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Modulation of cisplatin-induced DNA cross-links in human tumour cells by regulations of glutathione content

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Glutathione (GSH) has been shown to participate in the detoxification of cisplatin and to play a role in cisplatin resistance. This study aimed to investigate the effects of regulation of GSH content on cisplatin induced DNA cross-links which has been recognized as a major DNA damage in cisplatin cytotoxicity. Cisplatin cytotoxicity was measured by MTT method. DNA cross-links were determined by a fluorescent method. Cytotoxic effects of cisplatin on HepG2 cells were significantly potentiated by depletion of GSH by 0.5 mM buthionine sulphoximine (BSO), with IC_{50} values of 35.4 μM for cisplatin and 18.8 μM for cisplatin + BSO, repectively. While enrichment of GSH by 5 mM GSH monoethyl ester could protect the human tumor cells from cisplatin, with IC_{50} values of 35.4 μM for cisplatin and 88.8 μM for cisplatin + GSH monoethyl ester, respectively. At 6 h exposure, cisplatin induced interstrand DNA cross-links were potentiated by BSO by 98% and decreased by GSH monoethyl ester by 44%. The data showed that regulation of GSH content affected formation of cisplatin induced DNA cross-links and cytotoxicity of cisplatin on human tumor cells. This indicated the importance of contribution of cisplatin-induced DNA cross-links in cisplatin cytotoxicity.

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Various ph of nutrient medium changed the extent of spermine-fbs mediated cytotoxic effect on three neoplastic cell lines

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Spermine – serum amine oxidase (SAO) mediated cytotoxic action is proven on many normal cells and malignant cell lines. SAO is a copper dependent enzyme involved in the catabolism of polyamines. It is known that SAO activity changes with pH variation. The rate of oxidation for spermine at pH 6.2 is approximately 1.5 times greater than the rate at pH 7.2. The aim was to determine weather various pH of nutrient medium change the extent of spermine-SAO mediated cytotoxic effect on three different neoplastic cell lines. Target cell lines were human myeloid leukemia K562 cells, human cervix carcinoma HeLa cells, and human malignant melanoma Fem-X cells. Nutrient medium was RPMI1640, with additions of L-glutamine, antibiotics, and 10% of heat inactivated fetal bovine serum (FBS). pH of nutrient medium was adjusted by the additions of solutions of HCl, or of natriumbicarbonate. Investigated cells were seeded in 96 flat bottomed wells (10,000 cells per well) at four different pH of nutrient medium (from 6.45 to 7.56). After the three-hours incubation, different amounts of spermine were added to probes. Twenty-four hours later cell survival was determined using trypan blue exclusion test and/or MTT test. Results obtained showed higher survival score at lower pH for all investigated cell lines. This finding does not mimic the SAO activity dependence on pH. Results also showed that the effect of pH on SAO-mediated cytotoxicity does not depend on origin of malignant cells.

These results on the cell survival could be explained by the activation of protective cell survival mechanism during low pH cell preincubation. Considering all the mentioned factors: Spm, copper dependent AO and hydrogen ions are naturaly present in the organism, our results indicate that small variation in their mutual relation could have completely different effect; it can result in cell survival or in cell death depending on pH of surrounding in which cell is.

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Long term influence of fetal bovine serum (FBS) on ganglioside expression and sensitivity to chemotherapy and complement-dependent cytotoxocity (cdc) in vitro of a small cell line cancer (sclc) cell line, nci h69

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Introduction: Gangliosides are expressed at high levels, but heterogeneously, in SCLC and have been proposed as targets for immunotherapy. We have investigated the influence of long term exposure of SCLC cells to 2% and 10% FBS on ganglioside expression, and on the performance CDC- and chemosensitivity tests.

Material & Methods: NCI H69 cells were cultured with (H69VP) and without (H69 wt) etoposide (VP) 3 ug/ml in Iscoves with 2% or 10% FBS. Expression of GM2, GD2 and fucosyl-GM1 gangliosides was assessed by immunocytology using specific monoclonal antibodies (Mabs). Sensitivity to VP (96 h) and CDC with Mabs and 10% human serum as complement source (48 h) was assessed using the MTT-test.

Results: H69VP were more than 50× resistant to VP than H69 wt. The dose-response curves against VP were identical for cells cultured at 2% and 10% FBS. H69VP cells showed a higher resistance when tested in 10% FBS as compared to 2%. GD2 expression was seen on 10% of the cells, and GM2 on 60% of H69 wt and 100% of H69VP showed GM2 expression, regardless of FBS concentrations. Fucosyl-GM1 expression was seen on 90% of H69 wt cells cultured in 10% FBS, on 5% of H69 wt cells in 2% FBS, but not on H69VP cells. Change of FBS from 2 to 10% or 10 to 2% for 4 weeks had no effect. Anti-GD2 was unable to induce CDC. Anti-fucosyl-GM1 induced CDC of 40% of H69 wt cells cultured in 10% FBS, but not of any other cells. CDC of >75% of H69VP and 50% of H69 wt regardless of FBS-concentration was seen. FBS 10% in the test medium yielded higher Mab-induced CDC than 2%.

Conclusion: Exposure of SCLC cells to high serum concentration appears to favour expression of fucosyl-GM1, whereas low and VP-resistance favours GM2. Loss of fucosyl-GM1 appears to be irreversible. Ganglioside expression correlates to CDC. High serum concentration also seems to augment VP-resistance of VP-resistant cells and specific CDC. The mechanisms behind these effects remain to be elucidated, but have to be taken into account when designing immunotherapy against gangliosides in SCLC and and when testing therapy sensitivity in vitro.

769 PUBLICATION

Investigation of cellular metabolism using the tetrazolium salt WST-1

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Purpose: Different new colorimetric assays based on tetrazolium salts are used for the invetigation of cell proliferation and viability. The aim of the present study was to evaluate the relation between the cell number vs. cell diameter and the corresponding absorption change with the WST-1 assay. Furthermore it was of interest at which site of the respiratory chain the tetrazolium salt is reduced.

Methods: WST-1 is a tetrazolium salt which is cleaved to formazan by the mitochondrial succinate-tetrazolium reductase system. A mammalian cell line was propagated under standard tissue culture conditions. Within a time period of 17 days photometric measurements of differing cell suspensions were performed using the Erlanger micro-lightguide spectrophotometer. In additional experiments 50 μ1 of the WST-1 reagent was added to 100 μl cell suspension (4×10^5 cells) and exposed to rotenone or cyanide (1 mM/1).

Results: The reduction of WST-1 to the corresponding formazan was linearly related to the cell number. If cells with different diameters and thus cell volume were investigated, it became obvious that with increasing cell diameter less WST1 is reduced. With increasing cell diameter the absorption change per cell became smaller. The reduction of WST-1 seems to occur at cytochrome aa3 because both rotenone and cyanide had the same attenuating effect after 15 minutes.

Conclusion: In summary the measured absorption changes do not uniquely depend on the cell number only. Therefor, a change in optical densities using the colorimetfic assay based on WST-1 is not an indicator of cell proliferation alone.

770 PUBLICATION

Diverging effects of 5-HT3 receptor antagonists on cellular potassium transport

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We used the influx of 86Rb+ (K+ analogue) to study Na+, K+ ATPase and Na+, K+, Cl− cotransport activity during the interaction of 5-HT3 receptor antagonists ondansetron and granisetron with the cytotoxic effect of estramustine phosphate (EMP) on the P31 lung carcinoma cell line.

EMP per se blocked Na+, K+, Cl− cotransport activity and this blockage