In vitro study of heavy metal-based anticancer complexes: activity, neurotoxicity and photon activation therapy's effect.

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BACKGROUND

Cisplatin is one of the most efficient metal-based anticancer agents, targeting several solid tumours. Despite its efficacy, cisplatin treatment is still limited by severe side effects such as neuro-, hepato- and nephrotoxicity and by resistance phenomena, only partially overcome by the use of new platinum drugs (i.e. oxaliplatin and carboplatin). Among the several side effects induced by platinum drugs the chemotherapy-induced peripheral neurotoxicity (CIPN) can be dose limiting. Moreover, CIPN signs and symptoms can be permanent and severely impair the patients' quality of life even after drug withdrawal (Albers et al., 2011). These problems have stimulated the research and development of alternative therapeutic strategies based on different heavy metals. In this context, complexes of group 11 metals show encouraging perspectives. Specifically, structure-activity relationship for diphosphine and phosphonyridyl metal complexes suggested that the selectivity for cancer cells over normal cells can be tuned by adjusting their hydrophilic/hydrophobic balance (Humphreys et al., 2007). Furthermore, heavy metal-based anticancer complexes represent suitable candidates to be used in Photon Activation Therapy (PAT) (Ceresa et al., 2014).

RESULTS

NEUROTOXICITY

Neurotoxicity of the different water soluble compounds and of cisplatin (CDDP) are evaluated on rat embryonic dorsal root ganglia (DRG) organotypic culture model (Scuteri et al., 2006). Using this model, routinely used concentrations of the reference drug CDDP, known to be neurotoxic both in animal models and in humans, result neurotoxic also in our experimental setting. The measure of the longest neurite of DRG treated with different concentrations of the water soluble compounds tested demonstrates that:

- [Au(thp)][PF6] (Auhp), [Cu(thp)][PF6] (CuPATA) and [Cu(thp)][PF6] (CuPATA) compounds result NO NEUROTOXIC at concentrations much lower than Cipn, observed in A549 and IGROV-1 cancer cells.
- [Au(thp)][PF6] (Auhp) compound results NEUROTOXIC at concentrations lower than the IC50 of the cancer cells lines tested.

PROTEASES ACTIVITY

The proteasome inhibition is evaluated on embryonic DRG neurons and IGROV-1 human ovarian cancer cells treated for 48 h with bortezomib (BTZ), Auph, CuPATA and Cuthp. In both cellular models, neurotoxic concentration of the reference drug BTZ, inhibits the proteasome activity in a time-dependent way. Both embryonic DRG neurons and IGROV-1 cells exposed to the indicated concentration of Auph show a remarkable proteasome inhibition. On the other hand CuPATA and Cuthp compounds induce a significant proteasome inhibit in IGROV-1 cyh high proteasome activity at the concentrations used in all the proteasome assays. Rat DRG neurotoxicity is measured by the expression of neuronal filaments (Neurite length) in the cultured neuronal stem cells (in this case IGROV-1). RAD51 nuclear relocalization in IGROV-1 cells treated with CuPTA and exposed to SR, A, RAD51 foci in IGROV-1 cells treated with CuPTA 0.2 µM and irradiated with 30 kV energy (2 Gy). Nuclear reaction was counterstained with propidium iodide. B, percentage of cells with RAD51 foci positive nuclei scored in at least 200 cells treated or not with CuPTA and/or exposed to SR with 30 kV energy (2 Gy) (Bencosse et al., 2008).

PAT APPLICATION

PRINCIPLE

Tumor loaded with a high Z element (Pt, Cu, Au, Ag): higher probability for adsorbing radiation

- Irradiation with monochromatic X-rays beams (keV scale) tunable to the adsorption edges of the high Z element: induction of photoelectric effect

ADVANTAGES

- To reduce toxic effects on the surrounding healthy tissues
- To increase tumour control
- To reduce chemotherapy-induced side effects

MATERIAL AND METHODS

CYTOTOXICITY ASSAY. The Cu and Au complexes were dissolved in water just before the experiment. Work dilutions were performed in culture medium. Cytotoxicity was evaluated by SRB assay (Novo, 3.1 × 105 cells/well seeded in 96-well microplates in growth medium and then incubated at 37°C, 5% CO2. After 24h, the medium was removed and replaced with a fresh one containing the compound under investigation. Triplicate cultures were established for each treatment. For Auhp SRB assay was performed and IC50 (drug concentration that reduces the mean absorbance at 570nm to 50% of the untreated control wells) was evaluated.

NEUROTOXICITY EVALUATION. DRG from E15 Sprague-Dawley rats were asexually removed and cultured onto rat-tail collagen surfaces in Hanks’ base (4 DRG/dish). DRG were incubated in media (MEM plus 10% FBS, 100µg/ml ascorbic acid and 1.4mM L-glutamine, 0.66% glucose) added with 1mg/ml NGF for 2 hours in a 5% CO2 humidified incubator at 37°C. After 2 hours DRG were treated with the test drugs in media added with NGF and FBS 10-4M. Phase contrast micrographs of 6 dishes were made 48 hours after adding the test drugs. Measurements of neurite outgrowth were made by the program “Image J” for each DRG, the longest neurite was measured. Each experiment was carried out at least in triplicate for each of the drug concentrations tested. The data (expresses in percentage with respect to the control) shown in graphs are the means ±S.D of three independent experiments. The length of neurite were determined using ImageJ software, Image J (http://rsb.info.nih.gov/ij/). The neurite elongation was determined as the mean neurite length generated from the cleaved substrate in the reagent. Fluorescence generated from each reaction was detected with a fluorescence microscope. The proteasome activity (PA) was calculated as following: % A = 100 x [(CTC/FSUBSTRATE) -1 ] / (CTC/FSUBSTRATE) and the inhibition was obtained as 100-%A.

CONCLUSIONS

- Based on cytotoxicity and neurotoxicity profile copper compounds are promising anticancer drug
- Copper compounds induce a significantly higher proteasome inhibition in cancer cells with respect to the one observed in DRG neurons
- Our results suggest the potential use of [Cu(thp)][PF6] in PAT

REFERENCES