Combined Use of Etomidate and Dexmedetomidine Produces an Additive Effect in Inhibiting the Secretion of Human Adrenocortical Hormones

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: The direct effects of etomidate were investigated on the secretion of cortisol and its precursors by dispersed cells from the adrenal cortex of human of animals. Dexmedetomidine (DEX) is an anesthetic agent that may interfere with cortisol secretion via an unknown mechanism, such as involving inhibition of 11β-hydroxylase and the cholesterol side-chain cleavage enzyme system. The aim of this study was to determine whether dexmedetomidine (DEX) has a similar inhibitory effect on adrenocortical function, and whether combined use of etomidate (ETO) and DEX could produce a synergistic action in inhibiting the secretion of human adrenocortical hormones.

Material/Methods: Human adrenocortical cells were exposed to different concentrations of ETO and DEX. The dose-effect model between the ETO concentration and the mean secretion of cortisone (CORT) and aldosterone (ALDO) per hour was estimated.

Results: Hill's equation well-described the dose-effect correlation between the ETO concentration and the amount of ALDO and CORT secretion. When the DEX concentration was introduced into the model by using E_0 (basal secretion) as the covariate, the goodness of fit of the ETO-CORT dose-effect model was improved significantly and the objective function value was reduced by 4.55 points (P<0.05). The parameters of the final ETO-ALDO pharmacodynamics model were EC_{50}=9.74, E_{max}=1.20, E_0=1.33, and γ=18.5; the parameters of the final ETO-CORT pharmacodynamics model were EC_{50}=9.49, E_{max}=8.16, E_0=8.57, and γ=37.0. In the presence of DEX, E_0 was 8.57–0.0247×(CDEX–4.6), and the other parameters remained unchanged. All parameters but γ were natural logarithm conversion values.

Conclusions: Combined use of DEX and ETO reduced ETO's inhibitory E_0 (basal secretion) of CORT from human adrenocortical cells in a dose-dependent manner, suggesting that combined use of ETO and DEX produced an additive effect in inhibiting the secretion of human adrenocortical hormones.

MeSH Keywords: **Dexmedetomidine • Drug Interactions • Etomidate •** *In Vitro*

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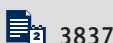
Background

Etomidate (ETO) is an intravenous anesthetic induction agent of imidazole derivatives. In 1983, Ledingham and 2 other research groups [1] found that ETO had the effect of inhibiting the synthesis and release of human adrenocortical hormones. Other researchers [2–6] found that the peak effect of ETO in inhibiting the adrenocortical function was 6–8 h after a single-dose injection of ETO, and restored to normal limits spontaneously within 24 h. Continuous injection of ETO could prolong this inhibitory effect beyond 24 h.

Dexmedetomidine (DEX) is a highly selective α2-adrenergic receptor agonist of imidazole derivatives [7–10]. It is currently speculated that DEX has some inhibitory effect on the secretion of adrenocortical hormones in animal and *in vitro* cell lines, but this inhibitory effect has not been demonstrated in clinical research. Therefore, Maze et al. [11] investigated the effects of dexmedetomidine on steroidogenesis, as well as on binding to glucocorticoid receptors, in a series of *in vitro* and *in vivo* animal studies in 1991.They found that at dexmedetomidine concentrations greater than 10^{-7} M, a dose-dependent inhibition of corticosterone release was detected in response to ACTH stimulation *in vitro*. When the drug was administrated with dexmedetomidine, the baseline of cortisol levels decreased and the cortisol response to ACTH was blunted for 3 h. More importantly, ETO and DEX are used alternatively in clinical practice, and therefore it is important to know whether they have a synergistic effect in inhibiting the adrenocortical function. To the best of our knowledge, there has been no applied research on the combination use of different concentrations of the 2 drugs, nor has there been systematic research on them in human primarily cultured adrenocortical cells. In the present study, we used 56 combinations of different concentrations of ETO and DEX (covering all possible clinical blood-drug concentrations) in a model of primarily cultured adrenocortical cells to clarify the nature of their interaction.

