The preclinical pharmacological study on HX0969W, a novel water-soluble pro-drug of propofol, in rats

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Background: Propofol is the most widely used intravenous sedative-hypnotic anesthetic in clinical practice. However, many serious side effects have been related to its lipid emulsion formulation. The pro-drug design approach was used to develop the water-soluble propofol, which could effectively resolve the limitations associated with the lipid emulsion formulation. Thus, the new water-soluble pro-drug of propofol, HX0969W, was designed and synthesized. The objective of this study was to conduct preclinical pharmacological studies on this novel water-soluble pro-drug of propofol.

Methods: The assessment of the loss of the righting reflex (LoRR) was used for the pharmacodynamic study, and liquid chromatography-tandem mass spectrometry and high-performance liquid chromatography- fluorescence were used for the pharmacokinetic study.

Results: The potency of HX0969W (ED_{50} [95% CI], 46.49 [43.89 - 49.29] mg/kg) was similar to that of fospropofol disodium (43.66 [43.57 - 43.75] mg/kg), but was lower than that of propofol (4.82 [4.81 - 4.82] mg/kg). Administered with a dose of 2-fold ED_{50}, propofol required a shorter time to cause LoRR than that of HX0969W and fospropofol. However, the LoRR duration was significantly longer in response to the administration of HX0969W and fospropofol disodium than that caused by propofol. In the pharmacokinetic study, the C_{max} of fospropofol was higher than that of HX0969W. HX0969W had a shorter mean residual time and a rapid clearance rate than that of fospropofol disodium. There was no significant difference between the T_{max} of the propofol whether it was released by HX0969W or fospropofol disodium; the C_{max} of propofol released by HX0969W was similar to that of propofol, which was higher than the propofol released by fospropofol disodium.

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22   **Abstract**

23   **Background:** Propofol is the most widely used intravenous sedative-hypnotic anesthetic in

24   clinical practice. However, many serious side effects have been related to its lipid emulsion

25   formulation. The pro-drug design approach was used to develop the water-soluble propofol,

26   which could effectively resolve the limitations associated with the lipid emulsion formulation.

27   Thus, the new water-soluble pro-drug of propofol, HX0969W, was designed and synthesized.

28   The objective of this study was to conduct preclinical pharmacological studies on this novel

29   water-soluble pro-drug of propofol.

30   **Methods:** The assessment of the loss of the righting reflex (LoRR) was used for the

31   pharmacodynamic study, and liquid chromatography-tandem mass spectrometry and high-

32   performance liquid chromatography- fluorescence were used for the pharmacokinetic study.

33   **Results:** The potency of HX0969W (ED_{50} [95% CI], 46.49 [43.89 - 49.29] mg/kg) was similar to

34   that of fospropofol disodium (43.66 [43.57 - 43.75] mg/kg), but was lower than that of propofol

35   (4.82 [4.81 - 4.82] mg/kg). Administered with a dose of 2-fold ED_{50}, propofol required a shorter

36   time to cause LoRR than that of HX0969W and fospropofol. However, the LoRR duration was

37   significantly longer in response to the administration of HX0969W and fospropofol disodium

38   than that caused by propofol. In the pharmacokinetic study, the C_{max} of fospropofol was higher

39   than that of HX0969W. HX0969W had a shorter mean residual time and a rapid clearance rate

40   than that of fospropofol disodium. There was no significant difference between the T_{max} of the

41   propofol whether it was released by HX0969W or fospropofol disodium; the C_{max} of propofol

42   released by HX0969W was similar to that of propofol, which was higher than the propofol

43   released by fospropofol disodium.

44 **1. Introduction**

45 General anesthesia is a combination of drugs, including sedative-hypnotic agents, analgesics, or

46 muscle relaxants, which put the patients in a sleep-like state before surgery or other medical

47 examinations. Propofol is the most widely used intravenous sedative-hypnotic anesthetic in

48 clinical practice (Hemphill et al. 2019). However, many serious side effects have been related to

49 its lipid emulsion formations, such as emulsion instability, injection pain, hyperlipidemia,

50 infection, fat metabolism disorder, and propofol-related infusion syndrome (Diaz et al. 2014; Lee

51 et al. 2014; Mirrakhimov et al. 2015; Pestana et al. 1996; Prankerd & Stella 1990; Singh et al.

52 2011; Wachowski et al. 1999; Wolf et al. 2001; Zhou et al. 2015). The pro-drug design approach

53 was widely used to develop the novel water-soluble propofol, which could effectively avoid the

54 limitations associated with its lipid emulsion formulation (Feng et al. 2017).

55   Fospropofol disodium is the first water-soluble pro-drug of propofol that has been approved

56 by the U.S. Food and Drug Administration to be administered as a bolus injection for adult

57 patients undergoing diagnostic or therapeutic procedures (Moore et al. 2009; Telletxea et al.

58 2012). Although it is confirmed that formaldehyde, which is one of the metabolites of

59 fospropofol disodium, does not accumulate after a single administration (Garnock-Jones & Scott

60 2010; Kumpulainen et al. 2008), it is still considered a potentially risk for systemic toxicity and

61 has limited the clinical indication of fospropofol disodium for continuous infusion. A new water-

62 soluble pro-drug of propofol was designed and synthesized in our laboratory (patent number,

63 WO2011160268; denoted as HX0969W), which was metabolized to propofol, γ-hydroxybutyrate

64 (GHB), and phosphate *in vivo*. GHB was found to be further converted into carbon dioxide and

65   water (Lang et al. 2014; Rousseau et al. 2012). In contrast to fospropofol disodium, HX0969W

66   cloud produce an effective sedative-hypnotic effect with lesser potential risks of systemic

67   toxicity.

