Eur J Cancer, Vol. 27, No. 9, p. 1182, 1991.
Printed in Great Britain
0277-5379/91 $3.00 + 0.00

Decrease of Cyclophosphamide Haematotoxicity by *Rhodiola rosea* Root Extract in Mice with Ehrlich and Lewis Transplantable Tumours

Sergey N. Udintsev and Vladimir P. Schakhov

BIOLOGICAL RESPONSE MODIFIERS may decrease haematotoxicity during cancer chemotherapy [1]. Many such modifiers are of natural origin [2]. One such is *Rhodiola rosea* root extract (RRRE), used in Siberian folk medicine for many diseases, including cancer [3]. Our aim was to study the effect of RRRE on neoplastic and normal haemapoietic cell precursors of mice with Ehrlich ascites tumour (EAT) and Lewis lung carcinoma (3LL) treated with cyclophosphamide 100 mg/kg. RRRE 0.5 ml/kg was given orally daily from 2–8 days after EAT and 3LL transplantation (7 doses). We evaluated the numbers of leucocytes and myelokariocytes, weight of tumours and extent of metastases in mice with 3LL, and relative number of viable EAT cells. Neoplastic (0.3×10^5) and haemapoietic (0.5×10^6) elements were placed in diffusion chambers and implanted intraperitoneally in syngenaic mice [4]. Colonies were counted on the 5th day.

Cyclophosphamide suppressed the growth of both tumours to 31–39% ($P < 0.05$) and extent of metastases of 3LL to 18%. Corresponding figures for RRRE were 19–27% and 16% ($P < 0.05$). However, while cyclophosphamide inhibited the numbers of leucocytes and myelokariocytes to 40–50% ($P < 0.05$) and 20–25%, respectively, RRRE had no effect on these indices. In combination, RRRE increased by 36% the antimetastatic activity of cyclophosphamide ($P < 0.05$) and enhanced the number of leucocytes and myelokariocytes by 30% ($P < 0.05$) and 16–18%, respectively.

Cyclophosphamide inhibited the proliferation of all the clonogenic cells assessed (Table 1); RRRE inhibited only tumour cells. The combination resulted in summation of these effects, and complete abrogation of the haematotoxicity of cyclophosphamide.

The results indicate that RRRE selectively inhibited the proliferation of tumour-disseminating elements, enhanced the antimetastatic and antitumour activities of cyclophosphamide, and decreased its haematotoxicity. This effect could be linked to different actions of RRRE on the clonogenic elements of both tissues.

Correspondence to S.N. Udintsev.
The authors are at the Research Institute for Pharmacology, Tomsk Scientific Centre, USSR Academy of Medical Sciences, 3 Lenin av., Tomsk 634028, USSR.
Revised 7 May 1991; accepted 16 May 1991.

Table 1. Effect of RRRE and cyclophosphamide on colony-forming activity of myelokariocytes and cells of EAT and 3LL mice tumours (% of control)

	CFA of tumour cells		CFA of myelokariocytes	
	EAT	3LL	EAT	3LL
Control	100	100	100	100
Cyclophosphamide	53.6*	52.6*	47.5*	42.9*
RRRE	67.9*	63.2*	103.8	104.2
RRRE + cyclophosphamide	43.4†	42.1	105.0†	124.3†

CFA = colony-forming activity.
* Statistically significant difference from non-injected (control) mice.
† Statistically significant difference from mice injected with cyclophosphamide.

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Eur J Cancer, Vol. 27, No. 9, pp. 1182–1183, 1991.
Printed in Great Britain
0277-5379/91 $3.00 + 0.00

In vitro Interaction between Retinoids and Cytokines in Human Myeloid Leukaemia Cells

Hassan Tawhid Hassan

PECK AND BOLLAG [1] reported that interferon-beta (INF-β) has no potentiating effect on retinoid-induced differentiation of human HL-60 and U937 cells after only 2 days of incubation. Previously, Ho [2] showed a significant synergistic anticellular effect between INF-β and tretinoin in U937 cells, but only after 4 and not 2 days of incubation. I have studied the effect of IFN-β with and without tretinoin on U937 cells.