Material and Methods

Human primarily cultured adrenocortical cells (HADCC, ScienCell, USA) were resuscitated, thawed, and cultured to 3–10 passages. Cells growing to 70–80% confluence were digested with 2 ml 0.25% trypsin+EDTA to single cells for sub-passage. The number of cells was counted using a cell-counting plate. After adjusting the concentration to 500 000~1 000 000/ml, cells were seeded to a 24-well plate at a concentration of 200 000 cells (about 250 μl)/well and cultured in 250 μl mesenchymal stem cell medium (MSCM) in a 95%O_2 + 5% CO_2 and 37°C incubator for 24 h. After secure confluence of the cells, the medium was sucked up gently, and the remaining cells were added with 250 μl fetal bovine serum (FBS)-free medium (Hyclone) and

pre-cultured for 2–3 h. After sucking up the medium, cells were added with FBS-containing MSCM and appropriate amounts of ETO (0, 10, 30, 100, 1000, 3000, 10 000 and 100 000 nM) and DEX (0, 10, 30, 100, 1000, 3000 and 10 000 nM). The volume of each well was finally adjusted to 1 ml, and the drug concentration of each well was 1 of the combinations of the 2 drugs, totaling 56 concentration combinations. After 24-h culture, cell confluence was observed under a phase-contrast microscope. The cell supernatant (250 μl) was stored at –80°C for detection of ALDO and CORTISOL within 2 months. Cells with the supernatant removed were used for counting the relative number of cells by MTT, using the standard curve.

Logarithmic growth phase cells were harvested. The cell suspension was assigned to 8 groups with each group containing 5 repeated wells. The cell concentration in each group was $1×10^4$, $2×10^4$, $4×10^4$, $8×10^4$, $16×10^4$, and $32×10^4$. To each well was added 100 μl cell suspension and 100 μl culture medium in a 96-well plate. The plate was then cultured in the incubator until cell confluence. After 24-h culture, to each well was added 20 μl MTT reaction liquid, cultured in a 95%O_2+5%CO_2 and 37°C incubator for 4 h, and 20 μl formazan was added again, and cultured overnight until the formazan crystals in each well were thoroughly dissolved. The 96-well plate was then moved from the incubator, placed horizontally on the ELISA analyzer, shaken for 10 s, and assayed for optical density (OD) of each well at 570 nm, using the zero-setting well (medium, MTT, and dimethyl sulfoxide in the same well) as control. The OD of each well was measured 6 consecutive times by ELISA to obtain the mean value of each well by deleting the maximum and minimum values. Using the OD value as the horizontal ordinate and the number of cells (10^4) as the longitudinal ordinate, a standard curve was mapped.

Using chemiluminescence immunoassay (CLIA) [12–14], the concentration of ALDO and CORT was detected with the ALDO assay kit (Tianjin Bioscience Diagnostic Technology Co., Ltd., Tianjin, China) and Access Cortisol kit (Beckman Coulter, Inc, USA).

Using the NONMEM Version VI Software (GloboMax LLC, Ellicott City, MD, USA), a pharmacodynamics model of ETO and DEX concentrations versus the amount of ALDO and CORT secretion was established using the nonlinear mixed-effects model. First of all, a set of pharmacodynamics parameters was fitted by using the nonlinear mixed-effects model to ensure the minimal error between the fitted value and the actual observation value (the least-square method), based on which the effect of population parameter variation (inter- and intra-individual variation) and covariant on pharmacodynamics parameters (fixed-effects), residual variability (random-effects), and pharmacodynamics parameters were calculated. Based on the natural logarithm of the actually measured ALDO or CORT concentration and the natural logarithms of the drug

concentration (nM), the pharmacodynamics model of either ALDO or CORT was calculated to obtain the parameters of the model. The first approach is based on the Sigmoid-E_{max} model, a commonly-used name for the Hill model (Hill, 1910) [15]. The Sigmoid-E_{max} model can be represented by the following equation for an inhibitory drug.

