

Supplementary Materials

Genotoxic Effects of Etoposide, Bleomycin and Ethyl Methanesulfonate on Cultured CHO cells: Analysis by GC-MS/MS and Comet assay

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GC-MS/MS Analysis

We utilized gas chromatography-tandem mass spectrometry (GC-MS/MS) with isotope dilution using stable isotope-labelled analogues of analytes as internal standards to positively identify and accurately quantify the following DNA base lesions in genomic DNA: 8-OH-Gua, 5-OH-MeUra, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 4,6-diamino-5-formamidopyrimidine (FapyAde), 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd), 5-OH-Cyt, 5-OH-Ura and 8-OH-Ade. Aliquots of 8-OH-Gua-¹⁵N₅, 5-OH-Me-Ura ¹³C, ¹⁵N₂, FapyGua-¹³C, ¹⁵N₂, FapyAde-¹³C, ¹⁵N₂, ¹⁵N₂, 5-OH-5-MeHyd-¹³C, 5-OH-Cyt-¹³C, ¹⁵N₂, 5-OH-Ura-¹³C₄, ¹⁵N₂ and 8-OH Ade ¹³C, ¹⁵N₂ were added as internal standards. The following mass transitions were used in mass spectrometric analysis: m/z 455 \rightarrow m/z 440 and m/z 460 \rightarrow m/z 445 for 8-OH-Gua and 8-OH-Gua-¹⁵N₅, respectively; m/z 353 \rightarrow m/z 343 and m/z 262 \rightarrow m/z 347 for 5-OH-MeUra and 5-OH-MeUra ¹³C, D, respectively; m/z 457 \rightarrow m/z 368 and m/z 460 \rightarrow m/z 371 for FapyGua and FapyGua-¹³C, ¹⁵N₂, respectively; m/z 369 \rightarrow m/z 354 and m/z 372 \rightarrow m/z 357 for FapyAde and FapyAde- ¹³C, ¹⁵N₂, respectively; m/z 346 \rightarrow m/z 331 and m/z 349 \rightarrow m/z 334 for 5-OH-5-MeHyd and 5-OH-5-MeHyd-¹³C, ¹⁵N₂, respectively; m/z 343 \rightarrow m/z 342 and m/z 346 \rightarrow m/z 345 for 5-OH-Cyt and 5-OH-Cyt-¹³C, ¹⁵N₂, respectively; m/z 344 \rightarrow m/z 343 and m/z 350 \rightarrow m/z 349 for 5-OH-Ura and 5-OH-Ura-¹³C₄, ¹⁵N₂, respectively; m/z 367 \rightarrow m/z 352 and m/z 370 \rightarrow m/z 355 for 8-OH Ade and 8-OH Ade ¹³C, ¹⁵N₂ respectively. GC-MS/MS measurements were conducted as described previously (see main text Materials and Methods).

Figure S1: Complete GC-MS/MS data comparing the induction of the DNA lesions by etoposide, bleomycin and EMS. The first three lesions (8-OH Gua, Fapy Gua and 5-OH MeUra), show increasing trends with bleomycin (bleo) compared to the lack of any significant increases after treatment by etoposide (etop) and EMS (ems). The remaining lesions (FapyAde, 5-OH-5-MeHyd, 5-OH-Cyt, 5-OH-Ura, and 8-OH-Ade), did not show significant increases after treatment by etoposide, bleomycin and EMS. Data is presented as mean and standard deviation of 4 replicates.