68   In the present study, we evaluated the median effective dose (ED_{50}) of HX0969W,

69   fospropofol disodium, and propofol for the loss of righting reflex (LoRR) in rats using the up and

70   down method. Then, we measured the onset time and duration for HX0969W, fospropofol

71   disodium and propofol after single intravenous injections. Furthermore, the pharmacokinetic

72   parameters of the drugs were assessed in rats.

73   **2. Material and Methods**

74   **2.1. Materials**

75   HX0969W and fospropofol disodium were synthesized at Yichang Humanwell Pharmaceutical

76   Co., Ltd. (Yichang, China). Propofol was purchased from AstraZeneca (Shanghai, China). 7-

77   Hydroxycoumarin, thymol, and ammonium acetate were purchased from Sigma-Aldrich Co., Ltd.

78   (MO, USA). Acetonitrile and methanol were obtained from ROE Scientific Inc. (DE, USA).

79   Ultrapure water was produced using Milli-Q® integral water purification system (Merck

80   Millipore, Germany).

81   **2.2. Experimental animals**

82   Adult Sprague-Dawley rats (age: 8 – 10 weeks, body weight: 220 - 350g) were purchased from

83   Dossy Biological Technology Co., Ltd. (Chengdu, China) and housed in polypropylene cages

84   (less than 5 animals per cage) at the Experimental Animal Center of Sichuan University

85   (Chengdu, China) at an ambient temperature of 25 ± 1°C, a controlled humidity of 50% - 70%,

86 and a 12-h light-dark cycle (7 a.m. to 7 p.m.). The rats were provided with a radiation-sterilized

87 commercial diet and filtered water *ad libitum*. The rats were subjected to fasting for 12 h with

88 uncontrolled water supply prior to initiation of dosing; food was supplied immediately after

89 dosing. The experimental interventions and sample collections in this study were accomplished

90 through the tail vein after venipuncture (Terumo ® intravenous catheter; 24G, Tokyo, Japan).

91 Thus, no anesthetic procedure was used in this study. At the end of experiments, all rats were

92 euthanized *via* overdose of pentobarbital sodium. All experiments were performed with the

93 approval of the Committee of Scientific Research and the Institutional Animal Experimental

94 Ethics Committee of West China Hospital, Sichuan University, Chengdu, China (2015015A).

95 **2.3. Measurement of the hypnotic median effective dose**

96 The ED_{50} values of HX0969W, fospropofol disodium, and propofol were determined by the up

97 and down method (Dixon 1991; Glen & Hunter 1984; Kilpatrick et al. 2007). The tail vein was

98 cannulated with a Terumo ® intravenous catheter (24G; Tokyo, Japan) for drug administration.

99 After administered the drug, the rats were assessed one by one in separate box for the LoRR

100 effect (once the rat lay prone and no reaction to mild stimuli, the rat was gently changed to the

101 supine position recognized as LoRR), and were provided with warm blanket and oxygen

102 inhalation to reduce external stimulation. If the duration of LoRR after drug administration was

103 more than 30 s, the next rat was administered with a lower dose. On the contrary, if the duration

104 of LoRR was less than 30 s (none-LoRR), a higher dose was injected in the next rat. Recording

105 the LoRR to none-LoRR as a cross, the measurement of the ED_{50} value was terminated once

106 more than five crosses occurred. An accurate ED_{50} value with 95% confidence intervals (CI) was

107   calculated using standard computing equations (Dixon 1991; Glen & Hunter 1984; Kilpatrick et

108   al. 2007).

2.4. Pharmacodynamic study in rats

110   Thirty adult Sprague-Dawley rats were randomly divided into three groups to evaluate the

111   efficacy of HX0969W, fospropofol disodium, and propofol (n = 10 in each group, 5 males and 5

112   females). The drugs were administered *via* tail vein through the Terumo ® intravenous catheter

113   (24G; Tokyo, Japan) at equivalent doses (2-fold dose of ED_{50} for LoRR in rats) at an injection

114   speed of 0.25 mL/s. Then, each rat was placed in a separate box with a warm blanket and oxygen

115   inhalation. For the pharmacodynamic study, the time to LoRR (onset time) and time to recovery

116   from LoRR (duration) were recorded. All rats were observed closely for any mortality,

117   behavioural changes, and clinical symptoms of toxicity.

2.5. Pharmacokinetic study in rats

119   Before the experiment, the rats were subjected to fasting for 12 h with uncontrolled water supply;

120   food was supplied immediately after drug administration. The tail vein was cannulated with a

121   Terumo ® intravenous catheter (24G; Tokyo, Japan) for drug administration and sample

122   collection. HX0969W, fospropofol disodium, and propofol were injected at their equivalent

123   doses in the rats through their tail veins (n = 10 in each group, 5 males and 5 females). The blood

124   samples from the rats were collected at different times, which were determined based on the

125   results of the preliminary experiments. For HX0969W and fospropofol disodium, 50 µL blood

126   samples were collected at 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min after

127   drug administration. For propofol, 50 µL blood samples were collected at 0.5, 1, 2, 3, 5, 7, 10, 15,

128   20, 30, 45, 60, 90, 120, and 180 min after drug administration.