The model used the Hill's equation-fitted sigmoid curve on the basis of the drug concentration and generation effect (the log-transformed value of ALDO or CORT secretion). The equation is as follows:

$$E = E_0 + \left(E_{max} - E_0\right)\frac{C^\gamma}{C^\gamma + EC_{50}^\gamma}$$

where E is the predicted effect (the amount of ALDO or CORT secretion); E_0 is the effect in the absence of the drug (the amount of ALDO or CORT secretion); E_{max} is the maximum effect of the drug (the maximum inhibition on ALOD or CORT secretion); EC_{50} is the drug concentration causing 50% of the maximum inhibitory effect; and γ is the drug concentration-effect slope. The drug concentration and the amount of ALDO or CORT secretion in the equation are expressed as their corresponding natural values.

The 7 ETO concentrations and the 8 DEX concentrations constituted 56 different concentration combinations. After fixation of the effect model, another drug concentration was used as the covariate to the effect on pharmacodynamics parameters. Uncertainty of the pharmacodynamics parameters was tested and calculated by the bootstrap method. The 5th percentile and 95th percentile were used as the lower limit and upper limit, respectively, of 95% confidence interval (CI) of the pharmacodynamics parameters and variability of the new sample set to compare the difference between the fitted parameters and the parameters of the original sample set [16–19].

Results

General description

The 56 concentrations of each cell batch were cross-combined in the model, totaling 19 batches of cell culture and concentration combination for drug stimulation test. The number of cells was calculated using the following equation:

Y(OD number)=−0.0002×X^2 +0.0362*X+0.0694, R^2=0.9927.

In the absence of drug stimulation, the amount of ALDO secretion was 5.65±0.53 pg/h per 10 000 cells, and the amount of CORT secretion was 12678.59±1965.13 pg/h per 10 000 cells.

When the different concentrations of either ALDO or CORT was used alone, the secretion showed a normal distribution in both groups, and the homogeneity of variance was P=0.598 and P=0.842, respectively (both larger than 0.05). All data were in a normal distribution, and ANOVA showed a P value=0.675 and 0.624 respectively, suggesting that there was no statistically significant difference between the 2 groups of data.

Methodology of CORT determination

The content of CORT was determined using the Access Cortisol kit, showing a correlation coefficient between 0.967 and 0.973. After diluting 2 times for samples with relatively high concentrations (>60 µg·dl⁻¹), the mean recovery rate was 98.3–109%. The result of 3 repeated measurements using this method showed an intra-day variability of 4.4–6.7% and an inter-day variability of 6.0–7.9%. According to 95%CI, the lowest detectable level of cortisol against the zero standard was 0.4 µg/ml (11 nmol/L).

Methodology of ALDO determination

The correlation coefficient of the quantitative determination of ALDO was 0.964–0.976. The result of 3 repeated measurements using this method showed an intra-day variability of 3.2–4.3% and an inter-day variability of 3.1–4.9%. According to 95%CI, the lowest detectable level of cortisol against the zero standard was 20 pg/ml, and the sensitivity was as high as 10–16 mol/L.

Pharmacodynamics analysis

Model fitting process

A drug concentration-effect correlation (Hill equation) model was established in 2 steps: ETO concentration-ALDO secretion and DEX concentration-ALDO secretion; and ETO concentration-CORT secretion and DEX concentration-CORT secretion. Once both drugs can establish dose-effect correlation with 1 effect successfully, a response surface model suggested by Minto was adopted to analyze and define interactions between 2 drugs. If only 1 of the drugs established dose-effect correlation with 1 effect successfully, the other drug served as a covariate to analyze impacts on the former drug dose-affect model.

The results of primary analysis showed that dose-effects models cannot be established successfully in DEX concentration-ALDO secretion and DEX concentration-CORT secretion, but dose-effects models can be established in ETO concentration-ALDO secretion and ETO concentration-CORT secretion according to Hill equation. Thus, DEX was set as a covariate of the former 2 models to be introduced into model parameters in linear and non-linear ways, separately. The model-establishing process of NONMEM is shown in Table 1.

