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Vinblastine, an anticancer drug, causes constipation and oxidative stress as well as others disruptions in intestinal tract in rat

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ABSTRACT

The purpose of this study is to examine the gastrointestinal disorders after injection of vinblastine (2 mg kg^{-1} *b.w. i.v.*) in rats. Animals were divided into two equal groups: Group 1 was considered as a control group (NaCl, 0.9%). Group 2 was treated with intravenous injection of vinblastine for 7 days. Loperamide (2 mg kg^{-1}) was injected in a saline solution subcutaneously to induce constipation in another group of rats during the same period. Fecal parameters of the different groups have been determined. At the end of the experiment, animals were anaesthetized and sacrificed by decapitation. The intestinal mucosa specimens were examined for lipid peroxidation, sulfhydryl groups (−SH) and protein carbonylation as well as antioxidant enzyme activities and intracellular mediators. Gastrointestinal motility was realized by the test meal (10% charcoal in 5% gum arabic). In result, statistically significant decreases in the fecal number and water content collected during 24 h were detected in the vinblastine group, but less important than loperamide control group. The animals treated with vinblastine, showed also a significant decrease (13%) of GIT, lower than that of loperamide (34%). The intestinal tissues from vinblastine-treated rats were showed a significant increase in lipoperoxydation and H_2O_2 production as well as a significant depletion of enzymatic and non-enzymatic antioxidants. Added to that, a disruption of intracellular iron and calcium levels was observed. Therefore, the present study provide the first strong evidence that vinblastine induced numerous disruptions in gastrointestinal which are related to oxidative stress and intracellular mediators disorders.

1. Introduction

The magnitude of use of chemotherapeutic agents has demonstrated therapeutic benefits in a wide variety of cancers including gastrointestinal (GI) cancers from the esophagus to the large intestine [1]. However, the gastrointestinal disturbance produced by these xenobiotics is occurring more frequently and with greater toxicological significance than previously thought. Although there are some preliminary clinical studies and reports, there does not appear to be an extensive examination of gastrointestinal damage of numerous chemotherapeutic agents in the rat [2]. Among these agents, include vinblastine which is an anti-cancer or chemotherapy drug used to prevent and treat numerous cancers such as lung cancer, breast cancer and lymphoma. However, it is well known to be associated with various side effects, with toxicity to the GI tract being a major clinical concern. In addition, the chemotherapy can result in the generation of excess ROS/RNS in the cytochrome P_{450} monooxygenase system. On another hand, the enzyme systems, such as the xanthine-oxidase system, and non-enzymatic mechanisms also play a role in creating excess oxidative stress during chemotherapy [3,4].

Constipation is a common problem in public health which marked with feces remain in the colon for prolonged periods of time, leading to water absorption, hardening of stool and excessive straining [5]. It is a risk factor of colorectal cancer [6]. As a drug inducing constipation, the loperamide inhibits intestinal water secretion [7] and colonic peristalsis [8], which extends the fecal evacuation time and delays intestinal luminal transit [9].

The present research was therefore undertaken to study the effect of vinblastine on gastrointestinal motility in relation to oxidative stress in rat.

Abbreviations: CAT, catalase; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; NaCl, sodium chloride; ROS, reactive oxygen species; –SH, sulfhydryl groups; SOD, superoxide dismutase

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2. Materials and methods

2.1. Chemicals and reagents

Charcoal meal, Gum arabic, Hydrochloric acid (HCl), trichloroacetic acid (TCA), 2-Thio-barbituric acid (TBA), nicotinamide adenine dinucleotide, sodium pentobarbital, *p*-hydroxybenzoic acid and butylated hydroxytoluene (BHT) were procured from Sigma chemicals Co (Germany). The vinblastine and loperamide were purchased from local pharmacy. All other chemicals used were of analytical grade.

2.2. Experimental animal groups and ethic statement

Wistar rats weighing 200–230 g (SIPHAT, Ben-Arours, Tunisia) were kept in cages under standard laboratory conditions with tap water and standard ad libitum, in a 12-h/12-h light/dark cycle at a temperature between 21 and 23 °C. All experiments were performed according with the local ethics committee of Tunis University for the use and care of animals in accordance with the NIH recommendations. All experiments were performed at the same time of day (9 h), to avoid the circadian rhythm impacts.

To evaluate the effect of vinblastine on small intestinal motility, the animals were randomly divided into a control group (group I, 10 mL kg^{-1} of saline solution) and two other experimental groups, a standard (loperamide) and vinblastine groups. There were eight animals assigned to each group. The rats (group II) were injected with loperamide (2 mg kg^{-1} of body weight) in a saline solution subcutaneously once per day for 7 days. In parallel, animals of group III were injected by vinblastine (2 mg kg^{-1} of body weight) in a saline solution intravenously under the same conditions as loperamide. We measured the body weight gain, food intake and water intake during the same period.