129   **2.5.1. Sample preparation and quantitative method**

130   Blood samples from the HX0969W and fospropofol disodium groups were deproteinized by

131   methanol solution with 7-Hydroxycoumarin (internal standard, IS). The mixture was centrifuged

132   for 10 min at 25,000 × g at 4 °C after vortexing. The supernatant was then subjected to

133   determination and quantification by liquid chromatography-tandem mass spectrometry (LC-

134   MS/MS). The LC-MS/MS analysis system consisted of an Agilent 6460 triple quadrupole mass

135   spectrometer (Agilent Technologies, CA, USA) with an electrospray ionization source.

136   Chromatographic separation was performed using a Zorbax eclipse plus C8 column (100 mm ×

137   2.1 mm, 3 μm) at 12 °C with 5 mM ammonium acetate in deionized water and acetonitrile at a

138   volume ratio of 65 : 35 for HX0969W and 70 : 30 for fospropofol disodium, at a flow rate of 0.3

139   mL/min. The mass spectrometry conditions were as follows: negative ionization mode; the

140   sheath gas flow rate, 5.0 l/min; sheath gas heater temperature, 350 °C; nebulizer pressure, 30 psi;

141   capillary voltage, 4,500 V; m/z 343.0→177.0 for HX0969W, m/z 287.00→177.00 for

142   fospropofol disodium, and m/z 160.7→89.0 for IS.

143   For propofol, the samples were deproteinized with a methanol solution with thymol (IS).

144   The mixture was centrifuged for 10 min at 25,000 × g at 4 °C after vortexing. The supernatant

145   was then subjected to determination and quantification by high-performance liquid

146   chromatography- fluorescence. The analysis system consisted of an Agilent Zorbax XDB

147   (Agilent Technologies, CA, USA) equipped with a C18 column (150 × 4.6 mm, 5 μm) and a

148   fluorescence detector. The liquid chromatography conditions were as follows: mobile phase

149 solvent, acetonitrile and water at the volume ratio of 40 : 60 at a flow rate of 1.2 mL/min;

150 fluorescence detector, the wavelength of excitation and emission at 276 and 310 nm, respectively.

151   The method validation results were detailed in the supplementary material includes the

152 following parameters: specificity, linearity, lower limit of quantitation (LLOQ), precision,

153 accuracy, matrix effect, extraction recovery, stability and dilution integrity.

154 **2.5.2. Pharmacokinetic analysis**

155 Noncompartmental pharmacokinetic methods were used for pharmacokinetic analysis of

156 HX0969W, fospropofol disodium, and propofol using the Phoenix Winnonlin [®] software

157 (version 6.3, NJ, USA). The pharmacokinetic parameters used in this study were as follows:

158 maximum concentration (C_{max}) and the time to acquire (T_{max}), area under curve (AUC) from zero

159 to the last time point (AUC_{0-t}), mean residual time (MRT), half-time ($t_{1/2}$), and clearance (CL).

160 **2.6. Data Analysis**

161 The statistical analyses were performed using SPSS (version 21, IL, USA). The ED_{50}, presented

162 as mean and 95% CI, was calculated using the up and down method. The *Kruskal- Wallis* test

163 followed by the Mann-Whitney U test was used for the data that did not fit normality, and the

164 one-way *ANOVA* followed by Tukey's test was used for the data that did not fit normality and

165 equality of variance. The level of statistical significance was set at $p < 0.05$.

166 **3. Results**

167 **3.1. Measurement of the median effective dose for the sedative-hypnotic effect**

168 Following a single intravenous administration of the drug, each rat was placed in a separate box

169 to record the time to LoRR and time to recovery from LoRR. Then, the median effective dose to

170   achieve the sedative-hypnotic effect (Table 1) was calculated using the up and down method

171   (Dixon 1991; Glen & Hunter 1984; Kilpatrick et al. 2007). The potency of HX0969W in rats

172   (ED_{50} [95% CI], 46.49 [43.89 - 49.29] mg/kg) was found to be similar to that of fospropofol

173   disodium (ED_{50} [95% CI], 43.66 [43.57 - 43.75] mg/kg), but lower than that of propofol (ED_{50}

174   [95% CI], 4.82 [4.81 - 4.82] mg/kg). Due to the pro-drug design, HX0969W and fospropofol

175   disodium are metabolized to propofol, which produces the sedative-hypnotic effect. Thus, the

176   dosages of HX0969W and fospropofol used for general anesthesia were higher than those of

177   propofol.

178   **3.2. Pharmacodynamic study in rats**

179   After administering a 2-fold ED_{50} dose for LoRR, the time to LoRR (onset time) and time to

180   recovery from LoRR (duration) were measured in the rats. No mortality or significant clinical

181   symptoms of toxicity were observed in these rats. As for the onset time (Fig. 1A), propofol (0.4

182   ± 0.1 min) required a shorter time to cause LoRR than that required for HX0969W (1.8 ± 0.4

183   min, p = 0.017) and fospropofol disodium (2.1 ± 0.7 min, p = 0.006). However, the duration of

184   LoRR for HX0969W (75.8 ± 9.6 min, p = 0.036) and fospropofol disodium (68.5 ± 18.4 min, p =

185   0.041) were significantly longer than that of propofol (27.1 ± 6.0 min, Fig. 1B).

