ROLE OF NUCLEOSIDE TRANSPORT IN GLUCOCORTICOID-INDUCED REGRESSION OF MOUSE LYMPHOMA P1798

J. Stevens, Y. W. Stevens, U. Behrens and V. P. Hollander

Research Institute, Hospital for Joint Diseases, Mount Sinai School of Medicine, 1919 Madison Ave., New York, N. Y. 10035

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Summary

Exposure of corticoid-sensitive P1798 lymphocytes to cortisol results in inhibition of uridine uptake and incorporation with no effect on 2-deoxyglucose transport. Nucleoside uptake by corticoid-resistant cells is not affected by the hormone. Inhibition of uridine transport may play a key role in tumor regression and does not depend on reduced availability of glucose.

Introduction

The beneficial effects of prednisone in the treatment of acute lymphoblastic leukemia have been known for many years (1) but the mechanism of glucocorticoid-induced lymphocytolysis is still poorly understood. Exposure of normal or malignant lymphocytes to glucocorticoids results in decreased incorporation of radioactive precursors into RNA, DNA and protein (2,3,4,5). These changes occur prior to detectable tumor regression or cell lysis. Munck and colleagues demonstrated that inhibition of glucose transport is the most rapid metabolic alteration caused by exposure of thymocytes to cortisol (6). They concluded that the catabolic effects of the glucocorticoids were secondary to their earlier actions on carbohydrate metabolism. In a preliminary report, we suggested that the sequence of events observed after *in vivo* treatment of lymphosarcoma P1798 with glucocorticoids may differ from that which occurs in thymus (7).

Interpretation of these experiments was complicated by possible extra-tumoral effects of the steroid in the intact animal. It is now shown that *in vitro* exposure of P1798 lymphocytes to physiological concentrations of cortisol causes pronounced inhibition of uridine uptake with no concomitant effect on 2-deoxyglucose transport. These results indicate that inhibition of uridine uptake cannot be explained solely in terms of reduced availability of glucose.

Materials and Methods

Lymphosarcoma P1798 was maintained by serial subcutaneous transplantation as described previously (4). The corticoid-sensitive tumor regressed almost completely after three days' treatment with steroid, whereas the resistant strain grew as rapidly as the control.

Uridine-5-^{3}H (specific activity 28.5 Ci/mmole) and 2-deoxy-D-glucose-1-^{14}C (specific activity 54.6 mCi/mmole) were obtained from New England Nuclear (Boston, Mass.). Purity of uridine-^{3}H was checked by thin layer chromatography, using Randerath's solvents 1 and 2 (8). Purity of 2-deoxyglucose was monitored by thin layer chromatography (8) and descending paper chromatography (9). Attempts to prepare cell suspensions from our tumor as described by Rosen *et al* (5) proved unsuccessful because of substantial cell breakage and inability to maintain appropriate pH during incubation. Therefore, the following technique was developed. Glassware was siliconized and procedures carried out at 4° unless otherwise indicated. Tumors (1.0-1.5g) were

trimmed, cut into narrow strips and gently teased with glass spatulas in 15ml of Dulbecco's modified Eagle's medium containing 3.5g/l $NaHCO_3$ and 1g/l glucose. The pH was maintained at 7.2-7.3 by gassing with 95% air - 5% CO_2. Aliquots of the cell suspension were diluted with fresh medium to a final concentration of 2 to 5×10^6 cells/ml in a total volume of 10 ml. Incubation was carried out in 50 ml Erlenmeyer flasks at 37° under 95% air - 5% CO_2 with constant shaking (45 rpm). Cortisol was added to a final concentration of 1×10^{-6}M in Hanks balanced salts. Control vessels received vehicle only. Fifteen minutes prior to the end of incubation, isotopes (0.2μCi uridine-^{3}H plus 0.5μCi 2-deoxy-D-glucose-1-^{14}C) were added in 0.5 ml of Dulbecco's modified Eagle's medium. After incubation, the cells were immediately separated from the medium by centrifuging 2 min at 165 x g. The cells were washed twice with ice-cold medium and counted in a hemocytometer. Viability was greater than 99% (Trypan blue exclusion) both prior to and after 3 hr exposure to cortisol. The cells were broken in 0.27 M $HClO_4$ and aliquots of the acid-soluble and acid-insoluble fractions counted as previously described (10). Efficiency was 48% for ^{14}C and 31.5% for ^{3}H. Tritium counts were corrected for ^{14}C spillover (37%).

Results

Fig. 1 shows uptake of radioactive uridine and deoxyglucose was proportional to time for at least 30 min. The only product formed from 2-deoxyglucose was 2-deoxyglucose-6-P. No free deoxyglucose could be detected. Table 1 shows that glucose

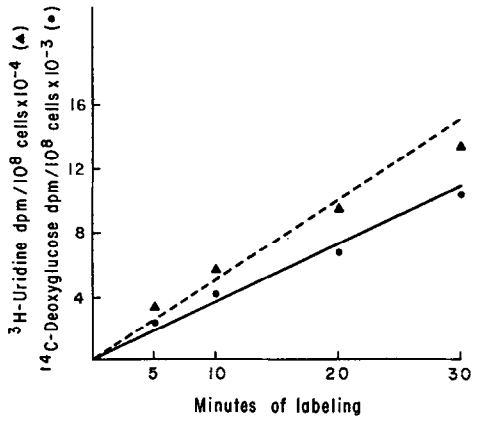

Fig. 1. Time course of uridine-^{3}H and 2-deoxyglucose-1-^{14}C uptake by P1798 lymphocytes. Cell suspensions were prepared as described in Materials and Methods, and incubated for 2.5 hr at 37°. A mixture of uridine-^{3}H and 2-deoxyglucose-1-^{14}C was then added to each vessel and duplicate flasks withdrawn after 5, 10, 20 and 30 min. Radioactivity in the acid-soluble fraction is expressed as dpm/10^{8} cells.

