

TEMPERATURE INFLUENCE ON CELLULAR UPTAKE, DNA PLATINATION AND ANTIPROLIFERATIVE ACTIVITY OF PLATINUM(II) DRUGS

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Cellular uptake is the main factor influencing the efficacy of the drugs acting via non-receptor mediated mechanisms. Platinum(II)-based antiproliferative drugs are thought to enter cells mainly by passive diffusion, although some assumption are still in discussion and need further demonstration. In recent years, beyond the passive diffusion mechanism, the hypothesis of active transport of cisplatin mediated by copper influx transporter (namely CTR1) has been proposed, mostly on the basis of the similar uptake and resistance mechanisms between the two metals in selected cell lines. Thermal enhancement of cytotoxicity has been widely proved for several drugs and clinically adopted for the combined treatment of various malignancies. Although there are a number of hypotheses to justify this clinical approach, the experimental bases for this synergistic effect remain yet to be fully elucidated. Over the past few years, our laboratory has focused on the study of platinum fate after cisplatin, carboplatin and oxaliplatin treatments. In this study we describe how temperature influences the following parameters, cellular accumulation, DNA platination and the dose-dependent antiproliferative effect for these drugs. We determined the accumulation ratio (AR) for short time continuous treatments (2 h), the initial rate constant of the cellular uptake (k). This procedure gave the activation parameters of uptake mechanisms, namely ΔH^\ddagger and ΔS^\ddagger to set up plots based on Eyring's equation. AR values measured within 2 h reflected the crossing ability of cellular membranes of the Pt-drugs, their aquation in the cytosol and their coordination to DNA. Interestingly, only cisplatin showed a true synergistic effect of its cytotoxic activity with hyperthermia, and this effect was maximum for treatments near to its IC_{50} . For carboplatin and oxaliplatin, cell viability of the drugs alone and drugs plus hyperthermia were quite similar, thus suggesting no synergistic effect. Among the three drugs, cisplatin only can undergo aquation within 2 h time scale and then DNA coordination, where the rate is obviously increased by temperature. This was confirmed from the behavior of DNA platination, where cisplatin only gave roughly a 5 times increase in the platination of the DNA with an increase of the temperature from 37 to 43 °C, and where carboplatin and oxaliplatin (whose hydrolysis half-times are of the order of days) only partially increased the platination. Good correlation has been found between antiproliferative effect and DNA platination, but not with the overall drug uptake for cisplatin. These data show that the chemical differences among platinum derivatives strictly condition not only the pharmacokinetics but also their cell behavior and activity.