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ARTICLE

Long-term pretreatment with desethylamiodarone (DEA) or amiodarone (AMIO) protects against coronary artery occlusion induced ventricular arrhythmias in conscious rats[1]

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Abstract: The aim of this investigation was to compare the effectiveness of long-term pretreatment with amiodarone (AMIO) and its active metabolite desethylamiodarone (DEA) on arrhythmias induced by acute myocardial infarction in rats. Acute myocardial infarction was induced in conscious, male, Sprague–Dawley rats by pulling a previously inserted loose silk loop around the left main coronary artery. Long-term oral pretreatment with AMIO (30 or 100 mg·(kg body mass)$^{-1}$·day^{-1}, loading dose 100 or 300 mg·kg^{-1} for 3 days) or DEA (15 or 50 mg·kg^{-1}·day^{-1}, loading dose 100 or 300 mg·kg^{-1} for 3 days), was applied for 1 month before the coronary artery occlusion. Chronic oral treatment with DEA (50 mg·kg^{-1}·day^{-1}) resulted in a similar myocardial DEA concentration as chronic AMIO treatment (100 mg·kg^{-1}·day^{-1}) in rats (7.4 ± 0.7 μg·g^{-1} and 8.9 ± 2.2 μg·g^{-1}). Both pretreatments in the larger doses significantly improved the survival rate during the acute phase of experimental myocardial infarction (82% and 64% by AMIO and DEA, respectively, vs. 31% in controls). Our results demonstrate that chronic oral treatment with DEA resulted in similar cardiac tissue levels to that of chronic AMIO treatment, and offered an equivalent degree of antiarrhythmic effect against acute coronary artery ligation induced ventricular arrhythmias in conscious rats.

Key words: arrhythmias, survival, myocardial infarction, rats, amiodarone, desethylamiodarone.

Résumé : Les présents travaux de recherche avaient pour objectif de comparer l'efficacité d'un prétraitement à long terme par l'amiodarone et la déséthylamiodarone, son métabolite actif, sur les arythmies induites par l'infarctus aigu du myocarde chez le rat. L'infarctus aigu du myocarde a été provoqué chez des rats Sprague–Dawley mâles en appliquant une traction sur une soie placée au préalable autour de l'artère interventriculaire antérieure. Les prétraitements à long terme par l'amiodarone (30 ou 100 mg·(kg de masse corporelle)$^{-1}$·jour^{-1}, dose d'attaque de 100 ou de 300 mg·kg^{-1} pendant 3 jours) ou par la déséthylamiodarone (15 ou 50 mg·kg^{-1}·jour^{-1}, dose d'attaque de 100 ou de 300 mg·kg^{-1} pendant 3 jours) ont été administrés par voie orale tout au long du mois précédant l'occlusion coronarienne. Le traitement chronique par l'administration de déséthylamiodarone par voie orale (à 50 mg·kg^{-1}·jour^{-1}) a permis d'obtenir des concentrations similaires de déséthylamiodarone à celles obtenues par l'administration chronique d'amiodarone (à 100 mg·kg^{-1}·jour^{-1}) chez les rats (7,4 ± 0,7 μg·g^{-1} et 8,9 ± 2,2 μg·g^{-1}). Aux doses élevées, les deux traitements ont amélioré le taux de survie pendant la phase aiguë de l'infarctus du myocarde provoqué expérimentalement (82 et 64 % avec l'amiodarone et la déséthylamiodarone, respectivement p/r à 31 % dans le groupe témoin). Les résultats cette étude montrent que l'administrations chronique de déséthylamiodarone ou d'amiodarone par voie orale a permis d'obtenir des concentrations similaires de déséthylamiodarone dans le tissu cardiaque et des effets de degrés similaires contre les arythmies ventriculaires induites par l'occlusion coronarienne aiguë chez des rats à l'état conscient. [Traduit par la Rédaction]

Mots-clés : arythmies, survie, infarctus du myocarde, rats, amiodarone, déséthylamiodarone.

