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Repurposing of sodium valproate in colon cancer associated with diabetes mellitus: Role of HDAC inhibition

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ABSTRACT

Background and purpose: Diabetic patients are at greater risk for colon cancer. Histone deacetylases (HDACs) serve as common target for both. The key objective of the study was to evaluate the effect of sodium valproate in type 2 diabetes mellitus associated colon cancer.

Experimental approach: High fat diet and streptozotocin were used to induce type 2 diabetes. Following this, after diabetes confirmation, colon cancer was induced using 1,2 dimethylhydrazine (25 mg/kg, *s.c.*) once weekly from 7th week to 20th weeks. Sodium valproate (200 mg/kg) treatment was given from 20th to 24th week. At the end of 24 weeks, several enzymatic and biochemical parameters, were estimated. MTT, clonogenic and scratch wound healing assay were carried out in HCT-15 cell line.

Key results: Hyperglycemia, hyperinsulinemia, increase in cytokines (TNF-α and IL-1β) and carcinoembryonic antigen and presence of proliferating cells was seen in disease control animals which was prevented by sodium valproate treatment. Overexpression of relative HDAC2 mRNA levels was found in diseased control animals, which was reduced by sodium valproate treatment. IC_{50} of sodium valproate was found to be 3.40 mM and 3.73 mM at 48 h and 72 h respectively on HCT-15 cell line. Sodium valproate also dose dependently prevented colony formation and cell migration.

Conclusion and implications: Sodium valproate can be considered for repurposing in colon cancer associated with diabetes mellitus.

1. Introduction

As per international diabetes federation, one in 11 patient is suffering from diabetes, with > 425 million suffering globally in 2017 and is expected to increase to 629 million by 2045 (International Diabetes Federation). Colorectal cancer is third leading cause of cancer death accounting for nearly 774,000 deaths (WHO). Diabetes is associated with several long term complications including hypertension and cardiomyopathy (Patel and Mehta, 2012; Goyal and Mehta, 2013; Patel and Mehta, 2013). In addition to this, diabetes mellitus is one of the important risk factor for several cancers like pancreatic cancer, breast cancer and colorectal cancer (Giovannucci et al., 2010). It has been documented that type 2 diabetes mellitus in patients increases risk of proximal or distal colorectal cancer (Oh et al., 2008), or proximal and distal, or colonic and rectal cancers (Yang et al., 2005). The excess risk of colon cancer has reached to 30% in type 2 diabetic patients and the majority of the epidemiological data on colon cancer incidence suggest that mortality in type 2 diabetic patients occur due to colon cancer (Giouleme et al., 2011). In an observational population-based cohort study, in which a median follow-up of 4.5 years was carried out for 2759 cases of colorectal cancer, it was reported that there was a 1.3 fold increased risk of colorectal cancer in diabetic patients (Peeters et al., 2015). Hence, regulating diabetes and colon cancer becomes clinically imperious and it is high time to identify a single agent which can control both the diseases by acting on a common target.

Type 2 diabetes mellitus and colon cancer share several common risk factors like western diet, cigarette smoking, obesity, physical inactivity, visceral adiposity, hyperglycemia, hyperinsulinemia and increased oxidative stress are common risk for CRC & T2DM (Giovannucci, 2001). Out of several factors, hyperinsulinemia is the key

Abbreviations: ACF, aberrant crypt foci; ALP, alkaline phosphatase; ANOVA, one-way analysis of variance; CEA, carcinogenic embryonic antigen; DMH, 1,2 dimethylhydrazine; EDTA, ethylene diamine tetra acetic acid; ELISA, enzyme-linked immunosorbent assay; HbA1c, glycosylated hemoglobin; HDACs, histone deacetylases; HE, hematoxylin and eosin; IGF-1, insulin like growth factor-1; IL-1β, interluekin-1β; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NCCS, National Centre for Cell Science; PBS, phosphate buffer saline; PCNA, Proliferating Cell Nuclear Antigen; PCR, Polymerase Chain Reaction; Rb, retinoblastoma protein; STZ, streptozotocin; TNF-α, tumor necrosis factor-α