186   **3.3. Pharmacokinetic study of HX0969W and fospropofol disodium in rats**

187   After a single intravenous injection, there was no significant difference in the action onset time

188   and duration between HX0969W and fospropofol disodium. The efficacy of a drug is closely

189   related to its pharmacokinetic features. Therefore, we measured the concentration-time profiles

190   of HX0969W and fospropofol disodium in rats. The concentration-time curves are presented in

191 Fig. 2, and the calculated pharmacokinetic parameters are shown in Table 2. Following the

192 administration of equivalent doses (92.98 mg/kg for HX0969W, 87.32 mg/kg for fospropofol

193 disodium), the maximal concentration of fospropofol disodium (603.49 ± 411.29 μg/mL) was

194 found to be higher than that of HX0969W (321.30 ± 67 .22 μg/mL). In rat plasma, HX0969W

195 had a shorter mean residual time (3.67 ± 1.71 min) and a rapid clearance rate (89.97 ± 15.94

196 ml/min/kg) than those of fospropofol disodium (13.15 ± 5.45 min and 31.12 ± 19.09 ml/min/kg,

197 respectively). The computed AUC value of HX0969W from zero to the last time point was

198 1053.78 ± 214.37 min*μg/ml, which was less than that of fospropofol disodium (3804.92 ±

199 2091.75 min*μg/ml). Thus, we considered that HX0969W, after a bolus injection in rats, had a

200 faster metabolism *in vivo* as compared to fospropofol disodium. The pharmacokinetic parameters

201 of HX0969W and fospropofol disodium could not explain the lack of significant differences in

202 the onset time and duration. Therefore, we further evaluated the concentration of propofol

203 released by HX0969W and fospropofol disodium in rats.

204 **3.4. Pharmacokinetic study of propofol in rats**

205 Using an equivalent dose of HX0969W, fospropofol disodium and the parent drug propofol, the

206 concentration-time curves are presented in Fig. 3, and the calculated pharmacokinetic parameters

207 are shown in Table 3. The T_{max} of propofol released by HX0969W (propofol$_H$, 4.0 ± 0.47 min) or

208 fospropofol disodium (propofol$_F$, 4.5 ± 1.18 min) in plasma had no significant difference, which

209 was longer than that for propofol (0.5 min). However, the C_{max} of propofol$_H$ (24.26 ± 5.14 μg/ml)

210 was similar to the parent drug propofol (23.4 ± 4.69 μg/ml), which was higher than that for

211 propofol$_F$ (21.91 ± 4.98μg/ml). The AUC, MRT, and CL of propofol$_F$ (740.5 ± 186.33

212 min*µg/ml, 52.6 ± 2.90 min, and 54.67 ± 13.83 ml/min/kg, respectively) were similar to those of

213 propofol$_H$ (704.53 ± 226.25 min*µg/ml, 50.92 ± 4.40 min, and 68.12 ± 22.84 ml/min/kg,

214 respectively), but were higher than those of propofol (219.23 ± 42.21 min*µg/ml, 29.45 ± 4.63

215 min, and 43.28 ± 8.46 ml/min/kg, respectively). In this pharmacokinetic study, the longer onset

216 time and duration of HX0969W and fospropofol disodium, than those of the parent drug

217 propofol, were due to their pharmacokinetic features, which included a larger AUC and a longer

218 MRT. Furthermore, the onset time and duration of HX0969W and fospropofol disodium, which

219 showed no significant difference, could be related to the undifferentiated pharmacokinetic

220 features of propofol$_H$ and propofol$_F$, including T_{max}, C_{max}, AUC, MRT and CL.

221 **4. Discussion**

222 In this study, we have demonstrated that HX0969W requires a longer time to induce the

223 sedative-hypnotic effect, but with longer duration than propofol in rats. HX0969W had a large

224 C_{max}, a short MRT, and a rapid CL than fospropofol disodium in rats. The T_{max} of propofol

225 released by HX0969W or fospropofol disodium showed no significant difference, and the C_{max}

226 of propofol released by HX0969W was similar to that of propofol, which was higher than that of

227 the propofol released by fospropofol disodium.

228   The main objective of this study was to develop a new water-soluble pro-drug of propofol

229 with non-toxic metabolites. In the pharmacodynamic study, the potency of HX0969W was found

230 to be similar to that of fospropofol disodium, but lower than that of the parent drug propofol.

231 Similar to fospropofol disodium, the onset time and duration of HX0969W after single

232 intravenous administration, were longer than those of propofol. Thus, HX0969W is the new

233   water-soluble pro-drug of propofol that can be used for induction and maintenance of general

234   anesthesia. In contrast to fospropofol disodium, which is metabolized into a toxic metabolite,

235   HX0969W was metabolized to propofol, GHB, and phosphate *in vivo*; GHB is further converted

236   into carbon dioxide and water (Lang et al. 2014; Rousseau et al. 2012). Previous studies have

237   demonstrated that GHB is mainly used as a sedative drug for children suffering from burns or for

238   the treatment of alcohol and opioid addiction (Brambilla et al. 2012; Gallimberti et al. 1993;

239   Rousseau et al. 2012). However, it was reported that GHB produced an obvious hypnotic effect

240   after oral administration at least 800 mg/kg (Lettieri & Fung 1979). In our published study,

241   HX0969W produced a sedative-hypnotic effect after oral administration with 193.08 mg/kg in

242   rats (Wang et al. 2015), which was far less than 800 mg/kg. Meanwhile, there is no data to show

243   if there is any synergy between the effects of propofol and GHB. Therefore, the LoRR caused by

244   HX0969W administration was mainly caused by the propofol released by HX0969W. As

245   compared to fospropofol disodium, HX0969W cloud be an effective sedative-hypnotic agent

246   with lesser potential risks of systemic toxicity.