Introduction

Cardiovascular diseases are the leading causes of death in developed countries, and arrhythmias are common complications of cardiovascular diseases. Antiarrhythmic drugs today are recommended as an adjuvant treatment to patients receiving an implantable cardioverter defibrillator (ICD) for the prevention and treatment of ventricular arrhythmias (for a recent review see Bunch and Anderson 2014).

Chronic treatment with amiodarone (AMIO) is the most effective pharmacologic tool for treating arrhythmias, with less proarrhythmic risk than other antiarrhythmic drugs. Both dofetilide and d-sotalol (selective I_{Kr} blockers) can provoke Torsade de Pointes (TdP) arrhythmias with an incidence of approximately 3%. AMIO in this respect is better tolerated, having a proarrhythmic incidence of less than 1% (Doval et al. 1994; Singh et al. 1995; Waldo et al. 1996; Torp-Pedersen et al. 1999). This effect of AMIO has been attributed to its sodium channel blocking properties and beta adrenoceptor blocking effects, leading to decreased dispersion of repolarization and triggered activity, despite the fact that AMIO prolongs repolarization by inhibiting a number of different cardiac potassium channels. In addition, the sodium channel block caused by AMIO is different compared with those selective Class

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I/C sodium channel blockers flecainide and encainide, which were proven to be proarrhythmic (The Cardiac Arrhythmia Suppression Trial (CAST) Investigators 1989). AMIO has Class I/B sodium channel blocking activity, leading to effectively blocked sodium channels at high frequency and (or) in case early extrasystoles develop. The calcium channel blocking property of AMIO also contributes to decreased triggered activity following its administration. In addition, experimental studies also support the hypothesis that selective Class III agents cause significantly higher incidence of TdP compared with AMIO (Farkas et al. 2002; Thomsen et al. 2004).

However, AMIO exerts serious extracardiac adverse effects, e.g., pulmonary fibrosis, hepatotoxicity, photodermatosis, and cornea deposits (Tisdale et al. 1995). The unique pharmacokinetic properties of AMIO (which include an extremely long elimination half-life as well as its metabolic interactions with several other drugs) may also contribute to the problems during its long-term use.

AMIO is a representative of Class III antiarrhythmic drugs, with multiple electrophysiological effects, inhibiting various cardiac voltage dependent potassium-, sodium-, and calcium-channels, as well as a reduced β-adrenoceptor response in the heart (reviewed by Kodama et al. 1999). Acute and long-term AMIO treatment influences transmembrane ionic currents in ventricular myocytes differently. After acute administration, the sodium and calcium channel blocking properties dominate, whereas after long-term use, the inward rectifying potassium channel and the transient outward potassium current (I_{to}) are depressed, with reduced inhibition of the inward calcium current (Varró et al. 1985, 1996; reviewed by Kodama et al. 1999).

During chronic AMIO treatment, an electrophysiologically active metabolite, desethylamiodarone (DEA; 2-*N*-butyl-3-(3,5-diiodo-4-ethylaminoethoxy-benzoyl)-benzofuran), is produced and accumulates in the plasma and tissues, including the myocardium (Flanagan et al. 1982).

DEA produces very similar pharmacological effects when compared with AMIO. Acute administration of both the AMIO and DEA significantly prolonged the QRS, QT, and QTc intervals of the electrocardiogram in rats. DEA showed a significantly greater potency in this respect (Nattel 1986). Acute treatment with either AMIO or DEA suppressed ventricular arrhythmias after a 2-stage coronary artery ligation in dogs (Nattel et al. 1988). Long-term administration of AMIO or DEA in rabbits prolonged the action potential duration and the effective refractory period (Kato et al. 1988).

There are some in vivo and ex vivo electrophysiological investigations on the effect of long-term desethylamiodarone treatment; however, there are no data available comparing long-term treatment with amiodarone and desethylamiodarone on arrhythmias in experimental animals, in vivo. Therefore the aim of this investigation was to compare the effectiveness of long-term pretreatment with AMIO and its metabolite DEA on ventricular arrhythmias induced by acute coronary artery occlusion in conscious rats. We also measured the plasma and myocardial concentration of AMIO and DEA after the long-term pretreatments.

