ABSTRACT

Relative antiinflammatory and immunosuppressive potencies of gluco-
corticoids (GC) were previously well defined. Nonetheless, GC also regu-
late cell proliferation and programmed death (apoptosis). The aim of this
study was to determine the relative potency of different GC on the
modulation of cell survival. The GC-sensitive lymphoblast cell line CEM-
c7/14 was submitted to 48h-exposure to GC (dose-response curve from
10^{-8} to 10^{-5}M). Cell survival was analyzed employing the DimethylTiazol-
Tetrazolium (MTT) test. For each GC at least 4 experiments were perf-
formed in quadruplicate. Responses to different GC at the same molarity
were analyzed by ANOVA on Ranks. Cell responses to the same GC in
different concentrations were tested by repeated measures ANOVA. The
EC50 for each GC was calculated with the GraphPad Prism 3.0 software.
The use of low concentrations (10^{-8} and 10^{-7}M) of hydrocortisone and
methylprednisolone determined a similar effects on cell survival, which
was less prominent than that observed with betamethasone, budesoni-
de or momethasone. Momethasone was the most potent GC, inducing
the most intense dexamethasone reduction on cell survival at the lowest
concentration (10^{-8}M). Momethasone and methylprednisolone were the
two GC with the strongest impact on cell survival. Our findings suggest
that antiproliferative and apoptotic potencies of GC are different from
those previously reported antiinflammatory and immunosuppressive
actions. (Arq Bras Endocrinol Metab 2005;49/3:378-383)

Keywords: Glucocorticoid; Cell survival; Cell death; Apoptosis

RESUMO

Potências Antiproliferativa e Pró-Apoptótica dos Glicocorticóides: 
Discordância com as Propriedades Anti-inflamatórias e Imunossupressoras.

As potências antiinflamatória e imunossupressora dos glicocorticóides
(GC) já foram bem estabelecidas previamente. No entanto, os GC tam-
bém possuem atividade reguladora da proliferação celular e da morte
celular programada (apoptose). O objetivo deste estudo foi determinar
a potência relativa de diferentes GC na modulação da sobrevivência
celular. Linfoblastos cortico-sensíveis (linhagem celular CEM-C7/14)
ficam mantidos em cultura prolongada e submetidos ao tratamento
com GC por 48h, em doses variando entre 10^{-9} e 10^{-5} molar. O índice de
sobrevivência celular foi quantificado pelo teste MTT (DimetilTiazol-
Tetrazolium). Para cada GC avaliado, foram realizados pelo menos qua-
tro experimentos em triplicata. A resposta celular aos diferentes GC foi analisada através do teste estatístico ANOVA on Ranks;
enquanto a resposta ao mesmo GC em concentrações diferentes foi
analisada pelo teste ANOVA for repeated measures. O EC50 de cada
GC foi calculado utilizando-se o software GraphPad Prism 3.0. Durante
o uso de concentrações baixas (10^{-8} e 10^{-7} molar), observou-se sobrevivência
semelhante dos linfoblastos após tratamento com hidrocortisona ou
methylprednisolona. Nestas mesmas concentrações baixas, a sobrevivência
celular foi menor quando utilizou-se dexametasona, betametasona,
budesonida ou mometasona. A mometasona e a methylprednisolona
foram os dois GC que determinaram maior redução da sobrevida linfoblástica. Nosso resultados sugerem que as potências antiproliferativa e pró-apoptótica dos GC sejam diferentes dos efeitos antiinflamatórios e imunossupressores previamente estabelecidos para estes GC. (Arq Bras Endocrinol Metab 2005;49:378-383)

**Descritores:** Glicocorticóide; Sobrevida celular; Morte celular; Apoptose

**CORTISOL, THE ENDOGENOUS GGLUCOCORTICOID (GC),** is secreted basally and during stress and modulates the amplitude of defensive responses. Cortisol and a variety of synthetic glucocorticoid agonists are able to control carbohydrate, protein and lipid metabolism, and to regulate immune and cardiovascular functions (1,2). GC suppress innate inflammatory responses, as well the cellular immunity (3). One of the major effects of GC is their ability to exert anti-proliferative and apoptotic actions both in vivo as in vitro cell culture (4). Glucocorticoid-induced apoptosis is an active, ATP-dependent phenomenon characterized by cellular and mitochondrial membrane changes, and alterations in calcium and potassium compartmental distributions (5). Programmed cell death depends on the activation of nuclear proteases, generating DNA, RNA and protein fragmentation, genomic instability and failure of DNA repair. The antiproliferative and apoptotic actions of glucocorticoids mediate their therapeutic effects in several autoimmune and lymphoproliferative diseases.