247   In the open-label, single-arm, phase 3 clinical trial (Gan et al. 2010), five (4.1%) patients

248   administered with fospropofol experienced the adverse events: hypoxemia (n = 1, 0.8%),

249   hypotension (n = 4, 3.3%), bradycardia (n = 1, 0.8%). No patient experienced apnea during the

250   procedure. In a randomized, double-blind, phase 3 study (Cohen et al. 2010), hypotension

251   occurred in two patients (1.3%) in the fospropofol group, and one patient (0.6%) experienced

252   hypoxemia that resolved after repeated verbal stimulation. Undergoing flexible bronchoscopy

253   (Silvestri et al. 2009), the most common cardiopulmonary adverse events were hypoxemia (n =

254   36, 14.3%) and hypotension (n = 8, 3.2%). In another clinical trial (Cohen 2008), four patients in

255   fospropofol group experienced sedation-related adverse events: mild hypotension (n = 2, 2%)

256   and hypoxemia (n = 2, 2%). Only one patient of hypoxemia required the airway assistance

257   (verbal stimulation). Thus, fospropofol showed lower incidences of hypotension, respiratory

258   depression, apnea, and loss of airway patency because of its slower onset of action (Mahajan et

259   al. 2012). On the basis of the findings in this study, there is no significant difference between

260   HX0969W and fospropofol disodium in pharmacodynamic and pharmacokinetic studies.

261   Therefore, it would be reasonable to consider the possibility that HX0969W could reduce the

262   cardiopulmonary side effects by a slow-released propofol as fospropofol disodium. In the

263   subsequent experiments, we will focus on evaluating the safety of HX0969W and propofol in the

264   cardiovascular and respiratory systems.

265   Propofol has been the most widely used intravenous anesthetic in clinical practices due to

266   its rapid onset and recovery from sedation (Hemphill et al. 2019). However, the pharmacokinetic

267   features of HX0969W, including a longer MRT and $t_{1/2}$, and a larger AUC, have superseded the

268   advantages of propofol. Therefore, HX0969W is more appropriate for patients undergoing

269   longstanding surgeries or a long-term sedation in intensive care unit, and its use can avoid the

270   side effects of propofol by its lipid emulsion preparation. Meanwhile, HX0969W generated a

271   hypnotic effect with rapid onset and shorter duration than fospropofol disodium and propofol

272   after oral administration in rats (Wang et al. 2015). Therefore, there might be a new clinical

273   indication for HX0969W for use in patients undergoing pre-operative sedation, transitory

274   diagnostic or therapeutic procedures by oral administration. Above all, our results show that

275 HX0969W can avoid the disadvantages associated with the lipid emulsion formulation of

276 propofol. Thus, HX0969W is more suitable for long-term sedation and pre-operative preparation

277 for children who do not cooperate with venipuncture.

278 **5. Conclusions**

279 With the equivalent dose, HX0969W and fospropofol disodium had a longer time to cause LoRR

280 with longer duration than propofol in rats. In pharmacokinetic study, the C_{max} of fospropofol

281 disodium was higher than HX0969W. HX0969W had a shorter MRT and a rapid CL than

282 fospropofol disodium. Due to the pro-drug design, HX0969W and fospropofol disodium are

283 metabolized to propofol, which produces the sedative-hypnotic effect. The T_{max} of propofol

284 released by HX0969W and fospropofol disodium had no significant difference, which was

285 longer than that for propofol. The C_{max} of propofol released by HX0969W was similar to the

286 parent drug propofol, which was higher than that for that of propofol released by fospropofol

287 disodium.

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361

Figure 1

Figure 1 The onset time (A) and duration (B) of hypnotic behavior observation after intravenous administration of 2-fold ED_{50} drugs (n = 10 in each group).

Data are expressed as mean ± SD. * $p < 0.05$; # $p < 0.01$.

