39

DEVELOPMENT OF A NEW RECOMBINANT ANTI-CD30 IMMUNOTOXIN (CD30L-ETA') FOR THE TREATMENT OF HODGKIN'S LYMPHOMA

Barth S., Matthey B., Schön G., Diehl V., Engert A.
Med. Universitaetsklinik I, Cologne, Germany

Though most patients with Hodgkin's disease (HD) can be cured by standard polychemo- or radiotherapy, fewer than 30% of those who relapse attain durable disease-free remissions after second-line treatment. Thus, alternative tumor-specific strategies using cytokines, soluble receptors or monoclonal antibodies to deliver immunotherapeutic agents to the cancer site are being developed.

Hodgkin's lymphoma is ideally suited for the use of immunotoxins (ITs) for the following reasons: 1. the lymphocyte activation marker CD30 is expressed in high copy numbers on H-RS cells, 2. the number of malignant cells to be destroyed is low, 3. Hodgkin's lymphoma are well vascularized, suggesting sufficient access of the IT to most or all target cells, 4. Hodgkin's lymphoma are very sensitive to conventional therapy, allowing substantial debulking before ITs are used. Since recombinant DNA technology allows more readily the production of large amounts of ITs, we have developed a new CD30L-based fusion toxin (CD30L-ETA'). Human CD30L-cDNA was ligated into pET-based expression plasmid pBM1.1 and fused to a modified *Pseudomonas aeruginosa* Exotoxin A (ETA') lacking its cell binding domain I. After IPTG-induced expression in *Escherichia coli*, the 60 kDa His-tagged fusion protein (CD30L-ETA') was isolated from inclusion bodies in Tris buffer supplemented with 6 M guanidinium hydrochloride, 0.3 M dithiothreitol and 2 mM EDTA. Denatured protein was renatured in the presence of 0.4 M arginine and a glutathione redox system. Refolded protein was purified and concentrated by ion-exchange chromatography on a HiTrap Q column. The binding properties of (CD30L)-ETA' were evaluated by ELISA and FACS analysis on different cell lines expressing CD30. The *in vitro* toxicity of the fusion protein was tested on several Hodgkin-derived cell lines and the Burkitt lymphoma cell line BL38. CD30L-ETA' exhibited specific cytotoxicity against L540Cy cells as determined by [^{3}H]-leucin-uptake and colony assays. Competition experiments and analysis of the *in vivo* potency in animal models are under way.

40

THE COMPLEX TREATMENT OF PATIENTS WITH RECTUM CANCER - A RANDOMISED STUDY

[1]G. Bondar, [1]A. Borota, [1]Y. Yakovets, [2]J. Nowicky, [1]S. Zolotukhin
[1]*Clinic of Proctology, Regional Anticancer Center, Donetsk, Ukraine*
[2]*Ukrainian Anti-Cancer Institute, Vienna, Austria*

Purpose: Investigation of the results of therapy with Ukrain in patients with rectum cancer.

Methods: 48 patients with rectum cancer in stages T2-4N0-3M0 were included in a randomised study. Group I: High-fractional X-ray therapy + chemotherapy with Fluorouracil + operation. Group II: Monotherapy with Ukrain (10 mg every 2nd day, up to 60 mg) + operation + 40 mg of Ukrain in the postoperative period. 6 months after the operation these patients received another course of Ukrain therapy with a total dose up to 100 mg. Thereafter examinations of the immune system state (IgA, IgM, IgG), of T- and B-lymphocytes' number, phagocytic activity as well as CIC, AFP and CEA were carried out.

Results: In group I heavy symptoms of intoxication were observed. Postoperative complications like inflammatory processes were distinctly higher in group I (35,6%) than in group II (patients with Ukrain therapy) (11,6%). The Ukrain therapy resulted also in increased T- and B-lymphocytes number and immune globulin content as well as in phagocytic activity. After 9 months there were 6 cases of tumor process relapses in patients of group I and 1 case in group II, i.e. one patient treated with Ukrain.