On day 5 and during 24 h, the wet and dry weights of fecal pellets of the animals were collected and measured. The total number, wet weight and water content of the fecal pellets were determined. The water content was determined by drying fecal pellets at 70 °C for 24 h in an oven and calculating the difference between the weight before and after drying [10]. The water content was calculated as follows:

Fecal water content (%) = [(fecal wet weight − fecal dry weight)/fecal wet weight] × 100.

On the last day, animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (40 mg kg^{-1}) and sacrificed by decapitation. The mucosal intestinal specimens were scraped and then placed in a phosphate buffered saline (PBS) solution, homogenized and centrifuged for 15 min at 9000 × *g*. Supernatants were stored at −80 °C for the determination of biochemical parameters.

2.3. Gastrointestinal transit

The effect of vinblastine and loperamide on movement rate in the intestinal tract was evaluated by the method elucidated by Yu et al. [11] with some changes. Animals fasted for 16 h prior the experiment, but consumed water ad libitum. At 15 min after treatment, different groups of rats received the charcoal meal (10% charcoal in 5% gum arabic). After 30 min, the rats were scarified and the intestinal tract was excised. The distance traveled by the charcoal meal from the pylorus was measured and expressed as a percentage of the total length of the small intestine from the gastro-pyloric junction to the ileocecal junction as follows:

T (%) = B/A × 100

Where T is the intestinal tract motility percent, A is the total length of the intestinal tract, and B is the moving distance of the most distal end portion of the charcoal.

2.4. Serum lipids

To evaluate the content of lipids in the serum such as triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol, the experimental animals were anesthetized on the last day of the experiment, and blood was collected from the abdominal vein via laparotomy. Serum was obtained from the collected blood by centrifugation to 2000 × *g* for 10 min. Triglycerides, total cholesterol, and HDL cholesterol were measured using commercially available kits from Biomaghreb, Tunisia.

2.5. MDA, −SH and protein carbonyls measurement

In intestine mucosa the MDA level was measured according to the double heating method [12]. Briefly, the aliquots from intestine tissue homogenates were mixed with BHT–TCA solution containing 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000 × *g* for 5 min at 4 °C. The supernatant was blended with 0.5 N HCl and 120 mM TBA in 26 mM Tris and then heated at 80 °C for 10 min. Afer cooling, absorbance of the resulting chromophore was determined at 532 nm using a UV–vis spectrophotometer (Beckman DU 640B). MDA levels were determined by using an extinction coefficient for the MDA–TBA complex of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Thiol groups (−SH) level was performed according to Ellman's method [13]. The results were expressed as nmol of thiol groups per mg of proteins. Oxidative damage to proteins induced by vinblastine in mucosal intestine was assessed by estimating the protein carbonylation according to Levine et al. [14]. Results were expressed as μmol carbonyl residues/mg proteins.

2.6. Antioxidant enzyme activities estimations

The activity of superoxide dismutase (SOD) was determined by the method of inhibition of the nicotinamide adenine dinucleotide (reduced) phenazinemetho-sulphate-nitroblue-tetrazolium reaction system as adapted by Kakkar et al., [15] and the results have been expressed as units (U) of SOD activity per mg proteins. Catalase (CAT) activity was estimated by the method of Aebi [16] and the results are expressed as nmol $min^{-1}\ mg^{-1}$ proteins. GPx activity was measured by the procedure of Flohé and Günzler [17] and the results are expressed as nmol GSH $min^{-1}\ mg^{-1}$ proteins.

2.7. Intracellular mediator determinations

H_2O_2 level in small intestine mucosa was performed according to Dingeon et al., [18]. The non-haem iron was measured using ferrozine as described by Leardi et al., [19]. Calcium level was measured using a colorimetric method according to Stern and Lewis [20].

2.8. Statistical analysis

The results were analyzed by one-way analysis of variance (ANOVA) and were expressed as means ± standard error of the mean (S.E.M.). All statistical tests were two-tailed, and a p value of 0.05 or less was considered significant.

3. Results

3.1. Effects of vinblastine and loperamide on body weight and food and water intake

As shown in Table 1, a statistically significant (0.05) decrease in weight gain was detected in the loperamide group (31.97%), when compared with the vehicle groups. But, no significant change in this last was detected when comparing the vinblastine-treated groups (4.30%) with the vehicle groups. The same results were found for food intake. While there was no difference observed concerning the water intake