Materials and methods

This study protocol was approved by the local animal care committee of the University of Szeged and conforms with the *Guide for the Care and Use of Laboratory Animals* (NAC 2011).

Animals

Male CFY (remote Sprague–Dawley) rats, weighing 260–300 g were used. The animals were housed 5 per cage and were maintained on a 12 h (light) – 12 h (dark) cycle. The animals were fed standard laboratory rat food and allowed to drink tap water ad libitum.

Coronary artery ligation induced arrhythmias in conscious rats

Coronary artery occlusion in conscious rats was performed as described earlier (Leprán et al. 1983). In a preliminary surgery, under ether anesthesia, the chest was opened in the 4th intercostal space. A loose silk loop (5-0; Ethicon) was applied around the left main coronary artery, and then the chest was closed. Seven to 8 days after the preliminary surgery, and after complete recovery and healing, the loose silk loop was tightened to occlude the coronary artery in conscious, freely-moving animals. During the first 15 min of myocardial infarction a bipolar ECG was recorded continuously (PowerLab 8SP; ADInstruments).

We followed the survival rate during the acute phase (first 15 min) and during the following 16 h after coronary artery occlusion. The incidence and duration of arrhythmias in the acute phase were evaluated according to the Lambeth Conventions (Walker et al. 1988), i.e., ventricular fibrillation, ventricular tachycardia, and other types of arrhythmias, including ventricular extrasystoles, bigeminia, and salvos. An arrhythmia score was used, including the incidence and duration of different arrhythmias by giving a grade to each animal as follows: 0 = no arrhythmia; 1 = <10 s ventricular ectopic beats (VEB), bigeminia, salvos, and (or) ventricular tachycardia (VT); 2 = 11–30 s VEB, bigeminia, salvos, and (or) VT; 3 = 31–90 s VEB, bigeminia, salvos, and (or) VT; 4 = 91–180 s VEB, bigeminia, salvos, and (or) VT; <10 s reversible VF; 5 = >180 s VEB, bigeminia, salvos, and (or) VT; >10 s reversible VF; 6 = irreversible VF. The size of the myocardial infarction was measured in the animals surviving for 16 h after coronary artery occlusion using nitrotetrazolium-blue dye staining to demonstrate the appropriate production of myocardial infarction. Animals with a scar tissue area of <5% were excluded from the final evaluations.

Table 1. Influence of long-term amiodarone (AMIO) or desethylamiodarone (DEA) pretreatment on the heart rate 5 min before (Baseline) and after coronary artery occlusion in conscious rats.

		Time after coronary artery occlusion (min)						
Group	Baseline	1	3	5	7	10	12	15
Vehicle								
Mean	327	402	395	394	390	380	382	370
SE	5.3	9.4	7.5	8.9	11.7	15.5	15.1	11.2
n	39	37	36	26	12	12	12	12
AMIO 30 mg·kg^{-1}								
Mean	343	415	401	385	393	360	364	382
SE	8.1	12.7	17.4	14.7	17.7	21.3	18.9	23.4
n	23	23	19	15	9	9	9	9
AMIO 100 mg·kg^{-1}								
Mean	321	373	363	356	365	357	348	340
SE	5.1	12.2	12.9	13.8	9.9	12.1	9.3	7.9
n	22	22	22	17	17	17	18	18
DEA 15 mg·kg^{-1}								
Mean	343	420	400	397	410	393	386	375
SE	7.9	15.7	14.9	14.8	16.1	11.9	13.2	12.0
n	23	21	20	15	8	8	8	8
DEA 50 mg·kg^{-1}								
Mean	330	398	382	369	365	363	359	350
SE	8.9	11.4	10.3	9.9	8.5	10.8	9.4	6.2
n	22	21	22	18	15	15	14	14

Note: Values are mean ± SE of *n* animals at the given time point.

