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Exacerbation of intestinal brush border enzyme activities and oxidative stress in streptozotocin-induced diabetic rats by monocrotophos

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ABSTRACT

The present study was undertaken to investigate the potential of monocrotophos (MCP), one of the widely used broad spectrum systemic organophosphorus insecticides (OPI) in India, to alter small intestinal structure and function. Further, its potential to exacerbate diabetes induced alterations in intestinal structure and function was also studied in experimentally induced diabetic rats. Rats were rendered diabetic with an acute dose of streptozotocin (60 mg/kg b.w.). MCP was orally administered at a sublethal dose (1/20 LD_{50} i.e. 0.9 mg/kg b.w./d) for 15 days to both normal and diabetic rats. MCP significantly increased unit weight of intestine in diabetic rats. MCP alone increased (up to 57%) the activities of intestinal brush border disaccharidases in normal rats and further augmented the enzyme activities in diabetic rats. Similar results were found with intestinal alkaline phosphatase activity. In addition, Na^+/K^+-ATPase activity was found to be aggravated in diabetic rats by MCP treatment. Oxidative stress markers showed similar degree of change in both MCP and diabetic rats while MCP aggravated oxidative stress condition in diabetic rats. Scanning electron microscopy and histological analysis of the small intestine revealed increased length of villi, congestion, goblet cell hyperplasia and infiltration of inflammatory cells in MCP and diabetic rats while MCP also induced necrotic lesions in diabetic rats. Collectively, our findings provide evidence that multiple doses of MCP has the propensity to augment diabetes associated intestinal dysfunctions in rats.

1. Introduction

The intestine is a major organ of the digestive system and is the primary site of exposure to nutrients/toxicants due to its extensive surface area and physiological properties [1]. The intestinal epithelium is one of the most important cellular membranes due to its role in digestive and absorptive functions. The intestine mucosal cell monolayer is also the first barrier that either allows or prevents the entry of food proteins, toxins, commensal gut microorganisms and pathogens, into the underlying tissues. Any alteration in the functional/structural features of the intestine can have a tremendous impact on the overall health of the organisms since this could lead to an inadequate nutrient supply to the organism or result in certain disease pathology or aggravate any pre-existing disease pathology.

The prevalence of diabetes mellitus in humans of all age groups is currently at alarming proportions, and it is estimated that it will increase even further over the next decades. Diabetes mellitus (DM) is a chronic disorder, and besides hyperglycemia, several other factors including dyslipidemia or hyperlipidemia contribute to the development of micro and macrovascular complications of diabetes, which are the major causes of morbidity and death [2]. In addition to the major complications such as cardiovascular disease, nephropathy, neuropathy, retinopathy and diabetic cataract, gastrointestinal (GI) disorders are also common in diabetic patients [3]. Structural and functional alterations in the gastrointestinal tract of diabetic patients are often accompanied by an increase in absorption of intestinal glucose and activities of brush-border disaccharidases. Small intestine of diabetics also exhibit numerous structural changes including hyperplasia and hypertrophy of epithelial cells, increased absorption of sugars and amino acids, and increased endogenous cholesterol and triglyceride synthesis [4]. The pathogenesis of the GI abnormalities is complex of nature, multi-factorial (motor dysfunction, autonomic neuropathy, glycemic control, psychological factors, *etc.*) and is not well understood [5].

Although the etiology of diabetes is not well defined, viral infection, autoimmune disease and environmental factors have been suspected to play major roles. Genetic predisposition and lifestyle choices are generally accepted reasons for the occurrence of type 2 diabetes, it has recently been suggested that environmental pollutants are additional risk factors for diabetes development [6]. Since the withdrawal of organochlorine pesticides from use, organophosphorus insecticides (OPI) have become the most widely used

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insecticides in today's world. Besides exerting neurotoxicity, OPI are also demonstrated to cause a variety of physiological abnormalities including alterations in glucose homeostasis [7], oxidative stress [8] and hyperglycemia [9]. Among these, hyperglycemic potential of OPI has been subjected to intense scientific scrutiny and several mechanisms, including altered gluconeogenesis, are believed to mediate OPI-induced hyperglycemia [10]. Recent evidences point to their lasting effects on metabolism and association with increased risk of developing diabetes [11]. Recent studies from our laboratory have demonstrated that OPI such as dimethoate and acephate disrupt glucose homeostasis, which lead to hyperglycemia in experimental animals [7,9].

Monocrotophos (dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) (MCP) is a broad spectrum systemic insecticide. As a result of its widespread use, monocrotophos has been detected in ground, surface and rain water [12]. Exposure to monocrotophos is known to produce a variety of biochemical dysfunctions in mammals and other experimental animals [13]. Earlier studies from our laboratory had also shown that MCP possesses the potential to intensify the pre-existing disrupted glucose homeostasis and the hepatic/renal oxidative stress among diabetic rats [14] and also alter the lipid profile in normal as well as experimentally induced diabetic rats [15]. Hence, the present work has been envisaged to investigate the impact of monocrotophos on intestinal functions in experimentally-induced diabetic rats. Blood glucose, intestinal disaccharidases, alkaline phosphatase, redox state markers and ultrastructure of intestine were studied in normal and experimentally-induced diabetic rats exposed to repeated oral doses of MCP.