Table 1. Establishing process of NONMEM pharmacological model.

No.	Description	OBJ	ΔOBJ	Compared with No.	df	P Value
	ETO concentration-CORT dose-effect correlation model					
1.0	Hill dose-effect correlation model*	210.152				
1.1	1.0 + DEX×EC_{50} (linear)	206.660	−3.492	1.0	1	>0.05
1.2	1.0 + DEX×γ (linear)	209.960	−0.192	1.0	1	>0.05
1.3	1.0 + DEX×E_{max} (linear)	209.251	−0.901	1.0	1	>0.05
1.4	1.0 + DEX×E_0 (linear)	205.606	**−4.546**	1.0	1	**<0.05**
2.1	1.0 + DEX×EC_{50} (non-linear)	209.148	−1.004	1.0	1	>0.05
2.2	1.0 + DEX×γ (non-linear)	209.308	−0.844	1.0	1	>0.05
2.3	1.0 + DEX×E_{max} (non-linear)	213.558	+3.406	1.0	1	>0.05
2.4	1.0 + DEX×E_0 (non-linear)	207.255	−2.897	1.0	1	>0.05
	ETO concentration-ALDO dose-effect correlation model					
3.0	Hill dose-effect correlation model*	−81.810				
3.1	1.0 + DEX×EC_{50} (linear)	−81.827	−0.017	3.0	1	>0.05
3.2	1.0 + DEX×γ (linear)	−81.809	−0.001	3.0	1	>0.05
3.3	1.0 + DEX×E_{max} (linear)	−82.025	−0.215	3.0	1	>0.05
3.4	1.0 + DEX×E_0 (linear)	−81.810	−0.000	3.0	1	>0.05

* Final dose-effect correlation model.

During the establishment of dose-effect models of ETO concentration-ALDO secretion and ETO concentration-CORT secretion by Hill equation, objective functions (OBJ) were 210.152 and −81.810 points separately. After DEX was introduced as a co-variate of E_0 into ETO concentration-CORT secretion model in a linear way, OBJ decreased by 4.55 points (ΔOBJ >3.84, df=1, P<0.05). However, when DEX was introduced as a covariate into ETO concentration-ALDO secretion model in linear way, the model had no significant improvement (P>0.05). If it was introduced into the model in a non-linear way, the model was without convergence.

Model-fitting parameters

The final pharmacological dose-effect correlation parameters are shown in Table 2. ETO combined with DEX did not have significant impacts on inhibiting adrenocortical functions; the 50% effective concentration (EC_{50}) of ETO inhibiting CORT was EXP(9.49) [113226.8] nM, and the slope of dose-effect correlation curve was as high as 36.3. Compared with basic secretion levels, secretion of CORT decreased (equal to E_{max}− E_0) by 1720 pg/h/10 000 cells.

Model performance diagnostic

The internal validity of the final PD model was assessed by the bootstrap re-sampling 1000 times. The values of the parameters were compared with those obtained from the original dataset. The 2.5th and 97.5th percentiles of the sets of bootstrap parameter estimates defined the lower and upper limit of 95% CIs for each parameter. The software Wings for NONMEM (WFN v616, N. Holford, University of Auckland) was used to perform a bootstrap analysis. The results of the boot-strapping are listed in Table 2. Mean values of the bootstrap-ping were very similar to the parameter estimates of the orig-inal dataset, and the 95% CIs overlapped with those of the original datasets, indicating that the final model pharmaco-dynamic model was stable.

The results showed that: 1) it was unable to establish an ef-fective dose-effect model between the DEX concentration and the amount of ALDO secretion; and 2) it was possible to es-tablish an effective dose-effect model between ETO concen-tration-ALDO secretion and ETO concentration-CORT secretion by using the Hill's equation. Therefore, we used the DEX con-centration as the covariate of the 2 models and introduced it into the model parameters in a linear (concentration depen-dent) and non-linear (non-concentration-dependent) manner. When we used the Hill's equation to fit the dose-effect model

Table 2. Pharmacological parameters and statistical results.