Determination of the plasma and tissue concentration of AMIO and DEA

At the end of the feeding experiments, the animals were anesthetized with pentobarbitone (60 mg·(kg body mass)$^{-1}$, by intraperitoneal (i.p.) injection; Sigma), then blood was taken from the abdominal aorta and the heart was excised. The ventricular myocardium was homogenized, with a tissue blender, in a 0.01 mol·L^{-1} mixture of KH_2PO_4 (pH = 4.3) and methanol (75:25; 1:4 *w*/*v*). Plasma and tissue homogenates were stored at –70 °C until later analysis. AMIO and DEA was determined according to the methods of Bolderman et al. (2009) using an HPLC apparatus (Shimadzu Cor-

Table 2. Influence of long-term amiodarone (AMIO) or desethylamiodarone (DEA) pretreatment on the survival rate and the incidence of arrhythmias during the first 15 min after coronary artery occlusion in conscious rats.

				Incidence of arrhythmias												
		Survived		None		VF		VT		Other		Br			Survived for 16 h	
Group	*N*	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	Arrhythmia score	*n*	%
Vehicle	39	12	31	2	5	29	74	35	90	28	72	1	3	4.77±0.33	8	21
AMIO 30 mg·kg^{-1}	23	9	39	1	4	16	70	20	87	13	57	6	26*	4.57±0.43	8	35
AMIO 100 mg·kg^{-1}	22	18	82*	10	45*	5	23*	9	41*	8	36*	2	9	2.05±0.52*	18	82*
DEA 15 mg·kg^{-1}	23	8	39	3	13	16	70	18	78	14	61	3	13	4.35±0.52	4	17
DEA 50 mg·kg^{-1}	22	14	64*	6	27*	10	45*	15	68*	13	59	2	9	3.27±0.56*	12	55*

Note: *N*, number of animals in the group; *n*, number of animals showing the given response; None, no arrhythmia occurred; VF, ventricular fibrillation; VT, ventricular tachycardia; Other, extrasystoles, bigeminia; Br, bradycardia; *, $P < 0.05$ compared with the vehicle treated group.

poration, Kyoto, Japan) equipped with a Kromasil Eternity C18 (5 μm, 150 mm × 4.6 mm) analytical column (AkzoNobel, Bohus, Sweden).

Pretreatment

Long-term oral pretreatment was applied for 1 month before the coronary artery occlusion. The animals were treated orally by gavage, and the last treatment was given 4 h before the coronary artery occlusion. The applied doses were as follows: AMIO (Sequoia Research Products, Pangbourne, UK) 30 or 100 mg·kg^{-1}·day^{-1} (loading dose 100 or 300 mg·kg^{-1} for 3 days); DEA (Szintekon, Debrecen, Hungary) 15 or 50 mg·kg^{-1}·day^{-1} (loading dose 100 or 300 mg·kg^{-1} for 3 days). Control animals were given the vehicle in a volume of 5 mL·kg^{-1}. The doses of AMIO were based on findings of Hu et al. (2004), and that of DEA on the findings of Varró et al. (1987).

Statistical analysis

The survival rate was compared by using the χ^2-method with Yates correction. All other parameters are expressed as the mean ± SEM, and after analysis of variance (ANOVA) were compared by means of the modified *t* test (Wallenstein et al. 1980).

Results

Neither AMIO nor DEA administration produced any visible behavioral changes of the animals during the long-term treatment, or any difference in the gaining of body mass.

There were no significant differences in the baseline heart rate before coronary artery occlusion, or the heart rate response after coronary artery occlusion among the control and AMIO- or DEA-treated animals (Table 1). The basal heart rate in our experiments at the end of the pretreatment period, before coronary ligation in non-stressed, conscious animals was relatively low (mean values in the different groups are between 321–343 beats·min^{-1}), and significantly elevated after coronary artery occlusion. This elevation was smaller in the AMIO- or DEA-treated animals (at 3 min after occlusion 68, 42, and 52 beats·min^{-1} in the vehicle, AMIO 100 mg·kg^{-1}, and DEA 50 mg·kg^{-1} treatment groups, respectively).

