The effects and mechanisms of action of diethylcarbamazine citrate in isolated rat hearts

Dietilkarbamazin sitrat'ın izole sıçan kalplerindeki etkileri ve etki mekanizmaları

Ziya Kaygısız, Bilgin Kaygısız, Mete Özkurt, Erkan Kılınç*

From Departments of Physiology and *Pharmacology, Eskişehir Osmangazi University Medical Faculty, Eskişehir-*Turkey*

The anthelmintic drug, diethylcarbamazine citrate (DECC), is a piperazine derivative (1). DECC has been used in the treatment of filarial infections. It has been demonstrated that intravenous DECC administration into anaesthetized cats induces hypotension, bradycardia and a reduction in $+dP/dt_{max}$ (the maximum rate of increase in the left ventricular pressure) followed by hypertension and an increase in $+dP/dt_{max}$ and the myocardial blood flow (2). It is possible that DECC affects cardiovascular functions, but little is known about its mechanisms of action during these cardiac effects. In isolated rat hearts, the possible action of DECC on the left ventricular developed pressure (LVDP), $+dP/dt_{max}$, the heart rate, the coronary flow, $-dP/dt_{min}$ (the maximum rate of decrease in the left ventricular pressure) and the left ventricular end-diastolic pressure (LVEDP) has not been investigated, and the signal transduction pathways mediating the actions of DECC are not known. Therefore, we studied the possible cardiovascular effects of DECC in isolated rat hearts. We postulated that the activation of sarcoplasmic reticulum Ca^{2+} ATPase inhibitor (SERCA) and the muscarinic receptors, as well as nitric oxide (NO) generation, may be responsible for the cardiac effects of DECC, and we also studied whether SERCA, muscarinic receptors or NO mediate the effects of DECC.

All procedures were approved by the local research ethics committee. Male and female Sprague-Dawley rats weighing 300-400 g were used. One hour after the administration of 1000 IU heparin ip., the heart was rapidly excised under the administration of light ether anesthesia. The aorta was immediately attached to a stainless steel cannula of the perfusion system and the hearts were perfused under a constant pressure. The perfusion solution was Modified Krebs-Henseleit solution and this solution was continuously oxygenated with 95% O_2 and 5% CO_2 (pH=7.4). The temperature was maintained at 37°C.

A liquid-filled latex balloon was connected to a pressure transducer (Isotec, Hugo Sachs Electronic, March-Hugstetten, Germany) and inserted into the left ventricle via the mitral valve. The peak systolic pressure and LVEDP were measured. LVDP (an index of cardiac contractility) was calculated as the difference between the systolic and the diastolic pressures, and this pressure was accepted as the contractile force. Furthermore, $+dP/dt_{max}$ (other index of contractility), heart rate and $-dP/dt_{min}$ (an index of relaxation) were determined from the left ventricular pressure signal using the data acquisition software. The coronary flow, which is an index of the coronary vascular tone, was measured from the timed collection of the coronary effluent in a graduated cylinder. The hearts were allowed to equilibrate for 30 minutes to establish a stable baseline. After the stabilization period, DECC (20, 100 and 500 µM) was infused to the hearts for 30 minutes using an infusion pump (Graseby Medical, Model 3400, Watford Herts, England). Ten nM thapsigargin (a SERCA inhibitor), 1 µM atropine (a muscarinic receptor blocker) and 100 µM L-NAME (a NO synthase inhibitor) and 500 µM DECC were used to investigate the mechanisms of DECC action.

The infusion of DECC significantly decreased the LVDP, $+dP/dt_{max}$, coronary flow and $-dP/dt_{min}$ in a dose-dependent manner. DECC (20 µM) did not alter the heart rate but at 100 and 500 µM significantly decreased the heart rate. Furthermore, DECC did not significantly affect LVEDP (Table 1). In accordance with our results, Abaitey and Parratt (2) observed that DECC (2.5 to 10 mg/kg) decreased $+dP/dt_{max}$, the heart rate, the systolic and the diastolic blood pressure in anaesthetized cats. Similar to our findings, DECC (1 nM-100 µM) also depressed the atrial contractility in the isolated left atria of guinea-pigs (3), and DECC (1000 mg/kg ip.) caused a precipitous decline in the heart rate in rats (4). It has been known

