

Cross-resistance to five glucocorticoids in childhood acute lymphoblastic and non-lymphoblastic leukemia samples tested by the MTT assay: Preliminary report[★][✉]

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In vitro antileukemic activity of five glucocorticoids and their cross-resistance pattern in childhood acute lymphoblastic and non-lymphoblastic leukemia were determined by means of the MTT assay in 25 leukemia cell samples of childhood acute leukemias. The equivalent antileukemic concentrations of the drugs tested were: 34 μ M hydrocortisone (HC), 8 μ M prednisolone (PRE), 1.5 μ M methylprednisolone

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Abbreviations: ALL, acute lymphoblastic leukemia; iALL, initial ALL; rALL, relapsed ALL; AML, acute myeloblastic leukemia; BET, betamethasone; BM, bone marrow; DX, dexamethasone; HC, hydrocortisone; MPR, methylprednisolone; PB, peripheral blood; PRE, prednisolone; RR, relative resistance.

(MPR), 0.44 μ M dexamethasone (DX) and 0.22 μ M betamethasone (BET). In comparison with initial ALL cell samples, the relapsed ALL group was more resistant to PRE (38-fold, $p = 0.044$), DX (> 34-fold, $p = 0.04$), MPR (38-fold), BET (45-fold) and HC (33-fold). The AML cell samples were even more resistant to: PRE (>85-fold, $p=0.001$), DX (> 34-fold, $p = 0.004$), MPR (> 69-fold, $p = 0.036$), BET (> 69-fold, $p = 0.038$) and HC (54-fold, $p = 0.059$) when compared with ALL on initial diagnosis. A significant cross-resistance among all the glucocorticoids used was found. Only in some individual cases the cross-resistance was less pronounced.

Glucocorticoids are important drugs in therapy of acute lymphoblastic leukemia (ALL) but resistance to those agents is a major obstacle in the progress of anti-leukemic therapy. On the other hand, this group of drugs is not used in therapy of acute myeloblastic leukemia (AML) due to clinical resistance of myeloblasts. Resistance *in vitro* and *in vivo* to glucocorticoids is nowadays one of the strongest adverse risk factors in the treatment of childhood acute lymphoblastic leukemia [1–2]. Based on the results obtained both with experimental cell lines and patients' leukemic cell samples, the most important resistance mechanisms to glucocorticoids include: abnormalities in number, morphology and function of glucocorticoid receptors [3–4], upregulation of transmembrane P-glycoprotein [5], intracellular level of glutathione and its *S*-transferases [6], changes in DNA repair processes [7], heat shock proteins expression [8], and apoptosis dysregulation, especially in the mitochondrial pathway [3]. Future clinical possibilities to overcome the resistance to glucocorticoids include the use either of resistance modifiers or a drug with the highest anti-leukemic activity for individual patient. Possible modulation of resistance, including administration of a modifier together with the glucocorticoid, is still far from clinical practice. On the other hand, the role and the use of prednisolone and dexamethasone in different therapeutic protocols for childhood ALL is not yet strictly established. Differential cytotoxicity of those drugs against human lymphoblasts has been reported in several studies [9–11].

The objective of the study was the determination of antileukemic activity and cross-resistance pattern among five various glucocorticoids in samples of childhood acute leukemia. In addition to prednisolone and dexamethasone, the basic drugs in the therapy of ALL, we tested three glucocorticoids which are in common use in treatment of numerous non-proliferative diseases, due to their anti-inflammatory properties: hydrocortisone, methylprednisolone and betamethasone.

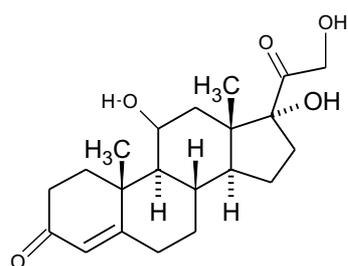
MATERIAL AND METHODS

Leukemic cells were from children with acute lymphoblastic leukemia on diagnosis (iALL, $n = 13$), rALL on relapse ($n = 6$) and with acute non-lymphoblastic leukemia on diagnosis (iAML, $n = 6$). The analyzed group of patients consisted of 17 boys and 8 girls, aged 0.6–18 yrs (median 9 yrs). Each patient was included only once in the study.

