The preclinical pharmacological study of a novel intravenous anesthetic, ET-26 hydrochloride, in aged rats

Pan Chang [Equal first author, 1] , YongWei Su [Equal first author, 1] , DeYing Gong [2, 3] , Yi Kang [2, 3] , Jin Liu [1, 2, 3] , YuJun Zhang [Corresp., 1, 2, 3] , Wen-sheng Zhang [Corresp. 1, 2, 3]

1 Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, China

2 Laboratory of Anesthesia and Critical Care Medicine, Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu, China

3 National-Local Joint Engineering Research Center of Translational Medicine of Anesthesiology, West China Hospital, Sichuan University, Chengdu, China

Corresponding Authors: YuJun Zhang, Wen-sheng Zhang
Email address: zhangyujun@scu.edu.cn, zhang_ws@scu.edu.cn

Background: ET-26 hydrochloride (ET-26HCl) is a novel analogue of etomidate approved for clinical trials. However, all results from recent studies were accomplished in young adult animals. The objective of this study was to evaluate the efficacy and safety of ET-26HCl in aged rats. **Methods:** The measurements of loss of the righting reflex and cardiovascular and respiratory function after injection at the two-fold dose of the median effective dose were used for evaluation of effectiveness and safety, and the modified adrenocorticotropic hormone-stimulation experiment was used to evaluate the inhibition effect of the drugs on the synthesis of adrenal cortical hormones. **Results:** There was no significant difference in the onset time among propofol, etomidate and ET-26HCl. The duration of propofol (850.5 ± 77.4 s) was significantly longer than that caused by etomidate (489.8 ± 77.0 s, p = 0.007) and ET-26HCl (347.3 ± 49.0 s, p = 0.0004). No significant difference was observed in the time to stand and normal activity among drugs. 66.7% of rats in the ET-26HCl group were evaluated to have mild hematuria. Then, etomidate and ET-26HCl had a milder blood pressure inhibition effect than propofol. Apnea was observed in all rats administered propofol and the duration for this side effect was 45.0 ± 9.0 s. For etomidate and ET-26HCl, no apnea was observed. No significant difference was observed between ET-26HCl and solvent group. However, rats administered etomidate had lower corticosterone concentrations than those administered ET-26HCl at 15, 30, and 60 min. **Conclusions:** ET-26HCl produced a desirable sedative-hypnotic effect with stable myocardial and respiratory performance and mild adrenocortical suppression in aged rats. Thus, these findings increase the potential for the clinical use of ET-26HCl in the elderly population.

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5 Pan Chang, M.D. [#, a], YongWei Su, M.D. [#, a], DeYing Gong, M.S. [b, c], Yi Kang, B.D. [b, c], Jin Liu, M.D. [a, b, c], YuJun

6 Zhang, M.D.[* a, b, c] WenSheng Zhang, M.D.[* a, b, c]

7 a: Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, China

8 b: Laboratory of Anesthesia and Critical Care Medicine, Translational Neuroscience Center, West China Hospital,

9 Sichuan University, Chengdu, China

10 c: National-Local Joint Engineering Research Center of Translational Medicine of Anesthesiology, West China

11 Hospital, Sichuan University, Chengdu, China

12 [#] These authors contributed equally to this work (co-first author).

13 [*] Correspondence:

14 WenSheng Zhang, M.D., Department of Anesthesiology, Laboratory of Anesthesia and Critical Care

15 Medicine, Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu, China.

16 E-mail: zhang_ws@scu.edu.cn.

17 YuJun Zhang, M.D., Department of Anesthesiology, Laboratory of Anesthesia and Critical Care Medicine,

18 Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu, China. E-mail:

19 zhangyujun@scu.edu.cn.

20   **Abstract**

21   **Background:** ET-26 hydrochloride (ET-26HCl) is a novel analogue of etomidate approved for clinical

22   trials. However, all results from recent studies were accomplished in young adult animals. The objective of

23   this study was to evaluate the efficacy and safety of ET-26HCl in aged rats.

24   **Methods:** Aged Sprague-Dawley rats were randomly divided into three groups (three males and three

25   females in each group) were given dose of two-fold of median effective dose (ED_{50}) of ET-26HCl,

26   etomidate and propofol: the measurements of loss of the righting reflex (LORR) and cardiovascular and

27   respiratory function after injection at the two-fold dose of the median effective dose were used for

28   evaluation of effectiveness and safety, and the modified adrenocorticotropic hormone-stimulation

29   experiment was used to evaluate the inhibition effect of the drugs on the synthesis of adrenal cortical

30   hormones.

31   **Results:** All Rats were observed for mortality and symptoms of toxicity. There was no significant

32   difference in the onset time among propofol, etomidate and ET-26HCl. The duration of propofol (850.5 ±

33   77.4 s) was significantly longer than that caused by etomidate (489.8 ± 77.0 s, p = 0.007) and ET-26HCl

34   (347.3 ± 49.0 s, p = 0.0004). No significant difference was observed in the time to stand and normal activity

35   among drugs. 66.7% of rats in the ET-26HCl group were evaluated to have mild hematuria. Then, etomidate

36   and ET-26HCl had a milder blood pressure inhibition effect than propofol. Apnea was observed in all rats

37   administered propofol and the duration for this side effect was 45.0 ± 9.0 s. For etomidate and ET-26HCl,

38   no apnea was observed. No other clinical signs of side-effect were observed, and no rats died. No significant

39   difference was observed in corticosterone concentrations between ET-26HCl and solvent group. However,

40 rats administered etomidate had lower corticosterone concentrations than those administered ET-26HCl at

41 15, 30, and 60 min.

