Estrogen Metabolism and Laryngeal Papillomatosis: A Pilot Study on Dietary Prevention

KAREN AUBORN\textsuperscript{1}, ALLAN ABRAMSON\textsuperscript{1}, H. LEON BRADLOW\textsuperscript{2}, DANIEL SEPKOVIC\textsuperscript{2} and VIRGINIA MULLOOLY\textsuperscript{1}

\textsuperscript{1}Department of Otolaryngology and Other Communications Diseases, Long Island Jewish Medical Center, The Long Island Campus of Albert Einstein College of Medicine, New Hyde Park, NY 11040; \textsuperscript{2}Strang Cancer Research Institute, New York, NY 10021, U.S.A.

Abstract. Evidence exists that estrogen metabolism has a role in the pathogenesis of recurrent respiratory papillomatosis (RRP). This disease has a papillomavirus etiology and is characterized by recurrent benign tumors with a significant propensity to become malignant. We have measured the systemic transformation of estrogen using an enzyme-linked-immunoassay to measure estrogen metabolites in the urine of patients with RRP and compared these ratios to the severity of RRP, a measure of the average growth rate of papillomas. Our results show an inverse relationship between the ratio of C-2 to C-16α-hydroxylated estrogens and the severity of RRP. In a pilot study, patients consumed cruciferous vegetables to induce C-2-hydroxylation. In this group of patients, an increase in the ratio correlated with an improvement in RRP. The ratio did not change in a subset of these patients, and their RRP did not improve. Regardless, the ratio correlated with severity of their RRP.

Recurrent Respiratory Papillomatosis (RRP) is a serious and potentially life-threatening disease which affects both children and adults. The benign tumors (papillomas) present as exophytic pedunculated masses, most often located on the true vocal cords and the epiglottis. These papillomas compromise the voice and can block the airway. These lesions usually remain benign, but malignant conversion can occur. Standard treatment for laryngeal papillomatosis is surgical ablation of all visible papillomata with a CO\textsubscript{2} laser (1). The etiologic agents of RRP are certain types of human papillomavirus (HPV), the same HPVs that cause exophytic lesions in the cervix (2). HPV DNA can be detected in tissue that is histologically normal throughout the respiratory or genital tracts (3,4,5). However, the larynx in the respiratory tract (6) and the cervix in the genital tract, are much more prone to develop papillomas than other areas that contain HPV DNA (7,8,9).

Hormones determine, at least in part, why the larynx and cervix are more likely to develop papillomas than other tissues infected with HPVs. Estrogen is known to exacerbate the HPV-induced lesions during pregnancy (10-11). In the larynx, estrogen binds cell membranes, and this binding is greater using membranes derived from papillomas (12). In the genital epithelium, most papilloma disease and subsequent cancers occur in the transformation zone of the cervix, the most estrogen responsive site (7). Moreover, estrogen increases expression of viral proteins (13).

As shown in Figure 1, the major metabolites of estradiol are those hydroxylated at either C-2 or C-16α positions, namely 2-hydroxyestrone (2-OHE1) and 16α-hydroxyestrone (16α-OHE1). C-2 and C-16α metabolism are competitive alternatives, and increases in the activity of one of these two oxidative pathways results in decreased activity of the other. The C-16α metabolites are fully estrogenic (14), whereas the C-2 metabolites are devoid of peripheral estrogenic activity. Additionally, 2-OHE1, the principal C-2 metabolite in humans is weakly antiestrogenic (15).

A concordance exists between the extent of 16α-hydroxylation of estradiol and the site of most papillomas in the genital tract (16). In larynx, 16α-hydroxylation of estradiol occurs at a high constitutive rate and is greater in papillomas (17) with no differences between men and women. Moreover, indole-3-carbinol (13C) an inducer of 2-hydroxylation, prevents papillomas in laryngeal xenografts in a mouse model (17). The relative use of these different types of estrogen metabolism is clear in the HPV induced pathology in the genital tract. The ratio of C-2/C-16α hydroxylated estrogens is decreased in women with cervical intraepithelia neoplasia (18). Together, estrogen and its metabolism contribute to the pathology of HPV infections.
In this study, we asked about the relative importance of C:2 and C-16α hydroxylation in RRP. We asked whether we could induce C:2 hydroxylation with a diet enriched in cruciferous vegetables and whether induction of 2-hydroxylation results in improvement in RRP.

**Patients and Methods**

*Assay for estrogen metabolites.* 2-Hydroxyestrone (2-OHE1) and 16α-hydroxyestrone (16α-OHE1) in urine were measured in an enzyme-linked-immunoassay as previously described (19). Urine was preserved with vitamin C and frozen until analyzed. Briefly, the assay is a competitive enzyme immunoassay in which binding of an antigen-enzyme conjugate to immobilized antibody is inhibited by addition of free antigen. Reagents were from Immunacare Corp, Bethlehem, PA.

*Evaluation of disease.* At each direct endoscopy, the extent of papilloma growth was measured using the numerical scoring system first described by Kashima et al (20) and modified by Abramson et al (21). Briefly, the five major anatomic sites in the larynx (epiglottis, false cords, ventricle, true cords and subglottis) subdivided into 23 sub-sites were evaluated according to the number of sites infected, the surface area involvement and extent of lumen obstruction. The severity of disease or average growth rate was calculated by this numerical score at the time of endoscopy divided by the number of days since the last surgery.

