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Archives of Biochemistry and Biophysics 434 (2005) 299–305
ABB
www.elsevier.com/locate/yabbi

Surgical stress-induced alterations in retinoid metabolism in the small intestine: role of oxygen free radicals

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Received 24 September 2004, and in revised form 8 November 2004
Available online 24 November 2004

Abstract

Oxidative stress in the small intestine can result in altered cell proliferation, migration, and differentiation of villus-crypt cells. Retinoid metabolism is recognized as an important mediator of cellular differentiation in the intestine. This study examined the effect of oxidative stress in retinoid metabolism in a surgical stress model. Surgical stress was performed by handling the intestine as done during laparotomy. Villus-crypt cells were isolated at different time periods and various retinoid concentrations in the cell homogenate and the retinoic acid forming enzymes were quantitated using HPLC. Surgical stress resulted in altered retinoid levels in various cell populations in the small intestine at 1 and 12 h. The activity of alkaline phosphatase and retinal oxidase was also altered at these time points and all these changes were prevented by inhibiting superoxide generation using xanthine oxidase inhibitor, allopurinol. These studies suggest that alterations seen in enterocytes during surgical stress may be mediated by changes in retinoid metabolism.

Keywords: Retinoids; Enterocytes; Surgical stress; Oxidative stress; Alkaline phosphatase; Retinal oxidase; Proliferation; Differentiation

The intestinal epithelium undergoes a continuous renewal process and has cells at various stages of differentiation. The integrity of the intestinal epithelium is critical to health [1] and any damage to the cells can affect proliferation as well as differentiation, leading to altered cell population and functional changes in the intestine. The intestine acts as a barrier to the luminal contents which include bacteria and endotoxins. The gut barrier is altered in certain pathological conditions such as shock, trauma or surgical stress, leading to bacterial or endotoxin translocation from the gut lumen into the systemic circulation [2]. This has been implicated in postoperative complications such as systemic inflammatory response syndrome (SIRS)[1] and multiorgan failure (MOF) [3]. Oxygen free radicals are known to play an important role in gut epithelial damage, which may alter gut barrier function and facilitate bacterial translocation and release of endotoxin. Our earlier work using a rat model has shown that laparotomy and intestinal handling, which could occur during any abdominal surgery, can result in increased intestinal permeability and oxidative stress in the enterocytes [4]. Oxidative stress in this system is mainly due to the generation of superoxide anion by xanthine oxidase and the damage is reversible with time [5]. The integrity and homeostasis of the intestinal mucosa are largely dependent on the continued

[1] *Abbreviations used:* SIRS, systemic inflammatory response syndrome; MOF, multiorgan failure; BSA, bovine serum albumin; EGTA, ethylene glycol-bis-(B-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid; DTT, dithiothreitol; NAD, nicotinamide adenine dinucleotide; ALP, alkaline phosphatase; RA, retinoic acid; RAR, retinoic acid receptor; GI, gastrointestinal tract.

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doi:10.1016/j.abb.2004.11.011

proliferation, migration, and differentiation of the crypt cells [6]. These multipotent stem cells located near the base of each intestinal crypt divide to produce daughter stem cells and more rapidly proliferating transit cell. Transit cells in turn undergo a number of rapid cell divisions and differentiate into mature epithelial cells, migrate to the villus, and either die or are extruded into the lumen [7].

