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High-dose glucocorticoids increases the expression of mineralocorticoid receptor in vascular endothelial cells

X.-Y. WANG, X.-L. CHEN, L. WANG, H.-W. CHEN

Department of Respiratory Medicine, General Hospital of Beijing Military Region, Beijing, China

Abstract. – OBJECTIVE: To explore the role of glucocorticoid new mechanism to observe the expression of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) with lipopolysaccharide (LPS) and dexamethasone (Dex) in human umbilical vein endothelial cells (HUVEC).

MATERIALS AND METHODS: LPS "injured" endothelial cells with Dex for "treatment", and then detected the expression of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in the endothelial cells by RT-PCR and immunohistochemistry.

RESULTS: With high dose (10-6 mol/L) of Dex to stimulate cell 3h, GRmRNA no significant changes in the expression, 6h began to decrease, 12h peak, 24h recovered nearly the level before stimulation. Using different concentrations of Dex and 100 ng/ml LPS stimulation, HUVEC MRmRNA expression was decreased, and high dose (10-6 mol/L) of Dex to stimulate cell 3h, MRmRNA no significant changes in expression, and GRmRNA The difference is that the expression began to increase 6h, 12h, peaked, 24h rebound near the level before stimulation. Immunohistochemistry results consistent with the RT-PCR.

CONCLUSIONS: Large dose of DEX (10-6 mol/l) up-regulated the expression of MR and GR in the reduction of the contrast exactly. GC induced the expression of GR and MR in different changes of stress injury of the body may be a regulatory mechanism, and indicate one new mechanism of glucocorticoid exist.

Key Words:
Glucocorticoid, Glucocorticoid receptor, Mineralocorticoid receptor.

Introduction

Vascular endothelial cells, which is a kind of important cell population, plays an important role in the vascular permeability barrier, immune defense, and inflammatory reaction. It is synthesized and secreted by many kinds of active factors, including IL-6 and ICAM-1, among which the IL-6 is considered as the best marker of endothelial inflammatory reaction. More and more studies have indicated that endothelial cells play a key role in lipopolysaccharide (LPS) induced sepsis, septic shock, multiple organ failure, and other pathological processes. At present, it is believed that endothelial dysfunction and apoptosis is not only a common link of cardiovascular disease, but also the important cause of ARDS and MODS in sepsis, shock, and trauma. In the process of ARDS, vascular endothelial cells are both target cells and effector cells, and its damage and activation are the basic link of the body's uncontrolled inflammatory reaction and high permeability pulmonary edema[1]. Glucocorticoid (GC) is a major target for the anti-inflammatory effect of ARDS, but it has been mainly focused on the study of ARDS in the treatment of vascular endothelial cells, and some studies on the adhesion and endothelial cell injury. In the treatment of ARDS, the mechanism of vascular endothelial protection, especially the mechanism of pre receptor regulation, has not been reported.

Recent evidence *in vivo* has shown that many effects of GC are developed through genic mechanism actived by combining with glucocorticoid receptor (GR). Intra-cellular GR down-regulated under LPS stimulation or GC and the down-regulation may induce a series of pathologic processes such as shock. Though the exact mechanism of down-regulation of GC whether related to GC *in vitro* is still unknown, we should pay much attention to the prereceptor regulation mechanism of GC. Recent study showed that GC may up-regulate GR by prereceptor regulation mechanism, thus enhancing the effect of GC[2]. This finding raised the question whether GC can induce changes by other mechanism under injured stimulation. Mineralocorticoid receptor (MR)

Corresponding Author: Xingyou Wang, MD; e-mail: wxy_med@163.com

and GR are highly homogenous, especially the structure of their ligand-binding domain (LBD). In addition, the affinities for MR combining with GC are 10 times greater than that of GR[3]. Thereby, the probability that GC combine with MR would higher than GR if they both existence in one cell. Apparent mineralocorticoid excess (AME) was characterized by excess GC combining with MR because of genetic deficiency[4, 5]. Owing to the above effect, the expression of MR when organism is injured has attracted the attention. The coexistence of GR and MR in human vein endothelial cells and the law of GR and MR variety that is stimulated by attack factors are both valuable to explore. Therefore, our purpose was to examine the expression variety of GR and MR after LPS injury in human vein endothelial cells, to elucidate the evidence of the prereceptor regulation mechanism of GC.