42 **Conclusions:** Our results indicate ET-26HCl in aged rats is an effective sedative-hypnotic with stable

43 myocardial and respiratory performance and also have mild adrenocortical suppression. Thus, these

44 findings increase the potential for the clinical use of ET-26HCl in the elderly population.

45 **Key words**: intravenous anesthetic, etomidate analogue, pharmacological study, aged rats, ET-26

46 hydrochloride

47 **1. Introduction**

48   A universal social situation is the aging global population. The number of aged people reached 600

49 million in 2000 and is predicted to reach 2.1 billion in 2050 (McNicoll 2002). Coexisting diseases,

50 especially in the cardiovascular system, are more prevalent in the elderly than in the young adult population.

51 A commonly used intravenous anesthetic, propofol, has extensive effects on the cardiovascular and

52 respiratory system (Sahinovic et al. 2018). The most prominent side-effect of propofol is cardiovascular

53 depression, especially for blood pressure (BP) reduction (Ebert 2005; Hug et al. 1993). Additionally,

54 propofol decreases the sensitivity of central chemoreceptors, reduces tidal volume, and leads to apnea

55 (Jonsson et al. 2005; Nieuwenhuijs et al. 2000; Nieuwenhuijs et al. 2001; Yamakage et al. 1999). Due to

56 its excellent cardiovascular stability and mild respiratory depression, etomidate is the most widely used

57 intravenous anesthetic for elderly patients (Giese et al. 1985; Tomlin et al. 1998; Ye et al. 2017). However,

58 previous studies have indicated that etomidate may even reduce the survival rate in the perioperative period

59 *via* the inhibition of the synthesis of adrenal cortical hormones (Chan et al. 2012; Fellows et al. 1983;

60 Forman 2011; Kamp & Kress 2007). Despite this severe adverse effect, etomidate is still the current optimal

61 option for the induction of anesthesia in elderly patients (Bovill 2006).

62   Etomidate is an imidazole-based agonist of the γ-aminobutyric acid type A receptor used for the

63 induction of general anesthesia and sedation (Valk & Struys 2021). Previous studies demonstrated that

64 etomidate-inhibited adrenal steroidogenesis was related to its ester side chain (Atucha et al. 2009).

65 Meanwhile, the primary metabolic production of etomidate, etomidate acid, had a lower inhibition of the

66 synthesis of adrenal cortical hormones (Zolle et al. 2008). Thus, a new etomidate analogue was designed

67 and synthesized to modify the ester side chain or metabolize to etomidate acid rapidly for reducing the

68 adrenocortical suppression effect of etomidate. ET-26 hydrochloride (ET-26HCl; patent number,

69 US9969695B2; Figure 1) was screened as a novel intravenous anesthetic with not lonely similar extent of

70 cardiovascular stability and respiratory depression comparing to etomidate, but also milder adrenocortical

71 inhibition (Wang et al. 2017c). ET-26HCl was approved by the China National Medical Products

72 Administration (NMPA) for clinical trials in 2019.

73   In non-clinical pharmacological studies, ET-26HCl produces a desirable sedative-hypnotic effect with

74 stable myocardial performance and mild adrenocortical suppression in young animals (Liu et al. 2018;

75 Wang et al. 2017c; Yang et al. 2017). In a rat uncontrolled hemorrhagic shock model, ET-26HCl shows

76 similar hemodynamic stability to etomidate (Wang et al. 2017a). Furthermore, in a rat lipopolysaccharide-

77 induced sepsis model, ET-26HCl induces a higher survival rate, a lower inflammatory reaction, and a

78 reduced organ injury than etomidate (Wang et al. 2017b). These findings indicated that ET-26HCl can be

79 an effectively sedative-hypnotic anesthetic with similarly excellent cardiovascular stability as etomidate

80 and milder adrenocortical inhibition than etomidate. However, all results from recent non-clinical

81 pharmacological studies were accomplished in young animals. Thus, there is currently no direct evidence

82 that shows the efficacy and safety of ET-26HCl in the elderly population.

83   In this study, we compared the pharmacodynamic properties of ET-26HCl, etomidate, and propofol

84 with the injection of the two-fold dose of the ED_{50} for LORR in aged rats. All rats were observed closely

85 for mortality and clinical symptoms of toxicity. We further evaluated the effect of these drugs on mean

86 arterial pressure, heart rate, respiratory rate, and adrenocortical suppression in aged rats.

2. Materials and Methods

2.1 Animals and Housing

All animal procedures were performed with the approval of the Institutional Animal Care and Use Committee of West China Hospital, Sichuan University, Chengdu, China (2015015A). All animals were housed according to the Guide for the Care and Use of Laboratory Animals by the West China Hospital, Sichuan University. Sprague-Dawley SPF rats (age: 20 months, body weight: 680 ± 23 g for male and 508 ± 59 g for female after 5 days adaptation) were purchased from Dossy Biological Technology Co., Ltd. (Chengdu, China). Rats (n = 5) were housed in polypropylene cages with *ad libitum* food and water supply, controlled room temperature (22 ℃ to 26 ℃) and humidity (40% to 60%), and under 12 h light-dark cycles (lights on at 7:00 a.m.). Prior to the experiments, animals were acclimated for a minimum of 5 days.