Vitamin A (retinol) plays an important role in the control of differentiation and proliferation of various epithelia of the body [8]. Absence of vitamin A can lead to uncontrolled proliferation of epithelial stem cells that fail to differentiate to the normal phenotype in many lining epithelia. Retinol and its metabolites are essential for growth and cell differentiation, particularly of epithelial tissue [9]. Restricted availability of retinol to the intestinal epithelial cells has been shown to result in impaired proliferation control of these cells [10,11]. To develop its biological function in these cells, retinol has to undergo cytosolic oxidation via retinal to (all-*trans*) retinoic acid [12,13], which is an active ligand for retinoic acid receptors [14,15]. Retinoic acid is the most physiologically active metabolite of vitamin A that modulates biological processes involved in embryogenesis, skeletal development, cellular differentiation, and growth, also it can modulate programmed cell death, may play an important role in the adapting intestine, and it alters intestinal adaptation after partial small bowel resection [16]. Since our earlier study had suggested that surgical stress leads to altered cell proliferation, migration, and differentiation of enterocytes [17] and retinoids play an important role in cell proliferation and differentiation, we hypothesize that changes in retinoid metabolism may play a role in mediating alterations seen in the villus-crypt cells after surgical stress. This was investigated in an animal model, using laparotomy and intestinal handling to induce surgical stress.

Materials and methods

Bovine serum albumin (BSA), ethylene glycol-bis-(B-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), dithiothreitol (DTT), nicotinamide adenine dinucleotide (NAD), allopurinol, *p*-nitrophenyl phosphate, *trans*-retinol, all-*trans*-retinal, and all-*trans*-retinoic acid were all obtained from Sigma Chemical (St. Louis, USA). All other chemicals used were of analytical grade.

Animals

Adult Wistar rats of both sexes (200–250 g) exposed to a daily 12 h light and dark cycle and fed water and rat chow ad libitum were used for the study. The rats were randomly divided into seven groups, group I ($n=4$), control (laparotomy alone without intestinal handling), group III ($n=4$), V ($n=4$), and VII ($n=4$) different time periods such as 1, 12, and 24 h after surgical stress (laparotomy with intestinal handling), group II, allopurinol control (laparotomy alone without intestinal handling after allopurinol treatment), group IV ($n=4$) and VI ($n=4$), 1 and 12 h after surgical stress (laparotomy with intestinal handling after allopurinol treatment). This study was approved by the Animal Experimentation Ethics Committee of the Institution.

Induction of surgical stress in rats

Surgical stress was carried out as described [18]. Briefly, overnight fasted rats were anaesthetized and the abdominal wall was opened by a vertical incision of approximately 4 cm. The intestine was gently moved and the ileocaecal junction was identified. The intestine was handled along its entire length from the ileocaecal junction proximally, simulating the "inspection" that occurs in a clinical setting. The intestine was then replaced back in the abdominal cavity and the whole process was completed within 1–2 min. Following this, the abdominal wall was sutured. The animals were killed by decapitation at 1, 12, and 24 h after the surgical procedure. This experimental model has been well established in our laboratory and many studies have been carried out using this model. For inhibition of XO activity, rats were given intraperitoneal injection of allopurinol (100 mg/kg body weight), 1 h before induction of surgical stress. Following this, the animals were sacrificed and various enterocyte populations were isolated from the intestine.

Isolation of villus and crypt cells from the small intestine

Care was taken both during cell isolation and retinoid extraction to avoid any light and all procedures were carried out under dim light. Following surgical stress, abdomen was opened, entire length of the small intestine was removed and washed gently with ice-cold physiological saline, pH 7.4, containing 1 mM DTT. Enterocytes of various stages of maturation (villus to crypt) were isolated by the metal chelation method as described [19]. Briefly, the intestine was filled with solution A (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, and 5.6 mM Na_2HPO_4, pH 7.3), clamped at both ends and incubated at 37 °C for 15 min in the beaker containing solution A. Following incubation, the luminal contents of the intestine were discarded and the intestine was filled with solution B (phosphate-buffered saline, pH 7.3, containing 1.5 mM EDTA and 0.5 mM DTT), and incubated at 37 °C for different time intervals of 4, 2, 2, 3, 4, 6, 7, 10, and 15 min in the beaker containing solution B. At the end of each time period, the incubated solution containing cells was collected in separate tubes. All these nine fractions were pooled into three. The first three fractions being the villus, the next three