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Inotodiol suppresses proliferation of breast cancer in rat model of type 2 diabetes mellitus via downregulation of β-catenin signaling

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ABSTRACT

Breast cancer is amongst the most common cancers causing death of women worldwide. Breast cancer occurrence is more prominent in people with diabetes. A recent trend is management of diabetes and cancer has evolved to be natural remedy including single molecule therapy or combination. In this study, we investigated the effect of inotodiol on breast cancer growth in diabetic conditions. Inotodiol is a lanostane triterpenoid found in natural resources like edible mushroom *Inonotus obliquus*. We established a rat model of diabetic-breast cancer by treating female Sprague-Dawley rats with streptazotocin (STZ) at 35 mg/kg followed by induction of breast cancer by administration of 7,12-dimethylbenz(a)anthracene (DMBA) at 10 mg/kg. Diabetes development in experimental rats was confirmed by measuring fasting blood glucose levels and oral glucose tolerance test (OGTT), and other biochemical assays were performed. Histological evaluation of pancreas was performed. The proliferation of breast tumor was measured by immunohistochemical staining for PCNA, cleaved-caspase-3 and TUNEL staining for apoptosis, and β-catenin. Results of the study demonstrate that inotodiol lowered the blood glucose levels in SD rats as well as reduced plasma levels of cholesterol, triglyceride, and high-density lipoprotein. The tumor proliferation marker PCNA was reduced by inotodiol. It downregulated the expression of β-catenin and its downstream targets (c-Myc and Cyclin D1) followed by apoptosis induction. Conclusively, results suggest that inotodiol regulates blood glucose levels in diabetic rats and then controls proliferation of breast tumor progression by inducing apoptosis via downregulation of β-catenin signaling. It further suggests that inotodiol can be a preventive approach in managing dietary chronic conditions like diabetic-breast cancer.

1. Introduction

Cancer and diabetes are major public health concerns worldwide. The major consequences of diabetes are blindness, kidney failure, and nontraumatic lower limb amputation [1]. While cancer remains to be a major cause of deaths. Recent reports indicate a potential link between diabetes and cancer especially breast cancer which is amongst the most common cancers causing death of women worldwide. Breast tumorigenesis is a multistep complex process involving role of growth factors, oncogenic signaling and various transduction events. Breast cancer is amongst the commonest malignancies in women worldwide, affecting 1 of every 8 women, with higher incidences in developed countries like USA as well as developing countries [2]. The other major health concern is type 2 diabetes, affecting about 7% of adults and about 15% of older people (over 60 years) [2]. Mammary malignancies are caused by several etiological factors mainly environmental, genetics, and immunological defects. Although the causative mechanisms regulating cancer progression in diabetic patients remain unclear, but a putative connecting link is hyperinsulinemia which is resultant of insulin resistance in type 2 diabetes. Amongst several dysregulated signaling and growth factor pathways, insulin-like growth factor (IGF) axis is fundamental that regulates proliferation, differentiation, migration, cell survival/apoptosis and transformation signaling events in breast carcinogenesis [3,4]. Both IGF1 and the receptor (IGF1R) has been reported to be strongly associated with the risk of breast cancer [5]. Insulin plays vital roles in cell proliferation via IGF1 and inhibits apoptosis which may play significant and direct role in the cancer development [6].

Studies have reported that a relatively strong correlation exists between diabetes and some types of cancers such as diabetic patients have relatively higher risk of hepatocellular carcinoma [7] and diabetic patients have showed an increased risk of developing pancreatic

Abbreviations: INO, inotodiol; STZ, streptazotocin; DMBA, 7,12-dimethylbenz(a)anthracene; OGTT, oral glucose tolerance test; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase

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cancer [8]. Diabetic patients have also shown as increased risk of developing cancers of some other tissues such as kidney, endometrial, colorectal, non-Hodgkin lymphoma, bladder, and breast [9]. The consequences of diabetes and cancer may be more fatal because mortality was found to be increased in cancer patients with type 2 diabetes especially increased mortality risk compared with patients with breast cancer without diabetes [10]. A connecting link between diabetes and cancer is hyperglycemia which may be induced by increasing levels of circulating levels of insulin and IGF1 and inflammatory cytokines [11]. The impact of high glucose levels cancer progression in diabetics still remain a relatively unexplored. Wnt/β-catenin signaling, is a key cancer-associated pathway which has been modulated by high glucose levels. In addition, elevated Wnt/β-catenin signaling has been correlated with higher frequencies of cancer in the diabetic population [12,13]. Thus, it is an active simultaneous area of research for using anti-diabetic modalities on breast cancer risk and prognosis.

Anti-diabetic preventive measures act mainly by reintroducing insulin production to reduce blood sugar levels, insulin sensitization to patients or a combination of both. Metformin is one of the most commonly prescribed oral medication for treating type 2 diabetes. It is reported that diabetic patients taking metformin have a lower incidence of invasive breast cancer compared those taking other anti-diabetic medications like sulfonylureas [14]. Sulfonyureas is another class of anti-diabetic drug used for treating type 2 diabetes which works by stimulating the pancreas to produce more insulin. However, a slightly higher risk of developing breast cancer has been observed with the use of sulfonylurea [15]. Even though sulfonyureas and metformin is often prescribed as a combination therapy. Thiazolidinediones are another class of anti-diabetic medication that promotes sensitive to insulin in patients yet its association with effects on breast cancer risk when used alone or in combination with chemotherapy and hormone therapy is not very clear [16,17]. Thus, a requirement remains for the safe and sustainable anti-diabetic drugs that may also help in protecting against breast cancer and lowering the risk of death from the disease in diabetic patients.

