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Alcohol intake and folate antagonism via CYP2E1 and ALDH1: Effects on oral carcinogenesis

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Abstract

The interaction of folate and alcohol consumption has been shown to have an antagonistic effect on the risk of oral cancer. Studies have demonstrated that increased intake of folate decreases the risk of oral cancer, while greater alcohol consumption has an opposite effect. However, what is poorly understood is the biological interaction of these two dietary factors in relation to carcinogenesis. We hypothesize that cytochrome P450 2E1 (CYP2E1) and the family of aldehyde dehydrogenase 1 (ALDH1) enzymes may play a causal role in the occurrence of oral cancer. Chronic and high alcohol use has been implicated in the induction of CYP2E1, which oxidizes ethanol to acetaldehyde. Acetaldehyde is a known carcinogen. As the first metabolite of ethanol, it has been shown to interfere with DNA methylation, synthesis and repair, as well as bind to protein and DNA to form stable adducts, which lead to the eventual formation of damaged DNA and cell proliferation. Studies using liver cells have demonstrated that *S*-adenosyl methionine (SAM), which is a product of folate metabolism, regulates the expression and catalytic activity of CYP2E1. Our first hypothesis is that as increased levels of folate lead to higher concentrations of SAM, SAM antagonizes the expression of CYP2E1, which results in decreased conversion of ethanol into acetaldehyde. Thus, the lower levels of acetaldehyde may lower risk of oral cancer. There are also two enzymes within the ALDH1 family that play an important role both in ethanol metabolism and the folate one-carbon pathway. The first, ALDH1A1, converts acetaldehyde into its non-carcinogenic byproduct, acetate, as part of the second step in the ethanol metabolism pathway. The second, ALDH1L1, also known as FDH, is required for DNA nucleotide biosynthesis, and is upregulated at high concentrations of folate. ALDH1L1 appears to be a chief regulator of cellular metabolism as it is strongly downregulated at certain physiological and pathological conditions, while its upregulation can produce drastic antiproliferative effects. ALDH1 has three known response elements that regulate gene expression (NF- κ B, C/EBP β , and RAR α). Our second hypothesis is that folate interacts with one of these response elements to upregulate ALDH1A1 and ALDH1L1 expression in order to decrease acetaldehyde concentrations and promote DNA stability, thereby decreasing cancer susceptibility. Conducting future metabolic and biochemical human studies in order to understand this biological mechanism will serve to

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support evidence from epidemiologic studies, and ultimately promote the intake of folate to at-risk populations.

Background

Previous studies have shown that the combined effect of folate deficiency and high alcohol consumption contributes to the risk of colorectal, breast, and oral cancer (1). Since folate is essential for maintaining normal function of nucleotide synthesis and DNA methylation, the disruption of folate metabolism can lead to abnormal cell activity and proliferation. Disruption of DNA repair and methylation, which are believed to enhance risk of carcinogenesis, have all been observed with folate depletion (2). Giovannucci found that folate deficiency is related to the misincorporation of uracil for thymidine during DNA synthesis and to an increased frequency of chromosomal breaks (3). In another study, Ulrich and Potter cite that disturbances in DNA methylation, where folate acts as the key methyl donor, are relevant in carcinogenesis (4).

In addition, alcohol has been shown to antagonize folate by interrupting intestinal folate absorption, increasing folate excretion, as well as cleaving serum folate. Nguyen and Dyer have demonstrated that chronic consumption of alcohol is associated with reduced folate carriers for intestinal uptake of folate in micropigs (5). Furthermore, Shaw et al. have shown that high acetaldehyde levels, the byproduct of high alcohol intake, disrupt available plasma folate levels by cleaving the folate to inactive forms via acetaldehyde/xanthine oxidase-generated superoxide (6). Other studies have reported that individuals who consume chronic high amounts of alcohol are marked by their low levels of serum folate (7).

