Mechanisms of N-acetyl Cysteine–mediated Protection From 2-Hydroxyethyl Methacrylate–induced Apoptosis

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Abstract

Resin-based materials are now commonly used in dentistry in restorative materials as well as in endodontic sealers. These materials have been shown to be cytotoxic. The mechanisms by which resin-based materials mediate their adverse effects have not been completely elucidated. Here we show that 2-hydroxyethyl methacrylate (HEMA) induces apoptotic cell death in oral keratinocytes and immune cells through the intrinsic cell death pathway. Functional loss and cell death induced by HEMA was significantly inhibited in the presence of N-acetyl cysteine (NAC) treatment. In addition, HEMA induced a decrease in mitochondrial membrane potential, and an increase in cleaved caspases was potently inhibited in the presence of NAC treatment. Overall, the results reported in this article indicate that NAC is an effective chemoprotectant that can safely be used to protect the pulp and the surrounding tissues from adverse effects of dental restorative and endodontic materials. *(J Endod 2008;34: 1191–1197)*

Key Words

2-hydroxyethyl methacrylate, caspases, mitochondria, N-acetyl cysteine

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Resin materials containing methacrylates are routinely used in dentistry in bonding agents, composite restorations, and endodontic sealers. One of the attractive features of the resin materials now in use in dentistry is that they can adhere to both dentin and enamel. Most dentin-bonding technologies use a primer containing the hydrophilic resin 2-hydroxyethyl methacrylate (HEMA, molecular weight 130) in combination with acid treatment to create a "hybrid layer" or "interdiffusion zone."

HEMA is also found in many medical devices and materials such as soft contact lenses, electrosurgical grounding plates, and drug-delivery systems (1). However, HEMA in such materials is polymerized and may not cause significant public health concern. In contrast, in dentistry, such materials require polymerization intraorally; thus, they may at least contain 30% unpolymerized monomers that may leach out to the surrounding area and the oral environment and they may cause significant adverse effects (2–4).

Recently, endodontic sealers have been developed to improve the sealing and bonding to root dentin. These improvements rely on the incorporation of resin monomers into the sealer or the application of resins during a conditioning step (primer) (5). The Epiphany obturating system uses Resilon points and is bonded to root dentin via a dual curing resin-based sealer (6). Although the use of these resin-containing materials is beneficial to patients, they carry the risk of local and systemic adverse effects. The potential risks are direct damage to the cells (cytotoxicity) and immune-mediated responses (allergy) (7, 8). We have previously shown that HEMA and triethyleneglycol dimethacrylate (TEGDMA) are released in vitro from many resin-based tooth restorative materials used in dentistry in microgram to milligram amounts in the first days after placement of clinically used amounts of the source materials (9–12), and they are cytotoxic to the pulp (13). In addition, Heitman et al (14) have shown Epiphany root canal sealer to be cytotoxic with an increase in concentration or exposure time.

To find novel strategies to minimize or significantly prevent future risks of adverse effects of dental materials, studies should be designed to (1) understand the exact mechanisms by which these materials induce cell death and (2) find strategies to decrease or eliminate their toxicities while preserving their beneficial effects.

We have previously shown that cell death induced by HEMA is apoptotic, and it occurs in a variety of cell types upon treatment with HEMA (15). Apoptosis or programmed cell death is a genetically controlled and evolutionary conserved process that ensures disposal of damaged or altered cells in diverse organisms. Most of the morphologic changes induced by apoptosis are brought about by activated caspases. The induction of apoptosis is initiated by two distinct pathways known as intrinsic pathway mediated by mitochondria and extrinsic pathway mediated by death receptors. Stress-induced apoptosis occurs when cells are exposed to genotoxic and/or cytotoxic drugs, γ-radiation, free radicals, metabolic toxins, endoplasmic reticulum stressors, and toxins that disrupt cytoskeletal structures. These stressors activate the intrinsic apoptotic pathway because of the perturbation of the mitochondrial membrane integrity. Mitochondria sequester a variety of proapoptotic proteins, and their release requires disruption of mitochondrial membrane potential. Once released from the mitochondrial compartment, proapoptotic effectors will, in turn, recruit and activate caspase 9, which then will activate downstream effector caspase 3 (16–18). Most if not all of the adverse effects of resin monomers could likely be prevented if cells are able to increase nuclear factor kappa B (NFκB) and are triggered to undergo differentiation (13, 19). Indeed,