Materials and Methods

Experimental Design

Human umbilical vein endothelial cells (HUVEC) were randomly divided into six groups in 6-well (6 wells per group, 3 wells in coverslip were prepared for immunohistochemistry analysis) as follows: group 1 (injured group): LPS (100 ng/ml); group 2 (injured control group): isodose RPMI-1640 culture medium (Roswell Park Memorial Institute-1640 medium, include solcoseryl) without endotoxin; group 3 (protected group): Made the concentration of dexamethasone (Dex) is 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-3} mol/L and mixed with HUVEC for 2h, then add LPS; group 4 (blocked group): RU486 (10^{-6} mol/L) cultured with HUVEC for 2h, then add Dex (10^{-6} mol/ L) and LPS (100 ng/ml); group 5 (protected control group): isodose PBS and LPS (100 ng/ml); group 6 (effective group): Dex (10^{-6} mol/L) cultured with HUVEC for 3, 6, 12 and 24 hours. After 24h treatment period, collected supernatant and stored at –20°C to detect the dose of IL-6, intercellular cell adhesion molecule-1 (ICAM-1), cultured HUVEC to determine the expression of GR and MR.

RNA Isolation

RNA was isolated from HUVEC after extraction in Trizol reagent (Roche, Mannheim, Germany), as suggested by the manufacturer. Total RNA was precipitated in isopropanol, washed with 75% ethanol and dissolved in nuclease-free water. RNA concentrations were measured by spectrophotometry and integrity was ensured by analysis on a 1.5% agarose gel at 18s and 20s scale before proceeding with cDNA synthesis.

Reverse Transcription Polymerase Chain Reaction (RT-PCR) Analysis

RT-PCR was performed according to Bland et al[6]. Primer Express (Applied Boya Biotechnology) and Primer 3 software were used to design primers for use in RT-PCR (Table I). Total RNA was (1 μg) was used for reverse transcription with reverse transcriptase. The products of cDNA were provided in Table II. PCR amplification was performed with reverse-transcribed DNA, the reaction was performed for 30 cycles as follows: GR: denaturation at 94°C for 1 min, annealing at 72°C for 1 min, and extension at 72°C for 5 min. MR: denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 2 min. β-Actin: 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 2 min. β-Actin: 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min.

Table I. Sequences for PCR primers and the product length.

Target genes	Primers	Size of products
GR	sense 5′ TCGACCAGTGTTCCAGAGAAC 3′ antisense 5′TTTCGGAACCAACGGGAATTG 3′.	693 bp
MR	sense 5′ AACTTGCCTCTTGAGGACCAA 3′ antisense 5′ AGAATTCCAGCAGGTCGCTC 3′.	450 bp
β-Actin	sense 5′ GTCACCAACTGGGACGACA 3′ antisense 5′ TGGCCATCTCTTGCTCGAA 3′.	468 bp

Table II. Reverse transcription reaction systems.

Reagents	Volume
ddH2O	20 μl
PCR mix	24 μl
Sense primer	1 μl
Antisense primer	1 μl
cDNA	4 μl
Taqase	1 μl
Total volume	50 μl

Evaluate PCR Products By Agarose gel Electrophoresis

Images collected by electrophoresis were analyzed in Bandscan mode to calculate the magnitude of the target gene mRNA expression.

Immunohistochemistry Analysis for the Expression of MR and GR

Flat preparations of the equatorial capsule (containing HUVEC) were prepared by HE stain. These were fixed for 5-10 min at room temperature in 4% Acetone, and were blocked in PBS for 3 min 3 times. Bound Ig was revealed with the appropriate ImmPRESS antirabbit Ig kit (Vector) according to the manufacturer's instructions and relevant records[7] negative control used PBS instead the first antibody. The immunoreactivity intensity was evaluated as brown-yellow nucleus and cytoplasm positive cells were quantified as a percentage of the total number of HUVEC in low power field (×200). HUVEC expression was scored semi-quantitatively using integral optical density (IOD) by Image-Pro Plus (version 4.5).