Address for Correspondence/Yazışma Adresi: Dr. Ziya Kaygısız, Eskişehir Osmangazi Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, 26480 Eskişehir-*Türkiye* Phone: +90 222 239 29 79-4575 Fax: +90 222 239 37 72 E-mail: ziyak@ogu.edu.tr

Accepted Date/Kabul Tarihi: 05.04.2013 **Available Online Date/Çevrimiçi Yayın Tarihi:** 24.07.2013

doi:10.5152/akd.2013.183

Table 1. The effect of the DECC on the LVDP, +dP/dt$_{max}$, heart rate, coronary flow, -dP/dt$_{min}$ and LVEDP

L VDP (mmHg)				
DECC	**Control**	**10 minutes**	**20 minutes**	**30 minutes**
20 µM (n=8)	84.0 (82.5-127.5)	78.5 (71.5-112.7)	69.5 (65.2-107.7)**	69.0 (61.7-99.7)***
100 µM (n=5)	118.0 (91.5-129.0)	57.0 (53.0-72.0)*	70.0 (59.0-85.5)	73.0 (62.0-83.5)
500 µM (n=6)	116.0 (96.5-142.0)	22.0 (18.0-30.0)**	24.5 (21.5-28.5)**	29.5 (24.5-38.0)
+ dP/dt$_{max}$ (mmHgs^{-1})				
20 µM (n=8)	3678.0 (3387.7-5057.0)	3283.0 (3016.0-4609.5)	3161.0 (2736.0-4329.0)**	2972.0 (2897.0-4266.0)*
100 µM (n=5)	4323.0 (3632.0-4606.0	2962.0 (2079.0-3539.0)*	3580.0 (2095.0-3763.0)	3580.0 (2095.0-3722.0)
500 µM (n=6)	4900.0 (3776.0-5041.0)	593.0 (569.5-740.7)*	645.5 (527.7-761.0)**	718.5 (681.0-793.0)
Heart Rate (Beats/min)				
20 µM (n=9)	258.0 (223.0-295.0)	268.0 (236.5-302.0)	273.0 (249.0-295.0)	265.0 (251.0-294.0)
100 µM (n=7)	260.0 (228.0-288.0)	225.0 (213.0-250.0)	221.0 (189.0-231.0)*	230.0 (228.0-240.0)
500 µM (n=7)	287.0 (230.0-306.0)	147.0 (131.0-200.0)*	153.0 (126.0-188.0)	122.0 (108.0-146.0)***
Coronary Flow (mL/min)				
20 µM (n=8)	13.5 (9.0-14.7)	12.0 (9.7-13.7)	11.5 (8.7-13.7)	11.5 (7.7-13.7)*
100 µM (n=5)	13.0 (8.0-3.0)	8.0 (7.0-11.5)*	8.0 (7.5-11.0)	8.0 (6.5-12.0)
500 µM (n=7)	10.0 (8.0-13.0)	5.0 (4.0-7.0)	4.0 (4.0-6.0)*	5.0 (4.0-6.0)
-dP/dt$_{min}$ (mmHgs^{-1})				
20 µM (n=9)	-2700.1±177.8	-2379.8±193.6***	-2291.3±122.3***	-2264.3±147.5***
100 µM (n=6)	-2493.8±100.6	-1872.0±157.1***	-2004.2±171.4***	-1887.8±123.5***
500 µM (n=7)	-2720.3±194.1	-637.0±37.8***	-602.3±32.4***	-616.1±38.5***
LVEDP (mmHg)				
20 µM (n=7)	8.2±0.6	8.6±0.7	8.6±0.6	8.8±0.5
100 µM (n=6)	8.4±0.5	8.7±0.5	8.9±1.1	9.1±0.6
500 µM (n=6)	8.0±0.8	8.4±0.8	8.5±0.8	8.8±0.7

The values were given as the mean±SEM or median (25%-75%). The values obtained prior to the addition of the drugs were considered as the control values.
Time-dependent effects of different doses of DECC were analyzed using repeated measures ANOVA and nonparametric Friedman test. Bonferroni test was used as a post hoc test
*p<0.05, **p<0.01 and ***p<0.001 significantly different from the control

that the increase in LVEDP is associated with decreased coronary flow (5) and in the present study, DECC did not change LVEDP but decreased coronary flow. Therefore, DECC-induced decrease in coronary flow might not be related to the LVEDP. It has been reported that decreased contractility contributes left ventricular (LV) dysfunction and LV dysfunction decreases cardiac output which in turn leads to global hypoperfusion (6). Thus, the decrease in LVDP may be responsible for the decrease in coronary flow.