U937 cells were cultured at 10^5 ml in 24-well plates containing RPMI 1640 supplemented with l-glutamine and 10% fetal calf serum for 4 days at 37°C in a humidified incubator in 7.5% CO_2. Recombinant human INF-β_2 (interleukin 6, IL-6) was added at 1–1000 U/ml either alone or with tretinoin at 1–100 nmol/l. After 4 days, viable cells were counted by trypan blue exclusion, and differentiation was assessed by nitroblue tetrazolium dye reduction; at least 500 cells were scored for positivity. IC_{50} was the concentration of tretinoin inducing either 50% inhibition of

Correspondence to H. T. Hassan, Leukaemia Research, Institute für Pharmazie, Freie Universität Berlin, Kelchstraße 31, 1000 Berlin 41, Germany.
Revised 7 May 1991; accepted 9 May 1991.

proliferation or 50% induction of differentiation on day 4 compared with control cultures, which contained neither tretinoin nor IL-6. The dose reduction index indicates how many fold of dose reduction is needed to achieve a given effect in combination compared with the agent alone [4] and was calculated by dividing the IC_{50} for tretinoin alone by the IC_{50} for tretinoin required in combination with IL-6. IL-6 enhanced the differentiation inducing and antiproliferation effects of tretinoin in U937 cells after 4 days (Table 1). IL-6 plus tretinoin significantly reduced the concentrations of tretinoin alone required to induce the same anti-proliferation and differentiation inducing effects in U937 cells (Table 1).

INF-β_1 and IL-6 had similar effects in human myeloid leukaemia cells after 5 days of incubation [3]. Moreover, the potentiation of the differentiation induced by retinoic acid in human myeloid leukaemia and abnormal cells by several cytokines, drugs and chemical agents in various studies [2, 4] was usually observed after 4 or more days of incubation.

The failure to detect the potentiation effect of INF-β on retinoids and the low number of cytokines (only four out of twelve) that significantly potentiated tretinoin-induced differentiation of HL-60 and U937 cells in Peck and Bollag's study [1] could be due to the short incubation (only 2 days). It is essential in the studies of interactions between retinoids and cytokines to allow adequate time for the potentiation of growth inhibition and differentiation of human myeloid leukaemia cells. To avoid problems with the limited *in vitro* stability of cytokines, fresh cytokines could be added to the cultures during the test period or a short incubation period of 4 days could be used.

Since retinoic acid stimulated the production of IL-1 and IL-3 from P388 D1 and WEHI-3 leukaemia cell lines [5], it is important to test the production of cytokines in cultures treated with retinoid as well as in cultures treated with retinoid plus cytokine(s). The assessment of secondary production of cytokines could also provide insight into the possible biological and molecular mechanisms of their interactions with retinoids.

In vitro studies have shown that many cytokines stimulate directly or indirectly the proliferation of myeloid leukaemia cells [6]. Also, disease progression indicated by an increase in leukaemic blast cells was observed in some patients with myelodysplastic syndrome following therapy with cytokines [6]. Despite the cooperation between tretinoin and granulocyte colony stimulating factor (G-CSF) in inducing differentiation of human myeloid leukaemia cells [1, 4], tretinoin inhibited the proliferative effect of G-CSF only at low dose [7]. Therefore, it is important to examine whether using retinoids, which are antiproliferative agents, in combinations with cytokines could limit the potentially hazardous proliferative effect of these cytokines on myeloid leukaemia cells. The predictive value of *in vitro* results in cloned haemopoietic cell lines, such at HL-60 and U937, for the treatment of myeloid leukaemia with retinoid plus cytokine, is undermined by the complex multiple cascades of cytokine/cell interactions. Therefore, it is important to test the interactions between retinoids and cytokines not only in cloned cell lines but also in mixed/heterogenous cell populations, i.e. in myeloid leukaemia and normal cells in primary cultures to detect secondary effects due to cytokines inducing the production of further cytokines via accessory cells. The importance of these investigations in primary cultures is emphasised by Peck and Bollag's findings [1] that some combinations of two cytokines resulted in antagonism in the presence of retinoids.

In addition to the successful role of tretinoin alone as remission induction therapy in patients with acute promyelocytic leukaemia, retinoids in combination with other drugs as maintainence therapy significantly increased the remission duration in both children [8] and adults [9] with acute myeloid leukaemia. The synergistic interactions between tretinoin and other drugs in inhibiting the proliferation of human myeloid leukaemia clonogenic cells [10] could prevent the regrowth of the residual leukaemic cells remaining after remission induction therapy and hence could be responsible for the increase of remission duration achieved in these patients. To explore the potential therapeutic role of combinations of retinoids and cytokines, more extensive *in vitro* investigations of the interactions between retinoids and cytokines in primary cultures of human myeloid leukaemic cells are required.

Table 1. Effect of IL-6 on the antiproliferation and differentiation inducing actions of tretinoin in U937 cells

	Antiproliferation effect		Differentiation induction effect	
Tretinoin	IC_{50} (nmol/l)	DRI (fold)	IC_{50} (nmol/l)	DRI (fold)
Alone	36.5*	—	76.0	—
+IL-6(U/mol)				
1	27.3	1.3	35.1	2.2
10	13.1	2.8	33.7	2.3
100	10.2	3.6	30.3	2.5
1000	8.5	4.3	15.9	4.8

DRI = dose reduction index.
*Mean values of three separate experiments with S.D. for each value ≤ 8% of mean.

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Acknowledgements—This work was supported by the Alexander von Humboldt Research Foundation.