Cell survival can be measured by the ability of live cells to metabolize MTT, a yellow tetrazolic salt, to its dark violet crystal product formazan. This conversion occurs after active enzymatic cleavage at the mitochondrial level, and the measurement of the final product can be used as a quantitative assay, reflecting the cell viability (5). Relative glucocorticoid potencies are well established for their anti-inflammatory and immunosuppressive effects. On the other hand, the pathways related to modulation of cell survival and death are unique, requiring additional studies to determine the relative potencies of new synthetic glucocorticoids. In this study, we compared the relative antiproliferative and apoptotic potencies of hydrocortisone against several other synthetic glucocorticoids.

**MATERIALS AND METHODS**

A GC stock-solution was prepared by diluting GC salts in absolute-ethanol to obtain a final concentration of 10^{-2} M. Working-solutions were obtained by subse-

---

Glucocorticoid and Lymphoblast Survival

Longui et al.

---

Arq Bras Endocrinol Metab vol 49 nº 3 Junho 2005
Statistical analysis employed the SigmaStat 2.03 software (SPSS, Inc.). Comparison of the same glucocorticoid at different molarities was performed applying the Friedman test, ANOVA for repeated measures. When significant difference was detected, the All Pairwise Multiple Comparison Procedures - Tukey Test was used to recognize each different pair concentration. For comparison among different GC at the same molarity the Kruskal-Wallis - ANOVA on ranks test was performed, followed by the All Pairwise Multiple Comparison Procedures - Dunn’s Method to verify the difference among two different glucocorticoids. The EC50 was calculated employing GraphPad Prism 3.0 software.

RESULTS

The most characteristic patterns observed for all tested glucocorticoids are shown in tables 1 and 2. Hydrocortisone decreased cell survival in molar concentrations $\geq 10^{-7}$M, with the maximum effect at $10^{-5}$M. The same pattern of cell survival reduction was observed with methylprednisolone, but the final effect was higher than the one observed with hydrocortisone (reduction of 60.9% and 36.7% respectively). Dexamethasone decreased cell viability at molar concentrations $\geq 10^{-8}$M, with maximum effect at $10^{-5}$M (49.6% of cell reduction). Betamethasone, budesonide and momethasone showed a pattern similar to that observed for dexamethasone. In a molar concentration as low as $10^{-8}$M, these three GC had an effect equivalent to that observed at $10^{-5}$M, and the cell survival was significantly lower than that observed for dexamethasone treated cells at $10^{-8}$M.

Comparing hydrocortisone to synthetic glucocorticoids at the concentration of $10^{-8}$M, we observed that all GC, but methylprednisolone, had significantly higher potency in decreasing cell survival.

The GC concentration necessary to obtain 50% of the maximal effect (EC50) was between $10^{-7}$M to $10^{-6}$M for hydrocortisone, $10^{-7}$M for methylprednisolone, $10^{-7}$M for dexamethasone, $10^{-8}$M for betamethasone, $10^{-8}$M for budesonide and $10^{-8}$M for momethasone.

