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Food-grade titanium dioxide exposure exacerbates tumor formation in colitis associated cancer model

Ismael M. Urrutia-Ortega [a, b], Luis G. Garduño-Balderas [a, c], Norma L. Delgado-Buenrostro [a], Verónica Freyre-Fonseca [a, d], José O. Flores-Flores [e], Arturo González-Robles [f], José Pedraza-Chaverri [g], Rogelio Hernández-Pando [h], Miriam Rodríguez-Sosa [a], Sonia León-Cabrera [a, i], Luis I. Terrazas [a], Henk van Loveren [j], Yolanda I. Chirino [a, *]

[a] Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, CP 54059, Estado de México, Mexico
[b] Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Mexico
[c] Programa de Maestría en Ciencias Biológicas, Universidad Nacional Autónoma de México, Mexico
[d] Doctorado en Ciencias en Alimentos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, CP 11340, México DF, Mexico
[e] Centro de Ciencias Aplicadas y Desarrollo Tecnológico, Universidad Nacional Autónoma de México, Circuito Exterior S/N, Ciudad Universitaria AP 70-186, CP 04510, México DF, Mexico
[f] Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies, CINVESTAV-IPN, Avenida Instituto Politécnico Nacional 2508, San Pedro Zacatenco, 07360, Mexico, DF, Mexico
[g] Laboratorio 209, Edificio F, Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México, CP 04510, México DF, Mexico
[h] Experimental Pathology Section, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran, Vasco de Quiroga 15, Colonia Sección XVI, Tlalpan, México DF, 14000, Mexico
[i] Carrera De Médico Cirujano, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, CP 54059, Estado de México, Mexico
[j] Department of Toxicogenomics, Maastricht University, PO Box 616, 6200MD, Maastricht, The Netherlands

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ABSTRACT

Colorectal cancer is the fourth worldwide cause of death and even if some dietary habits are consider risk factors, the contribution of food additives including foodgrade titanium dioxide (TiO2), designated as E171, has been poorly investigated. We hypothesized that oral E171 intake could have impact on the enhancement of colorectal tumor formation and we aimed to investigate if E171 administration could enhance tumor formation in a colitis associated cancer (CAC) model. BALB/c male mice were grouped as follows: a) control, b) E171, c) CAC and d) CAC + E171 group (n = 6). E171 used in this study formed agglomerates of 300 nm in water. E171 intragastric administration (5 mg/kg body weight/5 days/10 weeks) was unable to induce tumor formation but dysplastic alterations were observed in the distal colon but enhanced the tumor formation in distal colon (CAC + E171 group) measured by tumor progression markers. Some E171 particles were internalized in colonic cells of the E171 and CAC + E171 groups and both groups showed a decrease in goblet cells in the distal colon. However the CAC + E171 group showed a higher decrease of these cells that act as protection barrier in colon. These results suggest that E171 could worsen pre-existent intestinal diseases.

1. Introduction

Colorectal cancer is the fourth worldwide cause of death with 1,233,000 new cases according to the World Health Organization (Cancer Fact sheet N°297, 2015). The etiology of colorectal cancer is complex but it has been estimated that modifying dietary habits could prevent 70% of sporadic colorectal cancer cases. Then, concern for adverse effects after human consumption of some foods such as those containing nanoparticles (NPs), including food-grade titanium dioxide (TiO_2) has risen since NPs can have detrimental effects on experimental models. In this regard, oral

* Corresponding author. Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Av. de Los Barrios 1, Los Reyes Iztacala, Tlalnepantla, 54090, Estado de México, Mexico.
E-mail address: irasemachirino@gmail.com (Y.I. Chirino).

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consumption of TiO_2, known as E171 additive used as a whitening agent can be absorbed in the human gut according to a recent human volunteer study, which dosed TiO_2 at 5 mg/kg (Jones et al., 2015). Consumption by adults has been estimated at 1 mg/body weight/day while children can consume up to 2 mg/kg body weight/day since E171 is widely used for candies (Weir et al., 2012). In addition, the human TiO_2 average consumption can be estimated in 5.4 mg per adult person in the United Kingdom (MAFF, 1993). For this reason, several studies have been addressed to analyze the effects of TiO_2 by oral consumption and it has been demonstrated that TiO_2 dosed at 6.8–8.6 mg/kg body weight for 5 days can reach liver, spleen, mesenteric lymph nodes, however tissue absorption was very low (Geraets et al., 2014). Oral exposure dosed at 2 mg/kg body weight for five days in rats also showed TiO_2 particles deposition in the spleen causing necrotic cells in the cortex and pycnotic nuclei in the medulla of adrenal gland (Tassinari et al., 2014). However, effects of E171 in humans remain still unknown but suspected food-grade TiO_2 has been found in Peyer's patches in the ileum of healthy children and also in children having ulcerative colitis increasing the amount of black pigment with the age (Hummel et al., 2014). Similar deposits, which authors stated might be food-grade TiO_2, were also reported in Peyer patches of patients with colon adenocarcinoma, Crohn disease and non-Crohn disease colitis (Thoree et al., 2008). TiO_2 was also found in human gut associated lymphoid tissue from patients with Crohn disease, ulcerative colitis and colonic carcinoma (Powell et al., 1996). However, the role of food-grade TiO_2 in the development or the enhancement of other diseases by oral exposure has not been established. For these reasons we hypothesized that oral food-grade TiO_2, known as E171, exposure could enhance tumor formation during cancer development in the colon even if E171 is unable to promote tumor formation by itself. To test our hypothesis, we aimed to use a chemically colitis-associated colorectal cancer (CAC) model in mice (Tanaka et al., 2003) to investigate if intragastric E171 administration enhanced tumor formation in mice developing colorectal cancer and also to compare the effect of E171 intragastric exposure of tumor-free mice with CAC mice dosed with E171 on tumor progression markers.