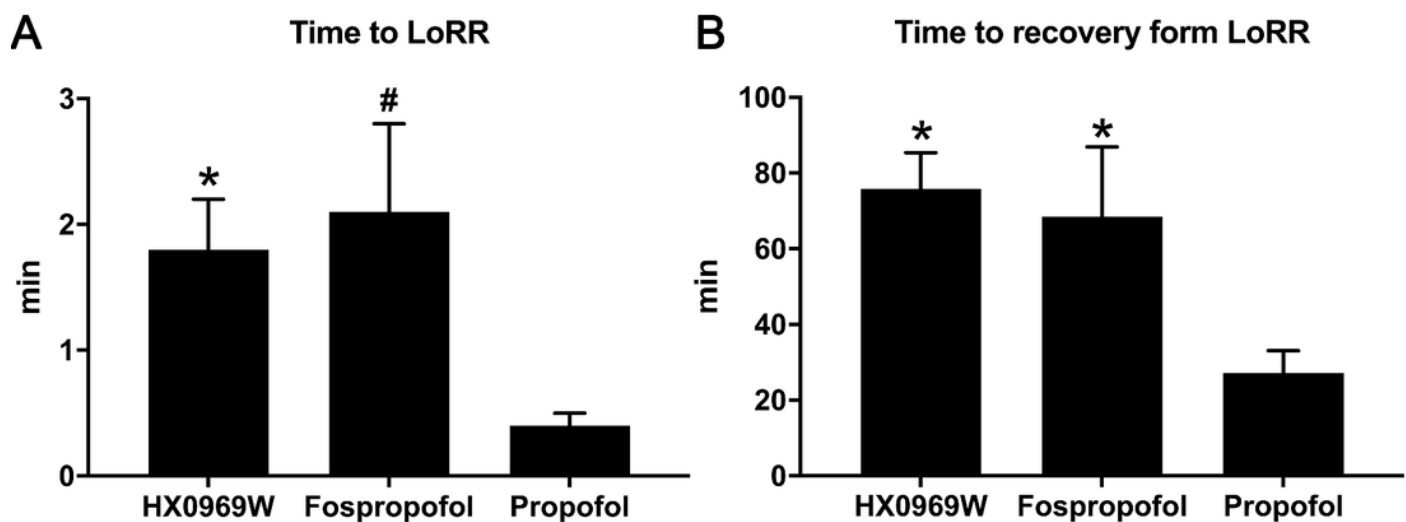



Figure 2

Figure 2 Mean concentration-time profiles of HX0969W (A) and fospropofol disodium (B) in plasma after intravenous administration with of 2-fold ED_{50} in rats (n = 10 in each group).

Data are expressed as mean ± SD.