Statistical Analysis

Analyses were performed with ± s, using SPSS version 11.0 software (SPSS, Inc., Chicago, IL, USA). The ratio of results measured as a Bivariate, were assessed using Pearson chi-square test for categorical variables. Significance was determined by an unpaired 2-tailed Student's *t*-test or by two-way ANOVA with statistical significance set at $p < 0.05$. As indicated in each figure $*p < 0.05$, $**p < 0.01$.

Results

Evaluation of Purity and Integrity for RNA

We checked out that the ratio of A260/A280 of isolated RNA was greater than 1.9. The strap at 5S, 18S, 28S were integrated and distinct by formaldehyde denature gel electrophoresis. The results indicate that RNA had no significant degradation and could use in RT-PCR.

Expression of GR mRNA, MR mRNA and the Role of GC by RT-PCR

RT-PCR showed that in the HUVEC, the basal levels of GR mRNA were higher than MR, and they both markedly down-regulated stimulated by LPS (100 ng/ml) and Dex of different density compared with basal expression (Table III, Figure 5). The expression of GR mRNA were reduced after 6h, at the peak after 12h and returned to the basal level after 24h stimulated by high dose Dex (10^{-6} mol/L) for 3h (Figure 1). RU486 could inverse the effect of Dex (Table IV, Figures 2 and 3). Under the same stimulation, MR mRNA expression and the effect of RU486 were opposite of the variety of GR mRNA (Tables IV, V and VI, Figures 4, 5 and 6).

Immunohistochemistry Analysis

The rule of GR and MR protein variety, determined by immunohistochemistry analysis, was

Table III. Expression of GR mRNA and the role of GC by RT-PCR.

LPS density (ng/ml)	LPS t (h)	Dex density (mol/L)	GR/β-Actin (mRNA)
0	0	0	0.65 ± 0.16
100	24	10□3	0.54 ± 0.12
100	24	10□5	0.51 ± 0.11
100	24	10□6	0.52 ± 0.08*§
100	24	10□7	0.36 ± 0.12
100	24	10□8	0.32 ± 0.11

*: compared with Dex 0, 10-8-10-7 mol/L, $p<0.05$; compared with Dex 10-5, 10-3 mol/L, $p>0.05$.

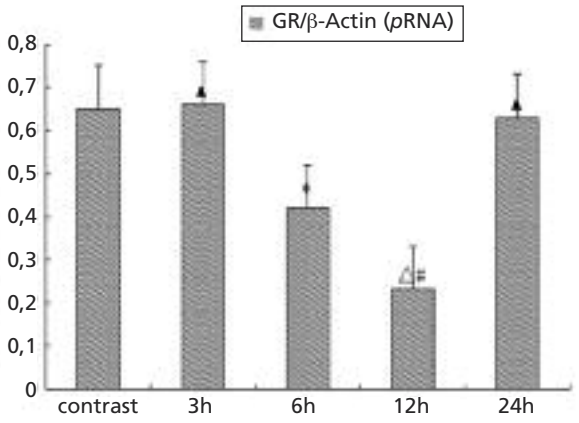

Figure 1. Influence of high dose Dex (10-6 mol/L) in different time to the expression of GR mRNA in HUVEC. *: versus contrast group, 3h, 12h and 24h, $p<0.05$, §Δ: $p<0.05$, versus 6h #: $p < 0.01$, versus contrast group, 3h, and 24h; ▲: $p > 0.05$, versus contrast group.

similar to the results obtained by RT-PCR (Tables VII, VIII, IX and X, Figures 7, 8, 9 and 10).

Discussion

GR is a member of nuclear receptor family, which is a crucial nuclear transcriptional factor and existing generally in organic cells. In addition, GR perform positive effect in anti-inflammation and stress. The experiment of part one demonstrates its anti-inflammatory action in HUVEC *in vitro*. Severe injury could down-regulate GR expression which inhibit cytokines including IL-1, IL-6 and TNF-β, and induce ARDS. GR protein and mRNA of cytoplasm in organic cells reduce under severe injury, we also observe that effect in HUVEC under LPS stimulation and GC.

Table IV. Influence of high dose Dex (10^{-6} mol/L) to the expression of GR mRNA in HUVEC.