Based on a more recent prospective cohort study examining folate-alcohol interactions in women's oral cancer risk using data from the Nurses' Health Study (NHS), high alcohol intake in a low folate environment appears to increase the risk of oral cancer in women, while high alcohol intake in a high folate environment appears to decrease the risk (8). Yet, low alcohol consumption along with a high or low folate environment does not seem to affect the risk. In order to understand these results, we need to investigate the interaction between the alcohol and folate pathways and its effect on oral cancer risk.

Other studies derived from the NHS have looked at the joint association of alcohol and folate on conferring the risk of major chronic disease in women, and a similar interaction between alcohol and folate has been observed in other types of cancer. Jiang et al. found that heavy drinkers (>30 g/day) with a lower folate intake (<180 μ g/day) had the highest risk of major chronic disease compared to abstainers with a folate intake of 400-599 μ g/day (Relative risk = 1.36; 95% confidence interval = 1.10-1.70) (9). Other studies, using different study designs and sample populations, appear to support these findings. A hospital-based case-control study from Italy and Switzerland, using 749 cases and 1772 controls, found that the combined effect of high alcohol consumption (≥ 38 drinks/week) and low folate intake (<235.9 μ g/day) led to a 22-fold increase in risk when compared to the combined effect of low alcohol consumption (<14.5 drinks/week) and high folate intake (>300.79 μ g/day) (1). Stolzenberg-Solomon et al. found positive associations between moderate alcohol consumption and breast cancer using a prospective cohort study. Alcohol intake was positive associated with breast cancer risk (highest versus lowest quintile: Hazard ratio = 1.37; 95% confidence interval = 1.08-1.76); women with lower total folate intake had the greatest risk (10). Low folate and excess alcohol consumption were also associated with increased risk of colon cancers with long interspersed nucleotide element 1 (LINE-1) hypomethylation based on an analysis of two previous prospective cohort studies (11).

Furthermore, earlier studies have established how each of these dietary factors, folate and alcohol, play a significant role in an individual's risk for cancer. As an important micronutrient, the metabolism of folate is essential for proper cell growth and function. Humans, however, cannot synthesize their own supply of folate (12). Consequently, folate is mainly provided through diet, particularly through the consumption of citrus fruits and dark-green vegetables. Previously, several studies have shown that high folate consumption through high fruit and vegetable consumption has a protective effect on cancer risk (13,14,15). In addition to folate, ethanol is an independent risk factor for cancer (16). Studies examining the association between alcohol consumption and oral and esophageal cancers have found that alcohol appears to increase the risk overall (17,18,19), although a j- or u-shaped relationship has been demonstrated in prior case-control studies (17).

As ethanol gets metabolized, the enzymes alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1) convert ethanol into its byproduct, acetaldehyde. Acetaldehyde dehydrogenase (ALDH) then breaks down acetaldehyde to acetate. Recent studies have shown that acetaldehyde rather than alcohol itself may be the critical factor to explain the carcinogenic effect of alcohol, affecting normal levels of DNA methylation and indirectly affecting DNA repair systems (20).

Many studies have shown that global and DNA hypomethylation of proto-oncogenes play a significant role in the development of cancerous tumors. Within the folate metabolic pathway, S-adenosyl methionine (SAM)-dependent methylation of specific CpG DNA sites is crucial for the regulation of gene expression (12). However, low dietary folate intake leads to lower levels of available SAM, resulting in decreased genome-wide methylation in the cell. Feinberg and Vogelstein found that hypomethylation in human colon cancer cells showed enhanced expression of specific proto-oncogenes, such as KRAS and c-MYC (12). Moreover, a greater percentage of hypomethylation of LINE-1, an emerging indicator of genome-wide DNA methylation level, has been associated with increased cancers of the colon, breast, stomach, and esophagus, as well as head and neck cancer (21).