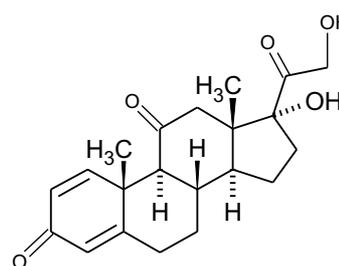
In vitro drug resistance was established by means of the MTT assay [12]. The following glucocorticoids were tested: prednisolone (PRE), dexamethasone (DX), betamethasone (BET), methylprednisolone (MPR) and hydrocortisone (HC) (Table 1, Scheme 1). Drug concentration lethal to 50% of tested cells (LC_{50}) was used as a measure of cytotoxicity. Differences between groups were compared with Mann-Whitney U test. Cross-resistance between the cytotoxicity of each pair of drugs was determined by Spearman's rho coefficient of correlation. The study was approved by the Local Ethics Committee, Medical University, Bydgoszcz.

Table 1. Drugs and concentrations tested

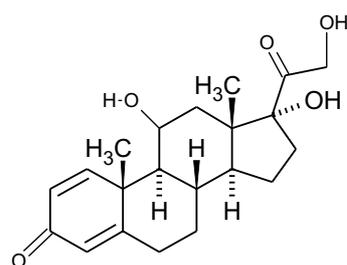
	Molecular mass	Concentration range	
	[g/mol]	[$\mu\text{g/ml}$]	[μM]
Betamethasone	392.0	0.000183–6	0.5 nM–15.3
Dexamethasone	392.0	0.000183–6	0.5 nM–15.3
Methylprednisolone	374.4	0.0012207–40	3.2 nM–106.8
Prednisolone	360.0	0.007629–250	0.0212–694
Hydrocortisone	362.5	0.0244141–800	0.0673–2.2 mM



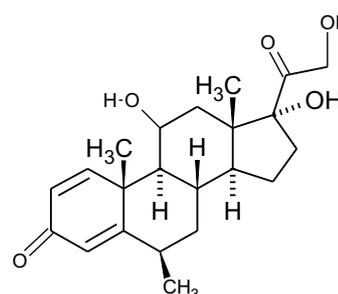
CORTISOL (HYDROCORTISONE)



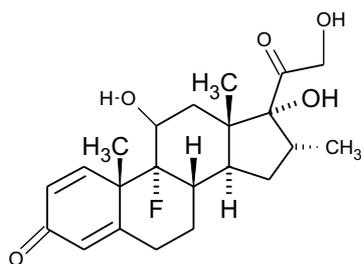
PREDNISONE



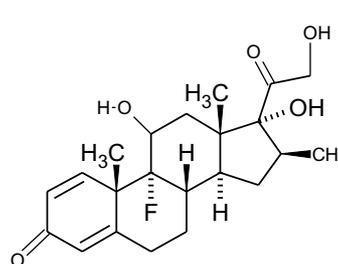
PREDNISOLONE



METHYLPREDNISOLONE



DEXAMETHASONE



BETAMETHASONE

Scheme 1. Chemical structure of glucocorticoids**RESULTS**

The equivalent antileukemic concentrations of the drugs tested were: 34 μM HC, 8 μM PRE, 1.5 μM MPR, 0.44 μM DX and 0.22 μM

BET. In comparison with initial ALL samples, the relapsed group of ALL was more resistant to PRE (38-fold, $p = 0.044$), DX (> 34-fold, $p = 0.04$), MPR (38-fold), BET (45-fold) and HC (33-fold). The AML group of patients was even

more resistant to PRE (> 85-fold, $p = 0.001$), DX (> 34-fold, $p = 0.004$), MPR (> 69-fold, $p = 0.036$), BET (> 69-fold, $p = 0.038$) and HC (54-fold, $p = 0.059$) compared with the ALL on initial diagnosis group (Table 2). A significant

lower than $1/2$ of the highest concentration tested. In 5/6 AML cases maximum resistance to PRE and DX was seen. In four cases, even a stimulation by at least one glucocorticoid of myeloblast growth was observed.

Table 2. Median cytotoxicity (LC₅₀) and relative resistance to glucocorticoids

	LC ₅₀ [μ M]			Relative resistance (RR)	
	iALL	rALL	iAML	RR(rALL vs iALL)*	RR (iAML vs iALL)**
Betamethasone	0.22	9.8	> 15.3	44.8; $p = 0.072$	> 69; $p = 0.038$
Dexamethasone	0.44	> 15.3	> 15.3	>34.2; $p = 0.040$	> 34.2; $p = 0.004$
Methylprednisolone	1.55	59.5	> 106.8	38.4; $p = 0.15$	> 69; $p = 0.036$
Prednisolone	8.1	308.3	> 694	38.0; $p = 0.044$	> 85; $p = 0.001$
Hydrocortisone	34.7	1155	1894	33.0; $p = 0.13$	54; $p = 0.059$

RR, relative resistance in comparison with iALL; (*) RR is the median LC₅₀ for rALL divided by the median LC₅₀ for iALL; (**) RR is the median LC₅₀ for iAML divided by the median LC₅₀ for iALL; RR > 1 indicates that for the given drug, rALL (iAML) is more resistant than iALL; p , Mann-Whitney U test.

cross-resistance between all glucocorticoids used was observed (Table 3). In some individual cases the cross-resistance was less pronounced.

