Int J Clin Exp Med 2017;10(8):12553-12558
www.ijcem.com /ISSN:1940-5901/IJCEM0048452

Original Article

Effects of ascorbic acid on etomidate-induced inhibition of adrenocortical function in rabbits

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Received October 15, 2016; Accepted June 21, 2017; Epub August 15, 2017; Published August 30, 2017

Abstract: Objective: To investigate the effect of ascorbic acid (AC) on the etomidate (ETO)-induced inhibition of adrenocortical function. Methods: The rabbits were randomly divided into the etomidate (ETO), ETO+AC1 [ETO+AC (275 mg/kg)] and ETO+AC2 [ETO+AC (550 mg/kg)] groups. The rabbits were anaesthetised by ear vein injection of ETO (1.0 mg/kg), and the anaesthesia was maintained by three ear vein injections of ETO (0.6 mg/kg/h) for 30 min once a week to induce the inhibition of adrenocortical function. The rabbits were injected with AC (275 or 550 mg/kg) by ear vein 20 min before the injection of ETO. Venous blood was sampled by the ear vein from each rabbit at 0, 2 and 6 h after final injection of ETO. The serum cortisol and adrenocorticotropic hormone (ACTH) levels were detected by enzyme-linked immunosorbent assay (ELISA). Results: Compared with the ETO group, serum cortisol levels in the ETO+AC1 and ETO+AC2 groups were significantly increased dose-dependently (all $P<0.05$). Compared with that measured at 0 h, serum cortisol levels in ETO+AC1 and ETO+AC2 groups at 2 and 6 h were significantly increased (all $P<0.05$). Compared with the ETO group, the variance ratios of serum cortisol concentration in the ETO+AC1 and ETO+AC2 groups at 2 and 6 h were significantly increased (all $P<0.05$). Compared with that at 2 h, the variance ratios of serum cortisol concentration in the ETO+AC1 and ETO+AC2 groups at 6 h were significantly increased (all $P<0.05$). Compared with the ETO group, serum ACTH levels in the ETO+AC1 and ETO+AC2 groups at 0, 2 and 6 h were significantly decreased dose-dependently (all $P<0.05$). Compared with that at 0 h, serum ACTH levels in the ETO+AC1 and ETO+AC2 group at 2 and 6 h were significantly decreased (all $P<0.05$). Compared with that in the ETO group, serum ACTH levels in the ETO+AC1 and ETO+AC2 groups at 6 h were significantly increased (all $P<0.05$). Compared with the levels at 2 h, serum ACTH levels in the three groups at 6 h were significantly increased (all $P<0.05$). Conclusions: Pretreatment with AC suppresses the ETO-induced inhibition of adrenocortical function in rabbits.

Keywords: Etomidate, ascorbic acid, cortisol, adrenocorticotropic hormone

Introduction

Etomidate (ETO) is frequently used clinically as an anaesthetic and sedation agent because of its favourable haemodynamic advantage [1]. Moreover, ETO can facilitate the intubation [2]. ETO presents advantages such as rapid onset, rapid metabolism and the especially unique function in stabilising cardiovascular condition, compared with other similar agents. Thus, ETO is widely used to maintain anaesthesia in patients with critical illness, cardiovascular surgeries and neurosurgeries, as well as for sedation in pain remission therapies [3, 4]. However, ETO application is limited, because it inhibits the synthesis of 11 β-hydroxylase, thus reducing the adrenal functions [5, 6]. Adrenal insufficiency (AI), whether ETO-induced or secondary to critical illness-related corticosteroid insufficiency, is a common and underappreciated problem in the intensive care unit (ICU) [7]. A single dose of ETO provides good intubation conditions with haemodynamic stability, but increases the risk for AI [8]. One meta-analysis shows that the administration of ETO for rapid sequence intubation is associated with the high rate of AI [9]. Another meta-analysis suggests a small trend towards an increased risk of death among ETO recipients, compared with non-recipients (OR 1.14, 95% CI 0.81-1.60) [10]. In recent years, although ETO alternatives with the same advantages as ETO have been investigated, only a few studies have focused on the reduction of adrenal inhibitory effects.

