

Antiarrhythmic mechanisms of Malbec wine and resveratrol in isolated rat heart

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Abstract

Oxidative stress during myocardial reperfusion contributes to ventricular arrhythmias onset. We aim to evaluate the antiarrhythmic effect of Malbec wine and resveratrol and compare them with the synthetic antioxidant tiron. Since alcohol use is controversial, we also assessed dealcoholized wine.

Isolated hearts from male Sprague Dawley rats were perfused with Krebs-Henseleit solution added with: Malbec wine (5 ml/L), resveratrol (10 μ M,) compared to controls with alcohol (0.5 ml/L); dealcoholized wine (5 ml/L), tiron (10 mM) were compared with controls without alcohol. Epicardial action potentials were analyzed during basal state, regional ischemia and reperfusion (10 minutes each period). The incidence of arrhythmias was determined. The antioxidant effect was assessed in left ventricle homogenates and expressed as a percentage of inhibition of the ABTS^{•+} radical.

Malbec wine and resveratrol reduced reperfusion arrhythmias in 56% and 50%, respectively, compared to 100% incidence in the control group with alcohol.

Dealcoholized wine reduced arrhythmias to 50% compared to non-alcoholic control (90.5%), but tiron did not protect (69%). The free radicals inhibitory effect increased with all the compounds (resveratrol 54.2%, tiron 43.2%, Malbec wine 42.9%, dealcoholized wine 40.2%) with respect to the control groups (with alcohol 23.5%, without alcohol 21.2%). Resveratrol shortened action potential duration and prevented ischemic depolarization. Malbec prevented ischemic-induced action potential shortening.

We conclude that Malbec wine and resveratrol are antiarrhythmic beyond their antioxidant properties. Alcohol content or was not essential. Protection from ischemic action potential changes could be relevant to the antiarrhythmic effect of both resveratrol and wine.

Introduction

Acute ischemic events are emergencies relevant for every physician [1]. The best and earliest treatment for myocardial ischemia is reperfusion [2]. However, during reperfusion, oxidative stress and metabolic/electrolyte imbalance contribute to ventricular arrhythmias onset [3]. Indeed, brief periods of ischemia and reperfusion due to coronary artery vasospasm could underlie sudden cardiac death [4,5].

Antioxidants are potential therapeutic strategies against cardiovascular diseases, including reperfusion injury [6–9]. Particularly, wine attracted much attention as cardioprotective due to the so-called French paradox [10–12]. Prospective studies suggest that wine is more protective against cardiovascular diseases than other alcoholic beverages like beer or liqueur [6,13,14]. Alcohol addictive properties and the damage associated with abuse make the wine difficult to recommend [15]. Besides alcohol, other components in wine support its protective properties [10,11,13,15,16]. However, the electrophysiological effects of wine are missing.

Resveratrol is one of the most studied wine components both *in vivo* and *in vitro* [12,17,18]. Clinical studies support discrete cardiovascular protection only after supplementation with high doses of resveratrol [19–21]. Its antioxidant, anti-inflammatory, fibrinolytic, and anti-atherogenic effects protect the endothelial organ [22–24]. High concentration of this stilbenoid is antiarrhythmic [25–30]. At micromolar concentrations, resveratrol inhibits voltage-sensitive calcium and potassium currents and activates ATP sensitive potassium channels (IK_{ATP}) [18,25,26,29–31].

We aim to evaluate the electrophysiological effects (action potentials and arrhythmias) of Malbec wine and resveratrol. To clarify the role of antioxidant properties as a potential antiarrhythmic mechanism, we compared them with the synthetic antioxidant tiron [32]. Since alcohol use is mechanistically and epidemiologically controversial, we also assessed dealcoholized wine.

Materials and methods

The animals were treated according to the local Committee on Ethics of Animal Experimentation recommendations, which are in agreement with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health.

Male Sprague Dawley rats weighing 260 to 320 g were used. Until euthanasia, animals were housed in metal cages with food and water at libitum and exposed to a cycle of 12/12h of light/darkness, respectively. After the deep anesthetic plane was achieved (by 80 mg/kg ketamine and 5 mg/kg xylazine i. p.), the hearts were removed and mounted on

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the aortic cannula of the Langendorff perfusion setup in less than 3 min. A modified Krebs-Henseleit (KH) perfusion solution contained (mM): 121 NaCl, 5 KCl, 2 CaCl₂, 1.2 NaHPO₄, 1.2 MgSO₄, 25 NaHCO₃ and 10 glucose. The pH was 7.4 ± 0.05 when equilibrated with 5% CO₂ in O₂, and temperature the perfusate was kept at $36 \pm 0.5^\circ\text{C}$.