### Table 1. Percentage of live cells expressed as mean (SD) after treatment with glucocorticoid for 48 hours.

<table>
<thead>
<tr>
<th>Glucocorticoid</th>
<th>GC (-)</th>
<th>$10^{-8}$M</th>
<th>$10^{-7}$M</th>
<th>$10^{-6}$M</th>
<th>$10^{-5}$M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>99.5 (5.0)</td>
<td>93.9 (7.6)</td>
<td>90.5 (5.9)$^a$</td>
<td>68.3 (10.0)$^a$</td>
<td>62.8 (10.3)$^a$</td>
</tr>
<tr>
<td>(n= 24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>105.1 (10.1)</td>
<td>102.1 (8.8)</td>
<td>76.0 (17.5)$^a$</td>
<td>49.5 (8.0)$^a$</td>
<td>44.2 (7.0)$^a,b$</td>
</tr>
<tr>
<td>(n= 24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>98.0 (9.4)</td>
<td>76.3 (11.0)$^a$</td>
<td>55.7 (7.7)$^a$</td>
<td>48.8 (8.2)$^a$</td>
<td>48.4 (10.4)$^a,b$</td>
</tr>
<tr>
<td>(n= 40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betamethasone</td>
<td>104.6 (7.7)</td>
<td>59.4 (2.9)$^a,c$</td>
<td>55.6 (2.3)$^a$</td>
<td>51.6 (3.0)$^a$</td>
<td>50.7 (8.2)$^a,b$</td>
</tr>
<tr>
<td>(n= 24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Budesonide</td>
<td>99.4 (5.8)</td>
<td>66.2 (5.0)$^a,c$</td>
<td>65.5 (7.0)$^a$</td>
<td>54.0 (14.9)$a$</td>
<td>53.3 (21.0)$^a$</td>
</tr>
<tr>
<td>(n= 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Momethasone</td>
<td>98.3 (6.7)</td>
<td>45.4 (2.2)$^a,c$</td>
<td>39.9 (13.1)$a$</td>
<td>39.7 (13.0)$a$</td>
<td>39.5 (11.3)$a$</td>
</tr>
<tr>
<td>(n= 38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* corticosensitive lymphoblasts (c7/14 cell line); GC (-): maximal cell survival without glucocorticoid; M = molar; n = total number of point-experiments performed for each glucocorticoid  
$^a$: significant reduction when compared to basal values, p<0.05 (Anova Repeated Measures)  
$^b$: significant reduction when compared to hydrocortisone $10^{-5}$M, p<0.05 (Kruskal-Wallis - Anova on Ranks test)  
$^c$: significant reduction when compared to dexamethasone $10^{-8}$M, p<0.05 (Kruskal-Wallis - Anova on Ranks test)

### Table 2. Major pharmacologic characteristics of glucocorticoids regarding their proapoptotic properties.

<table>
<thead>
<tr>
<th>Glucocorticoid</th>
<th>Start Effect</th>
<th>Max Effect</th>
<th>EC50</th>
<th>Max Cell reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>$10^{-7}$M</td>
<td>$10^{-6}$M</td>
<td>$5 \times 10^{-7}$M</td>
<td>37%</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>$10^{-7}$M</td>
<td>$10^{-6}$M</td>
<td>$10^{-7}$M</td>
<td>61%</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>$10^{-7}$M</td>
<td>$10^{-6}$M</td>
<td>$5 \times 10^{-8}$M</td>
<td>50%</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>$10^{-9}$M</td>
<td>$10^{-8}$M</td>
<td>$&lt; 10^{-9}$M</td>
<td>54%</td>
</tr>
<tr>
<td>Budesonide</td>
<td>$10^{-9}$M</td>
<td>$10^{-8}$M</td>
<td>$&lt; 10^{-9}$M</td>
<td>46%</td>
</tr>
<tr>
<td>Momethasone</td>
<td>$10^{-9}$M</td>
<td>$10^{-8}$M</td>
<td>$&lt; 10^{-9}$M</td>
<td>59%</td>
</tr>
</tbody>
</table>

