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Folic acid induces acute renal failure (ARF) by enhancing renal prooxidant state

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ABSTRACT

Systemic administration of folic acid (FA) in mice was used for studying the pathogenesis associated with acute renal failure (ARF). However, the mechanism by which FA induces ARF remains poorly understood. The present study therefore, was planned to investigate the effect of folic acid administration on prooxidant state and associated ultrastructural changes in renal tissue. Balb/c male mice of 4–6 weeks old were divided into control and two folic acid treatment groups (Groups A and B). The animals in group A were administered intraperitoneal injection of folic acid (100 mg kg^{-1} body weight) for a period of 7 consecutive days while the animal in group B were administered a single intraperitoneal dose of folic acid (250 mg kg^{-1} body weight). The renal tissues were collected and used for the analyses of lipid peroxidative indices and activities of antioxidant enzymes in renal tissues. To corroborate biochemical findings scanning electron microscopy (SEM) in renal tissue was studied. Folic acid treated animals demonstrated marked renal hypertrophy accompanied by severe impairment of renal function. Glutathione levels (GSH) and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) levels were significantly decreased and LPO levels increased following FA treatment. SEM results further substantiated the observed biochemical changes as evident by severe inflammation in glomeruli, swelling in primary and secondary pedicels, blebbing in villi, and tremendous deprivation of erythrocytes (RBCs) in FA treated kidneys. The present study therefore suggests that acute administration of folic acid leads to the generation of oxidative stress and altered membrane architecture responsible for folic acid induced ARF.

1. Introduction

Folic acid (FA) is one of the model compound commonly used to study the pathophysiology associated with acute renal failure (ARF) (Cheng et al., 2005; Ortega et al., 2005; Szczypka et al., 2005; Fiaschi-Taesch et al., 2004). Variety of nephrotoxins viz. mercury, gentamicin, glycerol, cisplatin, cyclosporine A is known to cause ARF (Baliga et al., 1999). As a member of Vitamin B complex, it is generally needed for cell replication and growth and helps forming building blocks of DNA, RNA. High concentration of folic acid has been found to be toxic to the various organs of the body especially kidney. Folic acid induced acute renal failure (FA-ARF) is associated with the rapid appearance of folic acid crystals within the renal tubules and subsequent acute tubular necrosis, followed by epithelial regeneration and renal cortical scarring (Bosch et al., 1993; Mullin et al., 1976). FA-ARF is characterized by tubular injury, including tubular cell apoptosis, as well as tubular cell proliferation, inflammatory cell infiltration, and mild fibrosis in the chronic phase (Ortega et al., 2005; Doi et al., 2006; Fang et al., 2005; Dai et al., 2002; Ortiz et al., 2000). Interestingly, all these features are also found in human ARF, suggesting that FA-ARF is an excellent model that mimics human ARF. But little is known of the mechanism(s) by which folic acid mediates its toxicity and its effect on the renal prooxidant state.

The involvement of oxidative stress in the progression in renal injury has been recognized a key player in pathophysiologic pathways of a wide variety of progressive and experimental renal diseases (Haugen and Nath, 1999). Kidney has a very active oxidative metabolism because of its transport function which results in the production of reactive oxygen species (ROS), which left unchecked, can damage all major cellular components and lead to a state of oxidative stress (Maser et al., 2002). Therefore, the present study was designed to investigate the effect of folic acid treatment under acute and short-term administration in mice renal prooxidant state. The study presented here demonstrates that following acute administration of folic acid induces a prooxidant state

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Table 1
Effect of folic acid administration on body weights in mice.

		Body weight (g)
Control		
Group A	7 days	29 ± 2
		20 ± 3*** (−31%)
Group B	12 h	27 ± 2 (−6.89%)
	24 h	26 ± 3 (−10.34%)
	36 h	27 ± 2 (−6.89%)

*$P<0.05$, **$P<0.01$, ***$P<0.001$ as compared to control (student t-test); $n=6$.

in renal tissue by increasing lipid peroxidation and reduced antioxidant enzyme protection which could be the key step in initiation of ARF.

2. Materials and methods

2.1. Chemicals

Folic acid was purchased from Sigma–Aldrich (St Louis, MO, USA). All other chemicals used were of analytical grade and purchased from local Sigma–Aldrich.

2.2. Animals and their treatment

Male balb/c mice of 4–6 weeks old weighing 20–30 g were purchased from a colony raised in the Central Animal House, Panjab University, Chandigarh, India. The animals were housed on husk bedding in clean polypropylene cages and were fed Hind Lever pellet diet and water *ad libitum*. The protocols used in the study were strictly according to the guidelines on the human use and care of laboratory animals and were approved by the ethical committee of the Panjab University. The mice were randomly segregated into various groups with each group having six animals.