Six experimental series were performed

- 1) Control: KH without additional components (n=21);
- 2) De-alcoholized Malbec, KH plus 5 ml/L of wine dealcoholized as described below (n=10);
- 3) tiron, 10 mM of the sintetic antioxidant (n=13);
- 4) Malbec, 5 ml/L of wine, (n=9) the composition of the Malbec is described below;
- 5) Resveratrol, 10 μM of resveratrol dissolved in ethanol to final concentration of 0.5 ml/L, (n = 18)
- 6) Controls Alcohol, KH with the addition of 0.5 ml/L ethanol (n=12)

The Facultad de Ciencias Agrarias (School of Agricultural Sciences, National University of Cuyo) provided the red wine from the Malbec grape variety. The characterization by high-performance liquid chromatography of Malbec was previously published [33]. The batch of Malbec wine contained 2.9 g/L of total phenols, 0.5 g/L of total anthocyanins and 3.8 g/l of total tannins. Resveratrol concentration was 1.1 mg/L. The other main components were (in mg/L): A) non-flavonoids: 18.2 gallic acid; 2 caffeic acid; 4.2 cis- caftaric acid, B) flavonoids: 24.1 catechin; 14.2 epicatechin; procyanidin (11.3 B1; 3.1 B3), C) flavonols: 4.9 quercetin, and D) anthocyanins: 344 malvidin-3-glucoside; 16.2 peonidin-3-glucoside; 60.3 delphinidin-3-glucoside.

Malbec wine was dealcoholized by rotary evaporation at low temperature and pressure as previously described [34]. To conserve the phenolic composition, we added a volume of water according to the alcohol evaporated.

Although resveratrol was not the most abundant phenolic component in the wine, we decided to use it as the comparator because resveratrol is the best electrophysiologically characterized. The concentration used in this study is also in agreement with previous publication showing its cardioprotective properties [25–30]. Unless specified, all the other reagents were from Sigma-Aldrich (Saint Louis MO, USA).

After 20 min of equilibration, action potential and ECG were recorded during three periods of 10 minutes: basal state, ischemia (by anterior descending coronary artery occlusion with a 6/0 silk thread), and reperfusion. Ischemia and reperfusion were efficiently confirmed by coronary flow measurements. A reduction of 25% or more, after occlusion was corroborated in all the hearts.

Transmembrane potential was recorded glass microelectrodes (filled with 3 M KCl) from epicardial ventricular cells and synchronously displayed with the electrocardiogram (ECG), obtained with a positive electrode in the apex and the negative close the right atria. Microelectrodes (10 to 15 M Ω resistances) were amplified five times with custom-made equipment. Both signals were photographed from the screen of the oscilloscope (Tektronix 565) with a C4 Grass camera. The following action potential characteristics were measured: resting membrane potential (RMP), action potential amplitude (APA), and action potential duration at 90% of repolarization (APD₉₀). The heart rate was measured from the ECG and the cell recordings. Arrhythmias were classified according to the Lambeth conventions [35]. A severity

score of the arrhythmias was determined every minute. The values of the scale were: 0 sinus rhythm, 1 premature ventricular beats or bigeminy, 2 Salvos, 3 nonsustained ventricular tachycardia (<30 s), 4 sustained ventricular tachycardia (>30 s), and 5 ventricular fibrillations. The hearts included in this study did not present sustained arrhythmias before reperfusion.

After of reperfusion, samples of 10 mg from the left ventricle free wall were transferred to Eppendorf tubes containing phosphate buffered saline, pH 7.4, and stored at -75°C . All samples were homogenized keeping the temperature low by immersion of the tubes in ice during the process. The total antioxidant capacity was determined as previously described [36,37]. In brief, the radical ABTS•+, monocation of 2,29-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) is generated by ABTS oxidation with potassium persulfate and read at 600 nm with a UV-visible Spectrometer (Helios Gama, Helios Delta, Unicam instruments, UK). In the presence of hydrogen-donating antioxidants the cation is reduced and the color inhibited. The changes in the color induced by the samples were measured after 18 min of incubation at 37°C . The results are expressed as ABTS• + radical inhibition in percentage.