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factor unifying type 2 diabetes mellitus and colon cancer. Currently several classes of drugs are available for treatment of type 2 diabetes like biguanides, sulphonylureas, thiazolidinediones and dipeptidyl peptidase-4 inhibitors. Although several anti-diabetic agents are beneficial in controlling diabetes, their effects with respect to anti-cancer activity are controversial. Metformin is reported to be beneficial in colorectal cancer as per meta-analysis (Meng et al., 2017); however, several confounding factors are associated with the clinical trials (Ioannou and Boyko, 2011). Hence, there is a dire need for identification of novel agents, which, are effective in both colon cancer associated with type 2 diabetes.

Histone deacetylases (HDAC) are group of such enzymes that play an important role in transcription regulation *via* influencing chromatin packaging status and HDAC inhibitors are implicated in several disorders including diabetes, cardiovascular diseases and cancer (Ye, 2013; Ceccacci and Minucci, 2016; Patel, 2018). HDACs have been suggested to play a regulatory role in physiological insulin signaling and HDAC inhibitors increase GLUT4 translocation and augment basal and insulin-induced glucose uptake in skeletal muscle (Takigawa-Imamura et al., 2003). HDAC inhibitors are also reported to play a role in preserving β-cell function and enhancing insulin actions both *in vitro* and *in vivo* (Larsen et al., 2007; Lewis et al., 2011). Additionally, several studies have reported increased expression of the class I HDACs, in colon tumors relative to adjacent normal mucosa (Giannini and Cavallini, 2005; Wilson et al., 2006). HDAC inhibitors have been reported to block cell proliferation, promote differentiation and induce apoptosis, any of which is desirable to impede the growth of rogue cells (Glozak and Seto, 2007). Anti-tumor effects for various HDAC inhibitors have also been documented (Xu et al., 2015; Zhao et al., 2014).

Valproic acid is a well known inhibitor of HDAC and it selectively inhibits the catalytic activity of class I HDACs and induces proteasomal degradation of HDAC2 (Kraemer et al., 2003). It has been reported that salts of valproic acid prevents cardiac complications in animal models of diabetes (Patel et al., 2014; Rabadiya et al., 2018). Moreover, various *in vitro* studies have reported that valproic acid exhibits anti-cancer potential in breast cancer (Li et al., 2012), non-small lung cancer (Du et al., 2013) and ovarian cancer (Yan and Zhang, 2012). Valproic acid is also reported to enhance cytotoxicity of doxorubicin in breast cancer (Tong et al., 2015), enhances efficacy of vinorelbine-cisplatin in non-small cell lung cancer cells (Gavrilov et al., 2014) and exerts synergistic effect with mitomycin in adenocarcinoma cell lines and in fresh tumor cells of patients with colon cancer (Friedmann et al., 2006). However, till date the role of valproic acid in colon cancer associated with diabetes mellitus is not available. Thus, in current investigation, we have studied the effect of sodium valproate in colon cancer accompanying diabetes mellitus and have also attempted to govern its probable mechanism.

2. Material and methods

2.1. In vivo studies

2.1.1. Study protocol

The ethics committee approval was taken from the ethics committee (protocol number: IP/PCEU/FAC/15-1/034).

We used male Sprague Dawley rats (200–280 g in weights). The animals were kept under normal conditions of temperature (25° ± 2 °C), humidity (55 ± 5%) and 12/12 h light/dark cycle. They were fed the normal laboratory rodent diet and UV filtered water *ad libitum*. After fifteen days of familiarization period, the animals were randomized and divided into four groups with eight rats in each group. Group 1 served as normal control group (CON), group 2 served as control group administered sodium valproate (COV), group 3 served as disease control groups (DCC) and group 4 served as diseased group, administered sodium valproate (DCV). Sodium valproate was dissolved in distilled water, and administered orally at a dose of 200 mg/kg/day, *p.o.* and this dose was selected based on our previous reports (Raghunathan et al., 2017).