2.2 Materials

ET-26HCl was synthesized by Humanwell Pharmaceutical Co., Ltd. (Yichang, China). The solution of ET-26HCl (10 mg/mL) was dissolved in 35% (*v/v*) propylene glycol and sterile injection water, and this specific solvent was prepared by Humanwell Pharmaceutical Co., Ltd. Propofol (Diprivan, 10 mg/mL) and etomidate (10 mg/mL) were purchased from AstraZeneca (Shanghai, China) and Nhwa Pharmaceutical Co., Ltd. (Jiangsu, China), respectively. Isoflurane was purchased from Yipin Pharmaceutical Co., Ltd. (Hebei, China). Dexamethasone sodium phosphate injection and normal saline were purchased from Tianyao Pharmaceutical Co., Ltd. (Hubei, China) and Qingshanlikang Pharmaceutical Co., Ltd. (Chengdu, China), respectively. Adrenocorticotropic hormone (ACTH) fragment 1-24 (human and rat) was purchased from Sigma-Aldrich (MO, USA) and dissolved with dimethyl sulfoxide (20 μg/mL).

107   Corticosterone and corticosterone-d8 were purchased from the Dr. Ehrenstorfer GmbH (Augsburg,

108   Germany) and C/D/N Isotopes Inc. (Quebec, Canada), respectively. Acetonitrile, formic aid, and dimethyl

109   sulfoxide were purchased from Sigma-Aldrich. Ethyl acetate, ethyl alcohol and methanol were obtained

110   from Fisher Scientific (NJ, USA). All reagents are HPLC grade solvents. Ultrapure water was generated by

111   the Milli-Q ® water purification system (Merck Millipore, Darmstadt, Germany).

112   **2.3 Experimental Design**

113   Eighteen aged Sprague-Dawley rats were randomly divided into three groups (three males and three

114   females in each group). According to a previous study (Wang et al. 2017c), the ED_{50} values for LORR in

115   rats are 2.35 mg/kg, 0.73 mg/kg, and 5.87 mg/kg for ET-26HCl, etomidate and propofol, respectively. Rats

116   were injected intravenously with the two-fold dose of ED_{50} for LORR *via* the tail vein in an injection speed

117   of 0.1 mL/s.

118   **2.4 LORR measurement**

119   Rats were separately measured for LORR in a transparent box with a thermostatically heated pad (37

120   ℃) to avoid external stimuli. The hypnotic depth was scored continuously until the rat was fully awake

121   according to the following criteria (Salamone et al. 1996; Shimoyama et al. 1999; Wang et al. 2017c): 0 =

122   normal activity; 1 = decreased activity or remained in same position; 2 = could not balance on hind limb; 3

123   = ataxia; 4 = lay prone and no reaction to mild stimuli; 5 = LORR. Once the score achieved 4, the rat was

124   gently changed to the supine position (recognized as LORR). The time to LORR, time to recovery from

125   LORR, time to stand up, and time to normal activity represented the onset, duration, and recovery of

126   sedative effect. All rats that recovered from LORR were observed closely for mortality and clinical

127   symptoms of toxicity.

128   **2.5 Cardiovascular and respiratory function measurement**

129   Rats were anesthetized with 2% isoflurane in oxygen using a mask that was placed in the supine

130   position on a thermostatically heated pad (37 ℃). The tail vein was cannulated with a Terumo ® intravenous

131   catheter (24G; Tokyo, Japan) for drug administration. Then, the left femoral artery was isolated and

132   cannulated for monitoring BP. A three-lead electrocardiogram (ECG) was monitored by the subcutaneous

133   placement of an electrode at the left upper limb, right upper limb, and left lower limb. Thoracic movement

134   was measured by a tension sensor for respiratory rate (RR). The ECG, BP and RR were recorded by the

135   BL-420 Biological Signal Acquisition and Analysis System (Techman, Chengdu, China). Rats were

136   acclimated for 20 min prior to the initiation of dosing. In the adaptive phase, the parameters were collected

137   every 4 min and averaged as the baseline. Then, rats were injected with the two-fold dose of the ED_{50} for

138   LORR as described in 2.3. The biological parameters were used to compare the degree of cardiovascular

139   and respiratory system inhibition by drugs at the following time points: 0.5, 1 min, and every 1 min to 15

140   min. The mean arterial pressure (MAP) was calculated according to the equation below:

141    $$Mean\ arterial\ pressure = (systolic\ pressure + 2 \times diastolic\ pressure)/3$$

142   **2.6 Serum corticosterone measurement**

143   **2.6.1 Modified adrenocorticotropic hormone (ACTH)-stimulation experiment**

144   The modified ACTH-stimulation experiment was used to evaluate the inhibition effect of the drugs on

145   the synthesis of adrenal cortical hormones (Cotten et al. 2009; Pejo et al. 2012; Wang et al. 2017c). Drug

146 administration and blood sample collection were completed *via* the tail vein with a cannulated catheter

147 (Terumo ®, 24G; Tokyo, Japan). Dexamethasone (0.5 mg/kg) was injected to inhibit corticosterone

148 synthesis. Two hours after the administration of dexamethasone, ET-26HCl and etomidate were

149 administrated at 4.70 mg/kg and 1.46 mg/kg (two-fold dose of the ED_{50} for LORR), respectively. In the

150 control group, 35% (*v/v*) propylene glycol was injected at 0.47 mL/kg (the same volume of ET-26HCl).

151 Fifteen min later, ACTH (25 μg/kg) was administered to accelerate corticosterone production. The

152 concentration of corticosterone was compared at the baseline, 2 h after dexamethasone injection, 15 min,

153 30 min, and 60 min after ACTH administration. Blood samples (0.2 mL each) were centrifuged at 870 g

154 for 10 min. Serum was collected after clotting within 1 h and was stored at -20 ℃.