It is found that ascorbic acid (AC) can accelerate the transformation and synthesis of 11 β-hydroxylase. Another study has shown no evidence of a clinically relevant attenuating effect of AC or xylitol on ETO-induced adrenocortical suppression in humans, but AC is administered after surgery [11]. Therefore, AC can theoretically prevent the inhibitory effects of ETO on adrenal functions. This study preventively injected AC into rabbits and then induced and maintained ETO anaesthesia. The serum cortisol and ACTH levels at different time points were tested to explore whether the prophylactic application of AC could reduce the ETO-induced inhibition of adrenocortical function. Moreover, whether the impacts of AC on these effects may change depending on the dose within the therapeutic range were determined. The objective was to provide a simple and feasible method to reduce the ETO-induced inhibition of adrenocortical function for clinical application.

Materials and methods

Animals

A total of 18 healthy male New Zealand white rabbits [ordinary level, Jinan Xilingjiao Breeding Centre, license number SCXK (Lu) 20100005] were used in this study. The rabbits weighed 1.6-2.5 kg and were not previously used for any other experiment. The rabbits were housed under a 12-h dark and 12-h light cycle with free access to water and food. The animal experiments were approved by the Committee on the Ethics of Animal Experiments of the Weifang Medical University.

Animal groupings

The rabbits were randomly divided into ETO (1.0 mg/kg ETO), ETO+AC1 (ETO, 1.0 mg/kg; AC, 275 mg/kg) and ETO+AC2 (ETO, 1.0 mg/kg; AC, 550 mg/kg) groups. AC (batch number: 1310107) was purchased from Shandong Xinhua Pharmaceutical Co., Ltd. (Zibo, China), and ETO (batch number: 20131206) was purchased from Jiangsu Nhwa Pharmaceutical Co., Ltd. (Xuzhou, China).

Induction of adrenocortical function inhibition and treatment with AC

All experiments were conducted at the same time in the morning to avoid the effect of daily rhythm on the study results. The inhibition of adrenocortical function was induced by anaesthetising the rabbits by ear vein injection of ETO (1.0 mg/kg). The anaesthesia was maintained by three ear vein injections of ETO [0.6 mg/(kg·h)] for 30 min once a week. The rabbits in the ETO+AC1 and ETO+AC2 groups were injected with AC (275 and 550 mg/kg, respectively) by ear vein 20 min before the injection of ETO.

ELISA

Venous blood (2-3 mL) was sampled by ear vein from each rabbit at 0 h ($T_{0\,h}$), 2 h ($T_{2\,h}$) and 6 h ($T_{6\,h}$) after the final injection of ETO. Then, the blood samples were naturally coagulated at room temperature for 10-20 min and centrifuged for 20 min (2000-3000 rpm) to collect the supernatant. ELISA kits were used to detect the serum concentrations of cortisol and adrenocorticotropic hormone (ACTH). Rates of change in the serum cortisol and ACTH levels were calculated as follows: $T_{2\,h}$ (%) = ($T_{2\,h}$-$T_{0\,h}$)/$T_{0\,h}$ × (100) and $T_{6\,h}$ (%) = ($T_{6\,h}$-$T_{0\,h}$)/$T_{0\,h}$ × (100).

Statistical analysis

SPSS 17.0 statistical software was used for the statistical analysis. Measured data were expressed as mean ± standard deviation. The homogeneity of variance and normal distribution of all data were tested before the statistical analysis. One-way ANOVA was used to compare the three doses and data among different time points for repeated measurements, followed by Student-Newman-Keuls post test. $P<0.05$ indicated statistical significance. The measured serum cortisol and ACTH levels at $T_{2\,h}$ and $T_{6\,h}$ were compared with those detected under basic condition, and the increased or decreased values were expressed by the rate of change.

