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Induction of genomic instability in cultured human colon epithelial cells following exposure to isocyanates

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Abstract

The toxic response of cultured human colon epithelial-FHC cells to methyl isocyanate was investigated with regard to genomic instability. Qualitative and quantitative assessments of the extent of phosphorylation of DNA damage signaling factors such as ATM, γH2AX and p53, was increased in treated cells compared to controls. At the same time, many treated cells were arrested at the G2/M phase of the cell cycle, and had an elevated apoptotic index and increased inflammatory cytokine levels. Cytogenetic analyses revealed varied chromosomal anomalies, with abnormal expression of pericentrin protein. Analysis through ISSR PCR demonstrated increased microsatellite instability. The results imply that isocyanates can cause genomic instability in colonocytes.

Keywords: Genomic instability; DNA damage; Apoptosis; Inflammation; Isocyanates

1. Introduction

Environmental chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs), probably have a prominent role in colon cancer etiology (Lichtenstein et al., 2000). Isocyanates are able to modulate biomolecules, resulting in a series of biotransformations (Shelby et al., 1987; Pearson et al., 1990; Slatter et al., 1991), which in turn may affect health adversely (Tamura et al., 1992), yet they have a wide array of industrial applications. MIC, a reactive byproduct, is detrimental to numerous organ systems (Worthy, 1985; Gupta and Prabha, 1996). It forms DNA cross links/adducts by reacting with exocyclic amino group of dNTPs (Segal et al., 1989), in turn contributing to cytotoxicity (Yoon et al., 2001). MIC intermediates (N-methylcarbamate) are also toxic to cultured mammalian cells (Hagmar et al., 1993; Kuo et al., 2008). Although the capability of isocyanates to induce carcinogenesis had been addressed in the past (Mikoczy et al., 2004), details of the complex molecular mechanisms underlying genetic hazards of occupational or accidental exposures to these chemicals are still unknown.

Colon epithelial cells are influenced constantly by many agents present in the natural environment via air, water, and soil exogenous or endogenous sources (Al-Saleh et al., 2008). Genomic instability in colon epithelial cells is an important event in the multistep progression of colorectal carcinogenesis (Grady, 2004) which is best studied by investigating both chromosomal and microsatellite instabilities. Chromosomal instability occurs early in colorectal cancer progression (Burum-Auensen et al., 2008) and is observed in 50–70% of

Abbreviations: MIC, Methyl isocyanate; ATM, Ataxia telangiectasia mutated; PIKK, Phosphoinositide-3-kinase-like kinases; DAPI, 4′, 6′-Diamidino-2-phenylindole dihydrochloride; FITC, Fluorescence isothiocyanate; IL, Interleukin; TNF, Tumor necrosis factor.

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colorectal cancers (Kargozaran et al., 2008). MIN, accounting for 10–15% of these cancers, is the expansion or contraction of short nucleotide repeats (Samowitz et al., 2007).

Genome stability depends on the accurate regulation of cellular responses to DNA damage and on the integrity of DNA repair systems. Cells use many DNA damage checkpoint pathways to maintain both cell viability and genomic stability (Bartek and Lukas, 2007). Since the broken DNA template is processed during replication, the repair mechanism requires arrest of cell cycle progression to avoid further damage (Bakkenist and Kastan, 2004; Kurz and Lees-Miller, 2004; Shiloh and Lehmann, 2004). DNA double-strand breaks (DSBs,) activate numerous signaling molecules, including ATM, a member of PIKK family (Abraham, 2004). DSBs trigger auto-phosphorylation of ATM at serine 1981 (ATM pSer1981), which results in the subsequent phosphorylation of H2AX at Ser139 (γH2AX), and initiates cell signaling events to induce cell cycle arrest or apoptosis through phosphorylation of p53 at Ser 15 (Kang et al., 2005; Rios-Doria et al., 2006).

Persistent inflammation may have a multifaceted role in onset of carcinogenesis (Mantovani et al., 2008). It may induce DNA damage, apoptosis, over-expression of cytokines and genomic instability (Kundu and Surh, 2008). Therefore down regulation of components of the DNA damage response mechanisms could enhance genomic instability and facilitate the proliferation of pre-cancerous cells (Gorgoulis et al., 2005). As MIC can initiate DNA damage, apoptosis, oxidative stress and inflammation in cultured human lymphocytes (Mishra et al., 2008), it is very likely that it is linked to genomic instability.

