers for cancer. Bioactive compounds like RSV have multiple mechanisms of action. It is vital to discover novel targets/biomarkers of chemopreventive agents like RSV that has multiple mechanisms of action so that those targets could be harnessed to develop targeted therapies.

Researchers have earlier reported that at concentrations >100 μM RSV suppressed colon cancer proliferation and up-regulated apoptosis even in the presence of IGF-1, elevated during obesity and that has shown to enrich colon cancer stem cell populations (Vanamala *et al*., 2010; Hart *et al*., 2011).

Cell division is an energy-demanding process and its correct progression depends on sufficient metabolic resources to support a doubling of cell mass. Though nutrient availability is a key factor for cell proliferation, nucleotide synthesis is a rate limiting step in cancer cell replication (Ramos-Montoya *et al*., 2006). Ribose-5-phosphate which is a key nucleotide component is synthesized from glycolytic intermediates in the PPP. PPP is considered important in tumor proliferation processes because of its role in supplying tumor cells with reduced NADP and carbons for intracellular anabolic processes (Boros *et al*., 1998). In particular, the two key enzymes G6PDH and TKT have been shown to play a critical role in cancer cell cycle progression in the HT-29 cell line (Vizan *et al*., 2009; Ramos-Montoya *et al*., 2006).

It has been demonstrated that using two different experimental approaches that the PPP which is specifically elevated during cell cycle progression in the highly proliferating advanced human adenocarcinoma cell line HT-29 (Vizan *et al*., 2009; Ramos-Montoya *et al*., 2006) is further elevated by IGF-1 but suppressed by the bioactive compound RSV.

Emerging evidence suggests that members of the Insulin-like Growth Factors (IGFs) family including IGF-I, IGF-II, IGF-IR and the IGF-Binding Proteins (IGFBPs) play a central role in the development and progression of a variety of cancers during obesity including colon cancer (Dupont *et al*., 2003). IGF-1 binding to IGF-1R stimulates downstream proliferating pathways such as the PI3K/Akt (Laurino *et al*., 2005) and Rassignaling (Desbois-Mouthon *et al*., 2001) resulting in increased human colon cancer cell proliferation thus suppressing IGF-1R might attenuate proliferation. Resveratrol suppressed IGF-1 stimulated HT-29 colon cancer cell proliferation.

Talin acts as one of the differentially expressed proteins in IGF-1 and RSV treatments. RSV elevated talin levels at low concentration (50 μM) and suppressed talin and concurrently elevated apoptosis at high concentration (150 μM). This may be due to the action of RSV as an antioxidant at low concentrations and pro-oxidant at high concentrations (Mukherjee *et al*., 2010; De la Lastra and Villegas, 2007; Dudley *et al*., 2009). Anti-oxidant action at lower doses could protect DNA damage via scavenging of ROS whereas at high concentration RSV acts as prooxidant leading to oxidative breakage of cellular DNA in the presence of transition metal ions such as copper causing apoptosis (De la Lastra and Villegas, 2007; Dudley *et al*., 2009). This could possibly explain differences in talin activity at low vs high concentrations of RSV (Juan *et al*., 2008). However, RSV was effective in suppressing IGF-1 stimulated talin expression, irrespective of concentration used.

CONCLUSION

Researchers have shown resveratrol to suppress IGF-1 induced cell proliferation and elevate apoptosis in rats' colon cancer cells and elucidated the mechanisms of action using IGF-1R siRNA.

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