A significant correlation was observed between the cytotoxicities of structurally similar drugs, such as DX and BET (Table 3). In 3/6 AML cases maximum resistance to all five glucocorticoids was observed; in all other cases, except one, the LC₅₀ values were not

In one case, however, good sensitivity to BET was observed.

DISCUSSION

Resistance to glucocorticoids can play a role in pathogenesis of various diseases, such as autoimmune disorders, AIDS, Nelson syndrome, sclerosis multiplex and leukemias

Table 3. Correlation matrix of glucocorticoids cross-resistance

	PRE	DX	BET	MPR	HC
PRE		0.625	0.550	0.407	0.525
		0.001	0.008	0.043	0.007
DX	0.625		0.681	0.352	0.639
	0.001		< 0.001	0.084	0.001
BET	0.550	0.681		0.461	0.690
	0.008	<0.001		0.031	< 0.001
MPR	0.407	0.352	0.461		0.693
	0.043	0.084	0.031		< 0.001
HC	0.525	0.639	0.690	0.693	
	0.007	0.001	< 0.001	<0.001	

Upper value in each area is the value of Spearman's rho coefficient of correlation of LC₅₀ values for a pair of drugs, lower value - p , value of this coefficient. PRE, prednisolone; DX, dexamethasone; BET, betamethasone; MPR, methylprednisolone; HC, hydrocortisone.

[13]. Recent studies demonstrate that primary (hereditary) abnormalities in the glucocorticoid receptor gene make 6.6% of the normal population relatively 'hypersensitive' to glucocorticoids, while 2.3% are relatively "resistant". These abnormalities might explain the well-known phenomenon that some individuals develop severe adverse effects during therapy with a low dose of glucocorticosteroids, while others do not develop side effects even during long-term therapy with a much higher dose [14]. In childhood ALL *in vitro* glucocorticoid resistance is observed in 41–48% patients on diagnosis. After repeated cycles of chemotherapy including glucocorticoids, the rate of glucocorticoid resistance reaches 60–90% patients at relapse [1, 15]. The sensitivity to glucocorticoids is thought to be one of the most important prognostic factors in various therapeutic protocols for children with ALL. Prednisolone resistance is thought to be the strongest single adverse prognostic factor [1–2, 9–11, 15]. Glucocorticoids are the most important cytotoxic drugs in the therapy of ALL. Recent studies have shown that the response of ALL to glucocorticoid therapy increases with drug dose. High-dose corticosteroid treatment abrogates the effect of relative drug insensitivity and of low glucocorticoid receptor number on peripheral blasts [16].

In our study, based solely on clinical material, we determined *in vitro* cross-resistance pattern between prednisolone, dexamethasone, betamethasone, methylprednisolone and hydrocortisone in samples from childhood acute leukemia. We showed that betamethasone, methylprednisolone and hydrocortisone cannot circumvent resistance to prednisolone and dexamethasone in childhood ALL and even showed worse activity against AML, with a possible exception of betamethasone. We observed that each of the glucocorticoids tested could stimulate *in vitro* proliferation of myeloblasts in 4-day culture, similar to the results of another study [17]. We noticed, however, that in individual pa-

tients cells could be identified as sensitive to betamethasone but resistant to prednisolone or dexamethasone. An additional observation of the present study is that DX has a 17-fold higher antileukemic potency than PRE, which was suggested earlier [10]. The activity of BET against leukemic cells was found to be 2-fold higher than that of DX. These results suggest that antileukemic properties of the glucocorticoids tested may differ from their anti-inflammatory activity, as DX is usually thought to be 7-fold more active than PRE, and BET about 1.5-fold more active than DX. In conclusion, the results of the study suggest that a subgroup of patients with leukemia can be recognized, for whom the individual profile of cytotoxicity to glucocorticoids can help to choose the drug with the highest antileukemic potency and possibly more favourable pharmacokinetics.

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