# Can bulky adducts be detected by employing the comet assay along with DNA repair inhibitors?

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The standard alkaline comet assay is a simple and economical genotoxic test widely used in genetic toxicology for the detection of strand breaks and alkali labile sites in the DNA. With several modifications it can also detect altered bases such as oxidized and alkylated bases and cross-links. However, it cannot detect bulky adducts, an important DNA lesion in which a chemical is bond to the DNA. Since DNA bulky adducts are mainly repaired by nucleotide excision repair (NER), the comet assay has been modified using NER inhibitors, such as the combination of hydroxyurea (HU) and cytosine arabinoside (Ara-C) or aphidicolin (APC), that blocks reparation process and causes incision breaks intermediates to accumulate. These modifications have been used without a proper validation study.

An internal validation study has been carried out using TK6 cells treated with a compound which causes DNA bulky adducts, six genotoxic agents with different mechanisms or two cytotoxicity controls, together with the DNA repair inhibitors. The MTS assay and comet assay were performed to determine cytotoxicity and DNA damage respectively.

Although more data is needed for a final conclusion, the use of HU/Ara-C or APC in combination with the comet assay increases the sensitivity of the comet assay for the detection of DNA damage, despite being nonspecific for the detection of DNA bulky adducts. Moreover, these results challenge the concept that different lesions in the DNA are repaired by different mechanisms or the specificity of the NER inhibitors.

**Funding:** Spanish Ministry of Sciences, Innovation and Universities (BIOGENSA2, PID2020-115348RB-100; FPU21/03187).