Coronary artery occlusion in conscious rats within 4–6 min resulted in various arrhythmias, frequently leading to irreversible ventricular fibrillation of the control animals. Both of the pretreatments, in the larger doses, significantly improved the survival rate during the acute phase of experimental myocardial infarction (Table 2). Significantly fewer animals developed ventricular fibrillation or tachycardia. Moreover, some animals did not develop arrhythmia during the first 15 min after coronary artery occlusion, despite the characteristic ECG changes and developing myocardial damage 16 h later. Bradycardia and atrioventricular-blockade developed in some animals transiently, after coronary artery occlusion in the animals treated with AMIO at 30 mg·kg^{-1}. The arrhythmia score, representing the incidence and duration of various arrhythmias and the survival as a single number, also significantly decreased after pretreatment with the larger doses of AMIO and DEA (Table 2). The time-lag to the appearance of the first arrhythmias was significantly delayed by the larger doses of both pretreatments. On the other hand, the mean duration of the arrhythmic attacks in the surviving animals did not change significantly (Table 3).

The infarct size was measured in the animals surviving for 16 h after coronary artery ligation. We could not measure the size of damage during the acute phase of myocardial infarction, which is when most of the deaths occurred. The infarct size ranged between 20.7% ± 5.48% and 22.9% ± 3.83% in the DEA- and vehicle-treated animals, respectively. There were no significant differences in infarct size among the groups we investigated, and no correlation was observed between the severity of arrhythmias during the first 15 min and infarct size measured 16 h later.

At the end of the pretreatments we also determined the concentration of AMIO and DEA in the plasma and the myocardium. After AMIO (100 mg·kg^{-1}·day^{-1}), the plasma concentration of its metabolite (i.e., DEA) was about 1/4 that of the parent molecule (AMIO). In the myocardium, the tissue concentration of AMIO was significantly higher (about 10-fold, $P < 0.05$) than in the plasma, and its main metabolite, DEA, was at the same high level (Table 4). Chronic oral administration of DEA (50 mg·kg^{-1}·day^{-1}) produced a concentration of myocardial DEA (7.4 ± 0.7 μg·g^{-1}) similar to that measured after AMIO (100 mg·kg^{-1}) pretreatment.

Discussion

Anesthetized animal models with acute surgical intervention are commonly used for the investigation of the acute phase of myocardial infarction. In such conditions the anesthetic agent, artificial ventilation, and the acute surgery may greatly and variably influence the results (Baczkó et al. 1997). Therefore, it is especially important to use experimental conditions where the acute phase of myocardial infarction develops in conscious conditions, as was achieved in our current investigation.

Our experiments demonstrate that after chronic oral treatment the main metabolite of amiodarone, i.e., desethylamiodarone, produced antiarrhythmic effects in a rat model of acute coronary artery ligation induced ventricular arrhythmia that were similar to its parent compound.

These experiments using long-term treatment with DEA supports and extends previous investigations in a similar model of arrhythmia in rats, i.e., acute administration of 50 mg·kg^{-1} desethylamiodarone, i.p., improved the survival during the first 20 min after coronary artery ligation in rats (Varró et al. 1987). In the present experiments, DEA in a smaller dose (50 mg·kg^{-1}) produced the same plasma concentration as is measured after the double dose of AMIO (100 mg·kg^{-1}). This dose of DEA, on the other hand, offered an antiarrhythmic effect that was comparable with that of the higher dose of AMIO. The smaller dose of DEA may produce

Table 3. Influence of long-term amiodarone (AMIO) or desethylamiodarone (DEA) pretreatment on the appearance and duration of arrhythmias during the first 15 min after coronary artery occlusion in conscious rats surviving the acute phase of experimental myocardial infarction.