Decreased folate metabolism from increased acetaldehyde interference also inhibits nucleotide synthesis, contributing to diminished DNA repair. Low cellular folate inhibits conversion of deoxyuracil monophosphate (dUMP) to deoxythymidine monophosphate (dTTP), leading to higher uracil and lower thymidine concentrations (22). Since uracil and thymidine differ only by a single methyl group, uracil can be misincorporated into DNA in place of thymidine in DNA synthesis. Although DNA repair enzymes quickly remove uracil, the excision leaves a single-stranded break in the DNA molecule. If folate is constantly limited, this cycle of DNA breakage continues, eventually leading to DNA double-stranded breaks and malignant chromosomal aberrations. Additionally, acetaldehyde directly inhibits O6-methylguanylintransferase, an enzyme critical to the repair of adducts caused by alkylating agents (23).

Other mutagenic effects associated with acetaldehyde include the induction of sister chromatid exchanges, gross chromosomal aberrations, as well as causing point mutations in the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus in human lymphocytes (24,25). HGPRT is an important enzyme involved in purine metabolism and synthesis. Acetaldehyde also binds to DNA and protein to form stable adducts, representing a mechanism by which acetaldehyde could increase the occurrence of replication errors and/or mutations in oncogenes or tumor suppressor genes. Acetaldehyde-induced adducts have been found in different organs such as liver and oral tissue (26).

By studying the enzymes that produce and catabolize acetaldehyde, CYP2E1 and ALDH1, respectively, we aim to understand more about how acetaldehyde accumulates in tissues and

can lead to carcinogenic and mutagenic consequences. Furthermore, we hope to illustrate the important role of folate and the expression of CYP2E1 and ALDH1, which may provide us insight on the interaction between folate and alcohol, and the risk of oral cancer.

CYP2E1 expression and products of the folate metabolism pathway

Chronic and high alcohol use has been implicated in the induction of CYP2E1, which oxidizes ethanol to acetaldehyde. While alcohol dehydrogenase (ADH) normally metabolizes ethanol to acetaldehyde, CYP2E1 is induced only at high levels of alcohol consumption and contributes to 30% of acetaldehyde production (27). Acetaldehyde, the first metabolite of ethanol, has been shown to interfere with DNA methylation, synthesis and repair, as well as bind to protein and DNA to form stable adducts, leading to the eventual formation of damaged DNA and cell proliferation (20,28). Activation of CYP2E1 also results in the production of reactive oxygen species (ROS) and oxidative stress, like nitrosamines from tobacco smoking (20). While there have been several studies examining the link between CYP2E1 induction and risk of alcohol liver disease, association between CYP2E1 induction and build-up of acetaldehyde and ROS may be applicable for potential causes of oral cancer risk.

Interestingly, studies have shown that products of folate metabolism, especially in the SAM pathway, regulate the expression and catalytic activity of CYP2E1 (29). In the folate metabolism pathway, a methyl group from folate is used for the conversion of methionine to SAM, the principal methyl donor in the body (1). Studies in mouse liver have shown that folate deficiency is sufficient to induce CYP2E1 expression; however, the mechanism behind the induction is still unclear (30). Caro et al. cite that SAM acts as a non-competitive, irreversible inhibitor of CYP2E1's catalytic activity (31). From *in vitro* models, SAM at pharmacological concentrations exhibited inhibitive binding activity with acetone- and isoniazid-induced rat liver microsomes and with human carcinoma liver tissue, Hep2G1 cells, engineered to only express human CYP2E1. Although the inhibition of SAM is relatively weak compared to the high catalytic activity of CYP2E1, this antagonistic effect of SAM on CYP2E1 activity may attenuate the effect of high ethanol consumption on CYP2E1 induction. Therefore, the overall amount of CYP2E1 produced may decrease or stay at normal levels, reducing the overall carcinogenic build-up of acetaldehyde and ROS. This data supports previous evidence, which suggests that high folate intake has a protective role against the effects of high alcohol exposure.

