766 PUBLICATION

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

Kai Zhang, May Chew, Peter Mack. *Department of Experimental Surgery, Singapore General Hospital, Singapore*

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.

767 PUBLICATION

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

Lj. Pantelic[1], Z. Juranic[1], T. Stanojkovic[1], S. Manic[1], S. Radulovic[1], J. Joksimovic[2], I. Juranic[3]. *[1]Institute for Oncology and Radiology of Serbia, Belgrade; [2]Institute for Biological Research, Belgrade; [3]Faculty of Chemistry, University of Belgrade, Belgrade, Yugoslavia*

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.

768 PUBLICATION

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

T. Brezicka[1]. *[1]Göteborg University, Respiratory medicine and allergology, Göteborg, Sweden*

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

Ludwig Plasswilm[1], Sabine Frenzel[2], Resit Demir[3], Jens Höper[4]. *[1]University Hospital of Tuebingen; [2]Erlangen; [3]Heidelberg; [4]Institute of Physiology and Cardiology, University of Erlangen, Germany*

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

K. Grankvist[1], P. Behnam-Motlagh[1], P.E. Sandstrom[2], R. Henriksson[3]. *[1]Umeå University, Clinical Chemistry, Umeå; [2]Umeå University, Pediactrics, Umeå; [3]Umeå University, Oncology, Umeå, Sweden*

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

was fully inhibited by the 5-HT3 receptor antagonists ondansetron but not by granisetron. Serotonin and the 5-HT3 receptor antagonists recovered the reduction of Na+, K+, ATPase activity by EMP when Na+, K+, Cl− cotransport activity was blocked by bumetanide. The data show that ondansetron possesses a distinct ability to regain Na+, K+, Cl− cotransport activity of cells exposed to EMP and that this property differs from that of granisetron. Thus, highly 5-HT3 receptor-specific antiemetic agents may have different effects on ion transport of tumour cells during treatment with cytotoxic drugs.

771 PUBLICATION

Immune implications of cytostatics loaded in drug carriers of second generation. nanoparticles

O. Balacescu, Ioana Berindan, Rodica Risca, E. Neagoe. *Tumor Biology Department, Oncological Institute, Cluj-Napoca, Romania*

Purpose: Nanoparticles represents the second generation of solid coloid transports carrier which are used in the systemic administration for chemotherapy. Our study followed to establish the effects of free epirubicin and loaded in nanoparticules on the mouse peritoneal macrophages (enzymatic activity).

Methods: The experiment was carried out on three groups of Swiss female mice: control free nanoparticles (FN) and epirubicin loaded in nanoparticles (EN). The tests were performed on peritoneal macrophages at 24, 48 and 72 h after the treatment. The peroxidase, acid phosphatase and alpha-naphtilacetate esterase of peritoneal cells were assayed using histochemical methods. The number of cells with intensive and moderate enzymatic activity was determined.

Results: The *peroxidase activity* is constantly low to control. For FN activity grow to 48 h and touch the maximum level at 72 h. For EN the activity decrease dramatically at 72 h after a high value at 24 h. The *esterase activity* is significant for free and EN at 24 h. The *acid phosphatase activity* has a high level in control group. FN induce high activity at 48 h and decrease at 72 h. EN induce high activity at 24 h and 48 h and a low level at 72 h.

Conclusion: The enzymatic reactions represents macrophages activation markers. The results almost heterogenous reveal 2 types of reactions of peritoneal macrophages under nanoparticles influence: the differentiated reactivity of lizozomal enzymes with low expression of esterase and epirubicin loaded in nanoparticles have a cytotoxic effect on peritoneal cells; the resting cells maintain a constant phosphatase level.

772 PUBLICATION

Energy status and mitochondria oxidative phosphorylation in doxorubicin-sensitive and doxorubicin-resistant solid Guerin's carcinoma

I.N. Todor, G.I. Kulik, V.F. Chekhun. *Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv, Ukraine*

Purpose: The cell energy status of any tissues influences their viability. The indices of energy status and mitochondria oxidative phosphorylation of solid Guerin's carcinoma with different sensitivity to doxorubicin were studied.

Methods: The ATP, ADP, AMP content and energy charge served as the indices of energy status. The ATP, ADP and AMP content was estimated by means of thin-layer chromatography. The mitochondria oxidative phosphorylation indices were studied by the polarographic method with using of the covered combined platinum electrode of the Clark type.

Results: The mitochondria oxygen consumption rate (in presence of succinate or glutamate) during phosphorylation of ADP in doxorubicin-sensitive tumours was higher than in drug-resistant tumours. The ATP/O ratio in mitochondria of both substrains of Guerin's carcinoma was practically equal. The ATP level and the energy charge were higher in doxorubicin-sensitive tumours as compared with drug-resistant variant. On the other hand the AMP level was higher in drug-resistant carcinoma than in sensitive substrain.

