Lym 16

EFFECTS OF CYTOTOXIC DRUGS ON THE LYMPHOMA CELL LINE JM. AN IN VITRO STUDY BY FLOW CYTOMETRY. G. Exeriede, M. Freund, G. Birk, S. Delorme and H. Poliwoda

The high grade malignant lymphoma cell line JM was used for in vitro studies with cytotoxic agents. Incubation was performed for 3 hours in different doses. Thereafter, cells were washed and cultivated in fresh medium. Flow cytometric studies were carried out at different intervals between 8 and 72 hours with ethidium bromide staining. Results are summarized below:

Drug	early effect at	late effect at	hr
AraC	drop in S-phase*	rise in S-phase	24
5-FU	no effect	rise in S-phase	24
MTX	drop in S-phase*	rise in S-phase#	24
BLM	no effect in DNA histograms		
MMC	no effect	block in S-phase*	24
Mitox.	no effect	block in S-phase*	24
ACM	block in S-phase*		
DNR	drop in S-phase*	rise in S-phase	24
		only present with low doses#	
VLB		arrest in M-phase	24
VCR		arrest in M (*)	48
VP16	no effect	block in early resp. late S-phase	48

*dose dependend #inversely dose dependend

Dose dependend drops in the S-phase may allow to quantify the effect of these drugs. Some agents e.g. prednisolone and alpha-interferon resulted in drops of the mitosis rates but in no effects in DNA histograms.

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Lym 17

DETECTION OF INSOLUBLE FIBRONECTIN IN LYMPHOMA-CELL-LINES
K. Bock, M. Freund and H. Poliwoda

Studies for cell associated insoluble fibronectin were performed on three Hodgkin derived cell lines L 428, L 540, L 591, on three cell lines derived from Burkitt NHL BJA-B, P3HR-1, RAJI, on the T-cell lymphoma lines L 735, JM, on the L 363, and U 266 myeloma derived cell lines and on U 937, a malignant histiocytosis derived cell line. For immunostaining a polyclonal rabbit antiserum (Behring Werke AG, Marburg) was used as well as a monoclonal IgM mouse antibody (Sera Lab, Crawley Down, Sussex RH104FF, cat.No.: MAS 037 c). Staining was done by the avidin biotin peroxidase method. Cultured fibroblasts served as positive, HeLa cells as negative control.

Correlation between reactions with monoclonal antibody and with polyclonal antiserum was 0,998. Cells positive for fibronectin were found as shown in the following table:

Cell-line	derived	%+ monocl.AB	%+ polyocl.AS
L 428	Hodgkin	2,2	2,7
L 540	Hodgkin	8,3	10,0
L 591	Hodgkin	1,2	1,0
BJA-B	Burkitt	9,6	8,1
P3HR-1	Burkitt	2,9	3,7
RAJI	Burkitt	5,8	7,0
L 735	T-ALL	9,5	8,3
JM	T-ALL	5,0	5,2
L 363	Myeloma	68,0	63,0
U 266	Myeloma	11,3	12,8
U 937	m.histiocytos.	2,8	2,3

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Lym 18

THE EFFECT OF 3',5'-C-AMP, 3',5'-C-GMP AND 3',5'-C-CMP ON CELL GROWTH OF THE T-LYMPHOBLASTIC JM CELL LINE. AN IN VITRO STUDY ON PARASYNCHRONIZED CELLS BY FLOW CYTOMETRY. M.C. Wang, M. Freund and H. Poliwoda.

Cyclic nucleotides may play a role in the regulation of tumor growth. The T-lymphoblastic JM cell line was used to study the influence of the cyclic nucleotides on cell growth. S-phase parasynchronization was induced by incubation with 0,2 mmol thymidin for 20 hrs. As determined by flow cytometry 11 hrs. after release only 14% of cells remained unsynchronized in G0/G1-phase. M-phase parasynchronization was induced by a two step method: Incubation with thymidin was performed first as described above. 11 hours after release colcemide 0,1 ug/ml was added for 6 hrs. 2 hours after release 61,8% of cells were in G2/M-phase. Incubation with cyclic nucleotides was performed at the time of release. Flow cytometry was performed by an Ortho Cytofluorograph System 30.

3',5'-cCMP inhibits cells in the development from S-phase to G2/M-phase and in the development from G1-phase to S-phase. In contrast 5'-CMP, 2',3'-cCMP and 5'-dCMP have no inhibiting effect on cell growth, but can stimulate cell growth in S-phase. Inhibiting effect of 3',5'-cCMP on cell growth was also present in asynchronous cell cultures. 3',5'-cGMP stimulates cell growth in the development from S-phase to G2/M-phase, whereas it inhibits cell growth on the way from G1- to S-phase. 3',5'-cAMP has no significant effect.

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AML 01

SERIAL DETERMINATION OF FIBRONECTIN AND HEMOSTATIC PARAMETERS IN ACUTE LEUKEMIA
M. Freund, M. Barthels and H. Poliwoda

Disposition for septic infections in patients with fibronectin deficiency is in discussion. On this background we performed serial determinations of fibronectin in patients with acute leukemia. In some patients, serial tests for thrombin-time, PT, aPTT, Fibrinogen, Factors II and V were done in conventional way, factor VIII-associated antigen and factor XIII A and S as well as FDP by Laurell - electroimmunoassay, AT III and plasminogen by chromogenic substrate methods additionally.

13 patients have been studied up to now, 10 AML, 2 ALL, 1 CML blast-crisis (lymphoblastic differentiation). In patients with high tumor burden and good response to therapy decreasing fibronectin levels were found in the time of cell destruction. Without massive cell destruction fibronectin levels had an inconclusive course. Low values were found for F V, F XIIIa, F XIIIb and plasminogen associated with cell destruction. F VIII:AG levels were high as a sign for endothelial damage in this phase. No conclusive results were found for AT III in those courses where serial determinations had been done. Over all, destruction of a high tumor burden was associated with considerable alterations in plasmatic hemostasis which contribute to clinical observed bleeding tendency in this phase. Opsonic deficiency associated with low fibronectin levels may contribute to subsequent infections.

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