2. Materials and methods

2.1. Chemicals

Standard chemicals used were obtained from Sigma Chemicals Co., USA. All other chemicals used were of analytical grade. Technical grade monocrotophos (78%) was a gift from Hyderabad Chemicals Ltd., Hyderabad, India.

2.2. Animals

Adult male rats (CFT – Wistar strain, 8 weeks old, 200 ± 10 g) were used for the study. They were housed in polypropylene cages at room temperature (25 ± 2 °C) with relative humidity of 50–60% and on a 12 h light–darkness cycle. They had free access to food and water ad libitum. The rats were acclimatized to the commercial diet (Saidurga Feeds and Food, Bangalore, India) for 7 days prior to the start of the experiment. All procedures with animals handling conducted strictly in accordance with guidelines approved by the Institute Animal Ethical Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering, and in addition, the number of rats used was kept to a minimum.

2.3. Induction of diabetes

Animals were administered a single dose of streptozotocin (STZ, 60 mg/kg b.w., i.p.) dissolved in 100 mM citrate buffer pH-4.5 [15]. Since STZ produces fatal hypoglycemia as a result of enormous pancreatic insulin release, STZ-treated rats were given 5% glucose water for 24 h. Blood glucose was monitored in rats after 48 h of STZ injection, by Accucheck Sensor Glucometer and rats showing blood glucose levels above 250 mg/dl were considered as diabetic rats.

2.4. Experimental design

All rats were grouped by randomized design into four groups of six animals each as follows:

Group A: Control, administered distilled water orally.
Group B: MCP (dissolved in distilled water) administered orally at 0.9 mg/kg b.w./d for 15 days.
Group C: STZ injected intraperitoneally at 60 mg/kg b.w.
Group D: STZ (60 mg/kg b.w., i.p.); 48 h later, MCP (oral, 0.9 mg/kg b.w./d for 15 days).
The dosage of MCP was equivalent to 1/20 LD_{50} and the criteria of selection of this dosage was based on preliminary dose-determination study [15].

During the treatment period change in body weight of rats was monitored at regular intervals. After 15 days of MCP treatment, rats of all the groups were sacrificed, blood was collected by cardiac puncture into tubes and serum was separated. Small intestine was taken out, cut into duodenum, jejunum and ileum. Each part was flushed with chilled 0.9% (w/v) NaCl solution. Intestine length and weight was measured.

Jejunum segment of the small intestine was selected for all biochemical, histological and scanning electron microscopic studies. Intestinal (jejunum) lumen was cut open; mucosa was scraped off using a glass slide. A 10% (w/v) homogenate was prepared in 100 mM phosphate buffer, pH 7.4 and centrifuged at 8000*g* for 15 min at 4 °C in a refrigerated centrifuge. The supernatant so obtained was used for disaccharidases and phosphatase assays. For the estimation of acetylcholinesterase activity and redox state markers, whole jejunum tissue was taken, homogenized in phosphate buffer (0.1 M, pH 7.4 – at 10%) or in trichloroacetic acid (at 20% for GSH), centrifuged at 10,000*g* and supernatants were used.

2.5. Estimation of blood glucose

Blood glucose was estimated using the commercial kit based on the GOD–POD method (Span Diagnostics Ltd., Mumbai) and the results were expressed as mg glucose/dl.

2.6. Disaccharidase activity

Disaccharidase activity was measured according to the method of Dalquist [16]. In brief, appropriate volume of homogenate (as the source of enzyme) was incubated with 56 mM substrates (sucrase, maltase, lactase and trehalase) in maleate buffer (0.1 M, pH 6.5) at 37 °C for 10 min. The reaction was stopped by the addition of 0.5 M Tris, and the liberated glucose was estimated using the commercial kit (Span Diagnostics Ltd., Mumbai) based on the GOD–POD method and results were expressed as nmol glucose released/min/mg protein.

2.7. Alkaline phosphatase (ALP) activity

Alkaline phosphatase was estimated using p-nitrophenyl phosphate (PNPP) as substrate [17]. In brief, brush border homogenate was incubated with 10 mM substrate in Tris buffer (50 mM, pH 10.1) containing 50 mM $MgCl_2$ for 10 min at 37 °C. Later reaction was stopped by adding 0.05 N NaOH. For blank, sample was added after NaOH addition. Absorbance was read at 405 nm against respective blanks and results were expressed as nmol p-nitrophenol (PNP) formed/min/mg protein.