Bowman's Gland in Nasal Tissues: As A Possible Biomarker for Vitamin A Nutritional Study

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ABSTRACT

We have investigated the Bowman's gland of the nasal cavity after ***in vivo*** *study in the Sprague-Dawley rats to asses its biomarker potential in vitamin A nutritional study. Fourty-eight male weanling Sprague-Dawley rats at 3-4 weeks of age were allotted to four groups of 12 each and were fed diets either vitamin A-deficient or supplemented with 30.000 IU vitamin A/kg for 150 days. Rats in their respective groups were also given fume of vehicle for 150 days. Fumigation was done 2x/day for 1 hour each. Rats given vitamin A-deficient diet and fume of vehicle had the most extensive inhibition of glycoprotein synthesis in cells of Bowman's gland in the olfactory region of the nasal cavities as determined by severe loss of Alcian blue-periodic acid-Schiff staining material. Histochemical lesions were not seen in nasal cavity of vitamin A-supplemented rats.*

INTRODUCTION

Good health is the foundation of human welfare and productivity. Health, nutrition and environment are interrelated. As an example of link between environmental condition and health include air pollution and the respiratory illnesses it causes. Vitamin A is known to be necessary for normal growth and differentiation of epithelial cells and bone (Wolbach, 1954; Anonymous, 1994). A deficiency of vitamin A results in squamous metaplasia in which a squamous keratinizing type of epithelium replaces the normal form of epithelium of a variety of organ, including respiratory tract (Wolbach and Howe, 1925; De Luca *et al.*, 1994) and in addition that the administration of vitamin A enhanced the capacity of those organs to regenerate (Anonymous, 1994; De Luca *et al.*, 1994).

In this study, we have used rats nasal epithelial cells of Bowman's glands in the olfactory region whose activity can be detected by histochemistry and have examined the differentiation capacity of these epithelial cells *in vivo* vitamin A nutritional-environment study.

MATERIALS AND METHODS

Experimental design

Fourty-eight male weanling Sprague-Dawley rats weighing approximately 60 g at 3-4 weeks of age were used. Rats were acclimated for 7 days prior to the study and fed a commercial pelleted diet and tap water *ad libitum*.

Rats were randomly allotted to four groups of 12 each and were fed diets either vitamin A-deficient (groups A and C) or supplemented with 30.000 IU vitamin A/kg (groups B and D) for 150 days. Rats in their respective groups were also given fume of vehicle for 150 days. Fumigation was done 2x/day for 1 hour each.

Rats were housed six per cage in stainless wire-top plastic cages, and bedding was changed twice weekly. Rats were observed daily for clinical signs. Moribund or dead rats were necropsied immediately after discovery.

Necropsy and histochemical procedures

The experiment was terminated on days 150. All rats were anesthetized with ether and killed by decapitation. The nasal cavities were collected and fixed in 10% neutral buffered formalin. Nasal cavities were decalci-

fied and sectioned according to the standard procedures (Young, 1981). Serial sections of the nasal cavities were embedded in paraffin, sectioned (6 µm), and stained with Alcian blue-periodic acid-Schiff (AB/PAS), pH 2.5 (Mowry and Winkler, 1956).

RESULTS AND DISCUSSION

Results of this study demonstrated that specific cells of the nasal cavity are highly susceptible to nutritional pathologic effects of vitamin A-deficient diet. These are determined by inhibition of glycoprotein synthesis in cells of Bowman's glands in the olfactory region of the nasal cavities. There appeared to be loss of Alcian blue periodic acid-Schiff (AB/PAS) staining material in some cells of Bowman's glands in the olfactory region of the nasal cavity.

In the present study, inhibition of AB/PAS staining material was not evident in the Bowman's glands of the nasal cavities in rats supplemented with vitamin A (Fig 1). The most dramatic histochemical changes, however, occurred in cells of Bowman's glands in the olfactory region of nasal cavities of rats given vitamin A-deficient diet and fume of vehicle. The combination treatment given to rats caused a dramatic decrease in the amount of glycoproteins in Bowman's glands as determined by the AB/PAS stain (Fig. 2). Therefore, It is demonstrated that the combined effect of a vitamin A-decifient diet and fume of vehicle adversely affected the glycoprotein synthesis in specific cells of Bowman's glands in the nasal cavity. The mechanism(s) whereby vitamin A-deficiency or a combination of vitamin A deficiency and fume of vehicle induced inhibition of glycoprotein synthesis in cells of Bowman's glands in the olfactory region of the nasal cavities is unknown. It is possible that fume of vehicles could cause defective absorption of nutrients from the gut based on the fact that tetrachlorodibenzo-p-dioxin (TCDD) was reported to impair active intestinal absorption of glucose and leucine in male Sprague-Dawley rats (Ball and Chhabra, 1981). Vitamin A deficiency impairs animal's ability to utilize, especially protein (Hayes, 1971).