2.1.2. Induction of T2DM and colon cancer

STZ and DMH induced diabetes mellitus and colon cancer was used as per previously described method (Patel and Shah, 2016). In order to induce type 2 diabetes mellitus, the animals of group 3 and 4 were given high fat diet comprising of 58% fat, 25% of protein and 17% of carbohydrate, as a percentage of total kcal, *ad libitum* for two weeks. Following this, streptozotocin (STZ) was administered in single dose of 35 mg/kg, *i.p.* After six weeks of STZ injection, diabetes was confirmed by measuring the serum glucose levels using available diagnostic kits acquired from Accucare using semi-automated biochemical-analyzer (Robonik India Pvt. Ltd.). Subsequently, 1,2 dimethylhydrazine (DMH) which is a carcinogen, was administered once weekly in the dose of 25 mg/kg, *s.c.* from 7th week to 20th weeks. The animals of group II and group IV were treated the sodium valproate (200 mg/kg, *p.o.*) from 20th week to 24th week.

2.1.3. Parameters evaluation

After the treatment period, after completion of 24 weeks, blood was collected from retro-orbital plexus using light ether anesthesia. 0.2 ml of blood was collected and stored in Ethylene diamine tetra acetic acid (EDTA) tubes at −20 °C until further analysis. The remaining blood was subjected to clotting for 30 min at room temperature following which, they were centrifuged at 4000 rpm for 20 min. The separation of serum was carried out and it was stored at −20 °C till further analysis. The fasting serum glucose levels and alkaline phosphatase (ALP) were analyzed using available kits procured by Accucare using semi-automated biochemical-analyzer (Robonik India Pvt. Ltd.) (Goyal et al., 2008; Goyal et al., 2009; Patel et al., 2013; Goyal et al., 2011a; Goyal et al., 2011b; Patel and Bhadada, 2014). The oral glucose tolerance test was also performed. Other parameters like tumor necrosis factor-α (TNF-α), iInterluekin-1β (IL-1β), insulin and carcinogenic embryonic antigen (CEA) levels were determined by enzyme-linked immunosorbent assay (ELISA) assay as per the manufacturer's instructions (Krishgen biosystems Mumbai, Version 4.1) using ELISA plate reader (Robonik Pvt. Ltd., version RBK 3.400A) (Patel et al., 2012). The whole blood was subjected for the estimation of glycosylated hemoglobin and mean blood glucose (Raghunathan et al., 2014) using ion exchange resin method as per the manufacturer's instruction by Accucare using U.V. spectrophotometer (SHIMADZU 2450). Subsequent to determination of blood parameters, the animals were sacrificed, and colon was located and dissected out from the rats. The colon tissues were cleaned and was either stored in 10% formalin for proliferating cell studies and histopathological studies or kept in trizol reagent at −20 °C for mRNA expression studies.

2.1.4. Histopathological studies

Colonic tissues were subjected to histopathological studies in which, the tissues which were previously stored in 10% formalin were used. The tissues were processed and paraffin blocks were prepared. The paraffin embedded tissues were cut into 4 mm thick sections on histology slides and the slides were in turn stained for hematoxylin and eosin (HE) staining. Proliferating Cell Nuclear Antigen (PCNA) immunostaining (Ki-67) using a streptavidin-biotin-immunoperoxidase complex method was carried out at Supratech Micropath Laboratory and Research Institute Pvt. Ltd. The nuclei stained in brown-yellow color were considered as positive. To calculate the proliferative index, area containing 100 cells was selected and positively stained nuclei were calculated and percentage index was determined in a blinded manner.

2.1.5. mRNA expression studies

Polymerase Chain Reaction (PCR) was carried out to determine HDAC 2 mRNA levels. Briefly, 50–100 mg of the colon specimen stored