Trials of Comet/Micronucleus Combined Assay

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Comet and micronucleus assays, having a different end point each other, usually get their specimens at a different time after administration. However, it is supposed that both end points can be evaluated concurrently when the test substance is administrated to animals once daily for 3 consecutive days, and the specimens for comet and micronucleus assays are prepared at 3 hours after the final administrations (hereinafter comet/micronucleus combined assay). Thus, a trial experiment of comet/micronucleus combined assay was conducted using rats. In this trial, ethyl methanesulfonate (EMS) and mitomycin C (MMC) were selected as a common reference substance for both assays to confirm its DNA damage and clastogenesis.

We obtained expected results on two model chemicals, EMS and MMC in the micronucleus assay and comet assay from the same animals. Though EMS induced early DNA damage in the first stage, it was comet and micronucleus-positive in this assay. MMC of crosslinking agent was comet-negative, but micronucleus-positive in this assay. A different mutagenic end point (DNA damage and clastogenesis) could be detected concurrently under this experimental condition. These results are well matched to our historical data, which these assays were performed independently. The combination of multiple genotoxicity assay, e. g., the micronucleus assay using young erythrocytes and the comet assay using the liver, can reduce the number of animals to be used in the genotoxicity evaluation. Furthermore, if we will use the transgenic animals, e. g., MutaMouse, we could obtain three main endpoints of the genotoxicity, i. e., DNA damage, gene mutation and chromosomal aberrations concomitantly. By using this comet/micronucleus combined assay, we evaluated the mutagenic potency of wheat extract. Furthermore, we got stronger antimutagenic activity of methylated catechins compared with catechins.