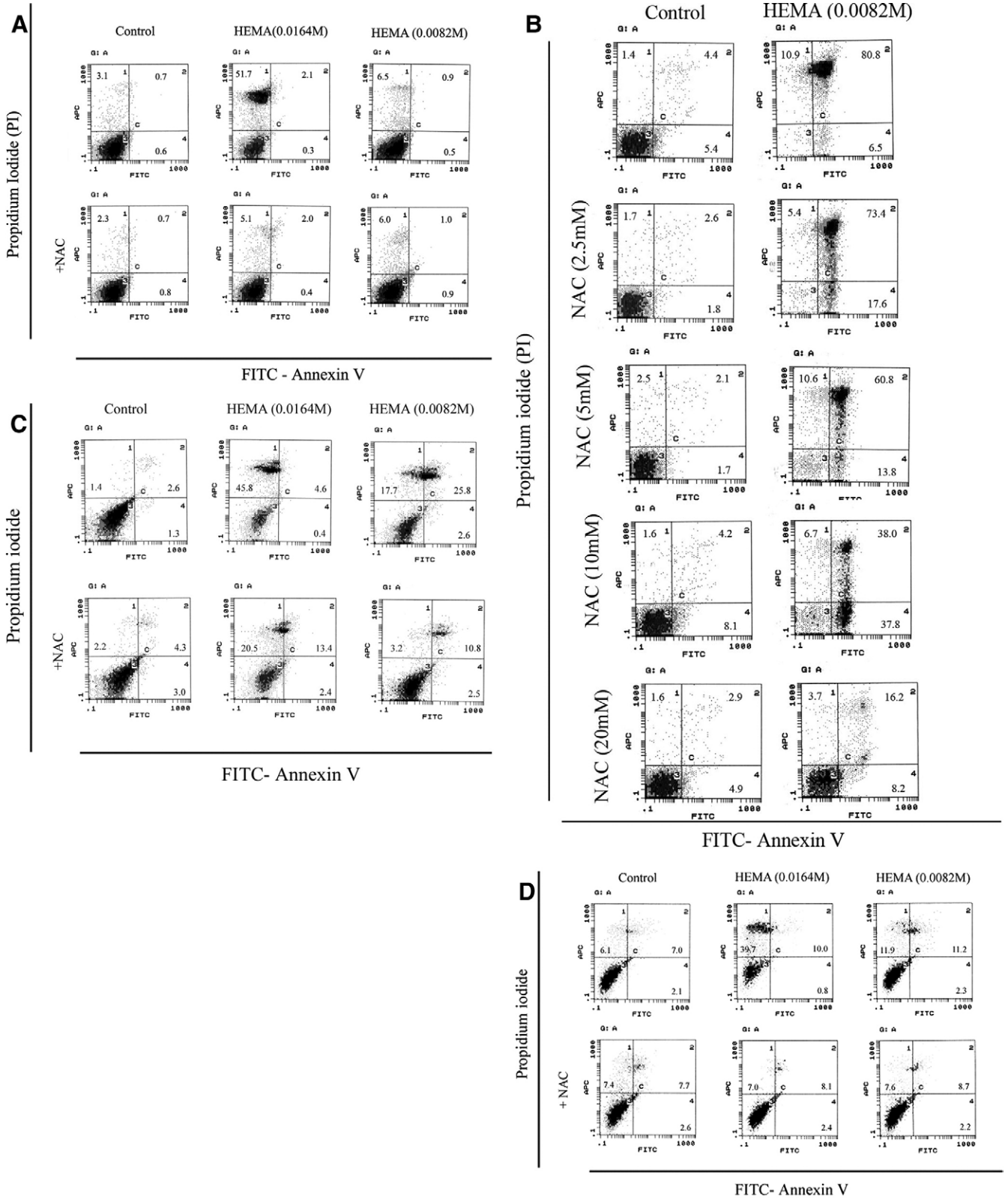

Figure 1. NAC inhibits HEMA-mediated cell death: (*A*) HEp2 cells, (*B*) Jurkat cells, (*C*) dental pulp cells, and (*D*) osteoblasts were treated with different concentrations of HEMA (as shown in the figures) in the presence and absence of NAC (20 mmol/L). After an overnight incubation, the levels of cell death were determined using propidium iodide (PI) and FITC-Annexin V staining. The numbers on the dot plots (*C*) are the percentages of cells stained in each respective quadrant. 10,000 events were analyzed for each sample.