155 **2.6.2 Sample preparation and liquid chromatography-mass spectrometry (LC-MS/MS) analysis**

156 For corticosterone measurement, 50 μL of sample was added into a polypropylene tube and 150 μL of

157 acetonitrile solution that contained corticosterone-d8 (20 ng/mL; internal standard, IS) was added to

158 deproteinize the serum. After centrifuging (10,300 g, 10 min, 4 °C), the supernatant was analyzed *via* LC-

159 MS/MS (Agilent Technologies 6460 triple quadrupole mass spectrometer with electrospray ionization

160 source). Chromatographic separation was carried out using Waters symmetry C18 column (3 mm × 100

161 mm, 3.5 μm) at 30 °C. The mobile phase consisted of 0.1% formic acid (A) and acetonitrile (B) in a gradient

162 elution: 0 min (A at 60%), 1 min to 1.4 min (A at 40%), and 4.5 min to 4.6 min (A at 10%) at a flow rate

163 of 0.3 mL/min. Mass spectrometry was in positive ionization mode: sheath gas flow rate, 11.0 L/min; sheath

164 gas heater temperature, 300°C; nebulizer pressure, 45 psi; capillary voltage, 3,500 V. MassHunter software

165 was used to analyze data (B.04.00 Build 4.0.479.0, Agilent Technologies, CA, USA).

166   **2.7 Data analysis**

167   For pharmacodynamic experiments, data were assessed by one-way analysis of variance (ANOVA)

168   followed by the Bonferroni post-hoc test ($\alpha = 0.025$). For comparison of cardiovascular function and adrenal

169   cortical hormones inhibition, data were assessed by two-way ANOVA followed by the Bonferroni post-

170   hoc test ($\alpha = 0.025$). For respiratory function measurement, data were assessed by Fisher's exact test ($\alpha =$

171   0.025). Data are reported as mean $\pm$ SEM, and the level of statistical significance was set at $P < 0.05$. Data

172   were analyzed using Statistical Package for Social Sciences version 23 (IL, USA).

173 **3. Results**

174 **3.1 Pharmacodynamic experiments**

175   After the injection of the drugs at the two-fold dose of ED_{50} for LORR, there was no significant

176 difference in onset time among those drugs (Figure 2A, p = 0.053). However, the duration of the sedative-

177 hypnotic effect (score of hypnotic depth = 5, Figure 2B) for propofol (850.5 ± 77.4 s) was significantly

178 longer than that caused by etomidate (489.8 ± 77.0 s, p = 0.007) and ET-26HCl (347.3 ± 49.0 s, p = 0.0004).

179 Regarding recovery from the state of sedation (Figure 2C), the time required to stand after administration

180 of propofol, etomidate, and ET-26HCl was 71.3 ± 33.5 s, 140.3 ± 42.6 s, and 129.0 ± 22.3 s, respectively.

181 The time required to normal activity after the administration of propofol, etomidate, and ET-26HCl was

182 393.0 ± 91.2 s, 382.0 ± 52.6 s, and 378.0 ± 47.8 s, respectively. However, no significant difference was

183 observed in the time to stand (p = 0.330) and normal activity (p = 0.987) among the drugs.

184   Regarding the clinical symptoms of toxicity, 66.7% (4 of 6) of rats in ET-26HCl group exhibited mild

185 hematuria (abnormal urine color - red), which was considered to be induced by the solvent. All rats

186 recovered from their symptoms of toxicity, no other clinical signs of side-effect were observed, and no rats

187 died.

188 **3.2 Cardiovascular and respiratory function measurement**

189   The advantages of etomidate are better cardiovascular stability and milder respiratory depression than

190 propofol. To test cardiovascular and respiratory inhibition in aged rats, BP, ECG, and RR were continuously

191 monitored to explore the e□ects of the drugs (Figure 3). In each group, MAP decreased after injection

192 (Figure 3A). The maximum value of MAP after the administration of etomidate, ET-26HCl and propofol

193   was decreased at 23.8 ± 3.1 mmHg, 30.9 ± 8.1 mmHg, and 44.2 ± 6.7 mmHg, respectively. In etomidate

194   and ET-26HCl groups, the inhibition of BP was temporary and it tended to recovery without any treatment.

195   However, etomidate (p = 0.0001) and ET-26HCl (p = 0.003) had a milder BP inhibition effect than propofol.

196   After drug administration, the etomidate-, ET-26HCl- and propofol-induced decrease in heart rate were not

197   significantly di□erent (Figure 3B, p = 0.12).

198   The respiratory depression of the aged rats was compared *via* the measurement of occurrence rate and

199   duration of apnea (Table 1). Apnea was observed in all rats that were administered propofol (6 of 6, 100%)

200   and the duration of this side effect was 45.0 ± 9.0 s. For etomidate and ET-26HCl, no apnea was observed

201   (p = 0.00016).

202   **3.3 Serum corticosterone measurement**

203   In the modified ACTH-stimulation experiment, the concentration of serum corticosterone in aged rats

204   is presented in Figure 4. The concentration of corticosterone was suppressed by dexamethasone to 37.4 ±

205   13.6 ng/mL in the ET-26HCl group, 86.7 ± 32.7 ng/mL in the etomidate group, and 39.8 ± 16.5 ng/mL in

206   the solvent group, and there was no significant difference among groups (p = 0.241). No significant

207   difference in the concentration of serum corticosterone was observed between ET-26HCl and solvent

208   groups. However, rats administered etomidate had lower corticosterone concentrations than were

209   administered ET-26HCl at 15, 30, and 60 min after the stimulation of ACTH (p = 0.016, p = 0.004, and p

210   = 0.002, respectively). These results demonstrated that ET-26HCl had no obvious inhibitory effect on the

211   synthesis of adrenal cortical hormones, whereas etomidate significantly decreased the synthesis of

212   corticosterone within 1 h in aged rats.