Parameter	ALDO		CORT	
	Estimate (%RSE)	Bootstrap mean (95%CI)	Estimate (%RSE)	Bootstrap mean (95%CI)
EC_{50}	9.74 (3.3%)	9.62 (9.11–10.13)	9.49 (6.0%)	9.39 (8.20–10.59)
Gamma	18.5 (14.6%)	98.7 (52.7–144.6)	37.0 (28.1%)	37.3 (8.8–65.9)
E_{max}	1.20 (11.3%)	1.17 (0.80–1.54)	8.16 (2.7%)	8.14 (7.92–8.55)
E_0	1.33 (13.5%)	1.28 (1.08–1.49)	8.57 (2.8%)	8.56 (8.14–8.99)
Dex on E_0			−0.0247 (48.2%)	−0.0252 (−0.0104, −0.0329)

E_{max} – denotes the maximal effects by maximal drug concentration; EC_{50} – means the drug concentration when 50% of the difference value between maximal effects and basic effects achieved; E_0 – means the drug effect value when the drug concentration was 0; γ – means the slope of dose-effect correlation curve. Effects and drug concentration were presented as their natural logarithm. %RSE – parameter estimate/standard error of estimate ±100%.

of ETO concentration-ALDO secretion and ETO concentration-CORT secretion and introduced DEX as the covariate of E_0 into the ETO concentration-ALDO secretion model parameters in a linear manner, the objective function of the model was reduced by 4.55 points (ΔOBJ >3.84, df=1, P<0.05). However, when we used DEX as the covariate of the other parameters of the model in a linear manner, no significant improvement was observed (both P>0.05). When these parameters were introduced into the model in a non-linear manner, there was no convergence. These results suggest that ETO inhibited ALDO and CORT secretion from human adrenocortical cells within the range of concentrations involved in this study. The use of DEX alone did not affect the secretion of ALDO and CORT, but there was a synergistic effect in the inhibitory effect of ETO on CORT secretion. In other words, ETO reduced the basal secretion of CORT in a dose-dependent manner. The dose-effect correlation equation indicating the inhibitory effect of ETO on ALDO and CORT secretion is shown as follows, based on which the amount of ALDO and CORT secretion at different concentrations of ETO could be predicted:

$$\text{ALDO}_{\text{secretion}} = 1.33 + \left(1.20 - 1.33\right) \cdot \frac{C_{\text{ETO}}^{18.5}}{C_{\text{ETO}}^{18.5} + 9.74^{18.5}}$$

$$\text{CORT}_{\text{secretion}} = 8.57 + \left(8.16 - 8.57\right) \times \frac{C_{\text{ETO}}^{37.0}}{C_{\text{ETO}}^{37.0} + 9.49^{37.0}}$$

When ETO was used in combination with DEX and the concentration of DEX was ≥EXP (4.6) [94.63] nM:

$$\text{CORT}_{\text{secretion}} = 8.57 + [8.16 - (8.57 + (C_{\text{DEX}} - 4.6) \times (-0.0247))] \cdot \frac{C_{\text{ETO}}^{37.0}}{C_{\text{ETO}}^{37.0} + 9.49^{37.0}}$$

Figure 1 indicates the correlation between the ETO concentration and CORT production. Given the range of concentrations involved in this study, only when the concentration of ETO was higher than about EXP (8.5) [5000]nM did the amount of CORT secretion begin decreasing. The inhibitory effect reached the peak at the concentration of EXP (10.5) [36 000]nM. When the DEX concentration change was used as the covariate and introduced into the ETO curve, no significant change in the configuration of the curve was noticed, but E_0 declined significantly (Figure 1). According to the principle of the Sarriselkä protocol, interactions between 2 drugs can be classified into 3 categories: additive, synergism, and antagonism. Therefore, ETO had a specific affect when used alone in this model. However, after DEX was added, the effect of DEX in inhibiting CORT increased, which is called synergism, while the effect of DEX inhibiting ALDO remained changed, which is called inertism.

