

CYCLOPHOSPHAMIDE METABOLITES PERFORATE THE APICAL PLASMA MEMBRANES OF UROTHELIAL CELLS IN RATS

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Abstract: The apical plasma membrane of differentiated superficial urothelial cells is characterised by the presence of asymmetric unit membrane (AUM). Cyclophosphamide (CP) metabolites cause perforation of these thickened membranes. In this study, apical plasma membranes were examined after CP injection by electron microscopy. The immediate effect of the CP metabolites was observed as small round holes appearing, first in the asymmetric apical plasma membrane of terminally differentiated superficial cells, and later in the symmetric apical plasma membrane of exposed undifferentiated intermediate and basal cells. Exposed cells which remained undamaged, immediately underwent maturation of the symmetric apical plasma membrane. These results indicate that CP metabolites perforate the symmetric and asymmetric membranes of most urothelial cells.

Key Words: Apical Plasma Membrane, AUM, Maturation, CP

INTRODUCTION

The apical plasma membrane of terminally differentiated superficial urothelial cells is composed of asymmetric unit membrane (AUM) that forms rigid plaques [1]. CP is metabolised, and approximately 70 per cent of the metabolites are excreted in the urine within 4 hours after administration [2]. The initial response to the CP metabolites seems to be perforation of the apical plasma membrane, followed by progressive detachment of the superficial urothelial cells and the exposure of undifferentiated cells [3].

Our study was conducted to clarify what kind of effect CP metabolites have on the apical plasma membranes of the urothelial cells.

MATERIALS AND METHODS

Male, 5-week-old F344 rats, housed at $23 \pm 2^{\circ}\text{C}$ and 50-60% relative humidity, were fed a basal diet and given water *ad libitum*. The rats received a single ip injection of CP (Sigma) at doses of 100 mg per kg body weight, or a placebo injection of distilled water. They were killed 1, 8, 18 and 24 hours after CP

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Abbreviations: AUM – asymmetric unit membrane, CP – cyclophosphamide

injection. The urinary bladders were processed for scanning electron microscopy by the standard technique.

RESULTS AND DISCUSSION

In untreated animals, the rigid, asymmetric apical plasma membrane of terminally differentiated superficial urothelial cells was undamaged. In treated animals, the immediate effect of CP metabolites was first observed as small round holes appearing in these membranes (Fig.1A).

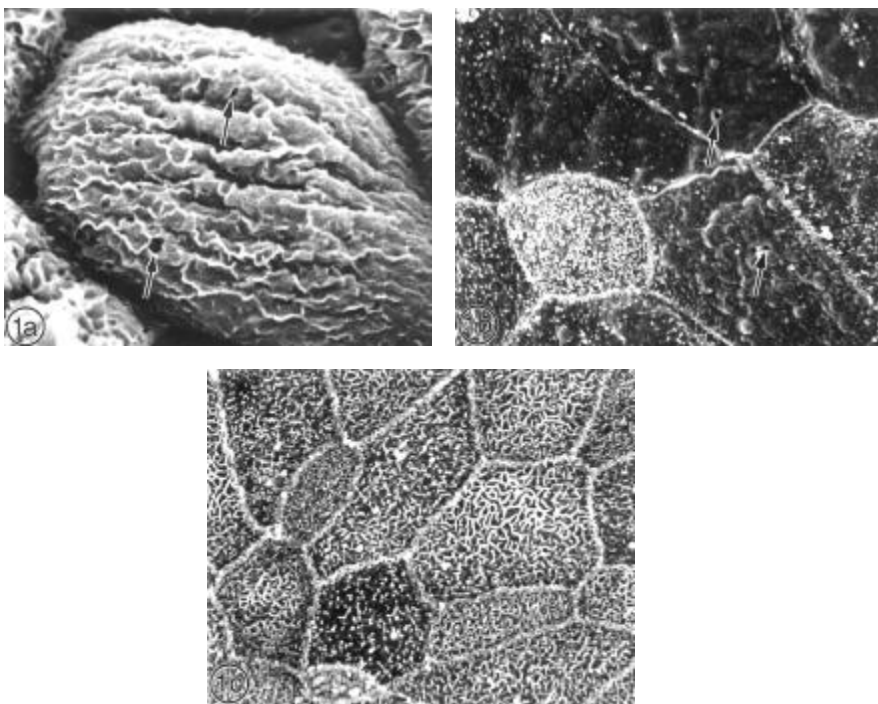


Fig.1. SEM micrographs. (a) small round holes (arrow) in the rigid, asymmetric apical plasma membrane of terminally differentiated superficial cells 8 hours after CP, (b) small round holes (arrow) in the smooth, symmetric apical plasma membrane of exposed undifferentiated cells and intact membranes with microvilli 24 hours after CP, (c) cells with short microvilli, pleomorphic microvilli, and rosy microridges 24 hours after CP. All magnifications $\times 3,000$.

Necrosis and detachment of superficial cells caused exposure of undifferentiated intermediate and basal cells with small round holes appearing in their symmetric apical plasma membranes. These holes always accompanied smooth membranes

without microvillar structures, whereby the membranes with microvilli remained intact (Fig.1B).

The membrane rupture probably caused necrosis and prevented maturation, while undamaged cells most likely immediately underwent signal-induced maturation of the apical plasma membrane, forming structures such as short microvilli, pleomorphic microvilli and ropy microridges (Fig.1C). These results indicate that CP metabolites perforate asymmetric membranes as well as symmetric ones, and so AUMs have no protective role against the immediate effect of CP metabolites. Exposed undifferentiated cells with undamaged symmetric membranes are capable of the maturation of these membranes.

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