Group	GR/β-Actin (mRNA)
LPS injured group	0.13 ± 0.04
Injured control group	0.65 ± 0.05
Protected group	0.52 ± 0.08*§
Protected control group	0.14 ± 0.06
Blocked group	0.16 ± 0.07

*: $p<0.01$, versus LPS injured group, protected control group, blocked group. §: $p<0.05$, versus injured control group.

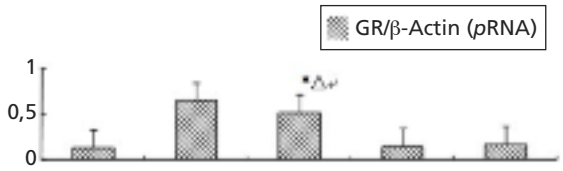

Figure 2. Influence of high dose Dex (10-6 mol/L) to the expression of GR mRNA in HUVEC. *: $p<0.01$, versus LPS injured group, protected control group, blocked group. Δ: $p < 0.05$, versus injured control group.

Meanwhile, the down-regulation of GR by GC (Dex) was accordant with traditional view. The expression of GR recover nearly the fundamental level about 24h after the stimulation from Dex. The result indicates that GC effect has time limit and the down-regulation of GR after a course of time may mediate by other pathways. So we should not ascribe the down-regulation completely to GC effect and scruple to use GC. On the contrary, we must elevate GC dose to enhance GC anti-inflammatory effect. In fact, the experiment reported demonstrate that high dose GC could induce high GR and the up-regulation emerge after transcription and translation.

GR, combining with molecular chaperone, exist in cytoplasm as usual. Once GR combining with GC, the compound enter the nucleus and educe GC effect that activating or inhibiting target gene by further combing with glucocorticoid responsive element (RE) or negative glucocorticoid responsive element (nGRE). The content of GR in nucleus determine GC effect for the most part because as a nuclear factor, GR only activated by hormone and enter the nucleus from cyto-

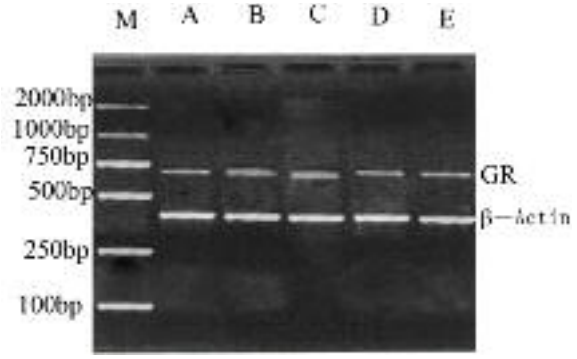

Figure 3. Influence of Dex (10^{-6} mol/L) to the expression of GR mRNA in HUVEC. M: Marker, A: LPS injured group, B: injured control group, C: protected group, D: protected control group, E: blocked group.

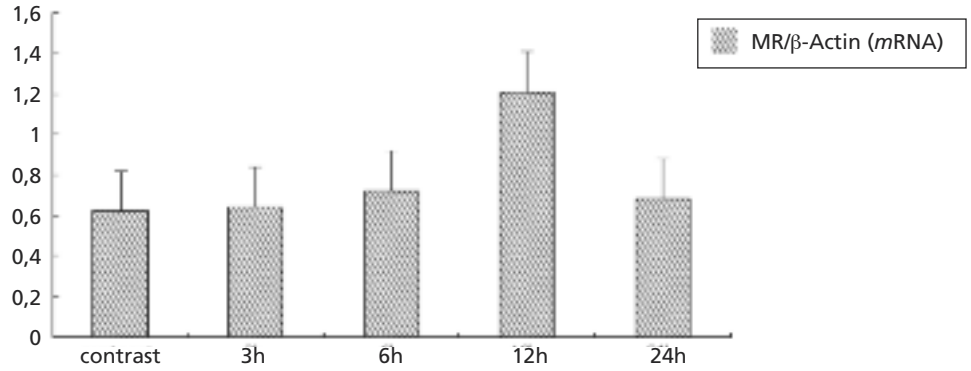

Figure 4. Influence of high dose Dex (10^{-6} mol/L) in different time to the expression of MR mRNA in HUVEC *: versus contrast group, 3h, 12h and 24h, $p < 0.05$, ▲: versus contrast group, $p > 0.05$.