Discussion

There are about 10 human adrenocortical cell lines, of which NCI-H295 is the most common cell line and can synthesize and secrete more than 30 steroid hormones. NCI-H295R is an adrenocortical cell line derived from NCI-H295. There are controversies over the use of NCI-H295R for ALDO secretion research. Some researchers [20–22] argued that NCI-H295R could not respond sufficiently to Ang II stimulation because the expression of Ang II receptor on the surface of NCI-H295R cell line was relatively low, and therefore was not suitable for ALDO secretion research. Miller et al. [23] obtained an NCI-H295A cell line by altering the composition of the medium and culture conditions, and found that this cell line did not respond to Ang II and ACTH stimulation, and its response to K+ was uncertain. In addition, as the expression of CYP11B2 mRNA in the nucleus is very low, it is not suitable for the present study, either. AR-47, ACT-1, and RL-251 cell lines are immortalized human adrenocortical carcinoma cell lines that can secrete cortisol in the early stage. But as generations pass by, they gradually loose the ability of secreting cortisol and can no longer secrete ALDO; therefore, they are currently used mainly in the
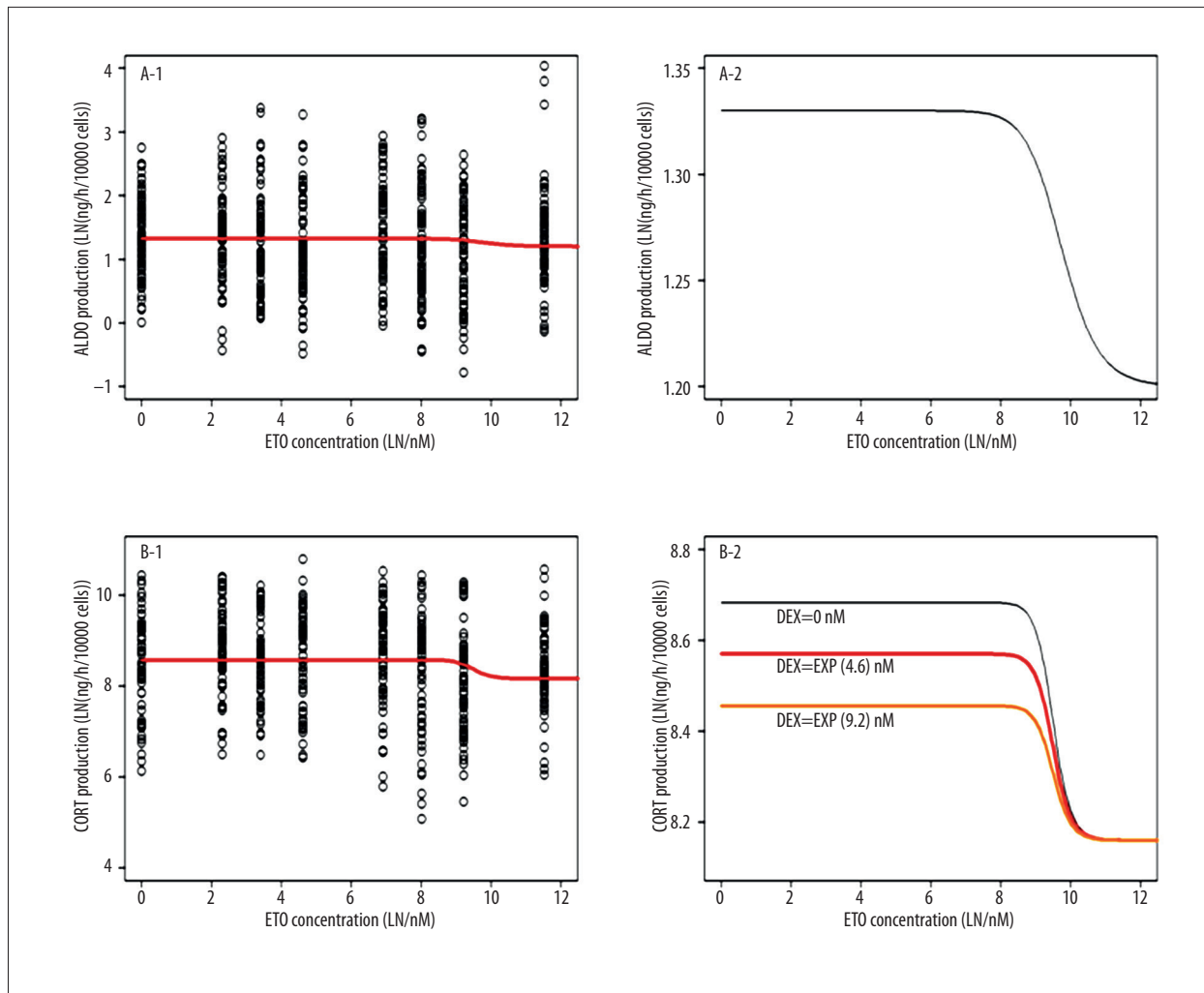
Figure 1. The inhibitory effect of ETO and DEX in ALDO and CORT secretion.

