Accelerated apoptosis in the zinc-deficient rat

Makoto NODERA, Hiroyuki YANAGISAWA and Osamu WADA

Department of Hygiene and Preventive Medicine, Faculty of Medicine, Saitama Medical School

Summary

To examine the effect of zinc (Zn) deficiency, we investigate the acceleration of apoptosis in Zn-deficient rat. Six-week-old male Sprague-Dawley (SD) rats were fed on diets supplemented 0 or 0.02% Zn. After 90 days from administration of the Zn diet, detection of apoptotic cells in skin, thymus and kidney was carried out using TUNEL (TdT-mediated dUTP-biotin nick end labeling) method. When compared to rat on 0.02% Zn diet, the number of TUNEL-positive cells in the thymus and skin was higher in the rat on 0% Zn diet. Furthermore, TUNEL-positive cells were detected in the renal cortex on the 0% Zn diet, however, none were detected in the rat on the 0.02% Zn diet. These findings suggest that Zn deficiency may affect the cell cycle.

Introduction

Zinc (Zn) is one of the essential trace elements for the maintenance of life, and is involved in the activation of many enzymes, including alkaline phosphatase (ALP) and carbonic anhydrase[1)]. Zn deficiency inhibits growth and causes dysgeusia or keratinization of the skin[1,2)]. However, the mechanism of these symptoms that can be attributed to Zn deficiency have no yet been fully clarified. In addition, the role of Zn in the body is not fully elucidated. In the present study, Zn deficiency rats were prepared to investigate the effect of long-term Zn deficiency with respect to apoptosis.

Metods

Two types of special diets supplemented different amounts of Zn were prepared: Zn-deficient diet (0% Zn diet) and Zn-adequate diet (0.02% Zn diet)[3)]. Two 6 week-old male Sprague-Dawley (SD) rats were administered either a 0% Zn diet or 0.02% Zn diet. The rats received distilled water ad libitum. Following administration of the respective diet for 90 days, the skin, thymus and kidney were extirpated under ether anesthesia. Just before the extirpation, blood sample was collected from the aorta to measure the serum concentration of Zn. The extirpated organs were fixed in 10% formalin for 48 hours and embedded

in paraffin. The paraffin block were cut into 4 μm sections, stained using a hematoxylin and eosin (H-E) stain and observed under a light microscope. The paraffin blocks were also cut into 6 μm sections to identify apoptotic cells by the TUNEL (TdT-mediated dUTP-biotin nick end labeling) method using TaKaRa In situ poptosis Detection Kit (Takara, Japan). TUNEL method was carried out as follows: the paraffin was first remove from the specimens, and the specimens were then immersed in proteinase K and 3% H_2O_2 at room temperature for five minutes each (3% H_2O_2 was used to block endogeneous peroxidase). The specimens were further subjected to labeling by TdT-enzym (37°C for 60 minutes) and an antibody procedure using anti-FITC HRP conjugate (37°C for 30 minutes). Apoptotic cells marked by DAB (Dako EPOS/HRP, Dako, Japan) were observed under a light microscope.

Result and Discussion

Body weight of the rat fed a 0% Zn diet did not increase throughout the experiment period (Fig. 1). This rat had skin erythema surrounding eyelids, nose and mouth after 30 days from administration of 0% Zn diet, and marked systemic hair loss was confirmed after 50 days. On the other hand, body weight of rat fed a 0.02% Zn diet increased 120g for 90days, and no abnormalities were observed. Histological examination did not reveal any abnormalities of the skin, thymus or kidney of the rat on the 0.02% Zn diet. However, the skin of the rat on the 0% Zn diet showed psoriasiform acanthosis consisting of hyperkeratosis with parakeratosis. These findings are typically seen in man with enteropathica acrodermatitis, which is one of the symptoms of congenital Zn deficiency[4]. In addition, the serum concentration of Zn was 31 μg/dl in the rat on the 0% Zn diet and 124 μg/dl in the rat on the 0.02% Zn diet. These results indicate that the rat on the 0% Zn diet is Zn deficiency state[3]. Furthermore, significant atrophy was confirmed in the thymic cortex of the rat on the 0% Zn diet, but no histopathologically significant changes were de-

Fig. 1 Zn-deficient (left) and -adequate (right) rats. Zn-deficient rat did not gain any body weight throughout the experimental period.

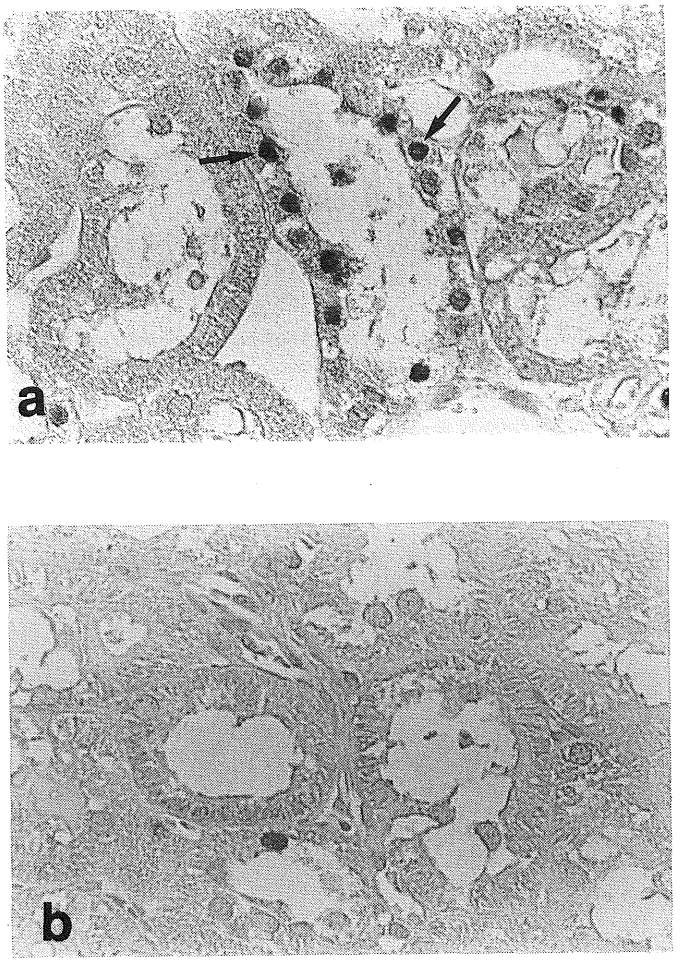

Fig. 2 TUNEL-positive cells (stained dark as indicated with arrows) in the distal renal tubules of the cortex in the rat on Zn-deficient diet (a) and Zn-adequate diet (b).

tected in the kidney.

The TUNEL method detects apoptotic cells by attaching fluorescent-dUTP to the free 3'-OH terminal ends of DNA fragments using terminal transferase, and then observing specimens under a microscope. In the present study, TUNEL-positive cells were defined as cells that were stained brown with DAB. When compared to the rat on 0.02% Zn diet, the number of TUNEL positive cells in the thymus and skin was higher in the rat on the 0% Zn diet. Furthermore, many TUNEL-positive cells were detected in the distal renal tubules of the renal cortex of the rat on 0% Zn diet, however, none were detected in the rat on the 0.02% Zn diet (Fig. 2). These findings suggest that although Zn deficiency may not cause significant pathological changes in tissue, it may affect the cell cycle. Further research is necessary to clarify this issue.

Acknowledgements

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