Cisplatin resistance analysis: insights on the combination of DNA adducts and DNA strand breaks detection in human cells

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Despite its wide use as a chemotherapeutic drug, cisplatin presents an important problem: resistance development. Although processes like chemical metabolism, DNA repair activity or cell uptake are known to be involved in this resistance, there is not a method to detect it in advance. In Drosophila the combined study of cisplatin induced adduct levels and their genetic consequences, in terms of DNA strand breaks, seemed a potentially useful tool in the identification of cisplatin resistance, since a significant correlation was found between adduct and DNA strand breaks levels, in different conditions of the nucleotide excision repair (NER) system. To check this possibility in human cells, two pairs of cell lines were chosen: 1) NER deficient (GM04321, *XPA* mutant) and efficient (A549) cell lines; 2) cisplatin sensitive (A2780) and resistant (A2780cis) cell lines. These cells lines were exposed to two different treatments: 3 h and 3 h plus 1 h recovery without cisplatin. DNA strand breaks were measured with the alkaline comet assay. Cisplatin induced adducts were quantified using HPLC, followed by ICP-MS. Intracellular Pt levels after 3 h treatment were measured by ICP-MS in all cell lines to estimate cellular uptake.

Total intracellular Pt results showed that there were no differences between the first paired cell lines, for any concentration, whereas total Pt in A2780 was 2-3 times higher than in those and 10 times higher than in A2780cis. Comet assay results, with the first pair of cell lines, showed some differences between them: i) cisplatin induced significant DNA strand breaks in GM04321 but not in A549; ii) after 1 h recovery, GM04312 showed two different responses: (A) double strand breaks were induced by cisplatin, and (B) treatment decreases the levels of spontaneous DNA breaks; iii) no differences between 3 h and 3 h plus 1 h recovery treatments were found for A549, whereas differences were found in the case of the B response for GM04321. For the second pair of cell lines, comet results showed no differences between them for 3 h treatment, although A2780cis showed two different responses. In the case of 3h plus 1 h treatment, a decrease of the spontaneous strand breaks was detected for the resistant cell line. Finally, the analyses of relationships between DNA adducts and strand breaks showed a statistically significant correlation for the first pair of cell lines, with 3 h plus 1 h treatment, considering the repair status.