TABLE 1

Glucose	2-deoxyglucose-1-C^{14}
7.5 mg%	30856 dpm/10^{8} cells
100.0	6194
450.0	2075

Competition between glucose and 2-deoxyglucose for uptake by P1798 lymphocytes. Cells were incubated with 0.5 μCi of 2-deoxyglucose-1-C^{14} for 15 min in the presence of different concentrations of nonradioactive glucose. Results are expressed as dpm/10^{8} cells in the acid-soluble fraction.

competes with 2-deoxyglucose for uptake in P1798. Helmreich and Eisen reported that glucose and 2-deoxyglucose are trans-

TABLE 2

Tumor	Steroid		Uridine-^{3}H Control dpm/10^8 cells x 10^{-3}	Uridine-^{3}H Treated (% Control)	2-Deoxyglucose-1-C^{14} Control dpm/10^8 cells x 10^{-3}	2-Deoxyglucose-1-C^{14} Treated (% Control)
Sensitive	Cortisol	1hr #1	141 ± 5[α]	99.8	5.3 ± 0.2[α]	105.5
		#2	198 ± 2	98.3	6.5 ± 0.1	101.7
	Cortisol	2hr #1	101 ± 0.7	82.9[b]	5.6 ± 0.1	101.2
		#2	66 ± 0.7	85.2[c]	4.7 ± 0.1	100.5
	Cortisol	3hr #1	118 ± 3	67.8[c]	6.2 ± 0.2	91.8
		#2	50 ± 2	72.5[b]	5.1 ± 0.2	100.0
		#3	95 ± 4	75.8[b]	4.9 ± 0.2	98.8
Sensitive	Epicortisol	3hr	164 ± 5	107.1	5.0 ± 0.2	108.9
Resistant	Cortisol	3hr #1	31 ± 0.3	90.7	8.4 ± 0.1	102.5
		#2	48 ± 4	93.0	9.6 ± 0.6	102.9
		#3	32 ± 0.3	91.8	7.1 ± 0.7	108.8

α) ± SEM
b) $P < 0.01$
c) $P < 0.001$

Effect of cortisol and epicortisol on uridine -H^3 and 2-deoxyglucose-1-C^{14} uptake by P1798 lymphocytes. Results are expressed as dpm/10^8 cells x 10^{-3} in the acid-soluble fraction (controls) or as % of control (treated).

planted by the same carrier in normal lymphocytes (11). Table 2 shows that exposure of corticoid-sensitive P1798 lymphocytes to cortisol resulted in marked inhibition of ^{3}H-uridine uptake (15% at 2 hr; 30% at 3 hr) with no significant decrease in 2-deoxyglucose transport. Inhibition of uridine incorporation into tumor RNA paralleled the reduction of nucleoside uptake and was of similar magnitude (not shown). The specificity of this decrease is demonstrated by the absence of inhibitory effects when the inactive isomer, epicortisol, is used instead of cortisol. Furthermore, cortisol did not inhibit uridine uptake in cell suspensions prepared from corticoid-resistant P1798, suggesting that inhibition of uridine transport plays an important role in glucocorticoid-induced lymphocytolysis.

Discussion

Our results clearly demonstrate that glucocorticoid-induced inhibition of uridine transport in corticoid-sensitive P1798 lymphosarcoma does not depend on prior reduction of glucose uptake. Gabourel and Aronow reported that mouse lymphoma cells cultured in the presence of cortisol show a decreased content of protein and DNA with no concomitant reduction in glucose utilization or lactate production (2). Stevens *et al.* (12) demonstrated that inhibition of *in vivo* thymidine incorporation into mouse lymph node DNA and of leucine incorporation into protein occurred prior to cortisol effects on glucose uptake. Drews and Wagner (13) in a comparative study on the effects of glucose deprivation and prednisolone treatment on rat thymocytes conclu-

ded that the effects exerted by the steroid were incongruent with those caused by reduced availability of glucose. Gray et al. (14) showed that growth of L cells, using fructose as a source of carbon, was inhibited to the same extent by triamcinolone acetonide as the growth of cells cultured in a glucose-containing medium. In contrast, Rosen et al. (5) reported that glucose transport was depressed as early as 1 hr after exposure of P1798 lymphocytes to cortisol and that this effect preceded inhibition of uridine uptake and incorporation. The discrepancy with the results presented here may be due to differences in the method used for preparing the cells or differences between the two tumor lines. It is noteworthy that both variants of corticoid-sensitive tumor are exquisitely sensitive to steroid treatment. The possibility that inhibition of nucleoside transport may play a key role in glucocorticoid-induced lymphocytolysis is currently under investigation.

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