In the present study, the thapsigargin or atropine infusion in combination with DECC partially abolished the negative inotropic and chronotropic effect of DECC. However, thapsigargin or atropine in the presence of DECC completely antagonized the decrease in the coronary flow induced by DECC. L-NAME in combination with DECC did not change the negative inotropy, negative chronotropy and the decrease in coronary flow induced by DECC. (Table 2). These results indicate that the activation of SERCA and

Table 2. The influence of the drugs on the LVDP, +dP/dtmax, heart rate and coronary flow when given alone or together with DECC

Drug	LVDP (mmHg)		
	Control	30 minutes	Δ% LVDP
DECC	118.3±11.6	18.8±1.5[c]	-84.0±5.4
Thapsigargin	110.8±6.8	95.7±8.9	
Thapsigargin+DECC	108.3±6.4	93.2±7.4	-14.0±1.3***
Atropine	109.8±10.8	78.0±7.5[a]	
Atropine +DECC	105.0±7.4	70.5±6.9[b]	-32.8±3.1***
L-NAME	104.5±7.7	72.2±5.7[a]	
L-NAME+DECC	97.3±5.5	25.3±2.3[c]	-74.0±3.7
Drug	**+dP/dt$_{max}$ (mmHgs^{-1})**		
	Control	**30 minutes**	**Δ% +dP/dt$_{max}$**
DECC	4751.2±416.7	517.8±49.1[c]	-89.0±5.0
Thapsigargin	4095.0±229.6	3714.7±324.8	
Thapsigargin+DECC	3735.5±321.0	2794.2±186.5[b]	-25.0±2***
Atropine	3893.0±349.1	2970.6±242.9	
Atropine +DECC	4218.6±428.4	2572.2±243.4[a]	-39.0±3***
L-NAME	4058.3±409.5	3000.8±304.4[b]	
L-NAME+DECC	3908.8±319.3	854.6±82.2[c]	-78.0±5.5
Drug	**Heart rate (Beats/min)**		
	Control	**30 minutes**	**Δ% Heart rate**
DECC	276.0±13.4	121.0±13.2[c]	-56.2±2.8
Thapsigargin	281.8±14.6	273.8±11.7	
Thapsigargin+DECC	270.2±13.9	232.2±20.4[b]	-13.8±1.0***
Atropine	281.8±13.0	277.6±9.7	
Atropine +DECC	295.0±16.6	234.2±20.4	-20.6±1.9***
L-NAME	285.6±14.8	242.6±18.2	
L-NAME+DECC	271.6±14.9	139.0±10.9[c]	-48.8±3.6
Drug	**Coronary flow (mL/min)**		
	Control	**30 minutes**	**Δ% Coronary flow**
DECC	10.0±1.1	5.1±0.7[c]	-48.7±3.8
Thapsigargin	13.3±1.0	13.0±1.1	
Thapsigargin+DECC	12.4±0.9	12.3±0.9	-1.1±0.1***
Atropine	12.7±1.7	11.6±1.1	
Atropine +DECC	11.5±0.9	11.3±1.1	-1.8±0.2***
L-NAME	12.8±0.9	8.3±0.8[a]	
L-NAME+DECC	10.8±0.8	5.8±0.4[a]	-46.1±4.2