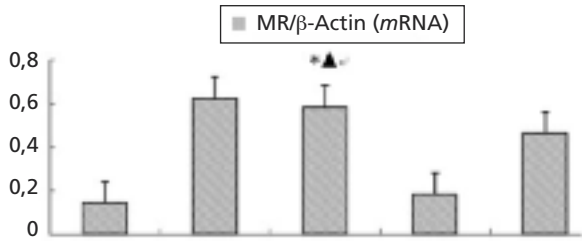

Figure 5. Influence of Dex to the expression of MR mRNA in HUVEC. *: versus LPS injured group, protected control group, $p < 0.01$, ▲: versus injured control group, blocked group $p > 0.05$.

plasm to exert effect. People lack for profound understanding of the GR expression variability in the nucleus. For the best chance of exogenous GC treatment to severe trauma patients, the researchers have not the same opinion. A previous study[8] detected the expression of the GR in the nucleuses of the rats liver during different phase after severe scalding using Western blot and immunohistochemistry and investigated the effect of dexamethasone on the transfer of the GR from cytoplasm to nucleuses. The result showed that the expression of the GR in the nucleuses of the liver significantly decreased from 15 minutes after severe scalding, reached the lowest at the 1st hour, resumed to normal at the 12h and was much higher than normal after 24 hours. Because GR of nucleus transferred from cytoplasm mostly, the result further indicated that the transfer of the GR from cytoplasm to the nucleuses significantly decreased during 24 hours after severe scalding (decreased within 1 hour, resumed to normal during 1-12 h). The decrease of GR in nucleus after severe scalding caused the ability of transcription and activation attenuated. Thus, the effect of anti-inflammation and stress reaction was weakened. Then, it could induce SIRS (systemic inflammatory response syndrome), cytokinemia and such secondary generalized disorders. The transfer of the GR to the nucleuse was evidently increased after intraperitioneal injection of high dose of dexamethasone during 4-12 hours after severe scalding. Combined the feature of GR expression variety, at the moment, the expression of the GR in the nucleuses resumed

Table V. Influence of high dose Dex (10^{-6} mol/L) in different density to the expression of MR mRNA in HUVEC.

LPS density (ng/ml)	LPS t (h)	Dex density (mol/L)	MR/β-actin (mRNA)
0	0	0	0.62 ± 0.13
100	24	10^{-3}	0.60 ± 0.14
100	24	10^{-5}	0.56 ± 0.09
100	24	10^{-6}	0.58 ± 0.11*§
100	24	10^{-7}	0.37 ± 0.08
100	24	10^{-8}	0.34 ± 0.09

*: versus Dex 0, 10-9-10-8 mol/L, $p < 0.05$, versus Dex 10-5 and 10-3 mol/L, $p > 0.05$.

Table VI. Influence of high dose Dex (10^{-6} mol/L) to the expression of MR mRNA in HUVEC.

Group	MR/β-actin (mRNA)
LPS injured group	0.14 ± 0.08
Injured control group	0.62 ± 0.13
Protected group	0.58 ± 0.11*§
Protected control group	0.18 ± 0.09
Blocked group	0.46 ± 0.12

*: versus LPS injured group, protected control group, $p < 0.01$, §: versus injured control group. Blocked group, $p > 0.05$.

and can be improved by high dose of dexamethsone which may promote the transfer of the GR from cytoplasm to the nucleuses and enhance organism self-restoration. There was no effect of dexamethasone on the transfer of the GR within 1 hour and over 24 hours after scalding. Since over 12 hours after scalding GR had resumed to normal and especially the level was much higher than normal after 24 hours. It indicated that GR had been activated thoroughly, high dose of exogenous GC haven't more powerful effect to promote the transfer of the GR. Wang[9] provide experimental evidence for clinical treatment of the severe traumas by using properly GR. But we found different result of the inflammatory injury model in HUVEC. The research exhibit that in prophase of HUVEC injury, the expression of GR in cytoplasm was higher than in nucleuses, that means compared with the transfer over 12h after injury, the transfer of GR within 12h was unobvious. The different results declared that GR expression regulation has species and cell variability. The study of GR expression regulation remains to be explored.