Conclusion: Thus, the mitochondria oxygen consumption rate during phosphorylation of exogenous ADP as well as the ATP level and energy charge are reliably higher in doxorubicin-sensitive Guerin's carcinoma than in drug-resistant tumours.

Breast cancer genetics & biology

773 POSTER DISCUSSION

Frequent loss of heterozygosity (LOH) & monoallelic expression of the *p73* gene, a *p53*-homologue, in inflammatory breast carcinomas

J.C. Ahomadegbe, S. Tourpin, M.C. Mathieu, M. Vayssade, D. Caput[1], L. Zelec, M. Spielmann, T. Tursz, G. Riou, J. Bénard. *Institut Gustave Roussy 94805 Villejuif; [1]Sanofi Recherche, 31676 Labège, France*

Inflammatory breast cancer (IBC), which accounts for 3–5% of BC, is a very aggressive form of the disease of a very poor prognosis which had been thoroughly defined in our institution by clinical criteria (Rouéssé *et al., JCO*, 1986). The *p73* gene, a *p53* homologue gene recently discovered, locates at 1p36-33 a chromosomal locus which is putatively imprinted in SK-N-SH cells (Kaghad et al Cell 1997) and submitted to LOH in breast carcinomas (BC). To study whether invalidation of this locus is associated with BC agressiveness, *p73* genomic and allelic status were determined in 61 invasive BC, including 41 NBC (non IBC) & 20 IBC.

Results: 1) Genomic DNA polymorphism analysis revealed a frequency of informativity of 39% (24/61). Among heterozygous tumors, 8 IBC and 16 NBC, *p73* LOH was found to be significantly higher in IBC than in NBC, respectively 5/8 (62%) versus 2/16 (12.5%) (Fisher' exact test, $P = 0.02$). 2) cDNA polymorphism study on 16 cases showed monoallelic expression in 4/5 (80%) IBC versus 2/11 (18%) NBC (Fishers' exact test, $p = 0.05$). 3) Semi-quantitive RT-PCR revealed that gene expression was lower in IBC than NBC and normal breast epithelium.

Conclusion: A p73 expression decrease (LOH or/and monoallelism) is associated with IBC aggressiveness: it could be a genetic marker of agressiveness of the disease. *Supported by CRC 98-20, IGR/Sanofi Recherche/Ligue, Comité des Hauts de Seines France.*

774 POSTER DISCUSSION

Defective iodination within the breast: A feature of breast carcinoma?

R. Dwyer[1], M.T. Kilbane[1], P.P.A. Smyth[3], R.A. Ajjan[2], A.P. Weetman[2], E.W.M. McDermott[1], N.J. O'Higgins[1]. *[1]UCD, Surgery, Dublin, Ireland; [2]University of Sheffield, Medicine, Sheffield, United Kingdom; [3]UCD, Medicine, Dublin, Ireland*

The thyroid and breast possess a common ability to actively transport and organify dietary iodide, a process recently demonstrated to be under the control of a specific transmembrane protein, the sodium iodide symporter (NIS). However little data exists on the involvement of iodination in the natural history of breast cancer. To study extrathyroidal control of iodide transport we investigated iodine content and NIS expression in human breast tissues and the ability of serum from patients with breast disease to modulate NIS activity. Mean tissue iodine levels measured by dry ashing (80.9 ± 9.5 ngI/mg protein in 22 benign tumours; fibroadenomata) were significantly higher than those in both breast cancer (18.2 ± 4.6 ng/mg) or in morphologically normal tissue (31.8 ± 4.9 ng/mg) taken from within the tumour bearing breast ($N = 17$; $p < 0.001$ in each case). The iodine content of normal breast was also significantly > that in breast cancer ($p < 0.01$). Breast tissue iodine was orders of magnitude < that in 2 thyroid tissues (704 and 850 ng/mg). RT-PCR showed NIS expression not only in thyroid but in breast tissues including fibroadenomata and breast carcinoma tissue isolates. In contrast, NIS was not expressed in control tissues. Significant inhibition (i.e. >mean + 3 S.D. of control sera) of 125 I uptake into NIS transfected CHO cells was observed in serum from 29/104 (27.9%) of Graves' patients. Such inhibition was only detected in 1/33 control sera but was present in sera from 20/105 (19.0%) breast carcinoma and 8/49 (16.3%) benign breast disease ($p < 0.05$). The coincidence of low tissue iodine with NIS blocking activity in breast carcinoma cohorts suggests a defect in iodine handling within the breast and supports the thesis for an as yet undetermined role for iodine in the natural history of breast carcinoma.