Start effect: minimal glucocorticoid concentration to start significant reduction on cell survival  
Max effect: minimal glucocorticoid concentration able to determine a maximal reduction on cell survival  
EC50: Concentration at which 50% of the glucocorticoid effect was observed  
Max cell reduction: maximal cell reduction (in percentage) related to basal nontreated cells
Glucocorticoids have specific biologic effects in several organ systems, depending on their pharmacokinetic characteristics and inherent actions exerted through their specific nuclear receptors (GR). Chemical changes in cortisol molecule can enhance glucocorticoid or mineralocorticoid activities, determining improved therapeutic properties and decreased side effects. Anti-inflammatory potencies have been defined in studies based on in vivo and in vitro methods. Relative anti-inflammatory potencies were previously reported for hydrocortisone, prednisolone and dexamethasone regarding inhibition of lymphocytes when stimulated by phytohemaglutinin (6). The anti-inflammatory effect was also related to other adrenal and gonadal steroids (7). It was observed that dexamethasone had the highest anti-inflammatory potency compared to hydrocortisone and prednisolone. Other studies have compared the relative potency of GC to inhibit the formation of granulomatous lesions, to

DISCUSSION

nisolone, between $10^{-8}$M to $10^{-7}$M for dexamethasone, and smaller than $10^{-8}$M for betamethasone, budesonide and momethasone (figure 1).

Figure 1. Dose response curve of cell survival after 48h of glucocorticoid treatment.
exert thymolytic actions (8), and to inhibit skin fibroblast growth rate (9).

There is a considerable variation between these previously described potencies. Additionally, just a small number of synthetic GCs were compared by the same technique, preventing direct comparison of the anti-inflammatory potencies among different glucocorticoids. Limited information is also available for comparison among recently synthesized novel glucocorticoids. Glucocorticoid receptor binding capability can be detected by radioreceptor-assay, and this characteristic has been correlated to the anti-inflammatory potency of these steroids. Using this method, anti-inflammatory potency observed for methylprednisolone, dexamethasone and betamethasone were considered higher than that established by other methods (10).

Studies evaluating GC antiproliferative effects are even more scarce. Using an MTT assay to compare prednisolone and dexamethasone on its relative antileukemic activity, a 16-fold higher potency was observed for dexamethasone (11). Another study evaluated the relative cytotoxicity of these two glucocorticoids by flow-cytometric analysis of cells from patients with acute lymphoblastic leukemia, and the authors concluded that dexamethasone had a cytotoxic activity five to six times higher than prednisolone (12). Despite the existing data evaluating and comparing the antiproliferative and apoptotic GC actions, these studies usually compare only two glucocorticoids (dexamethasone and prednisolone).

In this study, the relative potency of eight different glucocorticoids were compared regarding their antiproliferative and apoptotic activity, by examining cell survival. We described GC potencies considering the minimal glucocorticoid concentration able to start its effect on cell survival, the concentration at which the maximal reduction was obtained, the concentration at which 50% of the maximal effect was detected (EC50), and the maximal cell reduction observed after 48h of steroid therapy. This is the first report comparing multiple glucocorticoids in their effects or cell survival. As a group, hydrocortisone, methylprednisolone and dexamethasone started their antiproliferative and apoptotic effect at “physiological” concentrations (10⁻⁷M). The same effect was observed with budesonide and momethasone but at a 10-times smaller concentration. Momethasone was the GC able to induce the greater reduction on cell number and to require the smallest dose to start its effects.

We observed in this study, employing different glucocorticoids, that betamethasone, budesonide and momethasone have their EC50 at similar levels and under the 10⁻⁸ molar concentration, suggesting that further studies should evaluate even smaller doses of these compounds.

The discrepancy between anti-inflammatory and the cell proliferation and apoptotic potencies observed in this study are potentially related to the unique pathways involved in cell cycle control and apoptosis, different from pathways activated during inflammation. These discrepancies on relative glucocorticoid potency suggest that, if the regulation of cell number is the major target of therapy, specific dosage and type of glucocorticoid should be titrated for this specific effect. Future studies should determine the relative potency of new synthetic glucocorticoids and establish these effects for even smaller concentrations.

ACKNOWLEDGMENTS

We are deeply grateful to Dr. George P. Chrousos for his extensive collaboration and important suggestions for this study. This study was supported by a research grant of FAPESP - Process # 98/10680-7. We also thank the Editorial assistance offered by the Support Center for Scientific Publication of Santa Casa, SP - Faculty of Medical Sciences - Brazil.

REFERENCES