Mineralocorticoid are the major effectors of stress adaptation and modulating hormone levels after trauma[10] besides GC. A substantial amount of records shows that GR and the GC reactivity of target cells decreased during stress, burn and shock[11-15]. Receptors are the critical macromolecules which determine target cells reactivity. The decrease in GR during stress and shock might be a contributing factor in the pathogenesis of shock[16]. Many researchers have paid attention on

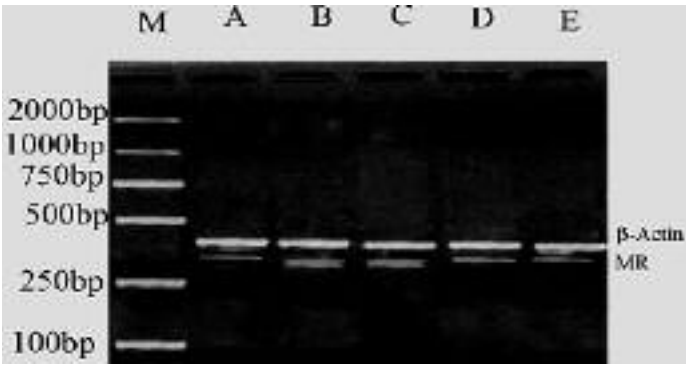

Figure 6. Influence of Dex to the expression of MR mRNA in HUVEC. M: Marker A: LPS injured group B: injured control group C: protected group D: protected control group E: blocked group

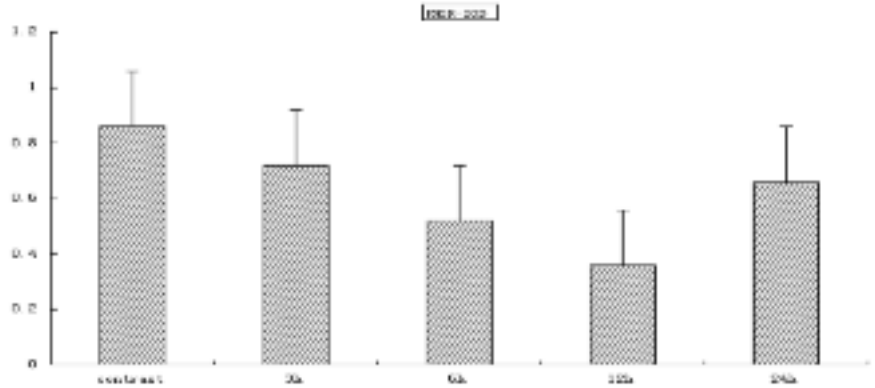

Figure 7. Influence of high dose Dex in different density to the immunohistochemistry stain result of GR in HUVEC . *: versus contrast group, $p < 0.05$, Δ: versus contrast group, $p < 0.01$, ▲: versus contrast group, $p > 0.05$.

Table VII. Influence of Dex in different density to the immunohistochemistry stain result of GR in HUVEC that stimulated by LPS.

LPS density (ng/ml)	LPS t (h)	Dex density (mol/L)	GR-IOD
0	0	0	0.86 ± 0.14
100	24	10^{-3}	0.65 ± 0.15
100	24	10^{-5}	0.68 ± 0.11
100	24	10^{-6}	0.66 ± 0.09*§
100	24	10^{-7}	0.43 ± 0.08
100	24	10^{-8}	0.36 ± 0.09

*: versus Dex 0, 10-8-10-7 mol/L, $p < 0.05$, §: versus Dex 10-5 and 10-3 mol/L, $p > 0.05$.

Table VIII. Influence of high dose Dex to the immunohistochemistry stain result of GR in HUVEC.

Group	GR-IOD
LPS injured group	0.32 ± 0.11
Injured control group	0.86 ± 0.14
Protected group	0.66 ± 0.09*§
Protected control group	0.34 ± 0.09
Blocked group	0.42 ± 0.12

*: versus LPS injured group, protected control group, blocked group, $p < 0.01$, §: versus injured control group, $p < 0.05$.