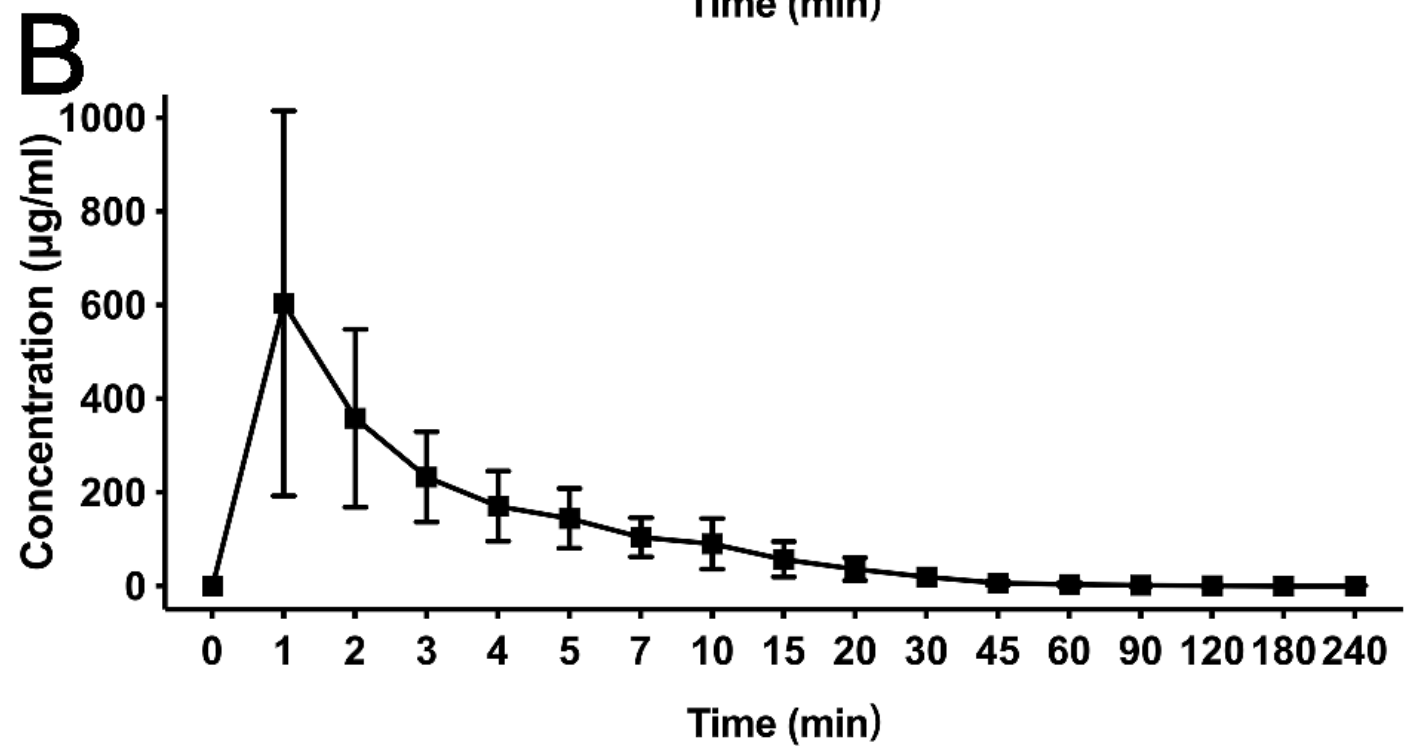
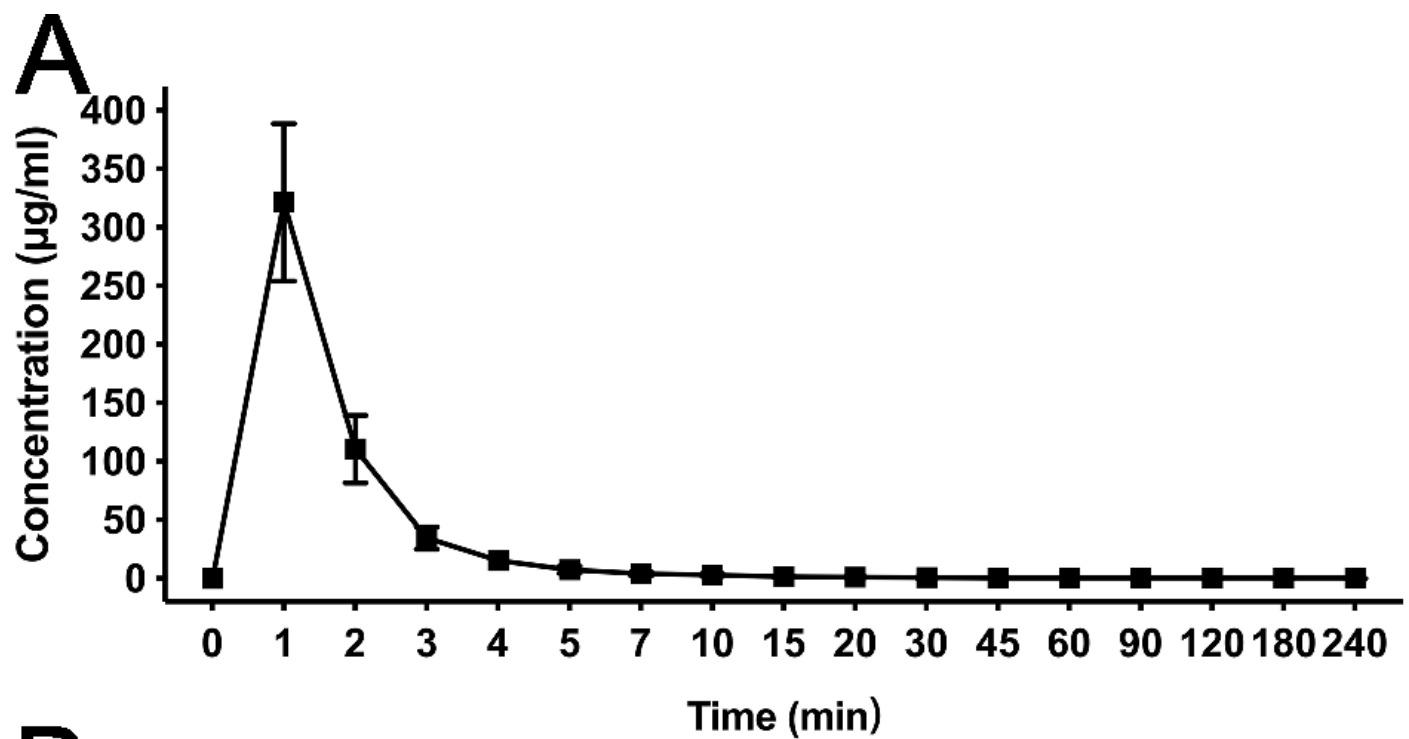
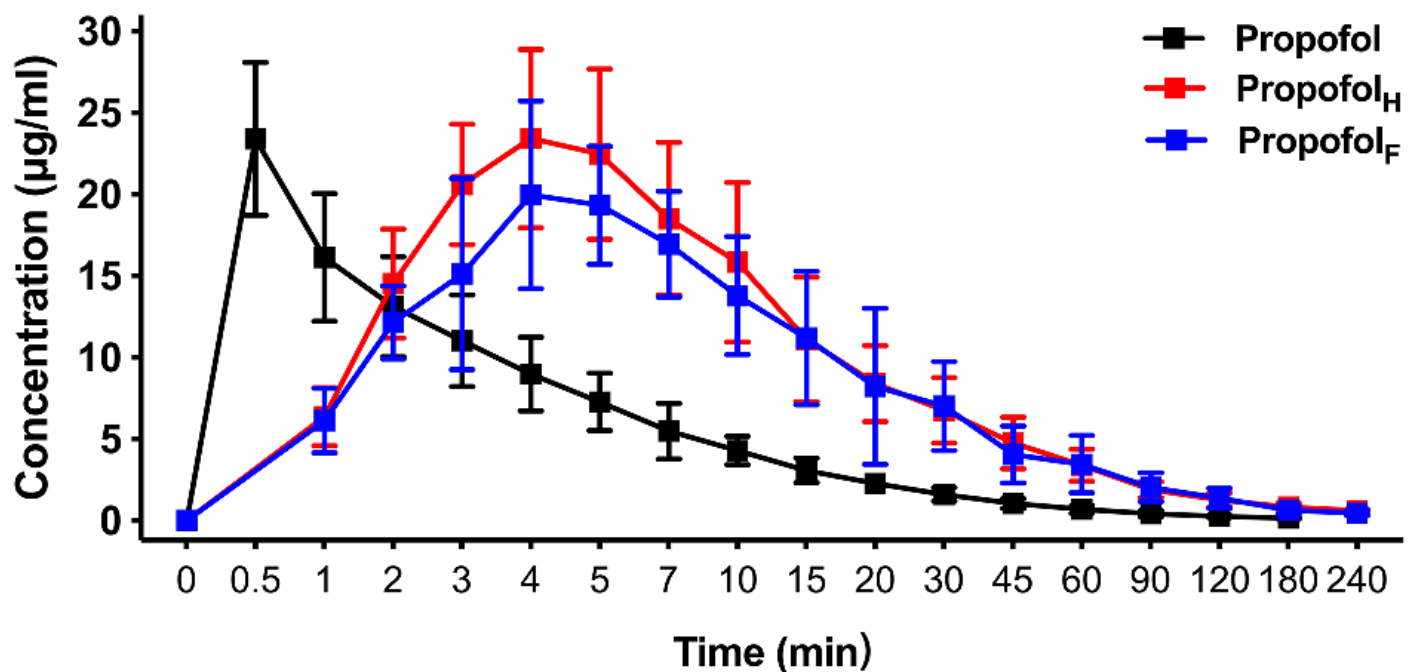



