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Mucin secreting cells in the stomach and colon are altered by combination antiretroviral treatment in an obese rat model

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ABSTRACT

Mucins, secreted by intestinal goblet cells, form an integral part of the intestinal biofilm, which is important for the functioning of a healthy gastrointestinal tract (GIT). This mucous layer is sensitive to factors such as diet, drugs and inflammation. Histochemically, mucins can be classified as neutral or acidic, where acidic mucins can contain sulphate groups (sulphomucins) or sialic acid (sialomucins). The aim of the present study was to determine the composition of various mucin secreting cells using histochemical stains in rats fed on a high calorie diet (HCD) treated with antiretroviral therapy (ART). Wistar rats (N = 24) were divided into a lean control group (C/ART−), high calorie diet group (C/HCD+), ART group (C/ART+) and HCD and ART group (HCD+/ART+). The body of the stomach as well as the colon were stained with Alcian Blue Periodic Schiff (ABPAS) to distinguish between neutral and acidic mucins and Alcian Blue Aldehyde Fuschin (ABAF) to distinguish between sialo-and sulphomucins. An increase of the total gastric mucous cells was observed in the HCD+/ART+ group compared to the C/ART− group using both ABPAS and ABAF. A decrease of neutral cells in the distal part of the colonic crypts in the C/HCD+ and C/ART+ groups compared to the C/ART− group were observed. Mixed goblet cells in the colonic crypts of the C/ART− and HCD+/ART+ groups were decreased in comparison to the C/ART+ group. The study showed that the total mean percentage of mucous cells in the stomach as well as the total amount of neutral goblet cells in the colon were most affected by ART and a HCD. These changes in a rat model suggest that the quality of the biofilm may be altered and should be considered when ART is prescribed to obese patients.

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1. Introduction

The intestinal biofilm is a viscoelastic layer found in the lumen of the gastrointestinal tract (GIT) and consists of composite microbial populations and the secretions of mucin secreting cells (Allen, 1981; Hollander, 1963; Kleessen and Blaut, 2005). Mucins are large carbohydrate-rich glycoproteins (Byrd and Bresalier, 2004) which provide the initial binding site for commensal micro-organisms to form an adhesive layer that ultimately forms microcolonies. The biofilm acts as the first defence against irritants and pathogens in the GIT and facilitates nutrient exchange between the lumen and the mucosa (Kim and Ho, 2010; Kleessen and Blaut, 2005). Using histochemistry, mucins can be classified as neutral or acidic. Furthermore, acidic mucins may contain sialic acid (sialomucin) or sulphate groups (sulphomucin) (Filipe, 1979). The distribution of these histologically identifiable mucin secreting cells can indirectly give an indication of the quality of the biofilm in a particular GIT segment as the biofilm is notoriously difficult to visualise using histology (Palestrant et al., 2004).

The types of intestinal mucins secreted at a given time in the GIT may vary depending on need and disease present (McGuckin et al., 2011). Neutral mucins are integral in buffering an acidic environment such as the stomach (Cao and Wang, 2009; Petrinec et al., 2005). Sialic acid is essential in the hydroxyl radical (•OH) scavenging properties of sialomucins whereas sulphomucin secretion results in an increased mucus viscosity and therefore tends to thicken the mucous layer (Croix et al., 2011; Ogasawara et al., 2007). A reduction of sulphomucins in relation to sialomucins may

Abbreviations: ABPAS, alcian blue periodic acid schiff; ABAF, alcian blue aldehyde fuschin; ART, antiretroviral therapy; C, control; EFV, efavirenz; FTC, emtricitabine; HCD, high calorie diet; TDF, tenofovir disoproxil fumarate.

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indicate inflammatory diseases and conditions such as colorectal carcinoma (Croix et al., 2011). Acute infections of the GIT cause mucin secreting cells to increase in order to aid with the elimination of pathogenic elements, while chronic infection results in a reduction of the amount of goblet cells. The latter is probably due to a depletion of mucin secreting cells over a period of time, which leads to subsequent damage to the protective mucosal layer (Croix et al., 2011; Kim and Ho, 2010).

Various studies have explored the immune responses and biochemical influences of antiretroviral medication on the intestinal mucosa of HIV positive patients (Braga Neto et al., 2010; Dikman et al., 2015; Schmidt et al., 2001; Sheth et al., 2008), but limited research is available regarding the histomorphology thereof (Dossou-Yovo et al., 2014). Furthermore, combination ART (cART) regimens have been shown to induce side effects which may mimic gastrointestinal symptoms of HIV infected patients. Such symptoms may include nausea, diarrhoea and vomiting (Bang and Scott, 2003; Max and Sherer, 2000). Research is thus necessary to distinguish between the symptoms caused by HIV and the side effects of cART.

Multiple studies suggest that the use of ART may cause significant weight gain as well as abnormalities such as lipodystrophy which may ultimately result in obesity in HIV positive patients (Beatriz and Marinho, 2008; Crum-Cianflone et al., 2008; Mariz et al., 2011; Roubenoff et al., 2002). The question begs whether the ingestion of ART in a rat model, which was already exposed to a high calorie diet, will exert additional side effects compared to an untreated high calorie diet model. According to various authors, the dietary intake of an individual alters the micro-organisms present in the GIT (de Heredia et al., 2012; Dibaise et al., 2008; Frazier et al., 2011). Specifically, high fat diets have demonstrated to affect intestinal permeability and cause inflammation (Frazier et al., 2011). The microbiota in the GIT affects the amount of nutrient and energy attainment from a diet and is an important factor in weight regulation (Frazier et al., 2011).