Results

Serum cortisol level

Table 1 showed that, compared with the ETO group, the serum cortisol levels in the ETO+AC1 and ETO+AC2 groups at all time points were significantly increased (all $P<0.05$). Compared with the ETO+AC1 group, the serum cortisol levels in the ETO+AC2 group at all time points were significantly increased (all $P<0.05$). Compared with that measured at 0 h, the serum cortisol levels in the ETO group at 2 and 6 h were significantly decreased (all $P<0.05$). Com-

Table 1. Serum cortisol levels ($\bar{x}$±s, pg/mL)

Group	*0 h*	*2 h*	*6 h*
ETO	67.60±9.52	58.74±3.34$^{▲}$	59.32±4.19$^{▲}$
ETO+AC1	72.82±4.65^{Δ}	90.55±10.57$^{Δ,▲}$	126.47±3.29$^{Δ,▲,◆}$
ETO+AC2	83.31±5.10$^{Δ,◇}$	99.87±9.22$^{Δ,▲,◇}$	141.03±2.18$^{Δ,▲,◇,◆}$

Data were expressed as mean ± S.D (n = 6 for each group). ^{Δ}P<0.05, compared with group ETO; $^{◇}P$<0.05, compared with group ETO+AC1; $^{▲}P$<0.05, compared with group T_{0h}; $^{◆}P$<0.05, compared with group T_{2h}.

Table 2. Variance ratio of serum cortisol concentrations

Group	T_{2h} (%)	T_{6h} (%)
ETO	13.2±2.5	13.1±3.0
ETO+AC1	24.3±1.8 (%)^{Δ}	73.6±4.3 (%)$^{Δ,▲}$
ETO+AC2	19.9±3.2 (%)^{Δ}	69.3±2.7 (%)$^{Δ,▲}$

Data were expressed as mean ± S.D (n = 6 for each group). ^{Δ}P<0.05, compared with group ETO; $^{▲}P$<0.05, compared with T_{2h}.

pared with that measured at 0 h, the serum cortisol levels in the ETO+AC1 and ETO+AC2 groups at 2 and 6 h were significantly increased (all *P*<0.05). No significant difference was found between the serum cortisol levels measured at 2 and 6 h in the ETO group (*P*>0.05). Compared with that measured at 2 h, the serum cortisol levels in the ETO+AC1 and ETO+AC2 groups at 6 h were significantly increased (all *P*<0.05).

Variance ratio of serum cortisol concentration

Compared with the ETO group, the variance ratios of serum cortisol concentration of the ETO+AC1 and ETO+AC2 groups at 2 and 6 h were significantly increased (all *P*<0.05). No significant difference was found between the variance ratios of serum cortisol concentration at 2 and 6 h in the ETO group (*P*>0.05). Compared with that at 2 h, the variance ratios of serum cortisol concentrations in the ETO+AC1 and ETO+AC2 groups at 6 h were significantly increased (all *P*<0.05) (**Table 2**).

Serum ACTH

Table 3 showed that, compared with that in the ETO group, serum ACTH levels in the ETO+AC1 and ETO+AC2 groups at all time points were significantly decreased (all *P*<0.05). Compared with that in the ETO+AC1 group, serum ACTH levels in the ETO+AC2 group at all time points were significantly decreased (all *P*<0.05). Compared with that at 0 h, the serum ACTH levels in the ETO group at 2 and 6 h were significantly increased (all *P*<0.05). Compared with that at 0 h, serum ACTH levels in the ETO+AC1 and ETO+AC2 groups at 2 and 6 h were significantly decreased (all *P*<0.05). Compared with that at 2 h, serum ACTH levels in the ETO and ETO+AC1 groups at 2 and 6 h were significantly decreased (all *P*<0.05). No significant difference was found between the serum ACTH levels measured at 2 and 6 h in the ETO+AC2 group (*P*>0.05).

Variance ratio of serum ACTH concentration

Compared with the ETO group, serum ACTH levels in the ETO+AC1 and ETO+AC2 groups at 6 h were significantly increased (all *P*<0.05). Compared with that at 2 h, the serum ACTH levels in the three groups at 6 h were significantly increased (all *P*<0.05) (**Table 4**).