213 **4. Discussion**

214   In this study, ET-26HCl was demonstrated to have similar advantages in aged rats as in young rats

215 (Liu et al. 2018; Wang et al. 2017a; Wang et al. 2017b; Wang et al. 2017c; Yang et al. 2017), including an

216 effective sedative-hypnotic effect, slight cardiovascular suppression, mild respiratory system and

217 adrenocortical inhibition. Therefore, results of the current study can be translated to predict the potential

218 advantages of ET-26HCl in the elderly population.

219   Aging induces pharmacokinetic and pharmacodynamic changes (Butterworth et al. 2018). With

220 decreases in muscle mass and increases in body fat, the total body water of elderly patients is decreased,

221 especially in women. The required dose of drug for the same anesthetic effect is lower in elderly patients

222 than that in young patients. In pharmacodynamic experiments, there is no significant difference between

223 young rats (Wang et al. 2017c) and aged rats in the onset time, duration, time to stand, and time to normal

224 activity after administration of the same dose of drug. Thus, as in young rats, ET-26HCl causes a shorter

225 duration with no significant difference in recovery than propofol in aged rats.

226   In young rats, ET-26HCl and etomidate produce a milder reduction in MAP than propofol at equal

227 hypnotic doses (Wang et al. 2017c). The good hemodynamic stability of ET-26HCl is maintained in aged

228 rats. ET-26HCl reduced the blood pressure with lesser reduce degree and shorter duration than propofol.

229 Meanwhile, propofol is more likely to cause apnea and hypotension in aged patients than in younger patients

230 (Butterworth et al. 2018). We further compared the depression effect on the respiratory rate. Apnea was not

231 observed in aged rats injected with ET-26HCl and etomidate. Then, we have demonstrated that ET-26HCl

232 has minimal adrenocortical suppression than etomidate in aged rats, as has already been shown in young

233   rats (Wang et al. 2017c). No significant difference in the concentration of serum corticosterone was

234   observed between ET-26HCl and solvent groups. However, etomidate still inhibited the synthesis of adrenal

235   cortical hormones in aged rats. All results indicated that ET-26HCl may be safer for elderly patients than

236   etomidate and propofol.

237   However, in the ET-26HCl group, mild hematuria was observed in 66.7% of rats, which could have

238   been induced by the solvent of 35% (*v/v*) propylene glycol. A previous study demonstrated that the

239   hemolytic effect of propylene glycol can be eliminated *in vivo* by the preparation of no more than 30% (*v/v*)

240   propylene glycol with saline or water (Ruddick 1972). However, the red blood cells of rabbit are not

241   affected after the intravenous injection of 50% propylene glycol in saline (Brittain & D'Arcy 1962).

242   Etomidate formulated in propylene glycol induces hemolysis after bolus injection in patients and healthy

243   volunteers (Doenicke et al. 1997; Doenicke et al. 1999). In the single dose and 14-days repeated-dose

244   toxicity study of ET-26HCl in rats (Zhang et al. 2020b) and beagle dogs (Zhang et al. 2020a), the occurrence

245   rate and severity of hemolysis are not significantly different between the treated and control groups (35%

246   propylene glycol). Therefore, we considered that this side-effect was related to the solvent, not ET-26HCl.

247   Furthermore, formulations of etomidate in 35% propylene glycol and water, such as Amidate ^{TM} (Pfizer Inc)

248   and Hypnomidate $^{®}$ (Janssen Pharmaceutica Ltd), have been used in hospitals. Thus, we believe that the

249   toxicity of this solvent should be noted in clinical trials; however, this toxicity is likely not severe.

250   There are still some limitations in this study. The required dose of drug for the anesthetic effect is

251   lower in elderly patients than that in young patients (Butterworth et al. 2018). The dosages in this study

252   were according to the previous study in young rats (Wang et al. 2017c), and we did not measure the ED_{50}

253   for LORR in aged rats. Besides, the telemetry method for cardiovascular and respiratory function

254   measurement in unrestrained conscious animal, which was more conform to the real clinical situation

255   without the interference of other drugs, such as 2% isoflurane in this study.

256   **5. Conclusions**

257   In this study, we demonstrated, for the first time in aged rats, that ET-26HCl reserves the advantages

258   of etomidate in aged rats, including a mild cardiovascular and respiratory system suppression than propofol.

259   ET-26HCl further eliminate the major disadvantage of etomidate, adrenocortical inhibition. Meanwhile,

260   ET-26HCl possesses the same sedative-hypnotic effect as etomidate. This key discovery can be used to

261   advise the use of ET-26HCl, rather than etomidate and propofol, for the induction of general anesthesia in

262   elderly patients. However, clinical trials that involve the use of elderly patients is needed before any

263   conclusions can be drawn.

264   **Declarations:**

265   **Ethics approval and consent to participate:** Experiments were performed with the approval of the

266   Institutional Animal Care and Use Committee of West China Hospital, Sichuan University, Chengdu, China

267   (2015015A) and were performed according to the Guide for the Care and Use of Laboratory Animals by

268   the West China Hospital, Sichuan University. The study was carried out in compliance with the ARRIVE

269   guidelines.

270   **Consent for publication:** Not applicable.

271   **Availability of data and materials:**   All data generated or analyzed during this study are included in this

272   published article.

273 **Competing interests:** none.

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378

379   **Figure legends:**

380   **Figure 1** Chemical structure of ET-26HCl.

381   **Figure 2** The onset time (**A**), duration (**B**) and recovery time (**C**) after treatment with two-fold dose of the

382   median effective doses for loss of the righting reflex (ET-26HCl, 2.35 mg/kg; etomidate, 0.73 mg/kg;

383   propofol, 5.87 mg/kg) in aged rats (n = 6 in each group). Data were presented as mean ± SEM. No

384   statistically significant difference in recovery time among the drugs.