the effects of aldosterone and Liu et al[17] used aldosterone as the ligand to observe the changes in aldosterone binding activity of kidney cytosols after pathological stress in rats and the regulation. They bound capacity (Rt) and apparent dissociation constant (K_d) of aldosterone binding activity of kidney cytosols in normal, low-degree or heavy-degree scalded rats were measured by radioligand binding assay. They found that the Rt of heavy-degree scalded rats was lower than that of the control group; while the Rt of low-degree scalded rats was not significantly different from that of the control group. Injection of anti-rat TNF-α and IL-1β antibodies, α-MSH and KPV prevented Rt of aldosterone binding activity from decrease in kidney cytosol of rats with heavy-degree scald. The results indicated that aldosterone binding activity as a significant aspect also has the possibility of down-regulation. The increase of vascular permeability, monocyte and neutrophilic exudate, vascular endothelium impaired induced by trauma and LPS released by bacterial infections result in activation of the hypothalamic-pituitary-adrenal axis. LPS induces the release of a number of proinflammatory cytokines, i.e. interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF), which activate the hypothalamic-pituitary-adrenal axis. The cascade by autocrine and paracrine secretion product a great quantity TNF, IL-1 and IL-6 entered circulation and activated hypothalamic-pituitary-adrenal axis on three unique levels[18-22]. We should take such conclusion: appropriate increased of TNF-α and IL-1β may activate hypothalamic-pituitary-adrenal axis and sympathetic nervous, inhibite parasympathetic nervous. Thus, enhance organism damage resistance ability. But continuing high level proinflammatory cytokine may dilute the protect effect on aldosterone binding activity level. We detected that MR expressed in HUVEC on mRNA and protein level. Both GR and MR existed

Table IX. Influence of Dex in different density to the immunohistochemistry stain result of GR in HUVEC that stimulated by LPS.

LPS density (ng/ml)	LPS t (h)	Dex density (mol/L)	MR-IOD
0	0	0	0.68 ± 0.13
100	24	10^{-3}	0.59 ± 0.12
100	24	10^{-5}	0.60 ± 0.13
100	24	10^{-6}	0.58 ± 0.08*§
100	24	10^{-7}	0.42 ± 0.09
100	24	10^{-8}	0.38 ± 0.07

*: versus Dex 0, 10-8-10-7 mol/L, $p < 0.05$, §: versus Dex 10-5 and 10-3 mol/L, $p > 0.05$.

Table X. Influence of high dose Dex to the immunohistochemistry stain result of MR in HUVEC.

Group	MR-IOD
LPS injured group	0.32 ± 0.08
Injured control group	0.68 ± 0.13
Protected group	0.58 ± 0.08*§
Protected control group	0.31 ± 0.07
Blocked group	0.46 ± 0.11

*: versus LPS injured group, protected control group, blocked group, $p < 0.01$, §: versus injured control group, $p < 0.05$.

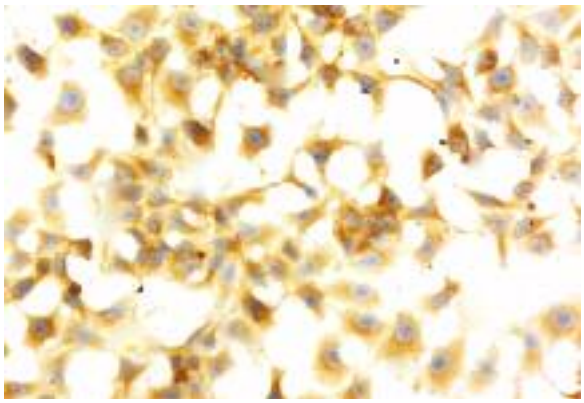

Figure 8. High stained of GR in cytoplasm of HUVEC by immunohistochemistry analysis (×400).

in HUVEC indicated that MR effect on Systemic Stress Response should be placed a high value. Compared with down-regulation of GR, we found that high dose GC (Dex10-6 mol/L) could up-regulate MR expression at the peak after 12h. In view of GC has greater affinity of the MR 10 times than GR, we infer that GC take effect through combining with MR during the period. Dex can rapidly inhibit ERK1/2 and stimulate p38 activation in GR-independent manner in HO-8910 cells, which might play a role in Dex-mediated growth inhibition in these cells. The regretful thing is the research hasn't showed whether Dex worked by combining with MR.