Discussion

ETO is a non-barbiturate short-acting intravenous anaesthetic and characterised by rapid onset, short duration, rapid and smooth recovery, mild respiratory depression, cardiovascular effects, etc [12, 13]. Thus, this compound is widely used in anaesthesia induction, ICU sedation and anaesthesia in ambulatory surgeries [14]. Almost all anaesthetic agents will produce cardiovascular depression [15], but ETO has significant advantages in haemodynamics. Thus, this drug is still the preferred anaesthetic drug for patients with poor cardiovascular reserve [16].

Inhibition of steroidogenesis is one potential fatal side effect of ETO, especially for critically ill patients. This inhibition is extremely strong and can occur even at doses lower than that necessary for general anaesthesia. Inhibition of adrenal functions results in the loss of response of patients to irritable stimuli. Ledingham et al [17] find that ETO can reduce the serum cortisol level, leading to AI and adrenal suppression [18]. ETO may cause transient adrenal dysfunction, resulting in water and salt imbalance, hypotension or even shock, as well as increasing the mortality in critically ill patients [17]. However, the correlations of ETO with mortality have not been precisely estab-

Table 3. Serum adrenocorticotropic hormone (ACTH) level ($\bar{x}$±s, pg/mL)

Group	*0 h*	*2 h*	*6 h*
ETO	60.64±3.33	67.77±3.15$^{▲}$	70.69±2.90$^{▲,◆}$
ETO+AC1	51.86±2.40^{Δ}	45.35±2.58$^{Δ,▲}$	37.44±1.71$^{Δ,▲,◆}$
ETO+AC2	33.82±3.43$^{Δ,◇}$	30.42±1.00$^{Δ,▲,◇}$	29.37±0.94$^{Δ,▲,◇}$

Data were expressed as mean ± S.D (n = 6 for each group). ^{Δ}P<0.05, compared with group ETO; $^{◇}P$<0.05, compared with group ETO+AC1; $^{▲}P$<0.05, compared with T_{0h}; $^{◆}P$<0.05, compared with T_{2h}.

Table 4. Variance ratio of serum ACTH concentrations

Group	T_{2h} (%)	T_{6h} (%)
ETO	11.5±1.3	16.4±1.2$^{▲}$
ETO+AC1	13.5±1.4	28.8±2.3$^{Δ,▲}$
ETO+AC2	11.7±1.9	14.7±2.2$^{Δ,▲}$

Data were expressed as mean ± S.D (n = 6 for each group). ^{Δ}P<0.05, compared with group ETO; $^{▲}P$<0.05, compared with T_{2h}.

lished. High-risk patients are often in high metabolic state, and the acute and chronic severe diseases will lead to changes in hormone levels. Studies have shown that approximately 50% of critically ill patients, such as patients with traumatic brain injury, have AI, which is related to age, injury severity and administration of ETO [19]. Thus, the clinical application of ETO is limited because of the cortisol suppression of this drug. Patients after surgery or with critical illness need to supplement the renocortical hormones because of the application of ETO. Moreover, the in-time monitoring and appropriate supplementation of ACTH in critically ill patients administered with ETO can reduce the complications and mortality caused by this drug [20, 21]. However, this method is not ideal because the dosage, timing and duration of steroid treatment to any given patient can only be obtained through speculation. In addition, administration of exogenous steroids itself can produce serious complications (especially in septicaemia), such as alteration of the glucose homeostasis, impaired wound healing or immunosuppression. Therefore, the ETO has limited application because of its inhibition of the adrenocortical functions [22].