	Arrhythmia		Duration of arrhythmic attacks (s)				
Group	First (min)	Last (min)	VF	VT	Other	Br	Total
Vehicle							
Mean	4.72	9.00	3	17	18	3	41
SE	0.44	0.98	1.8	4.7	5.1	3.3	9.8
n	12	12	12	12	12	12	12
AMIO 30 mg·kg^{-1}							
Mean	5.17	9.37	6	9	18	10	42
SE	0.52	0.87	3.8	2.6	4.3	6.5	6.0
n	9	9	9	9	9	8	9
AMIO 100 mg·kg^{-1}							
Mean	9.95*	12.75*	2	16	12	2	32
SE	1.10	0.70	1.7	10.1	5.0	1.7	12.4
n	18	18	18	18	18	18	18
DEA 15 mg·kg^{-1}							
Mean	5.86	11.06	10	4	15	11	40
SE	0.80	1.29	10.0	1.8	7.1	11.3	17.7
n	8	8	8	8	8	8	8
DEA 50 mg·kg^{-1}							
Mean	7.56*	11.30	5	16	19	6	46
SE	1.05	1.01	3.4	6.4	9.3	5.7	15.8
n	14	14	14	14	14	14	14

Note: For details see Table 2.

Table 4. Plasma and myocardial concentration of amiodarone (AMIO) or its metabolite, desethylamiodarone (DEA) after long-term oral administration of AMIO or DEA in rats.

	Plasma (μg·mL^{-1})		Myocardium (μg·g^{-1})	
Group	AMIO	DEA	AMIO	DEA
Vehicle				
Mean	0.00	0.00	0.00	0.00
SE	0.00	0.00	0.00	0.00
n	4	4	4	4
AMIO 100 mg·kg^{-1}				
Mean	0.68	0.15	7.91	8.95
SE	0.10	0.03	1.25	2.21
n	12	11	30	30
DEA 50 mg·kg^{-1}				
Mean	0.00	0.20	0.00	7.35
SE	0.00	0.02	0.00	0.73
n	16	16	27	27

Note: For details see Table 2.

fewer adverse effects as well as less of a problem with drug interactions during long-term use.

Chronic AMIO treatment is the most effective antiarrhythmic drug treatment today, producing negligible negative inotropic effect and a very low incidence of proarrhythmia, which is the major problem with the long-term use of other antiarrhythmic drugs. On the other hand, the extracardiac adverse effects and unique pharmacokinetic properties of AMIO create several problems during routine use. AMIO is extensively and variably metabolized to desethylamiodarone in the first pass during the absorption. The major enzymes responsible for this reaction in human beings are the cytochrome P450 1A1, 3A4, and 1A2 isoenzymes (Elsherbiny et al. 2008). AMIO and DEA are further metabolized by dealkylation, hydroxylation, and deamination in human beings (Ha et al. 2001). Enzyme polymorphism, as well as the co-administration of enzyme inhibitors or inducers, greatly influence the rate of conversion of amiodarone, and are responsible for the great individual variations in response and for the interactions with other drugs or food (Heimark et al. 1992; Libersa et al. 2000; Werner et al. 2004; Becquemont et al. 2007).

Our results demonstrate that the antiarrhythmic effect of DEA after chronic oral treatment was achieved with similar plasma and tissue levels of DEA, without however, the presence of the relatively high levels of AMIO that occurred during the double dose of AMIO during the chronic treatment.

These results also support the hypothesis that during long-term administration, amiodarone undergoes significant metabolism, and its main metabolite, desethylamiodarone, accumulates in the myocardium and may contribute to the antiarrhythmic effect. This finding may also suggest the long-term use of DEA instead of AMIO as an antiarrhythmic tool in human beings. Since the adverse effects of chronic AMIO therapy presumably depend on tissue accumulation of the drug in various organs, it could be expected that substituting DEA for AMIO in clinical practice would result in a better therapeutic option, resulting in similar effectiveness with less of the limiting drug toxicity. Using the active metabolite of AMIO, i.e., DEA, may exclude individual variations in the metabolism of AMIO to DEA, as well as the interaction of this metabolic reaction with other drugs and food, and may result in a more easily optimized dosage and fewer adverse effects.

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