Recently, several lines of experimental evidence demonstrate[23,24] that Aldosterone as a major ligand of MR display effect only combined with MR not GR when GC could integrate MR. It is more fascinating that aldosterone has an extensive effect which always anti GC besides water and salt metabolism regulation. The most distinct evidence is that aldosterone may promote fibrosis and inflammation while GC takes effect on anti-fibrosis and anti-inflammation. Therefore, GC and Ald, GR and MR constitute a contradictory system in cells and in response to injury stimulation and decided the ultimate consequences. In short, the feedback pattern which mediated GC through MR and GR make organism activity being properly level, the disorder induce occurrence and development of disease. So that future study of inflammation regulation will focus on the regulation not only between GC, GR and cytokine (TNF, IL-6, IL-10, etc.), but also between GC and Ald, MR. GC combine with GR or MR will determin the successor response of histiocyte. Recently research has recently been recognized that GC access to receptors is determined by the presence of tissue-specific 11β-hydroxysteroid dehydrogenases (11β-HSDs) that catalyze the metabolism and inversion of corticosteroids. 11β-HSD2, a determined factor of MR specificity, is a higher affinity exclusive dehydrogenase that rapidly inactivates GCs, thus, protecting otherwise nonselective MR from GCs and inducing that aldosterone combined with MR in the MR target region such as kidney endothelial cells. Conversely, 11β-HSD1 is a predominant 11β-reductase that regenerates active corti-

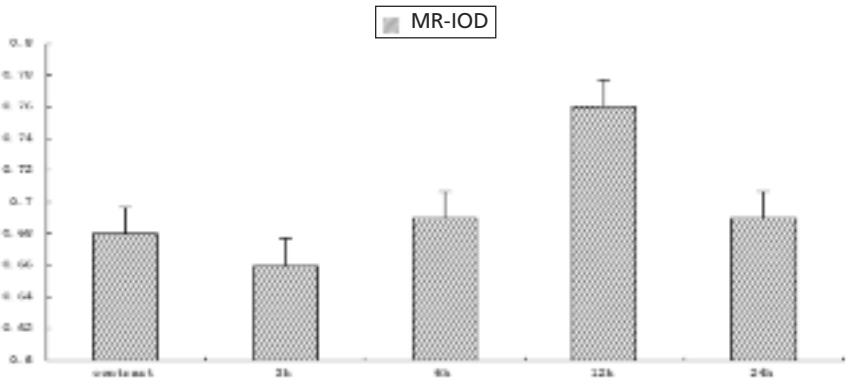

Figure 9. Influence of high dose Dex in different time to the immunohistochemistry stain result of MR in HUVEC (±s). *: versus contrast group, $p<0.05$, ▲: versus contrast group, $p > 0.05$.

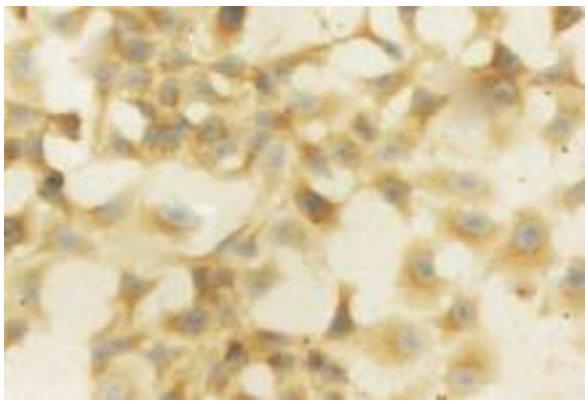

Figure 10. High stained but more pallid than GR of MR in cytoplasm of HUVEC by immunohistochemistry analysis (×400).

sol from cortisone. Interestingly, both MR and 11β-HSD1 were found highly expressed in the hippocampus where 11β-HSD2 was absent, that makes MR in the hippocampus exposure to the effect of GC. The existence of 11β-HSD1 in the hippocampus sends MR in the hippocampus lost optimal specificity of mineralocoticoid. GC in the hippocampus combining with MR and/or GR mediated and activated two pathways which co-regulated the HPA-axis[25-27].

Conclusions

A regulation model may also exist in HUVEC because we find MR and GR coexistence in HUVEC. 11β-HSD as a key substance of the prereceptor regulation mechanism of GC in endothelial cells which participating in inflammation regulation will be a challenge for future research.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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