The aim of the present investigation was to explain the effect of MIC on genomic instability parameters of human colon epithelial cells. The experiments were performed on the cultured human normal colon epithelial-FHC cell line using N-succinimidyl N-methylcarbamate, a surrogate chemical to MIC (Martinez et al., 1982). Qualitative and quantitative assessment of phosphorylation states of DNA damage signaling factors such as ATM, γH2AX and p53 were chosen as indicators of the kinetics of DNA damage. Cell cycle arrest, DNA ploidy apoptotic index and status of inflammatory cytokines prior to and following exposure to isocyanates were also evaluated. Cytogenetic studies, along with immunocytochemical analysis of centrosomal protein pericentrin and inter simple sequence repeat (ISSR) PCR were performed to indicate a potential instability of the recipient genome at both chromosomal and microsatellite level.

2. Materials and methods

2.1. Reagents

N-succinimidyl N-methylcarbamate [CAS No. 18342-66-0] (Sigma Aldrich Laboratories, St. Louis, USA) at a final concentration of 0.005 μM was used. The culture petri-dishes were procured from Nalgene–Nunc Inc., Roskilde, Denmark. Antibiotic–antimycotic solution was obtained from Hi-Media Labs Pvt. Ltd, Mumbai, India. Fetal bovine and calf serum were obtained from HyClone Labs, Logan, Utah, USA. Dulbecco's Modified Eagles Medium (DMEM) growth medium and phytohemagglutinin were procured from Gibco/BRL Life Technologies, Inc., NY, USA. The cell growth supplements hydrocortisone, HEPES and insulin were procured from MP Biomedicals, Solon, USA. DNA damage responses were assessed qualitatively by immuno labeling of ATM, γH2AX, pericentrin and p53 phosphorylation states, using antibodies from Abcam, Cambridge, UK and Calbiochem, Nottingham, UK with appropriate dilution in 1 X PBS prior to use. Apoptosis was quantified with an Annexin-V-FITC/PI assay kit from Roche Applied Sciences, Mannheim, Germany. Secreted levels of inflammatory cytokines were analyzed using a BD™ Multiplex Cytometric Bead Array (CBA) Human Inflammation kit from BD™ Biosciences, San Diego, USA. DNA cell cycle and ploidy were investigated using a BD™ Cycle TEST PLUS DNA Reagent Kit, BD Biosciences, USA. Microsatellite instability was assessed, using a Wizard® Genomic DNA Purification Kit and a PCR II Core System kit for PCR amplification from Promega Corporation, Madison, USA.

2.2. Cell lines and culture conditions

Normal human colon epithelial-FHC cells (CRL-1831) were obtained from the American Type Culture Collection (ATCC; Manassas, VA). The cells were seeded at 2×10^5/60 mm culture dishes in DMEM supplemented with 10% fetal bovine serum, 10 ng/ml cholera toxin, 0.005 mg/ml insulin, 0.005 mg/ml tranferrin, 100 ng/ml hydrocortisone and 10 mM HEPES at 37 °C in the humidified atmosphere of 5% CO_2 in air, according to ATCC catalogue instructions. Fully confluent cells were treated with, N-succinimidyl N-methylcarbamate for 6, 12, 24, 48 and 96 h from passages 1–5. At the onset of the experiments, the cells were growing exponential and asynchronously.

2.3. Study design

The study was conducted in 2 phases: (i) analysis of DNA damage, cell cycle arrest, apoptotic index and inflammatory cytokine release; and (ii) cytogenetic analysis for chromosomal instability, immunocytochemistry of anti-pericentrin antibody, DNA ploidy analysis and simple inter-sequence repeat PCR for evaluation of microsatellite instability. Different sampling intervals ($n = 3$) ranging from 6 to 96 h were chosen for the first phase, whereas cells were subjected to analysis at passages 1–5 for the second. The optimum N-succinimidyl N-methylcarbamate concentration for inducing DNA damage, apoptosis, oxidative stress and inflammation in cultured mammalian cells of 0.005 μM was used throughout (Mishra et al., 2008). Controls were untreated normal FHC cells.

2.4. Evaluation of DNA damage, cell cycle, apoptosis, and inflammation

2.4.1. DNA damage

DNA damage kinetics analyzed in qualitative and quantitative terms of ATM/H2AX/p53, according to the protocol of