AC is highly significant in improving renocortical hormone levels, improving therapeutic effects in acute ischemic renal injuries, which may initially be the major role in CI-AKI [23]. Study on healthy men has shown that continuous infusion of AC can promote the steady-state stability of the pituitary gland and secretion of ACTH, as well as increase the plasma ACTH and cortisol levels [24]. Brody et al [25] find that different doses and plasma concentrations of AC have distinct roles in stimulating and increasing the secretions of ACTH. However, sole consumption of food with high AC content is not sufficient to meet the hormone secretion level under strong stress. The synthesis of AC is inhibited when the imidazolyl of ETO binds with cytochrome P450, affecting the activities of 11β-hydroxylase. Clinical study has shown that AC can promote the transformation of 11β-hydroxylase and is closely related to the synthesis of adrenocortical hormones [26]. Hydroxylation during the synthesis of glucocorticoids consumes AC. Thus, AC deficiency results in weakened hydroxylation in cytochrome P450 monooxygenase system, leading to the suppression of cortisol [27, 28].

Prophylactic application of AC protects ETO-induced adrenocortical suppression. In this study, we found that the cortisol level in the ETO group was significantly reduced, but ACTH was significantly increased. This result indicates that ETO can significantly inhibit the adrenocortical functions. However, rabbits with injected with prophylactic AC immediately after ETO anaesthesia showed significantly increased serum cortisol concentration and significantly decreased ACTH concentration than the rabbits in the ETO group. These results indicate that prophylactic application of AC can prevent ETO-induced adrenocortical suppression. The serum cortisol levels in ETO+AC1 group at 0, 2 and 6 h were higher than those in ETO+AC2 group, but the serum ACTH concentrations were significantly lower than that in ETO+AC1 group. These results indicate that the protective effects of AC against ETO-induced adrenocortical suppression are dose-dependent.

This study also investigated whether the protective effects of AC would be continuously enhanced or rapidly weakened at a certain time period. Thus, the concentrations and changing trends of serum cortisol and ACTH at 2 and 6 h after ETO administration were observed. The results showed that, compared with 0 h, the

cortisol concentrations in the three groups at 2 and 6 h showed statistically significant increase, similar to the cortisol concentrations in ETO+AC1 and ETO+AC2 groups at 6 h than at 2 h. Meanwhile, compared with that at 0 h, statistically significant decrease in ACTH concentrations was observed in the three groups at 2 h and 6 h. By contrast, ACTH concentration in ETO+AC1 group at 6 h was significantly decreased compared with that at 2 h. Therefore, the protective effects of AC were basically continuously enhanced within 6 h of ETO injection, but some exceptions were also found.

Statistics of the changing rates of serum ACTH revealed that the intragroup comparison showed statistically significant reductions in ACTH. The ACTH concentrations in ETO+AC1 group at 2 and 6 h showed statistically significant reductions, contrary to those in ETO+AC2 group. This phenomenon suggests that high doses of AC effectively can protect the decreasing serum ACTH level in ETO-induced anaesthesia. However, the decreased level of ACTH in ETO+AC2 group at 6 h was statistically significant than in ETO group, indicating a limited protective duration of high doses of AC. The possible reasons may be that, the effect of AC begin to decrease with prolonged time. Additionally, ETO-induced adrenocortical suppression may have also started to decrease with prolonged time. Another probable reason will be the metabolism problem of ACTH itself. These factors need further investigation.

Protective mechanisms of the prophylactic application of AC on ETO-induced adrenocortical suppression still require further in-depth studies. These protective mechanisms, especially on issues in molecular biology, gene expression and pathophysiological changes, still require much investigation. Thus, we will focus on the possible mechanisms in our next study. Moreover, the rabbit adrenal cortex should be stained with HE and observed with electron microscopy at the same time points to determine the apoptosis of adrenocortical cells and alterations in the intracellular mitochondria and microsomes. Meanwhile, RT-PCR should be performed to detect the expression of apoptotic genes, such as bcl-2, bax and NF-κb. Urine from each group at a certain period should be collected to examine the levels of ACTH and COR, especially the free cortisol level in urine. These methods can effectively validate the experimental findings. In conclusion, prophylactic application of AC can impact the ETO-induced adrenocortical suppression and increase the cortisol level. The impacts of AC on ETO-induced adrenocortical suppression are dose-dependent and vary at different time points.

Disclosure of conflict of interest

None.

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