385   **Figure 3** The effect of ET-26HCl, etomidate, and propofol on mean blood pressure (**A**) and heart rate (**B**)

386   after treatment with two-fold dose of the median effective doses f of ET-26HCl, etomidate and control in

387   aged rats (n = 6 in each group). Data were presented as mean ± SEM. ** $p < 0.01$; *** $p < 0.001$. Etomidate

388   and ET-26HCl had a milder blood pressure inhibition effect than propofol.

389   **Figure 4** The concentration of serum corticosterone stimulated by ACTH after treatment with two-fold

390   dose of the median effective doses of ET-26HCl, etomidate and control in aged rats (n = 6 in each group).

391   Data were presented as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$, compared ET-26HCl group with etomidate

392   group; # $p < 0.05$, compared control group with etomidate group;

Figure 1

Chemical structure of ET-26HCl.

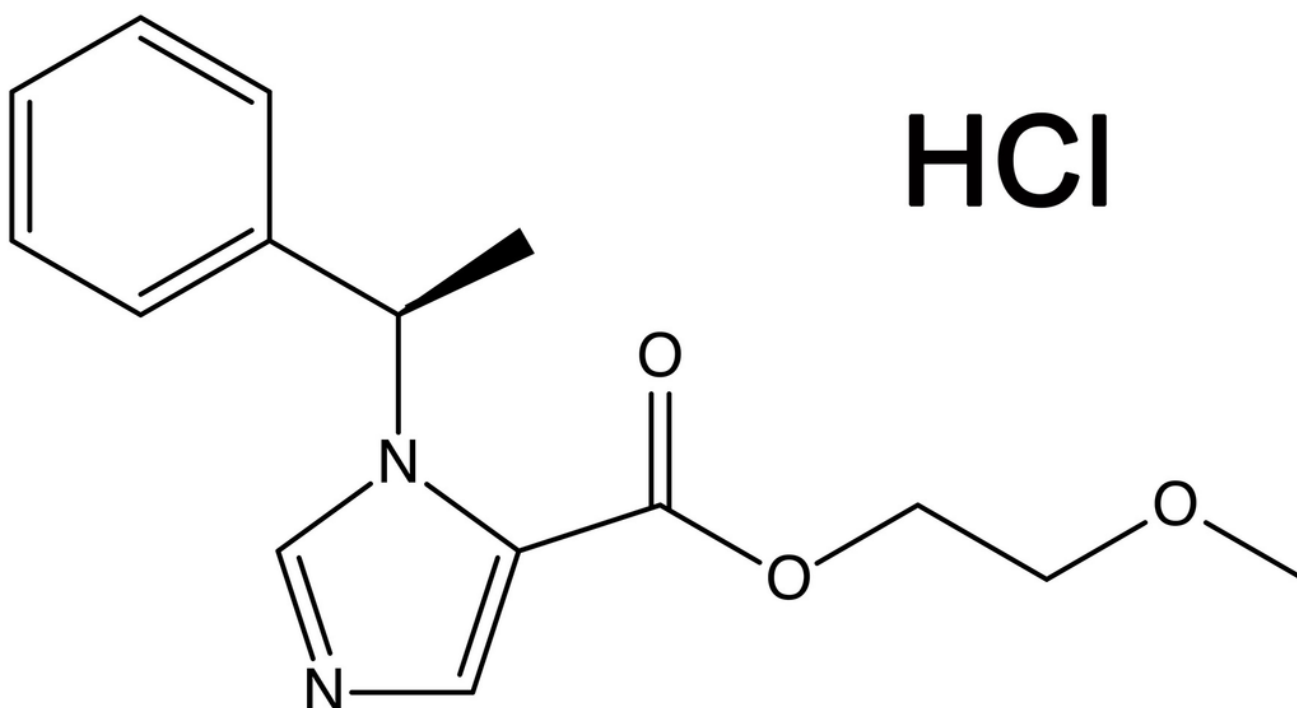



Figure 2

The onset time (A), duration (B) and recovery time (C) after treatment with two-fold dose of the median effective doses for loss of the righting reflex in aged rats (n = 6 in each group).

