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In vivo standard and Fpg-modified comet assay: study of lysis conditions

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Cell lysis is one of the steps in the comet assay. It is a popular belief that it should endure for at least 1 hour, and it is common practice to extend it, conveniently pausing the experiment, sometimes until next day (i.e. giving an overnight or a 24-hour lysis). It has also been published that it could be fully removed in the standard in vitro comet assay or reduced to 5 minutes regarding the enzyme-modified version. However, the influence of lysis length regarding the in vivo version of the assay is not well established.

For that purpose, a single oral dose of 200 mg/kg methyl methanesulfonate (MMS) to induce strand breaks, or 5 mg/kg MMS or 400 mg/kg potassium bromate (KBrO3) to induce Fpg-sensitive sites, was administered to male Wistar rats (n=3 rats/group). Negative control rats were dosed with saline solution.

After 3 hours, the animals were sacrificed. Liver, kidney and duodenum were removed from animals administered with 200 mg/kg MMS, while liver and duodenum were taken when the lower dose of MMS was used. In the case of the animals treated with 400 mg/Kg KBrO3 only the kidney was extracted. These tissues were processed using the standard alkaline or the Fpg-modified comet assay, as appropriate, and lysed for 5 minutes, 1 hour, overnight, or given no lysis. The influence of lysis pH was also studied in the Fpg-modified comet assay, using neutral (pH 7) and alkaline (pH 10) conditions for the 1 hour lysis timepoint.

Regarding the standard comet assay, no significant differences were found among different lysis lengths, including the absence of lysis. However, as expected, the lysis step is necessary to measure Fpg-sensitive sites. Concerning MMS, the levels of Fpg-sensitive sites detected increased along with lysis duration. In contrast, this effect was not found in rats treated with KBrO3. Moreover, decreasing lysis solution pH seems to have an impact on results obtained with MMS, reducing the Fpg-sensitive sites obtained, which is not the case for KBrO3-induced lesions. This could be due to the different nature of the Fpg-sensitive sites detected in animals administered with MMS or with KBrO3.

Financial support: Spanish Ministry of Science and Innovation (BIOGENSA2, PID2020-115348RB-I00).

Keywords:
Comet assay, *in vivo*, Fpg, lysis duration.