*Diets.* The diets were the patient's normal diet but included the daily consumption of cruciferous vegetables. For an adult weighing greater than 120 lbs, this amount was two cups of finely chopped vegetables or 16 oz. The vegetables could be consumed raw, cooked or juiced. Proportionally less was recommended for persons weighing less than 120 lbs.

**Results**

Estrogen metabolism was compared to the severity of RRP in patients to determine if there was any difference in metabolism in individuals with moderate disease compared to those with severe disease. Previous results indicated that the extent of 16α-hydroxylation was greater in cells grown from normal papillomas than normal larynx (17), but evaluation of systemic estrogen metabolism and RRP had not been evaluated. The patients were not on any adjunct therapy, but required periodic removal of papillomas. The amount of 2-hydroxyestrone (2-OHE1) and 16α-hydroxyestrone (16α-OHE1) in urine were determined and the ratio of 2-OHE1 to 16α-OHE1 calculated. The severity of papilloma disease in the respiratory tract was evaluated using the standard scoring described in methods. As shown in Figure 2, an inverse relationship between the ratio of 2-OHE1 to 16α-OHE1 occurred. Results show that systemic estrogen metabolism reflects severity of RRP.

In a subset of patients, the amount of 2-OHE1 and 16α-OHE1 were normalized to urine creatinine (Table I). The absolute amounts of either metabolite did not correlate with disease score. This result, together with the fact that most patients did not have lower amounts of estrogen, suggests that the metabolic ratio is the important parameter (rather than the absolute amount of a particular metabolite) in the correlation with pathogenicity of RRP.

We tested whether we could induce 2-hydroxylation in patients. Increasing the 2-OHE1 to 16α-OHE1 ratio should, in turn, improve their RRP. While 16α-hydroxylation is not readily altered, 2-hydroxylation can be induced by a number of ways (22). Indoles in cruciferous vegetables (cabbage, brussel sprouts, cauliflower or broccoli) induce 2-hydroxylation (23). Patients with moderate to severe RRP (needing surgery at least twice a year for the last 18 months and not currently on any adjunct therapy were eligible for this study. The diet was as described in methods. Patients were their own controls since an extensive history of each patient was available with disease scores. The amount of RRP was scored by direct laryngoscopy at the time of endoscopy prior to the start of diet. Urines were analyzed before diet and at two-week intervals. Diet was started immediately after clearing of papillomas with CO2 laser surgery. The amount of
disease scores was evaluated at regular intervals and compared to past history.

Induction of 2-hydroxylation occurred in some patients and not in others. In this small number of patients, most patients showed increases in the ratio of 2-OHE1 to 16α-OHE1 (Figure 3). This increase occurred in both males and females. In these patients, fluctuations in ratios occurred when using urines from different days were analyzed. No induction of 2-hydroxylation occurred in two patients (#5 and 6). The ratios were very constant when analyzed on different days. RRP did improve in patients when their ratios of the 2-OHE1 to 16α-OHE1 increased (patient 1, 2, 3, 4 and 7). Patients 1 and 7 have remained free of disease. The growth of papillomas in patients 2 and 4 slowed. Patient 3 was alternately free of disease or had very little papilloma growth. No improvement of disease was apparent in the patients 5 and 6, the same patients whose ratios did not change. Although improved, patient 2 has the worst disease and the least favorable 2-OHE1 to 16α-OHE1 ratio. Patients 1, 2, 3, 5 and 6 have been on the diet for greater than 2 years, and patient 4 has been on diet for greater than one year. The implication from this study is that induction of 2-hydroxylation promotes the improvement of disease, and higher ratios correlate with less disease.

**Discussion**

One treatment approach for RRP is preventing the regrowth of papillomas after surgical removal. The goal of this study was to manipulate estrogen metabolism to create a hormonal milieu in laryngeal cells that is less favorable to HPV expression and pathology. We were able to show that a higher 2-OHE1:16α-OHE1 ratio correlated with less severe disease. Moreover induction of a more favorable ratio improved disease in a pilot study.

In this pilot study, a diet rich in cruciferous vegetables was used to induce a more favorable 2-OHE1:16α-OHE1 ratio. The advantages are: 1. Induction is possible with diet. 2. Consumption of these vegetables have other health benefits such as decreasing cancer risk induced not only by papillomavirus but also other cancers. 3. Safety is indicated since consumption of these vegetables in these amounts is a normal part of diet in some parts of the world, e.g. Northern China. However, disadvantages are: 1. Some patients have difficulty complying with diet resulting in a dropout rate which was greater than 50% in this study. 2. The vegetables vary which content of indoles (24), the compounds that induce 2-hydroxylation, in these vegetables. We believe this is
papillomavirus tumors. Our studies are suggestive that patients with the more severe papillomavirus disease have more 16α-OHE1 relative to 2-OHE1 as determined by measurement of these compounds in urine. Our studies are suggestive that a diet heavily enriched with cruciferous vegetables can alter these ratios.

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References


