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DIFFERENTIAL EFFECTS OF AN IFOSFAMIDE TREATMENT ON INTRACELLULAR GLUTATHIONE (GSH) LEVELS OF LYMPHOCYTE SUBSETS CORRELATES WITH IMMUNOLOGICAL FUNCTIONS

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We studied the influence of 4-hydroxyifosfamide, the activated form of ifosfamide (4-OH-IF), on intracellular glutathione (GSH) levels concomitant with immunological functions (cytotoxic and proliferative capacity) in human $CD3^-$ natural killer cells (NK) and $CD3^+$ allogeneic, cytotoxic T lymphocytes (CTL). Similar to BSO treatment, an incubation of activated human peripheral blood lymphocytes (PBL) with 4-OH-IF results in a depletion of the intracellular GSH levels (GSH levels are determined by HPLC analysis) and a significant inhibition of the proliferative capacity (determined by a $[^3H]$-thymidine incorporation) in a dose dependent manner. The cytotoxic activity of separated $CD3^-$ NK cells and $CD3^+$ CTL, either untreated or pre-treated with 4-OH-IF at concentrations ranging from 25 μM to 100 μM, is compared in a standard ^{51}Chromium release assay (CML). The major findings are: a) The capacity of $CD3^+$ major histocompatibility complex (MHC) restricted CTL, to lyse their allogeneic target cells is substantially reduced (down to 1/3 of the initial level) after preincubation of the effector cells with 50 μM 4-OH-IF. This inhibition of the lytic activity in $CD3^+$ CTL correlates with a substantial depletion in the intracellular GSH levels (from 27.2 to 5.3 nmol GSH/mg protein) in these cells. Rapid reconstitution of depleted GSH levels (from 5.3 to 26 nmol GSH/mg protein) and restoration of cytotoxic activity up to initial levels of CTL was achieved by incubation of the effector cells with thiols, e.g. glutathione ester (0.4 mM GSH-ester) or 2-mercaptoethanesulfonate (0.4 mM mesna). b) In contrast, the lytic activity in $CD3^-$ NK cells is not substantially affected after incubation with 50 μM 4-OH-IF. This result correlates with the capacity of NK cells to maintain their intracellular GSH levels after ifosfamide treatment (from 36.5 to 27.0 nmol GSH/mg protein). c) In comparison to $CD3^+$ CTL, $CD3^-$ NK cells are more resistant to an ifosfamide treatment because they have higher initial GSH levels (27.2 compared to 36.5 nmol GSH/mg protein) and a 4-fold higher capacity to synthesize GSH. Our findings might have further clinical implications concerning treatments with oxazaphosphorines to maintain immunocompetence by thiols.

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Amifostine - Protection from Hematotoxicity and Nephrotoxicity induced by Chemotherapy

