As public interest in safety has increased the toxicity evaluation of chemicals become more important. In this study, the DNA-damaging effect of genotoxicants was examined in HepG2 cell line originated from human hepatocellular carcinoma by widely used genotoxicity assays: the comet assay and gammaH2AX immunostaining. Four different direct/indirect genotoxicants were tested in dose-/time-dependent manner. The comet assay and the gammaH2AX immunostaining enables detection of DNA damages in the form of DNA strand breaks with different sensitivity. Therefore, the combination of comet assay and gammaH2AX immunostaining will be complementary tool for evaluation of various forms and degree of DNA damage. Our result also suggested that HepG2 cells could be a suitable model for assessing the genotoxicity of various mutagens and for determining the lowest genotoxic concentration. Further analysis using a larger number of chemicals is warranted to determine the sensitivity and the specificity of HepG2 with in vitro genotoxicity test.

Keywords: Comet assay, GammaH2AX immunofluorescence, Cytotoxicity, Genotoxicity, HepG2

Introduction

The genotoxic potential of chemical substances is generally assessed, possibly enabling prediction of their carcinogenic potential in humans. In vitro mammalian genotoxicity assays have issued regarding the specificity, as unlike in vivo systems they yield false positive results. Several in vitro and in vivo genotoxicity assays are used to evaluate the genotoxic potential of chemicals. Commonly used assays include single-cell gel electrophoresis assay which has been termed comet assay as well as phosphorylated histone variant (gammaH2AX) immunofluorescence.

The comet assay which was first established by Östling and Johanson is now accepted worldwide as a rapid and sensitive method of detecting DNA damage, including strand breaks and alkaline labile sites (ALS). The method is currently employed in biomonitoring, mechanistic, and regulation studies in a wide range of species both in vitro and in vivo. Test guideline 489 on genotoxicity from Organization for Economic Co-operation and Development (OECD) have been developed for the alkaline version of in vivo comet assay (electrophoresis buffer pH > 13). In vitro assays are being improved with purpose of future validation. The recent international comet assay workshop included an overview of use of the comet assay in various genotoxicity studies in vitro and in vivo. Test guideline 489 on genotoxicity from Organization for Economic Co-operation and Development (OECD) have been developed for the alkaline version of in vivo comet assay (electrophoresis buffer pH > 13).