A

Onset time

B

Duration time

C

Recovery time

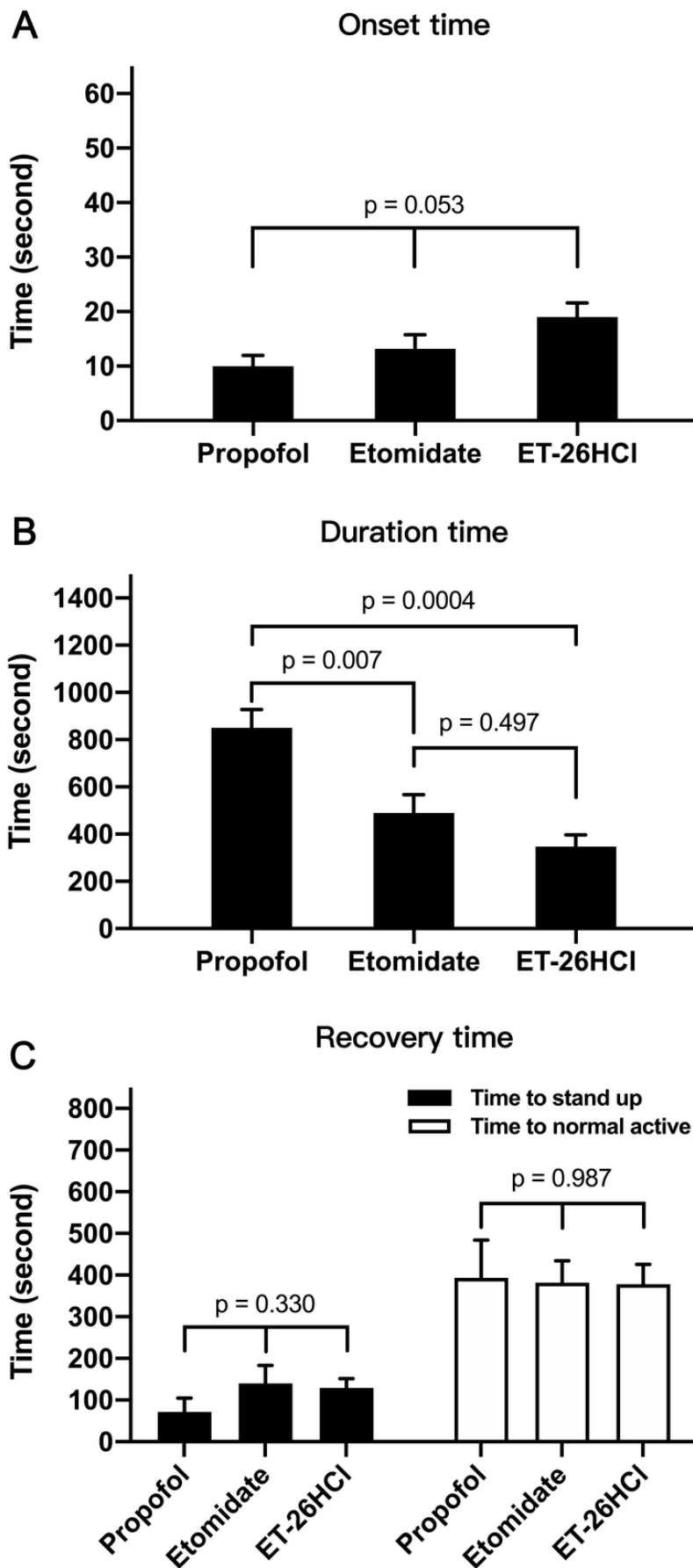



Figure 3

The effect of ET-26HCl, etomidate, and propofol on mean blood pressure (A) and heart rate (B) after treatment with two-fold dose of the median effective doses for loss of the righting reflex in aged rats (n = 6 in each group).