While most of these studies look at folate metabolism and CYP2E1 in liver cells, the interaction between folate metabolism and CYP2E1 could be applicable to cells in the upper aerodigestive tract. The next steps to take are to further investigate how folate deficiency is sufficient to induce CYP2E1 expression (Figure 1). Perhaps folate deficiency leads to high SAH and homocysteine levels, and these molecules positively interact with CYP2E1 transcription factors. Furthermore, while SAM is known to directly inhibit CYP2E1 activity, whether folate and/or molecules from the folate metabolism pathway affect CYP2E1 catalytic activity remains unknown. Lastly, finding out whether high folate along with high alcohol consumption decreases the risk of cancer in the liver may support the theory that the interaction among folate, alcohol, and CYP2E1 activity in liver cells is similar to the interaction of cells located in the oral cavity and upper aerodigestive tract. These questions will need to be examined using tissue and molecular studies from blood and oral biopsy samples in order to understand how folate affects the activity of CYP2E1 expression by utilizing assays to measure levels of key molecules mediating this proposed pathway.

The ALDH1 family: Enzymes implicated in folate and ethanol metabolism

The enzyme, aldehyde dehydrogenase 1 (ALDH1) plays an important role in both the folate one-carbon pathway and ethanol metabolism. It is found in high concentrations in liver and stomach tissue, but is also expressed in a variety of cells, including the oral cavity (32). Within the ALDH1 family, there are two sub-types that may shed some insight into the folate-alcohol interaction on oral cancer. ALDH1A1 has been found to convert acetaldehyde to acetate as part of the second step in the ethanol metabolic pathway. ALDH enzymes use NAD^+ as a cofactor in the oxidation of acetaldehyde (33). So far only ALDH1A1, ALDH1B1 and ALDH2 have been found to play a significant role in acetaldehyde oxidation (34), and the mitochondrial ALDH2 plays a central role in human acetaldehyde metabolism because of its very low K_m ($<5 \mu\text{M}$) for acetaldehyde whereas ALDH1A1 shows only moderate acetaldehyde catalytic efficiency ($K_m = 50\text{-}100 \mu\text{M}$) (35). ALDH1L1, also known as FDH, is required for DNA nucleotide biosynthesis by catalyzing 10-formyltetrahydrofolate to tetrahydrofolate (THF) via a NADP^+ -dependent reaction (36). ALDH1L1 is found in especially high levels in the liver and kidney, the two principal sites of folate metabolism (37). Although the exact biological role of ALDH1L1 is not yet fully understood, the enzyme appears to be a chief regulator of cellular metabolism as it is strongly downregulated at certain physiological and pathological conditions (37,38,39), while its upregulation can produce drastic antiproliferative effects (37).

ALDH1 has three known response elements that regulate gene expression (NF-Y, C/EBP β , and RAR α) (32,40,41). The next step involves studying the role that folate or any of its derivatives may have in influencing these transcription factors, whether by one of the possibilities suggested above, or by some combination of the three. We hypothesize that the ALDH1 gene is upregulated, and therefore expression of ALDH1 is increased, via a mechanism involving folate, or one of its derivatives (Figure 2). If the availability of ALDH1 is increased, then more acetaldehyde gets converted into acetate, which decreases the negative effect that acetaldehyde has on folate one-carbon metabolism, along with its carcinogenic effects. Moreover, greater expression of ALDH1 as ALDH1L1 within the folate one-carbon pathway leads to greater availability of THF, thereby decreasing cancer susceptibility by promoting the conversion of dUMP to dTMP and DNA excision repair, both of which would lead to a decrease in DNA strand breaks and chromosomal instability. What this mechanism that upregulates ALDH1 involving folate remains unknown.

