1204 Chlorophyllin modulates gene expression and DNA-adduct formation in normal human mammary epithelial cells (NHMECs) exposed to benzo(a)pyrene (BP)

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A panel of NHMEC strains (n=10), each derived from a different donor, was developed from tissue discarded at reduction mammoplasty (Cooperative Human Tissue Network, sponsored by the NCI and the National Disease Research Interchange) and used to study gene expression and DNA adduct formation following exposure to BP (4μM) in the presence or absence of chlorophyllin (5μM). Chlorophyllin treatment has been shown to reduce aflatoxin-induced DNA adduct formation and tumorigenesis, and we hypothesized that a similar chemopreventive mechanism might be demonstrable with BP. For the gene expression studies a two stage strategy was used. First, gene expression was monitored in 4 cell strains using high density oligonucleotide arrays. Second, genes for which expression was consistently altered were monitored by real-time polymerase chain reaction (RT-PCR) in 10 cell strains. Induction of CYP1B1 comprised the most consistent increase in gene expression [signal log ratio of 1.5, or 3-fold] in response to BP exposure. Subsequently, expression of CYP1A1 and CYP1B1 was monitored by RT-PCR in 10 BP-exposed NHMEC strains, in the presence and absence of chlorophyllin. The data showed a good correlation between CYP1A1 and CYP1B1 induction levels when oligonucleotide array data were compared to RT-PCR data for BP exposure (r^2 = 0.96 and 0.86, respectively) and for BP exposure in the presence of chlorophyllin (r^2 = 0.96 for both). By RT-PCR, there was wide inter-individual variation in induction of both CYP1A1 (7 to 96-fold) and CYP1B1 (8 to 43-fold) in cells exposed to BP alone. Lower induction levels of CYP1A1 (2 to 54-fold) and CYP1B1 (3 to 39-fold) were observed in cells pretreated for 24 hr with chlorophyllin followed by 24 hr treatment with BP plus chlorophyllin. In addition, the major BP-DNA adduct (7R)-N2-(10[7b,8a,9a-trihydroxy-7,8,9,10-tetrahydro-benzo[a]pyrene]yl)-deoxyguanosine (BPdG) was measured by r7,t8-dihydroxy-c9,10-epoxy-7,8,9,10-tetrahydo-benzo[a]pyrene (BPDE)-DNA chemiluminescence immunoassay. Similar to the profile observed with CYP1A1 and CYP1B1, there was significant reduction in BPdG adduct levels in the presence of chlorophyllin. BPdG adducts were reduced 48% to 86% in cells pretreated for 24 hr with chlorophyllin followed by 24 hr exposure to BP plus chlorophyllin. Overall the data show that chlorophyllin treatment of BP-exposed NHMECs reduces expression levels of the CYP1A1 and CYP1B1 enzymes required for metabolic activation of BP and reduces the formation of BPdG adducts. The data suggest that chlorophyllin may be a useful chemopreventive agent for human cancers attributed to polycyclic aromatic hydrocarbon exposure.

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