2. Material and methods

2.1. E171 particles characterization

Food-grade TiO_2 was from SENSIENT COLORS (Color index CI 77891 named as E171, 99% minimum of TiO_2). Size and morphology of E171 were characterized by scanning electron microscopy (JEOL 5800-LV, Japan, 5,000X, 15Kv). Agglomerate size and morphology were analyzed by transmission electron microscopy (JEOL JEM 1010, Japan, 75,000X 60Kv). Raman spectra of E171 were measured in the 300–2000 cm-1 spectral region with an Almega XR dispersive Raman spectrometer. Raman spectra were accumulated over 25 s with a resolution of ~4 cm-1, the excitation source was 532 nm radiation from a Nd:YVO4 laser (frequency-doubled) and the laser power on the sample was 2.5 mW.

2.2. Chemically colitis-associated cancer (CAC) mice model and E171 administration

Experimental work followed the guidelines of Norma Oficial-Please do not replace. Mexicana (NOM-062-ZOO-1999, NOM-087-ECOL-1995) and the Protocol for the Care and Use of Laboratory Animals (PICUAL). Six BALB/c male mice of 4–6 weeks old (Harlan Laboratories, México) were housed in a polycarbonate cages and kept in a housing room (21 °C, 50–60% relative humidity, 12 h light/dark cycles, air filtered until 5 μm particles and was exchanged 18 times/h, standard commercial rat chow diet from Harland Teklad, Madison, WI, USA). After one week of acclimation, the mice were randomly divided in the following groups: a) control, b) E171 group, c) CAC group and d) CAC + E171 group. The control group received a single intraperitoneal injection of saline solution. One milligram of E171 was sterilized, resuspended in 1 mL of water and sonicated for 30 min at 60 Hz. The E171 group of mice received an intragastric administration of 5 mg/kg body weight of E171 by a gavage from Monday to Friday during 10 weeks in a final volume of 100 μL. The CAC group received a single dose of 12.5 mg/kg body weight/ip azoxymethane (AOM) and 2% dextran sulfate sodium (DSS) in the third, sixth and ninth week in water *ad libitum*. The CAC + E171 group received a single dose of AOM and DSS was given in the same scheme of CAC group, and in addition, received intragastric administration of E171 according the same scheme as the E171 group (Fig. 1). After 11 weeks, mice were sacrificed in a humid chamber with sevoflurane and colon, kidney, liver, spleen, lung tissue and blood samples were collected.

2.3. Colorectal histology

The colon was dissected, opened and immediately fixed by immersion in 4% paraformaldehyde. Tissue samples were dehydrated in graded ethylic alcohol, embedded in paraffin and cut at 3-μm thickness. For histological examination, colon sections were stained with hematoxylin (SIGMA, cat. HHS16) and eosin (SIGMA cat. E4009).

2.4. Primary cell culture

Two square millimeters of excised distal colon from each group were washed with PBS and cultured using RPMI medium supplemented with 15% fetal bovine serum (FBS) and 10% antibiotic and subcultured for 7 days and then, fixed with 2% glutaraldehyde for 2 h and used for TEM analysis.

2.5. Tumor progression

Tumor progression was evaluated by the detection of COX2, β-catenin, and Ki67 (β-catenin, Abcam ab16501; COX2, Genetex GTX1591; Ki67, Biolegend 652402; and NF-κB was used as a key marker of inflammation (p65-NF-κB CST 3033) in colon tissue by immunohistochemistry. Cuts of 3 μm were incubate with primary antibody overnight at 4 °C, then, slides were incubated with secondary antibody (TRITC, Santa Cruz 111-025 and FITCI, Santa Cruz 64419) during 2 h at 37 °C. Samples were analyzed with a Zeiss Vert. A1 conventional epifluorescence microscope and with LEICA TCS SP2 confocal microscope (area of each sample was 2.8 mm^2 and 20 fields of 50 μm^2 were analyzed in each sample).

2.6. Goblet cell detection

Cuts of 3-μm were incubated in 1% alcian blue solution in acetic acid for 10 min, then with nuclear fast red 0.1% in 5% aluminum sulfate during 10 min. Images were obtained with an optical microscope at 63X. Goblet cells identified as stained blue cells were counted in each sample.

2.7. Tissue IL-2, TNF-α, IFN-γ, IL-10 and GM-CSF determination

Colon tissue (150 mg) was homogenized in RIPA buffer for interleukin detection using Bio-Plex multiplex MAGPIX with Bio-Plex Pro Mouse Cytokine 8-plex kit (BIO-RAD cat. M60-000007A). Briefly, 50 μL microsphere solution was used for each sample and incubated under stirring (850 ± 50 rpm) during 30 min and