Figure 3

Figure 3 Mean concentration-time profiles of propofol in plasma after intravenous administration of HX0969W, fospropofol disodium, and propofol at dose of 2-fold ED_{50} in rats (n = 10 in each group).

Data are expressed as mean ± SD. $Propofol_H$, propofol released by HX0969W; $Propofol_F$, propofol released by fospropofol disodium.

Calculation process of ED_{50} for HX0969W, fospropofol disodium and propofol

1, $X = lg(dose)$; $t =$ sum total of rat; $C = X \times t$; $M = X^2 \times t$.

2, +: rat with loss of the righting reflex; -: rat without loss of the righting reflex.

3, $95\%CI = lg^{-1}(lgED_{50} \pm 1.96slgED_{50})$; $SlgED_{50} = \{[\ \Sigma M - (\Sigma C)^2/\Sigma t]\ /(\Sigma t \times (\Sigma t-1)\}1/2$.

substance	dose (mg/Kg)	X	loss of the righting reflex (+/-)											t	C	M
HX0969W	59.17	1.772										+		1	1.772	3.140
	53.25	1.726				+			+	-		+		4	6.904	11.916
	47.93	1.681	+	+		-	+			-	-		-	7	11.767	19.780
	43.13	1.635	-	+	-		+	+	-					6	9.810	26.682
	38.82	1.589		-			-	-						3	4.767	7.575

$$ED_{50} = lg^{-1} (\Sigma C / \Sigma t) = 46.49 \text{ mg/Kg } (95\%CI = 43.894 - 49.293 \text{ mg/Kg})$$

substance	dose (mg/Kg)	X	loss of the righting reflex (+/-)									t	C	M
Fospropofol	61.80	1.791										0	0	0
disodium	55.62	1.745										0	0	0
	50.06	1.700	+		+							2	3.400	5.780
	45.05	1.654	+	-	+	+	+	+	+	+		8	13.232	21.886
	40.55	1.607	-		-	-	-	-	-	-		7	11.249	18.077

$$ED_{50} = lg^{-1} (\Sigma C / \Sigma t) = 43.66 \text{ mg/Kg } (95\%CI = 43.567 - 43.752 \text{ mg/Kg})$$

substance	dose (mg/Kg)	X	loss of the righting reflex (+/-)									t	C	M
Propofol	6.00	0.778		+				+				2	1.566	1.211
	5.04	0.702	-	+	+	+	+	-	+	+	+	8	5.616	3.942
	4.23	0.626	-		-	-	-	-		-	-	6	3.756	2.351
	3.56	0										0	0	0
	2.99	0										0	0	0

$$ED_{50} = lg^{-1} (\Sigma C / \Sigma t) = 4.82 \text{ mg/Kg } (95\%CI = 4.818 - 4.821 \text{ mg/Kg})$$

Pharmacokinetic parameters of HX0969W and fospropofol (mean ± SD).

AUC, area under the curve; MRT, mean residual time; CL, clearance.

Parameters	HX0969W	fospropofol
C_{max} (µg/ml)	321.3 ± 67.22	603.49 ± 411.29
$t_{1/2}$ (min)	71.79 ± 59.52	49.29 ± 40.31
$AUC_{0\sim t}$ (min*µg/ml)	1053.78 ± 214.37	3804.92 ± 2091.75
MRT (min)	3.67 ± 1.71	13.15 ± 5.45
CL (ml/min/kg)	89.97 ± 15.94	31.12 ± 19.09

Pharmacokinetic parameters of propofol (mean ± SD).

[1] Propofol$_H$, propofol released by HX0969W; Propofol$_F$, propofol released by fospropofol.

[2] AUC, area under the concentration; MRT, mean residual time; CL, clearance.

Parameters	Propofol	Propofol$_H$	Propofol$_F$
C_{max} (µg/ml)	23.4 ± 4.69	24.26 ± 5.14	21.91 ± 4.98
T_{max} (min)	0.5	4.0 ± 0.47	4.5 ± 1.18
$t_{1/2}$ (min)	54.24 ± 16.18	88.62 ± 77.35	78.97 ± 29.05
$AUC_{0 \sim t}$ (min*µg/ml)	219.23 ± 42.21	740.5 ± 186.33	704.53 ± 226.25
MRT (min)	29.45 ± 4.63	52.6 ± 2.90	50.92 ± 4.40
CL (ml/min/kg)	43.28 ± 8.46	54.67 ± 13.83	68.12 ± 22.84