Effects of dantrolene on calcium-induced calcium release from the sarcoplasmic reticulum in human skeletal skinned fibers

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Background

Dantrolene (Dan) is the only drug available to treat malignant hyperthermia (MH) crisis, a pharmacogenetic disorder caused by increased calcium (Ca) release from the sarcoplasmic reticulum (SR) via ryanodine receptor 1 (RYR1). The Ca-induced Ca release (CICR) rates from the SR were found to be accelerated in the skeletal muscles of patients with MH¹⁾. The effects of Dan on CICR in skinned fibers are controversial²⁾³⁾, and have not been studied in the skeletal muscles of patients with MH.

Goal of Study

We investigated the effects of Dan on the CICR rate in the skeletal muscles of patients predisposed to MH.

Materials and Methods

This study was approved of the local ethics committees (Hiroshima University and Hiroshima Prefecture Rehabilitation Center)
Muscle samples were obtained, with prior written consent, from 11 individuals to determine their predisposition to MH.

The CICR rates were measured in chemically skinned fibers achieved by Endo's method. Calcium ions [Ca²⁺] at five different concentrations (0, 0.3, 1.0, 3.0, and 10 μM), were used to induce Ca²⁺ stimulation via RYR1. The effects of Dan-treatment (50 μM) on CICR rates were assessed at five different Ca²⁺ concentrations, under magnesium-free conditions, at 20°C. Dan (50 μM) was added in 4-2 and 5 of CICR procedure (Table 1).

Fig. 1 A representative trace of time-tension curve

Table 1. Outline of CICR procedure and constitution of the solutions

CICR procedure	solutions	Mg ²⁺ mM	MgATP mM	Ca ²⁺ μM	EGTA mM	PIPESm M	Others mM
1. Relaxing	Relaxing solution	1.5	3.5	0	2	20	
2. Loading Ca ²⁺ into SR with ATP	Ca loading solution	1.5	3.5	0.2	10	20	
3. Sop loading Ca ²⁺ with high EGTA	relaxing solution high EGTA	1.5	3.5	0	10	20	
4-1. Washout ATP	ATP free solution	1.5	0	0	2	20	
4-2. Washout ATP and Mg ²⁺	Mg and ATP free solution	0	0	0	2	20	
5. Releasing Ca ²⁺ from SR with X (Ca ²⁺) μM for t** min	releasing solution	0	0	X*	10	20	
6. stop releasing Ca ²⁺ with Procaine	stop solution	10	0	0	10	20	Procaine 10
7. Re-introduce ATP	pre-assay solution	1.5	3.5	0	0.1	20	Procaine 5
8. Releasing all residual Ca ²⁺ from SR with 25mM caffeine	assay solution	0.1	1.0	0	0.1	20	Caffeine 25

A : 1→2→3→5→6→7→8
B : 1→2→3→4-1→4-2→5→6→7→8
C : 1→2→3→4-1→4-2→5→6→7→8

The area under time-tension curve is equal to Ca²⁺ amount releasing in CICR procedure 8
S_A: area of curve A, S_B: area of curve B.

$$\text{CICR rate (min}^{-1}\text{)} = - \{ \ln (S_A - S_B / S_A) \} / t$$

EGTA ; ethylene glycol-bis(β-amino ether) – N, N, N', N'-tetra acetic acid
PIPES ; piperazine –N, N'-bis(2-ethanesulfonic acid)
X* = free, 0.3, 1.0, 3.0, 10 ,
t** min ; time of releasing Ca²⁺ in CICR procedure 5

Statistical analyses : The values are expressed as mean ± SD. A paired t-test and a regression analysis were performed for statistical analysis. Significance was determined at p<0.05.

Results

Table 2. Demographic data

No.	Gender	Age * (y)	Indication for CICR test	Result of CICR test	CK* (IU/l)	Variants detected in RYR1
1	male	33	MH reaction (no data)	accelerated	391	p. Ala1449val
2	male	34	MH reaction (no data)	accelerated	267	p. Gly341Arg
3	female	56	MH reaction (CGS 30)	accelerated	204	p. Arg614Leu
4	male	12	Suspicion of muscle disease	accelerated	189	p.Thr84Met
5	male	52	MH reaction (no data)	accelerated	50	p. Ala5025Gly
6	female	73	Brother died in MH reaction	accelerated	297	p. Gln155Lys
7	female	65	Brother developed an MH	accelerated	98	No variant
8	male	40	Brother died in MH reaction	accelerated	276	No variant
9	male	37	MH reaction (CGS 30)	accelerated	322	No variant
10	male	42	MH reaction (CGS 48)	accelerated	305	p. Ser2345Thr
11	male	44	Idiopathic high CK	not accelerated	1135	Not done

* Age and CK at CICR test, CGS: Clinical grading scale

Table 3. Relative Ca²⁺ release rates

Ca ²⁺ (μM)	free	0.3	1.0	3.0	10.0
Relative Ca ²⁺ release rate	0.95±0.18	0.79±0.22	0.67±0.087	0.74±0.11	0.73±0.13
P value	0.0832	0.0087	<0.0001	0.0001	0.0006

Ten of the 11 individuals were diagnosed with predisposition to MH, based on the accelerated CICR rates (Table 2). Their CICR rates were increased by 2 to 8-fold compared to the normal rate. Dan-treatment significantly decreased the CICR rates at Ca²⁺ concentrations of 0.3, 1.0, 3.0 and 10 μM (Table 3). CICR rates, both pre- and post- Dan-treatment, exhibited a significant correlation (Fig 2).

Fig. 2 Correlation of CICR rates, both pre- and post- Dan-treatment

Discussion : It was therefore assumed that applying 50 μM Dan would decrease the CICR rates by 0.754-fold. The inhibitory effect of Dan on CICR may contribute to the suppression of myoplasmic elevation of Ca²⁺ in MH crisis.

Conclusion

We conclude that Dan is effective in the treatment of MH crisis via direct inhibition of Ca²⁺ release from the SR and reduction of Ca²⁺.

References: 1) Anesthesiology. 2006; 104: 1146-54, 2) Br J Pharmacol. 2001;134: 729-36. 3) Am J Physiol. 1989; 256 C 358-67.