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Amifostine (WR-2721) was originally developed by the US Army as a radioprotective agent. Of 4400 chemicals screened for this purpose, amifostine was selected as having the most effective radioprotective activity and the best safety profile. Subsequently it was recognized that it also protected normal tissue but not tumours against the toxic effects of various chemotherapeutic agents. Amifostine is a prodrug with little or no intrinsic protective effects. For the drug to become active, the phosphate group must be cleaved by the enzyme alkaline phosphatase to produce the free thiol, WR-1065. Inside the cell WR-1065 protects by scavenging oxygen-free radicals, and direct binding to the active species of alkylating and platinum agents. Other mechanisms include reversal of cisplatin-DNA adduct formation and chemical repair of DNA. The clinical development of amifostine focused on hematoprotection and nephroprotection, both of which are dose-limiting for regimens used in highly chemosensitive tumours. Several completed Phase II and Phase III trials with amifostine preceding chemotherapy allow conclusions regarding its efficacy and safety in clinical use. In a comparative trial in patients with advanced malignancies a single dose of cyclophosphamide (CTX) (1500 mg/m^2) was used in one cycle and the same dose in another cycle preceded by amifostine (740 mg/m^2). With CTX alone the median nadir neutrophil count was 400/mm^3 in contrast to 1157/mm^3 when the same dose of CTX was administered with prior amifostine ($p<0.001$). The percentage of patients with a neutrophil count <500/mm^3 was 67% vs 24% of patients receiving amifostine ($p<0.008$). Further evidence of the hematoprotective effects of amifostine is available from other studies, including a multicenter Phase III trial of CTX (1000 mg/m^2) and cisplatin (100 mg/m^2) without or preceded by amifostine (910 mg/m^2) for a total of 6 cycles in women (n=242) with ovarian cancer in stage III or IV. The following end-points were compared between both treatment groups: incidence of neutropenia with fever and/or infection ($p=0.001$), days in hospital ($p=0.003$), days on antibiotics ($p=0.002$), incidence of withdrawals for hematologic toxicity ($p=0.016$), percent of patients with grade 4 neutropenia following the last cycle of chemotherapy ($p=0.001$), percent of patients with grade 4 neutropenia whose neutrophil count failed to recover to $\geq$ 1500/mm^3 by day 22 ($p=0.008$). The ovarian cancer study as well as other confirmatory trials point out the capacity of amifostine to protect from cisplatin-induced nephrotoxicity. No patient had to discontinue therapy due to nephrotoxicity in the amifostine treatment arm ($p=0.004$). Baseline serum creatinine levels (day 22) over cycles showed highly significant differences between treatment arms ($p \leq 0.003$ in late cycles), favouring amifostine. Taken together amifostine is highly efficient to protect from chemotherapy-induced hematotoxicity and nephrotoxicity. All clinical trials were analyzed with regard to tumour-response and/or survival. None of the clinical studies revealed any evidence for a diminished treatment outcome. Survival follow-up in excess of 4 years showed super-imposable curves for both treatment groups in the ovarian cancer study. Dose-limiting toxicities of amifostine are nausea/vomiting and transient hypotension both of which are manageable when following proper handling procedures.

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EFFECTS PRODUCED BY A NEW BACTERIAL SUBSTANCE ON THE PROCESSES OF CELL PROLIFERATION.

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A new biologically active preparation "Blastolen" affecting the proliferative activity of hemopoietic cells and cells of the hemopoietic microenvironment has been developed. The efficacy of the preparation is due to its contained proteids, nucleotides and peptides derived from the walls of special strain of bacteria by means of enzymatic and chemical hydrolysis.
A series of in vitro culture experiments was performed in order to establish the way in which the preparation affect the functional activity of hemopoietic precursors in norm and in different disorders of blood system.
Analysis of results has shoun that "Blastolen"produces a dose-dependent hemopoiesis-stimulating effect which manifests itself in a differently disordered bone marrow hemopoietic function. In addition it was demonstrated in experiments on animals that "Blastolen" produces immunomodulating effects it stimulates a cellular and humoral immunity, protects against radiation and has an anti-tumoral effect.
The results of a detailed study of "Blastolen" suggests its possible application in the combination therapy of patients with leukemias and cancer

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EXPRESSION AND CLONING OF LST-1: A NOVEL GENE IN THE HUMAN TNF REGION

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The Leucocyte Specific Transcript - 1 (LST-1) represents the human homolog of the mouse B144 transcript, encoded within the tumor necrosis factor region of the human major histocompatibility complex class III interval. The gene is localized about 4 kb upstream of the lymphotoxin β gene, which constitutes the receptor for TNF-β . It spans a polymorphic genomic region encompassing the microsatellites TNFd and TNFe in intron three and a polymorphic Pvu II restriction site 260 bp downstream of the polyadenylation signal. Isolation of a full length cDNA clone revealed that LST-1 codes for interferon-γ inducible 800-nt transcripts, which are present in lymphoid tissues, T cells, macrophages, and histiocyte cell lines. We identified four different splice variants of the LST-1 mRNA encoding a soluble and a transmembrane protein. Its close linkage to the TNF genes and pattern of expression point toward a possible role for LST-1 in the immune response.