research of cAMP/PKA signaling pathways related to the development of adrenal tumors, or the efficacy and mechanism of anti-adrenal tumor agents [24–26]. Taking into account the ethical problems and reproducibility of the experiment, we used commercial HADCC in the present study.

During *in vitro* culture of young rat adrenocortical cells, some studies [27] found that Zona glomerulosa cells remained stable for as long as 3 weeks; with the lapse of culture time and addition of ACTH, these Zona glomerulosa cells gradually transformed into Zona reticularis cells. In addition, with alteration of the ACTH concentration, a biphasic effect of either inhibition or promotion was noticed. For this reason, ACTH was not used for cell stimulation for the sake of obtaining relatively stable cell differentiation and baseline. Jager et al. [28] found that the proliferation of *in vitro* cells was associated with the concentration of ETO added. A single-dose addition of 40×10^{-3} M ETO to fetal rat adrenocortical cells increased the proliferation rate by 5% of the total cell number. This figure increased to

about 7% at the concentration of 4×10^{-3} M, and was close to the control group at the concentration of 0.4×10^{-3} M. When ETO and ACTH were added in combination, the cell proliferation rate was about 18% versus 6% when ACTG was added alone. The concentration of 0.4×10^{-3} M is about 500-fold the effective dose (0.81×10^{-6} M) of ETO used clinically on humans. Based on the above consideration, we postulated that addition of this ETO concentration to the medium would not cause insufficiency of cell proliferation, nor would it affect the results of the experiment due to errors arising from insufficient cell proliferation. Maze et al. [10] found that the 50% concentration of inhibition (IC_{50}) of ETO was 10^{-6} M. When the concentration was larger than 10^{-7} M, the corticosterone secretion reaction induced by inhibition of rat cells to ACTH stimulation also increased with the increasing dose, suggesting that ETO may also have an inhibitory effect on the secretory function of adrenocortical cells. For this reason, we selected the concentration range of ETO from 10^{-8} to 10^{-4} M, and the concentration range of DEX from 10^{-8} to 10^{-5} M.

For the first time, we found that DEX inhibited the basal secretion of CORT in a dose-dependent manner, and that the relative pharmacologics effect was not observable until the concentration was higher than 94.63 nM, suggesting that only when the concentration of DEX reaches a certain level can it work with ETO to produce an additive effect on CORT secretion. The experiment provided evidence sufficient to develop a mathematical model that explains the experimental data. Compared with the previous findings, our mathematical model is more convincing to explain some phenomena that cannot be noticed in clinical practice [8]. This model can be explained simply through the view of mathematics. It was found that in the dose-effect model parameters, EC_{50} and γ represent the effective drug dose and the dose-response curve slope, respectively; when γ is relatively large, the concentration-effect curve is relatively steep, and even a subtle change in the concentration would induce a relatively large change in the effect. The effect-concentration relationship is intrinsically biphasic, or the pharmacologics effect presents in the "yes" or "no" form [29]. These results suggest that there is minimal probability of producing the effect when the ETO concentration is far below the EC_{50} value, while the probability of producing the maximal pharmacologics effect is high when the ETO concentration is far larger than EC_{50}. When γ is relatively small, the curve is relatively flat, when a small change in the concentration would not induce significant fluctuations of the pharmacologic effect. However, when the concentration-effect curve slope is relatively small, there is a good linear correlation between the effect and the concentration [30]. In other words, there is a concentration threshold for ETO to inhibit the secretion of adrenocortical hormones.

