Hydrocortisone does not influence glucocorticoid sensitivity of acute lymphoblastic leukemia cells

Lidewij T. Warris, 1,2 Marry M. van den Heuvel-Eibrink, 1,2 Ingrid M. Ariës, 4 Rob Pieters, 1,3 Erica L.T. van den Akker, 1,3 and Monique L. den Boer 1

1Department of Pediatric Hematology and Oncology, Erasmus MC- Sophia Children’s Hospital, Rotterdam; 2Department of Pediatric Endocrinology, Erasmus MC- Sophia Children’s Hospital, Rotterdam; and 4Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

Correspondence: m.m.vandenheuvel-eibrink@prinsesmaximacentrum.nl
doi:10.3324/haematol.2014.112177
Hydrocortisone does not influence glucocorticoid sensitivity of acute lymphoblastic leukemia cells.

Supplemental information

Figure S1. Hypothesis.
Dexamethasone treatment is known to deplete endogenous cortisol levels. This may cause the cerebral side-effects reported for this drug. Exogenous applied cortisol (hydrocortisone) may reduce these side-effects through binding to the mineralocorticoid receptor for which dexamethasone has no affinity. GR = glucocorticoid receptor, MR = mineralocorticoid receptor.
Figure S2. Correlation between microarray and RT-qPCR determined expression levels of MR and GR.
A. Correlation between microarray and RT-qPCR of MR, R=0.88, p<0.0001. B. Correlation between microarray and RT-qPCR of GR, R=0.48, p=0.02. MR = mineralocorticoid receptor, GR = glucocorticoid receptor. Spearman’s rank correlation coefficient was calculated to compare microarray gene expression results to RT-qPCR results.
Figure S3. Hydrocortisone does not induce resistance to prednisolone in leukemic cell lines.
A. Cytotoxicity of hydrocortisone in combination with prednisolone in a glucocorticoid resistant MHH-CALL4 cell line. B. Cytotoxicity of hydrocortisone in combination with prednisolone in a glucocorticoid resistant Reh cell line. C. Cytotoxicity of hydrocortisone in combination with prednisolone in a glucocorticoid sensitive MHH-CALL2 cell line.
Responsiveness of leukemic cell lines was determined by an MTT-assay. Sensitivity to prednisolone was corrected for cell death induced by hydrocortisone as single agent to determine the synergistic or antagonistic effect of the drug combination.
Figure S4. Hydrocortisone sensitizes leukemic patients’ cells of different subtypes which are intrinsic sensitive to prednisolone. Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on prednisolone sensitivity of glucocorticoid sensitive primary patients’ cells (hyperdiploid, ETV6-RUNX1+, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of prednisolone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.
Figure S5. Hydrocortisone sensitizes leukemic patients’ cells of different subtypes which are intrinsic sensitive to dexamethasone. Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on dexamethasone sensitivity of glucocorticoid sensitive primary patients’ cells (hyperdiploid, ETV6-RUNX1+, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of dexamethasone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.
Figure S6. Hydrocortisone does not induce more resistance to leukemic patients’ cells which are intrinsic resistant to prednisolone in different subtypes of ALL.
Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on prednisolone sensitivity of glucocorticoid resistant primary patients’ cells (hyperdiploid, ETV6-RUNX1+, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of prednisolone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.
Figure S7. Hydrocortisone does not induce more resistance to leukemic patients’ cells which are intrinsic resistant to dexamethasone in different subtypes of ALL.

Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on dexamethasone sensitivity of glucocorticoid resistant primary patients’ cells (hyperdiploid, ETV6-RUNX1+, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of dexamethasone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.
Methods microarrays
RNA was extracted by means of Trizol isolation (Invitrogen, Bleiswijk, Netherlands) according to the manufacturer’s protocol and RNA quality and integrity determined with the 2100 bioanalyzer (Agilent, Amstelveen, Netherlands). The Affymetrix One-Cycle cDNA Synthesis kit (Santa Clara, CA, USA) and the GeneChip IVT Labeling kit (Santa Clara, CA, USA) were used to synthesize cRNA. RNA processing and hybridization to the Affymetrix U133 Plus 2.0 GeneChip oligonucleotide microarray were performed according to the manufacturer’s protocol. Gene-expression values were calculated with Affymetrix Microarray Suite version 5.0. Expression signals were scaled to the target intensity of 500 and log transformed. Only arrays with scaling factor <10 and GAPDH cRNA integrity (3’/5’) <3 were used for subsequent normalization procedures and analysis of GR and MR expression levels. Normalization of Affymetrix U133 Plus 2.0 data was performed by Robust Multichip Average and variance stabilization and normalization 2 (VSN2) in the R environment (Huber et al. Bioinformatics 2002). Probe sets 232431_at for GR and 205259_at for MR were used to determine the expression levels of both receptors in leukemic cells.

Methods RT-qPCR
RNA was extracted using Trizol isolation (Invitrogen), where after cDNA was synthesized according to standardized procedures. (Ariës, Leukemia 2014) NR3C1 (GR) and NR3C2 (MR) mRNA levels were quantified by incorporation of SYBR Green (Thermo Scientific) by RT-qPCR (Applied Biosystems 7900HT). Primers for NR3C1 were; 5'-TGC-CAA-GGA-TCT-GGA-GAT-GA -3' (forward) and 5'-TGG-GAG-GTG-GTC-CTG-TTG-T-3' (reverse). Primers for NR3C2 were; 5'-TCC-CTT-CTG-CTA-TTG-TTG-3' (forward) and 5'-TCC-CCa-CAC-ACC-AAA-CAT-ATT-3' (reverse). Primers used for the reference gene RPS20, were 5'-AAGGGCTGAGGATTTTTG-3' (forward) and 5'-CGTTGCGGCTTGTTTAG-3' (reverse).