Protection of HLE B-3 Cells against Hydrogen Peroxide– and Naphthalene-Induced Lipid Peroxidation and Apoptosis by Transfection with hGSTA1 and hGSTA2

Yusong Yang1, Rajendra Sharma1, Ji-Zhong Cheng1, Manjit K. Saini1, Naseem H. Ansari1, Usha P. Andley2, Sanjay Awasthi3 and Yogesh C. Awasthi1

1 From the Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas; the
2 Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri; and the
3 Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, Texas.

PURPOSE. To investigate the physiological role of two major α-class glutathione S-transferases (GSTs), hGSTA1-1 and hGSTA2-2 in protection against oxidative stress and lipid peroxidation (LPO) in human lens epithelial (HLE B-3) cells.

METHODS. Total GSTs were purified from HLE B-3 cells by glutathione (GSH)-affinity chromatography and characterized by Western blot analysis, isoelectric focusing, and kinetic studies. The relative contributions of the α-class GSTs and the Se-dependent glutathione peroxidase (GPx)-1 in GSH-dependent reduction of phospholipid hydroperoxide (PL-OOH) were quantitated through immunoprecipitation studies using separately the specific polyclonal antibodies against human α-class GSTs and GPx-1. HLE B-3 cell membranes were prepared, peroxidized, and used to examine whether hGSTA1-1 and hGSTA2-2 catalyzes the reduction of membrane PL-OOH in situ using the microiodometric and spectrophotometric assays. The protective effects of the α-class GSTs against H2O2- and naphthalene-induced LPO and apoptosis were examined by transfecting HLE B-3 cells with cDNAs of hGSTA1 and hGSTA2.

RESULTS. HLE B-3 cells expressed only the α and π class GSTs. The Michaelis-Menten constant (k_m) and turnover number (k_cat) of purified total GSTs toward phosphatidylcholine hydroperoxide (PC-OOH) were found to be 30 ± 4 μM and 1.95 ± 0.26 seconds, respectively. The α-class GSTs accounted for approximately 65% of the total GPx activity of HLE B-3 cells toward PC-OOH. Our results demonstrate for the first time that hGSTA1-1 and hGSTA2-2 effectively catalyzed GSH-dependent reduction of membrane PL-OOH in situ in HLE B-3 cells. Transfection with hGSTA1 or hGSTA2 protected these cells
from H₂O₂- and naphthalene-induced LPO and attenuated H₂O₂- and naphthalene-induced apoptosis through inhibiting caspase 3 activation.

CONCLUSIONS. These results demonstrate that the α-class GSTs hGSTA1-1 and hGSTA2-2 play a major role as antioxidant enzymes and are the main determinants of the levels of LPO caused by oxidative stress in human lens epithelial cells.