Some other studies [31] found that IC_{50} of ETO in inhibiting the adrenocortical function was 1×10^{-6} M, which is close to the apparent dissociation constant of ETO binding to these cells, which is consistent with the results of our study. We found that the EC_{50} of ETO in inhibiting the secretion of ALDO and CORT from adrenocortical cells was EXP (9.74), which is equivalent to 1.7×10^{-5} M), and EXP (9.49), which is equivalent to 1.3×10^{-5} M, respectively. In a study using NCI-H295R cell line, Ullerås et al. [32] reported that the ETO concentration was 100 M (equivalent to 10×10^{-5} M) when the inhibitory effect on ALDO and CORT secretion reached 75~100%. Although this concentration was by far higher than that in our study, it is still very close to the extrapolated value of the result in the present study. However, our result is somewhat different from the result reported by Kenyon et al. [33] and Zolle et al. [34], who reported that the EC_{50} was 4.6×10^{-7} M, showing a significant difference from ours.

There have been some studies on the inhibitory effect of DEX on adrenocortical cells. Maze et al. [26] found that the amount of basal secretion of CORT decreased significantly 3 h after administration of DEX to hybrid dogs, and the increased amount of secretion upon ACTH stimulation was also decreased significantly. They further found that DEX also had the function of inhibiting the secretion of CORT in rats. However, Venn et al. [35] reported that continuous injection of 2~2.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ DEX to ICU patients could produce an inhibitory effect on adrenocortical function. They believed that short-term (<24 h) use of DEX would not affect adrenocortical hormone synthesis and ACTH release, nor would it inhibit the hypothalamus -pituitary axis. In addition, Aho et al. [36] found that the plasma CORT concentration of the patients was reduced after administration of 2.4 $\mu g \cdot kg^{1}$ DEX, suggesting that DEX could attenuate the response of the endocrine system to external stimuli. These 2 results are absolutely contradictory, and the result of our study seems to be a summary of their results. We found that DEX did not affect the dose-effect correlation of ETO in inhibiting the secretion of ALDO and CORT, but was able to reduce the basal secretion of CORT in a dose-dependent manner. Therefore, we conclude that the dose-effect of DEX on the inhibitory effect of ETO on CORT secretion is intrinsically an additive effect. In this study, DEX presented to affect ETO inhibiting CORT, which mainly focused at E_0, when DEX was over exp(4.6)94.63nM. This effect increased proportionately as the concentration of DEX increases. Thus, it was defined as a synergistic effect.

The present study has some limitations. First, we failed to fix the number of cells before determining the inhibitory effect on ALDO and CORT secretion, and therefore were unable to get a more comprehensive understanding about the interaction of DEX and CORT by means of the response surface model. In addition, we failed to set a blank control group for each batch of cells. The blank control group used in this study only used the relative cell number and, therefore, was unable to control 24-h cell proliferation precisely, nor was it able to define or evaluate the cell function after differentiation.

Conclusions

1) ETO exhibited an inhibitory effect on adrenocortical function at relatively high concentrations within the concentration range specified in the present study, and reduced the CORT and/or ALDO production; 2) administration of DEX alone did not exhibit a significant inhibitory effect on adrenocortical function; and 3) DEX, when used in combination with ETO, affected ETO's inhibitory effect on adrenocortical function (CORT secretion) in a dose-dependent manner.

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