** p < 0.01; *** p < 0.001

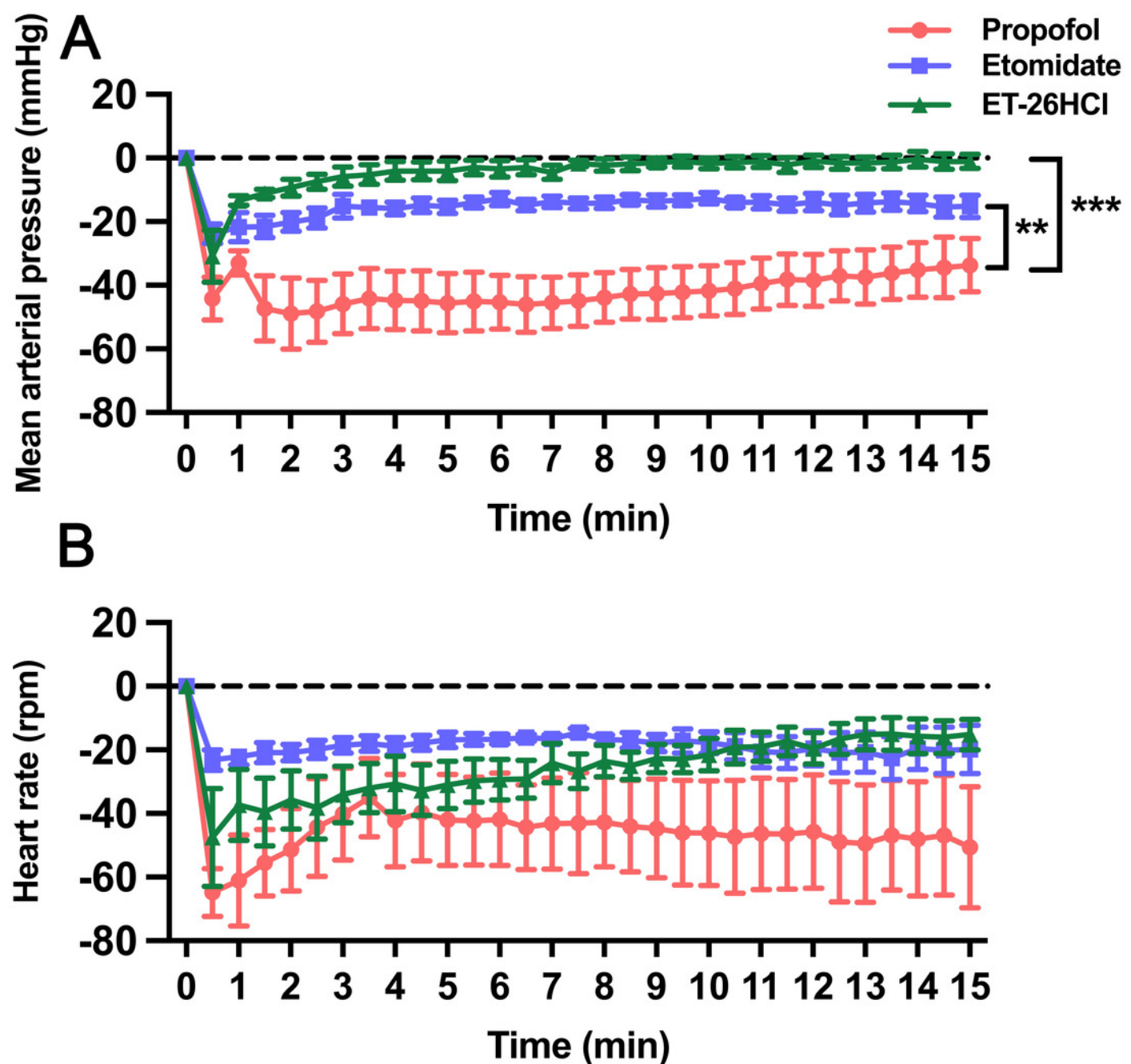
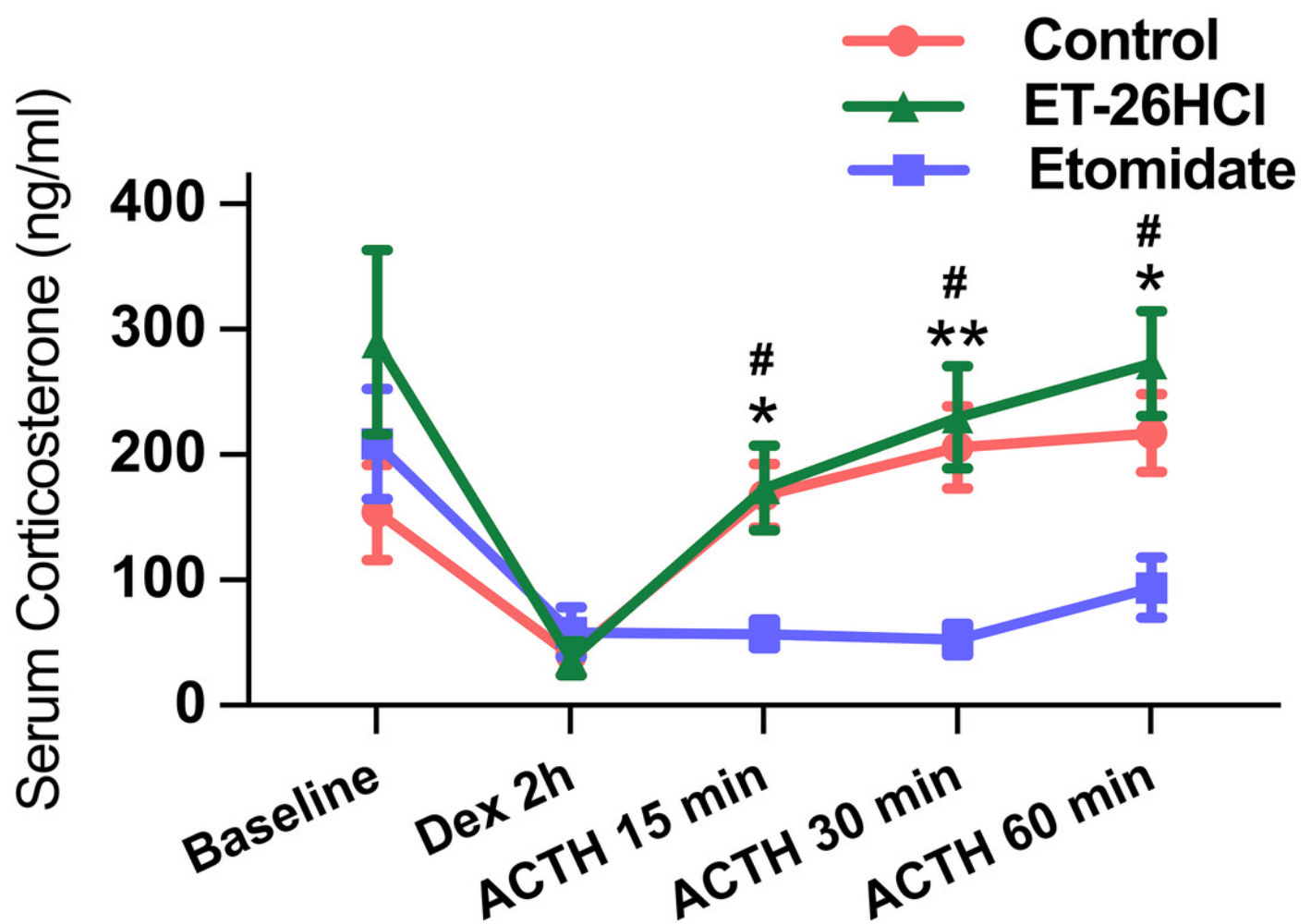



Figure 4

The concentration of serum corticosterone stimulated by ACTH after treatment with two-fold dose of the median effective doses for loss of the righting reflex in aged rats (n = 6 in each group).

Compared with etomidate, * p < 0.05 and ** p < 0.01, compared ET-26HCl with etomidate; # p < 0.05, compared control with etomidate

The information of apnea after single administration in aged rats (means ± SD).

a: compared with propofol, χ^2 = 16.5, p = 0.00016.

1   **Table 1** The information of apnea after single administration of propofol, etomidate and ET-26HCl in aged
2   rats (mean ± SD).

Agent	n	Number of aged rats with apneas	Apnea		Duration of apnea (s)
			onset time (s)	offset time (s)	
Propofol	6	6	7.5 ± 5.7	52.5 ± 9.2	45.0 ± 9.0
Etomidate	6	0 [a]	0	0	0
ET-26HCl	6	0 [a]	0	0	0

3   **a**: compared with propofol group, $\chi^2 = 16.5$, p = 0.00016. Apnea in all rats was observed in propofol group; no
4   apnea in etomidate and ET-26HCl groups.