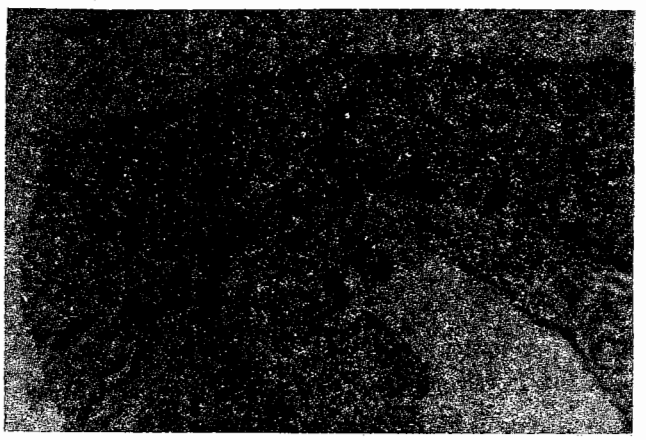

Figure 1. **Photomicrograph of nasal cavity from a rat supplemented with vitamin A. Notice glycoprotein staining as dark AB/PAS-positive granules in cells of Bowman's glands (AB/PAS, 100x).**

Figure 2. **Photomicrograph of nasal cavity from a rat fed vitamin A-deficient diet and given fume of vehicle. Notice a dramatic decrease in the amount of glycoprotein in cells of Bowman's glands (AB/PAS, 100x).**

As mentioned above, there was no apparent inhibition of AB/PAS staining material in cells of Bowman's glands in the olfactory region of the nasal cavity in rats supplemented with vitamin A. The mechanism by which vitamin A is able to elicit this effect may reside in its ability to regulate gene expression at specific target sites within the body (organs, e.g. nasal tissues) (Mangelsdorf, 1994). However, its mechanism of action in this process was unknown until 1987, when researchers discovered nuclear receptors specific for vitamin A metabolite (retinoids) (Petkovich, 1992). These receptors are activated by retinoids and regulate gene expression by binding to short DNA sequences in the vicinity of target

genes (Blomhoff, 1994). It appears that the diversity of effects by retinoids on cells can be explained in part by the diversity of all-*trans* retinoic acid (atRA) receptor.

The histochemical effects of vitamin A-deficient diet on the nasal tissues were similar to that previously reported (Jensen and Sleight, 1987; Wasito, 1987). They reported N-nitrosodiethylamine (NDEA) whether given to rats or hamsters caused a dramatic decrease in the amount of glycoproteins in cells of Bowman's glands in the olfactory region of nasal cavities. They hypothesized that the unique susceptibility of cells of Bowman's glands to the effects of NDEA may be due to the site of deposition of NDEA in nasal tissues and by the presence of metabolizing systems in Bowman's glands. When radiolabeled nitrosamines are administered intravenously, there is a localization of tissue-bound metabolites in cells of Bowman's glands of rats (Brittebo and Tjalve, 1983) and hamsters (Reznik-Schuller, 1983). For chemicals such as NDEA which require metabolism for toxic effects, nasal membranes of rats and hamster have cytochrome P-450-dependent monooxygenase activity (Hadley and Dahl, 1983). One site of cytochrome P-450 and associated enzymes in the nasal cavity is cells of the Bowman's glands (Voight *et al.*, 1985). Cells of Bowman's glands in rats were more susceptible to necrogenic effects of NDEA than those of hamsters (Jensen and Sleight, 1987). Since reactive intermediates are the proposed cause of toxicity (Kroeger-Koepke *et al*, 1981), species differences in the metabolism of NDEA could explain differences in the toxicity of NDEA.

The finding of histochemical changes in Bowman's glands of rats indicates that tissues of respiratory tract, especially the upper respiratory tract (the nasal cavity) can be a primary target organ not only for vitamin A and air pollution, but also for toxic chemical, such as NDEA. Whether the histochemical lesions are critical determinant of susceptibility to respiratory tract carcinogenesis remains to be determined.

CONCLUSION

Thus, this finding provides evidence of the importance of vitamin A for the survival of the function of specific cells in the nasal cavities and that Bowman's glands of the nasal cavity is important target organ in the nutritional pathology experiment in the present study.

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