In this purview, medicinal plants and their molecules may serve important roles in preventing diabetes as well as reducing the risk of breast cancer induction and progression. Inotodiol is a lanostane triterpenoid found in natural resources like edible mushroom chaga, *Inonotus obliquus*. The extracts of *I. obliquus* have been applied in various disease conditions for their antioxidant, antiviral, antifungal, anti-inflammatory, anticarcinogenic and anticancer properties [18,19]. An aqueous extract of *I. obliquus* suppressed intestinal inflammation and colorectal cancer by modulating NF-κB/β-catenin signaling [20,21]. The anti-inflammatory and anticancer effects of the extract was supposed to be mediated by ergosterol epoxide which blocked the entry of β-catenin in to nucleus [22]. In this study, we investigated the effect of inotodiol on growth of breast cancer in diabetic condition. The preventive effects of inotodiol on the levels of blood glucose levels, IGF signaling, pancreatic and liver function was assessed followed by Wnt/β-catenin signaling and apoptosis in breast cancer.

2. Materials and methods

2.1. Animals

All animal experimental procedures were performed in accordance with the guidelines of the Animal Ethical Committee of the Institute with approved ethical clearance for the study. Female Sprague-Dawley rats, aged 5 weeks, weighing 110–120 g included in the study were obtained from Shanghai Laboratory Animal Center, China. Rats were housed in a climate controlled room maintained at 22–24 °C temperature and 50–60% humidity with 12 h light and dark cycle.

2.2. Induction of breast cancer in diabetic rats

Inotodiol was procured from ALB Technology Ltd. (Hong Kong), streptozotocin and DMBA was obtained from Sigma-Aldrich (St. Louis, MO, USA). SD rats were randomly divided into six groups, each containing 10 animals: control (CON) group; STZ-induced diabetes (STZ) group; STZ-INO treated with inotodiol 10 mg/kg; DMBA group; DMBA-INO treated with inotodiol 10 mg/kg; STZ-DMBA group; STZ-DMBA-INO treated with inotodiol 10 mg/kg. Diabetes was induced in rats by intraperitoneal administration of STZ at 35 mg/kg dissolved in 10 mM sodium-citrate buffer pH 4.5. Rats in both CON and DMBA groups received only an equivalent volume of citrate buffer. Diabetes development in experimental rats was confirmed by measuring fasting blood glucose levels using a gluco-meter (Roche Diagnostics, Rotkreuz, Switzerland). Diabetes was established in rats only when their fasting blood glucose levels exceeded 16.67 mmol/L (300 mg/dL) after 1 week of STZ injection. Two weeks after STZ injection, rats in STZ-DMBA and DMBA groups were intraperitoneally injected with a single dose of 10 mg/kg DMBA dissolved in corn oil. At this age, rats in both CON and STZ groups received only an equivalent volume of corn oil. One week after DMBA administration, rats in STZ-INO, DMBA-INO and STZ-DMBA-INO groups were bi-weekly intravenously administered with 10 mg/kg inotodiol. Tumor appearance in rats was checked by weekly palpation and first detection of tumor was observed at approximately 84 and 91 days in STZ-DMBA and DMBA groups, respectively. Rats were treated with INO till total 126 days and then rats in all groups were euthanized under anesthesia. Blood was drawn under anesthesia for biochemical assays. Tumor shape and size was recorded and compared amongst groups. Pancreas and breast tissues were resected and stored accordingly for immunohistochemical and histological staining, biochemical and molecular assays.

2.3. Oral glucose tolerance test (OGTT)

The OGTT was performed two weeks after STZ or vehicle administration by keeping animals at fasting overnight and delivering an oral dose of glucose solution (2.5 g/kg). Blood for serum analysis was drawn from the tail vein under light ether anesthesia at each time point after glucose loading. The levels of glucose and insulin in serum were measured using a glucose test kit (Jiancheng Biotech Co, Nanjing, China) and an insulin radioimmunoassay kit (North Institute of Biotech Co, Beijing, China), respectively.

2.4. Biochemical assays

Blood samples were collected from experimental animals and plasma was isolated. The levels of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C); the activities of SOD, GPX, CAT and the concentration of MDA were determined from plasma using commercially available kits (Nanjing Jiancheng Bioengineering Institute, China) as per manufacturers' instructions. Antioxidant enzyme activities were also performed for superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

2.5. Histological evaluation of pancreas and breast

A splenic portion of pancreatic tissues were removed from sacrificed rats and rinsed with ice-cold PBS. Similarly, a breast tumor tissue portion was rinsed with ice-cold PBS. The tissue samples were fixed in neutral buffered paraformaldehyde solution (4%) for 24 h. Tissue sections were then embedded in paraffin and deparaffinized using standard procedures. Tissues were sectioned to 5 μm thickness followed by dewaxing and rehydration in a graded series of ethanol. Sections were then rehydrated and stained with haematoxylin and eosin (H&E). Sections were visualized under a light microscope (Leica, Germany) at 40× by two investigators blinded to the identity of samples.