P090 Establishment of a robust *in vitro* Comet Protocol using human lymphoblast TK6 cells

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The Comet assay has been widely used as a genotoxicity test for detecting initial DNA damage in individual cells. Its main advantages include: (a) simplicity and speed; (b) the small number of cells needed; (c) high sensitivity; (d) utilizability for any kind of eukaryotic cells, and it promises to be an excellent genotoxicity assay in the future. To simplify Comet methodology, we previously applied a special coated slide glass (Matsunami MAS coat) instead of bottom layer agarose. To establish a robust *in vitro* Comet protocol with this simpler method, we then examined procedural issues including cytotoxic parameters, fluorescent staining, and Comet scoring. Human lymphoblast TK6 cells were treated with chemicals (EMS, MMS, MNNG, H_2O_2, etc.) for 4h and subjected to a Comet assay. Relative survival and cell growth proved to be more suitable parameters for estimating cytotoxicity than dye-exclusion assay. SYBR-Gold staining was more visible than SYBR-Green, but this did not affect Comet scoring. For objective scoring, the Comet Ⅳ (Version 4.11, Perceptive Instrument) image analyzer proved to be very reliable, and the clearly dose-dependent, and varied smaller, increases were in the order of %DNA in tail > *tail moment* > tail length.

ヒトリンパ芽球細胞株（TK6）を用いた*in vitro* comet protocolのさらなる確立

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P091 Assessment of chromium compounds on dG→8OHdG oxidation with metabolic activation

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Chromium especially the hexavalent chromium compounds can pose an environmental health risk. Since 8-hydroxy 2'-deoxyguanosine (8OHdG), which is the oxidized form of 2'-deoxyguanosine (dG), is known to induce G:C→T:A transversion on the gene, the dG→8OHdG oxidation seems to represent the risk of DNA damage resulting in the mutagenic and/or carcinogenic reaction.
In this study, the effect of chromium compounds on dG→8OHdG oxidation has been investigated, using a novel assay system, which can measure both dG and 8OHdG, simultaneously (PCT/JP01/02095). We evaluated a risk of dG→8OHdG oxidation in chromic acid derivatives (potassium chromate (Ⅵ), potassium dichromate (Ⅵ), and chromium (Ⅲ) chloride hexahydrate) before and after S9 metabolic activation treatment. We prepared 0.5, 5, 50, 500, 5000 μM solution in each chemical. Without S9 treatment, 50 μM in potassium chromate and 500 μM in potassium dichromate showed dG→8OHdG induction, but chromium chloride hexahydrate did not. After S9 treatment for one hour, all chemicals did not show 8OHdG induction. The bio-hazard risk in chemicals produced by oxygen radicals can be evaluated by dG→8OHdG oxidative reaction in a dose-dependent manner in vitro.

dG の酸化誘導指標を用いた三価及び六価クロムの変異原リスクの検討

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