The possible role of the SERCA, muscarinic receptors and NO on the cardiac effects of DECC was investigated using thapsigargin, atropine and L-NAME, respectively. Each of thapsigargin, atropine or L-NAME was infused separately for 30 minutes prior to the addition of DECC. In another stage of the study, thapsigargin, atropine or L-NAME was infused in combination with DECC for 30 minutes. The % change in the LVDP, +dP/dt$_{max}$, heart rate and coronary flow as a percentage of the control after 30 minutes of drug infusion has been shown as Δ % LVDP, Δ % +dP/dt$_{max}$, Δ % heart rate and Δ % coronary flow, respectively.
Each value is the mean±SEM of 7 experiments. Independent samples t-test, paired sample t-test and Wilcoxon rank test. a: p<0.05, b: p<0.01, and c: p<0.001, significantly different from the control. ***p<0.001, significantly different from the Δ % LVDP of DECC, Δ % +dP/dt$_{max}$ of DECC, Δ % heart rate of DECC or Δ % coronary flow of DECC.

muscarinic receptors may play an important role in the effect of DECC on the cardiac contractility, heart rate and coronary flow. Furthermore, NO does not mediate the effects induced by DECC. In cardiac myocytes, the activation of SERCA can reduce the cytoplasmic Ca^{2+} concentration, and the decrease in the cytoplasmic Ca^{2+} causes the contractile dysfunction. Thapsigargin increases cardiac myocyte contraction (7). DECC inhibits acethylcholinesterase activity (8), resulting in the accumulation of acethylcholine (9). Activation of the muscarinic receptors produces negative inotropic and chronotropic effects on the cardiac muscle and decreases coronary flow (10). In our study, DECC mimicked the actions of muscarinic receptor stimulation.

We suggest that DECC causes a negative inotropic effect with a decrease in the coronary flow. We also suggest that DECC exerts a bradycardic effect. The activation of SERCA and muscarinic receptors mediates these effects, and NO is not involved in the cardiovascular effects of DECC.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - Z.K.; Design - Z.K.; Supervision - Z.K.; Resource -Z.K., E.K.; Data collection &/or processing - Z.K., M.Ö.; Analysis &/or interpretation - Z.K., B.K.; Literature search - Z.K., B.K.; Writing - Z.K., B.K..; Critical review- Z.K., B.K., M.Ö., E.K.

References

1. Sanchez Bruni SF, Jones DG, Mckellar QA. Pharmacological approaches towards rationalizing the use of endoparasitic drugs in small animals. J Vet Pharmacol Ther 2006; 29: 443-57. [CrossRef]
2. Abaitey AK, Parratt JR. Cardiovascular effects of diethylcarbamazine citrate. Br J Pharmacol 1976; 56: 219-27. [CrossRef]
3. Ojewole JA, Onejeme IV. Myocardial depressant effects of diethylcarbamazine citrate in vitro. Eur J Pharmacol 1983; 87: 245-52. [CrossRef]
4. Hunsinger RN, Jenkins RL, Brown AL, Belew DH. Studies on the acute lethality of diethylcarbamazine in the rat. Vet Hum Toxicol 1993; 35: 11-5.
5. Doi Y, Masuyama T, Yamamoto K, Mano T, Naito J, Nagano R, et al. Coronary back flow pressure is elevated in association with increased left ventricular end-diastolic pressure in humans. Angiology 1996; 47: 1047-51. [CrossRef]
6. Kemp CD, Conte JV. The pathophysiology of heart failure. Cardiovasc Pathol 2012; 21: 365-71. [CrossRef]
7. Zhang Q, Scholz PM, He Y, Tse J, Weiss HR. Cyclic GMP signaling and regulation of SERCA activity during cardiac myocyte contraction. Cell Calcium 2005; 37: 259-66. [CrossRef]
8. Fujimaki Y, Sakamoto M, Shimada M, Kimura E, Aoki Y. Diethylcarbamazine: inhibitory effect on acetylcholinesterase of Dirofilaria immitis and Brugia pahangi. Southeast Asian J Trop Med Public Health 1989; 20: 179-82.
9. Bhattacharya C, Singh RN, Misra S, Rathaur S. Diethylcarbamazine; effect on lysosomal enzymes and acetylcholine in Wuchereria bancrofti infection. Trop Med Int Health 1997; 2: 686-90. [CrossRef]
10. Nadler E, Barnea O, Vidne B, Isakov A, Shavit G. Positive inotropic effect in the heart produced by acetylcholine. J Basic Clin Physiol Pharmacol 1993; 4: 229-48. [CrossRef]