However, the concept of nutrigenomics – diet and gene expression – suggests three possibilities: 1) folate acts as a ligand for transcription factor receptors; 2) folate is metabolized by primary or secondary metabolic pathways thereby altering concentrations of substrates or intermediates; 3) folate alters signal transduction pathways (42). In recent years, studies have demonstrated the effect of folate in the cell cycle as it pertains to modulating gene expression and signal transduction pathways, with further implications on cancer development. A study done by Zhang et al. on neonatal rats showed that folate altered Notch signaling, which regulates the control of proliferation and differentiation in neural stem cells. Compared to the control, folate increased the rate of cell proliferation by increasing expression of *Notch1* and *Hes1*, while decreasing expression of *Mash1* (43). All three genes are components of the Notch signaling pathway. Another study examining human colonic epithelial cell lines showed that folate depletion modulated the expression of selected genes involved in cell cycle, intracellular signaling and folate uptake. Changes in the *Wnt/APC* pathway as well as genes involving DNA damage signaling, inhibition of cell cycle progression through checkpoints and apoptosis - p16, p21, p53, and β -catenin - induced by folate depletion may underlie observed relationships between folate status and cancer risk (44). Attias et al. observed that the ability of folic acid to downregulate insulin-like growth factor-1 (IGF-1) signal transduction pathway, which has a significant role in

cancer development and progression, may allow the micronutrient to have anti-cancer properties (45). Similar effects were observed by the folic acid metabolites dihydrofolic acid and tetrahydrofolic acid. With studies showing the effect of folate on gene expression related to cell cycle regulation, folate may exert a similar influence on the relationship between alcohol exposure and oral cancer via mechanisms involving modulation and regulation of transcriptional factors and intracellular signaling pathways.

One possible mechanism involves upregulation of cAMP response element-binding (CREB) protein via C/EBP β activation. The control of C/EBP β is exerted in many manners, which include phosphorylation, acetylation, activation and repression by other transcription factors, oncogenic elements or chemokines, and autoregulation. C/EBP β can interact with different proteins like CREB, NF- κ B and others leading to a trans-activation potential (46). Choline, a B-complex vitamin and a one-carbon donor similar to that of folate, has been shown to enhance CREB activation (47). Therefore, if folate can exhibit the same effect on CREB activation as choline does, then ALDH1 transcription will be upregulated given the interaction between C/EBP β and CREB, which leads to a trans-activation potential. As with our earlier CYP2E1 hypothesis, the ALDH1 and folate hypothesis needs to be tested from a molecular and genetic approach using assays qualitatively measuring expression of important intermediate molecules and transcription factors within the pathway. Correlating folate levels from blood samples to CREB and ALDH1 expression from oral tissue samples may be one method in which to evaluate this hypothesis. This potential pathway involving CREB may be just one of several possible mechanisms that activate C/EBP β expression. Furthermore, given that there are other gene regulatory elements as part of the ALDH1 gene promoter, more can be done to study what role, if any, folate may have on NF- γ and RAR α .

Conclusion

Epidemiological evidence suggests that folate may serve to decrease the risk of oral cancer even with high alcohol consumption. A biological understanding of this interaction, however, is necessary in order to provide further support for this finding. Thus, these proposed hypotheses may lead to greater insight as to how folate acts at the cellular and molecular level to reduce the carcinogenic effects of alcohol (Figure 3). We believe that CYP2E1 and ALDH1 serve as the intersection between the alcohol and folate metabolism pathways, and therefore it is crucial to understand their induction and regulation to help elucidate their molecular interactions. Further metabolic and biochemical studies among human subjects will help to test and confirm these hypotheses to determine what pathways folate works through to have its protective effect, thereby yielding essential information on the physiologic effect of folate. Since the oral cavity is the first site of nutrient and carcinogen exposure, understanding the local interaction of alcohol and folate in the oral cavity may provide greater knowledge into the systemic inhibition of folate against various cancers. Given that alcohol consumption is an established risk factor for oral cancer, the implications of these studies should emphasize the need to increase folate intake especially in combination with drinking alcoholic beverages in order to decrease the incidence of oral cancer. Public health recommendations should include eating folate-rich foods or taking folate supplements when consuming alcohol in order to mitigate the carcinogenic effects of alcohol to the general public.

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FIGURE 1.
FLOW DIAGRAM OF FOLATE AND CYP2E1 HYPOTHESIS

FIGURE 2.
FLOW DIAGRAM OF FOLATE AND ALDH1 HYPOTHESIS

FIGURE 3.
MODEL OF FOLATE-ALCOHOL AND CANCER PATHWAY HYPOTHESES