The stomach contains surface mucous cells and mucous neck cells; the secretions of which are integral in the maintenance of the mucous-bicarbonate barrier that protects the stomach from self-digestion (Kierszenbaum, 2007; Ross and Turnberg, 1983). The goblet cells in the colon secrete mucus for lubrication and provide a physical hindrance for attachment of pathogens (Kierszenbaum, 2007; McGuckin et al., 2011). Due to the prominent function of mucin secreting cells in the stomach and colon, the aim of the study was thus to compare the distribution of these cells in high calorie diet-fed rats exposed to ART.

2. Materials and methods

2.1. Animals and tissue preparation

Adult male Wistar rats (N = 24) were randomly divided into four experimental groups (n = 6/group) and housed in a controlled environment (temperature: 22 °C; humidity: 40%; circadian rhythm: 12 h of artificial light per day). Ethics approval for this study was obtained from the Committee for Experimental Animal Research of Stellenbosch University.

The experimental groups were: 1) a lean control group (C/ART−), 2) rats that were subjected to a high calorie diet (C/HCD+), 3) a lean group subjected to ART (C/ART+) and lastly 4) a group subjected to a high calorie diet and to ART (HCD+/ART+).

All four groups had free access to normal rat chow and water for 16 weeks. In order to induce obesity, the high calorie groups had additional access to high calorie food. The high calorie diet consisted of 11.5 g/100 g total fat, 7.6 g/100 g saturated fat, 13 mg/100 g cholesterol, 8.3% protein, 34.6% carbohydrate and 20.4% sucrose. The ART was administered via oral gavage at week 10 of the diet programme. A generic single-tablet of Odimune® containing 600 mg Efavirenz (EFV), 200 mg Emtricitabine (FTC) and 300 mg Tenofovir Disoproxil Fumarate (TDF) was ground into a powder and administered according to the body weight of each rat. The dose administration was conducted according to Food and Drug Administration (FDA) standards for human to rat conversion, which was six times the dose for humans per kg for rats.

The animals were anesthetized using an intraperitoneal injection of 160 mg/kg sodium pentobarbitone into the lower right abdomen and euthanized via exsanguination. Tissue samples of the body of the stomach and the proximal part of the descending colon of each animal were harvested, fixed in paraformaldehyde for 48 h and embedded in paraffin wax. Histological sections of the stomach and colon were cut at 5 μm. The sections were stained with Alcian Blue Periodic Acid Schiff (ABPAS) to distinguish between neutral (magenta), acidic (blue) and mixed (purple) mucin secreting cells. To distinguish between the two types of acidic mucins, Alcian Blue Aldehyde Fuschin (ABAF) stained mucin secreting cells containing sialic acid (blue), sulphate (purple) or both (dark purple/blue) (Bancroft and Gamble, 2008; Bancroft and Stevens, 1990). For the purposes of this article, mucin secreting cells containing both neutral and acidic granules will be referred to as mixed cells and the mucin secreting cells containing both sialomucins and sulphomucins will be referred to as mixed acidic cells. Mucin-secreting cells containing sialic acid and sulphate will be referred to as sialomucins and sulphomucins, respectively.

2.2. Histochemical method

The staining protocol for ABPAS and ABAF was carried out according to Bancroft and Gamble (2008). Briefly, for both stains slides were deparaffinised with Xylene and ethanol (70%, 90% and 99%), then hydrated with distilled water. Slides allocated for ABPAS were treated with Alcian Blue (pH = 2.5) (1% Alcian Blue in 3% Acetic Acid, (8GX, Colour Index 74240, product 34089, Gurr Microscopy Materials, BDH Chemicals Ltd.,Poole, England) for 15 min and then thoroughly rinsed with tap water and there after distilled water. Slides were then immersed in 1% Periodic acid for 5 min (SAARCHEM 4946180, UNI-VAR, Muldersdrift, RSA), where after it was rinsed with running tap water. Schiff's reagent was then applied for 15 min and then rinsed in lukewarm tap water for 10 min and counterstained in Meyer's Haematoxylin (SAAR2822001LC, Merck Chemicals (Pty.) Ltd. Gauteng, RSA) for 60 s. The excess was washed off with running tap water for 5 min after which the slides were then dehydrated in ethanol and cleared in xylene before it was mounted with DPX mounting medium.

For the ABAF slides, Aldehyde Fuchsin with a pH of 1.7 was made by dissolving 1 g basic Fuchsin in 200 ml 70% ethanol with 2 ml concentrated hydrochloric acid and 2 ml paraldehyde. The solution was left for 3 days at room temperature, filtered and refrigerated. Slides were immersed in Aldehyde Fuchsin for 20 min, rinsed in 70% ethanol and then rinsed in running tap water. There after the slides were stained with Alcian blue for 30 min and rinsed in running tap water for 2 min. The slides were dehydrated in ethanol and cleared in xylene before it was mounted with DPX mounting medium.

2.3. Quantification of mucin secreting cells

2.3.1. Stomach

To quantify the mucin secreting cells of the stomach, multiple photographs were taken with a Zeiss Axioscop2 microscope using a 2.5× objective lens (25× magnification) and stitched with Hugin 2014.0.0 (version 0.2.11.0). The total length of the stomach tissue on the slide was divided into three equal parts to ensure equal distribution of the glands examined. Ten gastric glands per part (30 glands

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