41

LYSIS OF HUMAN CARCINOMA CELLS BY NK CELLS CORRELATES WITH HSP72 CELL SURFACE EXPRESSION

C. Botzler[1], R. Issels[1,2] and G. Multhoff[1]
[1)] GSF - Institut für Klinische Hämatologie, Munich, Germany;
[2)] Klinikum Großhadern Med. Klinik III, Munich, Germany

The cell surface expression patterns of major histocompatibility complex (MHC) class I, class II and HSP72 molecules was measured on human lung (LX-1) and mammary (MX-1) carcinoma cells. No major differences were found in the MHC cell surface expression pattern of both cell lines. However, they differ significantly in their capacity to express HSP72 on their cell surface. Under physiological conditions LX-1 cells express HSP72 molecules on more than 90% of the cells, whereas MX-1 cells exhibit no significant HSP72 cell surface expression (less than 5%). These expression patterns remained stable in all further tested cell passage. The sensitivity to lysis by NK cells could be correlated with the amount of cell surface expressed HSP72 molecules. By antibody blocking studies using HSP72 specific monoclonal antibody (mAb) a strong inhibition of lysis was only found with LX-1 cells but not with MX-1 cells.

In contrast to the cell surface expression, the cytoplasmic amount of HSP72 in MX-1 cells was twice as high compared to LX-1 cells under physiological conditions, whereas after nonlethal heat shock the rate of induction and the total cytoplasmic amounts of HSP72 were comparable in both cell lines. The clonogenic cell viability of LX-1 cells after heat incubation at temperatures ranging from 41°C to 44°C was significantly elevated compared to MX-1 cells.

In conclusion we can state the following: i) HSP72 cell surface expression on human carcinoma cells is independent of the cytoplasmic amount of HSP72. ii) The cell surface expression of HSP72 is associated with an increased sensitivity of tumor cells to lysis by NK cells. iii) Thermoresistance might be related to the amount of HSP72 expressed on the cell surface.

42

COMPARATIVE STUDYING OF RADIOMODIFYING ACTIVITY OF IMMUNOMODULATOR UKRAIN AND OTHER CYTOSTATICS.

V.Boyko, S.Smirnova, A.Petrov, S.Pavlov, I.Voltchek
Scientific Research Institute of Military Medecine, Defence Ministry,
St.Petersburg, Russia

Aim: The wide usage of such anti-cancer drugs as immunomodulator Ukrain and cytostatics Methotrexat, Cyclofosfan, Vinblastin, Vinkristin in medical oncology makes it actual to study their side pharmacological effects. Another problem follows in that anti-cancer drugs may be used before, in the course of, and following adjuvant therapy, so their influence on normal and malignant radiosesitivity should be evaluated.

Methods: These studies were carried out with inbreeding, CBA and BALB/c male mice. Short-term whole-body gamma-irradiaton of mice at doses ranging from 5.25 Gy to 7.5 Gy was performed with the IGUR device using 137 Cz as a sorce of radiation. The drugs were administrated one hour before irradiation and 30 minutes after irradiation at doses as follows: Methotrexat - 2.5 mg/kg, Cyclofosfan - 25 and 50 mg/kg, Ukrain - 0.2, 1.4 and 10 mg/kg, Vinblastin - 100 mg/kg and Vinkristin 20 mg/kg.

Results: From our studying it becomes evident that Methotrexat, Vinblastin and Vinkristin are not capable of modifying the effect of irradiation. The survival rate of the irradiated animals was inverse to the Cyclofosfan increased it by 30-40% and the administration of the Ukrain increased it by 50-60% at the irradiation doses from 5.25 - 6.75 Gy, with no significant effect at 7.5% Gy.

Conclusion: Ukrain and Cyclofosfan are capable of modifying the effects of irradiation, and we mention that Ukrain s capability is higher than that of Cyclofosfan.