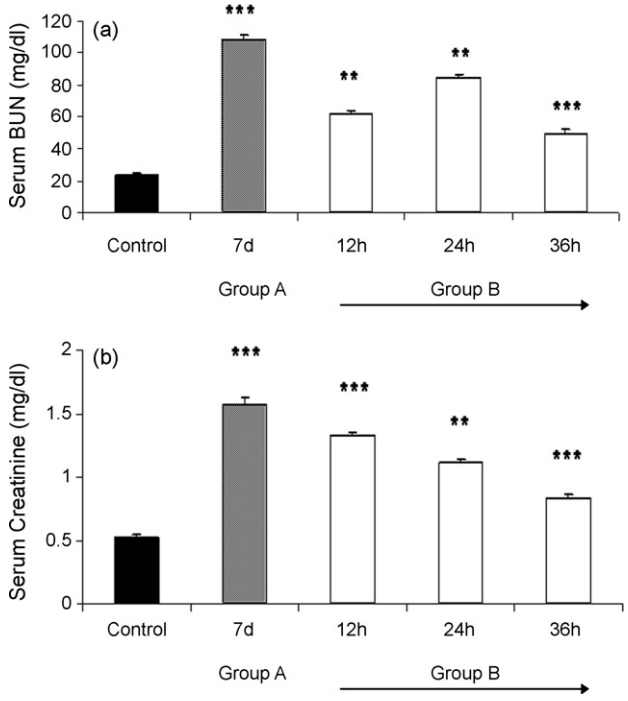

Fig. 1. Effect of folic acid administration on (a) serum blood urea nitrogen (BUN) and (b) serum creatinine in group A (100 mg/kg i.p.) and group B (250 mg/kg i.p.). *$P<0.05$, **$P<0.01$, ***$P<0.001$ as compared to control (student t-test); $n=6$.

3. Experimental design

Animals were divided into two groups, control (C) and folic acid treatment group. FA treated group was further subdivided into two groups, group A and group B. The animals in group A were administered intraperitoneal injection of folic acid (100 mg kg^{-1} body weight) for a period of seven (7) consecutive days, and were sacrificed on day 8th. The animals in group B were administered a single intraperitoneal dose of folic acid (250 mg kg^{-1} body weight) and were sacrificed after 12, 24 and 36 h intervals. Folic acid was dissolved freshly in double distilled water each time before administration. Body weights were also measured every day before administering folic acid. These doses of folic acid though induced nephrotoxicity but were also associated with a morbidity rate of <5% for the experimental period.

3.1. Renal function

To carry out renal function studies the serum was prepared after collecting the blood samples through the cardiac puncture of the animal following overnight fasting. Renal function was assessed by measuring the levels of creatinine and blood urea nitrogen (BUN) using a commercially available kit (Reckon Diagnostics Pvt Ltd, Baroda, INDIA). The level of serum creatinine and BUN was expressed as milligrams per 100 ml (mg/dl).

3.2. Sample preparation and biochemical assays

Animals were euthanized post-dosing (7 days in group A and 12, 24 and 36 h interval in group B after the last injection) by cervical

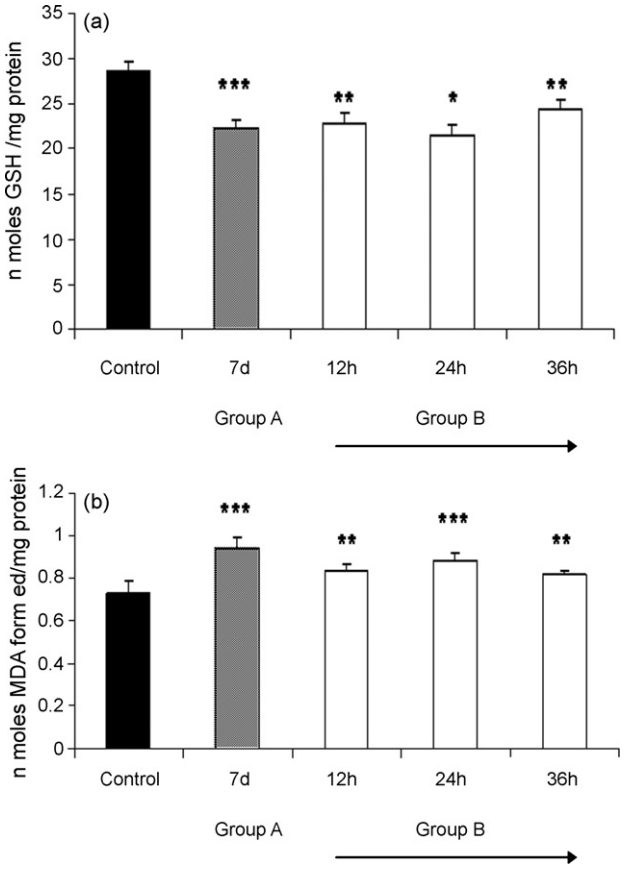

Fig. 2. Effect of folic acid administration on (a) GSH levels and (b) MDA levels in group A (100 mg/kg i.p.) and group B (250 mg/kg i.p.) in mice kidney homogenate. *$P<0.05$, **$P<0.01$, ***$P<0.001$ as compared to control (student t-test); $n=6$.