Effect of chronic exposure to dexamethasone and its withdrawal on rocuronium-induced neuromuscular block and sugammadex reversal in *in vitro* study of rats

Seok Kyeong Oh¹, Il Ok Lee^{1*}, Young Sung Kim¹, Hong-Seuk Yang², Junyong In³, Hey-Ran Choi⁴

Department of Anesthesiology and Pain Medicine, ¹Korea University Guro Hospital, ²Asan Medical Center, ³Dongguk University Ilsan Hospital, ⁴Seoul Paik Hospital, Seoul, South Korea

Background Chronic exposure to dexamethasone is associated with resistance to non-depolarizing neuromuscular blocking agents. Previous studies have reported that chronic dexamethasone treatment induced alteration and upregulation in immature subunits of nicotinic acetyl-choline receptor in diaphragm of rats. However there was no previous report about the effect of chronic exposure to dexamethasone on sugammadex (SGX) reversal in rat diaphragm.

Methods Male Sprague-Dawley rats (7-8 week-old; 202 to 271 g) were randomized to receive a daily intraperitoneal injection of dexamethasone (500 µg/kg) in dexamethasone group or same amount of saline (0.9% NaCl) in control for 14 days. At 1,3,7 days after the last dexamethasone (group D1,D3,D7) (given the withdrawal time) or 1 day after saline (C), the rats were anesthetized. The hemi-diaphragm with attached phrenic nerve intact was immediately obtained.

After the elicited twitch tension by phrenic nerve had been stabilized, Train-of-four (TOF) was determined. Rocuronium 250 µg was then applied to the organ bath. Following stabilization, TOF was again determined with repetitive administration of cumulative dose of Rocuronium 250 µg. After complete block (T1 height <5%), SGX twice as much as rocuronium-equimolar dose was applied. Recovery profiles (time to TOFRatio[TOFR]>0.9, recovery index [Dur25to75%]) were observed. Expression of α7-nAChR subunit was assessed using immunohistochemistry. The correlation for the time to recovery and the other variables was conducted using Spearman’s test.

Conclusions Chronic exposure to dexamethasone causes the resistance of the rat diaphragm to rocuronium. Furthermore, the pharmacodynamics change in dexamethasone treatment was recovered within 3 days after withdrawal (in 7-8 week-old rats). TOFR > 0.9 when reversed with SGX was not affected by dexamethasone administration, however the recovery index in group D7 was shorter than group C and D1. The chronic exposure to dexamethasone seemed not effect on sugammadex (SGX) reversal in rat diaphragm.

Table 1. IC50 values and slopes of the rocuronium dose-response curves.				
	Control	Dexa 1	Dexa 3	Dexa 7
IC50	14.62 (13.66-15.65)	17.42* (16.31-18.61)	16.02 (15.05-17.06)	15.77 (15.10-16.47)
LogIC50	1.165 (1.135-1.194)	1.241* (1.212-1.270)	1.205 (1.178-1.232)	1.198 (1.179-1.217)
Hillslope	-2.867 (0.280)	-3.551 (0.389)	-3.507 (0.372)	-4.910*#▲ (0.489)
IC50 ratio		1.197 (1.138-1.256)	1.092 (1.039-1.145)	1.081 (1.028-1.133)
IC50 = 50% inhibitory dosage. IC50 ratio = (IC50 of the dexa group dose-response curves/IC50 of the control group dose-response curve). Values are means with 95% confidence intervals and mean (SE). *P<0.05 vs the control group; #P<0.05 vs the Dexa 1 group; ▲P<0.05 vs the Dexa 3 group				

Reference Chen D, Yang MR, Huang LN, Qiu YW, Li ST. Genet Mol Res 2014; 13: 5892-900.

Table 2. Recovery after sugammadex administration				
	Control	Dexa 1	Dexa 3	Dexa 7
Dur 25% (s)	138.9 (101.5-176.2)	199.2 (140.8-257.5)	119.0 (70.9-167.1)	121.1 (84.8-157.3)
Dur 25 to 75% (s)	321.6 (262.7-380.5)	350.2 (235.7-464.6)	228.0 (187.5-268.5)	187.2*# (149.6-224.8)
Dur 95% (s)	693.5 (552.1-834.9)	692.8 (543.1-842.5)	583.2 (473.7-692.8)	445.8*# (372.7-518.9)
DurMax T1 (s)	1010.7 (852.9-1168.5)	962.2 (719.6-1204.9)	773.6 (694.6-852.6)	704.4* (606.4-802.4)
DurTOF ratio >0.9 (s)	769.0 (614.7-923.2)	900.1 (653.5-1146.7)	612.0 (505.2-718.9)	606.8 (486.8-726.8)
Does of sugammadex (µL)	88.1 (7.6)	102.02 (13.0)	88.1 (12.1)	81.4 (13.0)
Values are mean (95% CI) or mean (SD).				
*P<0.05 vs the control group; #P<0.01 vs the Dexa 1 group				

Results There was no significant difference between groups in diaphragm size and weight. Dose-response curve of rocuronium in group D1 was shifted to right compared to group C (p < 0.05), but curves in D3, D7 groups were not significantly different. IC50 in group D1 is significantly larger than group C.

(Table 1) Recovery time to TOFR > 0.9 and recovery index were 12.8 ± 4.9, 15.0 ± 7.1, 10.2 ± 3.3, 10.1 ± 3.8 and 5.36 ± 1.9, 5.8 ± 3.3, 3.8 ± 1.3, 3.12 ± 1.2 (min) in group C and group D1, D3, D7 respectively. It was not statistically significant in time to TOFR > 0.9, but recovery index in group D7 were significantly shorter than group C and D1. **(Table 2)** The expression of α7-nAChR were detected on the membrane of skeletal muscle cell, which appeared on the all Dexa groups, peaked on the Dexa 1, and declined gradually on Dexa 3 and Dexa 7 group.

optical microscope (BX51T-32F01; Olympus Corporation, Tokyo, Japan) upper row , x100, scale bar=200 µm; lower row , x400, scale bar=50 µm

Recovery profiles were positively correlated with IC50 value and amount of drugs. The time to recovery of NMB by SGX seemed to be correlated with the binding time of the NMBAs and SGX, not with the resistance property of receptors. **(Table 3)**

Table 3. Correlation between recovery profiles with other variables.			
	logIC50	hillslope	Dose of sugammadex
DurTOF ratio >0.9	r=0.566 p<0.001	r= -0.085 p=0.510	r=0.551 p<0.001
Dur 25 to 75%	r=0.436 p<0.001	r=0.169 p=0.184	r=0.490 p<0.001
Dur 95% (s)	r=0.364 p=0.006	r=0.068 p=0.621	r=0.442 p=0.001
Values are Pearson’s coefficient of correlation. IC50 = 50% inhibitory dosage. Amount of drugs are the total amount of administered rocuronium and sugammadex into the organ bath.			

Contact Address : Korea Univ. Guro Hospital, Gurodong-ro 148, Guro-gu, Seoul 08308, South Korea
The Corresponding author (IO Lee) iloklee@korea.ac.kr
The first author (SK Oh) nanprayboy@korea.ac.kr