Data are expressed as means \pm SEM. The results were analyzed with two-way ANOVA followed by the Student Newman Keuls posttest. The incidence of arrhythmias was tested with contingency tables using the Fisher exact test. The statistical level of significance was $P < 0.05$.

Results

Arrhythmias

Brief ventricular tachycardia developed during the first seconds of flow restitution in control group regardless the presence of alcohol. One example of this arrhythmias is shown in Figure 1. In both control groups (with and without alcohol), the tachycardia persisted during reperfusion and alternated with episodes of ventricular fibrillation. The severity score of arrhythmias persisted high through the ten minutes evaluated (Figure 2).

Malbec wine prevented the onset of reperfusion arrhythmias (Figures 1 and 2). However, when arrhythmias developed in wine treated hearts, the severity persisted high like in the controls. Therefore, the severity score reflects the combination of hearts in sinus rhythm and hearts with sustained arrhythmias.

Resveratrol antiarrhythmic effect was like the one observed with Malbec wine. The hearts that suffered arrhythmias recovered sinus rhythm in less than 60 seconds. At the end of the ten minutes of reperfusion, the proportion of hearts without arrhythmias was the same that the Malbec group.

The synthetic antioxidant tiron did not reduce ventricular arrhythmias. Ventricular fibrillation developed during the first minute of reperfusion in most of the hearts and persisted during the following ten minutes.

Dealcoholized wine was as protective as Malbec and resveratrol. The preventive action was present in half of the hearts and persisted through the reperfusion period.

Action potentials

The morphology of action potentials during the different phases of the experiment showed the same pattern for both control groups (Figure 3). Therefore, we decided to show representative traces from the control with alcohol group to compare them with Malbec and resveratrol-

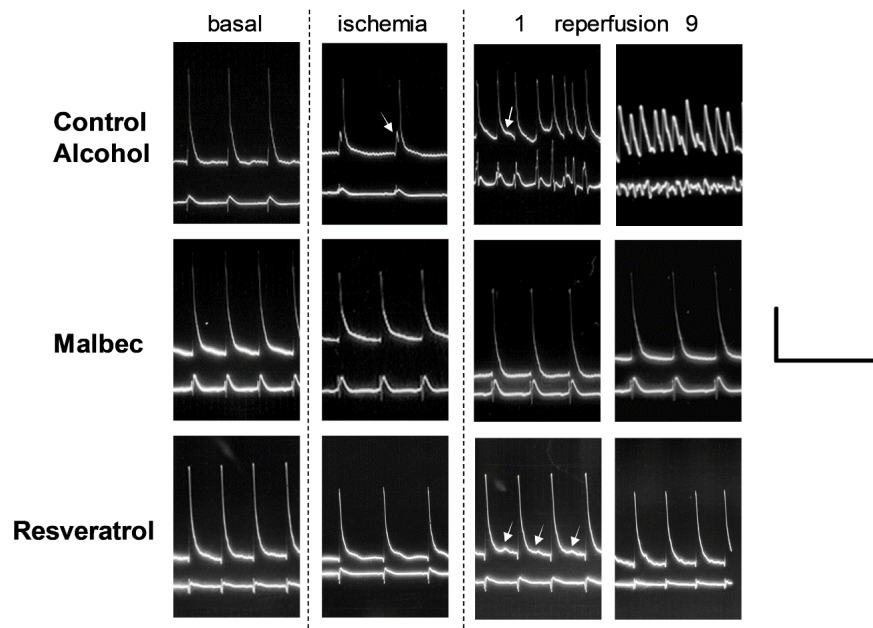


Figure 1. Representative action potentials and ECG from Control Alcohol, Malbec and Resveratrol groups. The columns indicate the experimental period; 1 and 9 correspond to the minute of reperfusion. The horizontal scale on the right corresponds to 0.5 seconds and the vertical scale correspond to 40 mV for action potentials and to 2 mV for ECG signal. Arrow in Control Alcohol during ischemia indicates the delay in activation and during reperfusion, the oscillation of membrane potential without reaching a resting state. Arrows during Resveratrol reperfusion indicate delay after depolarizations.

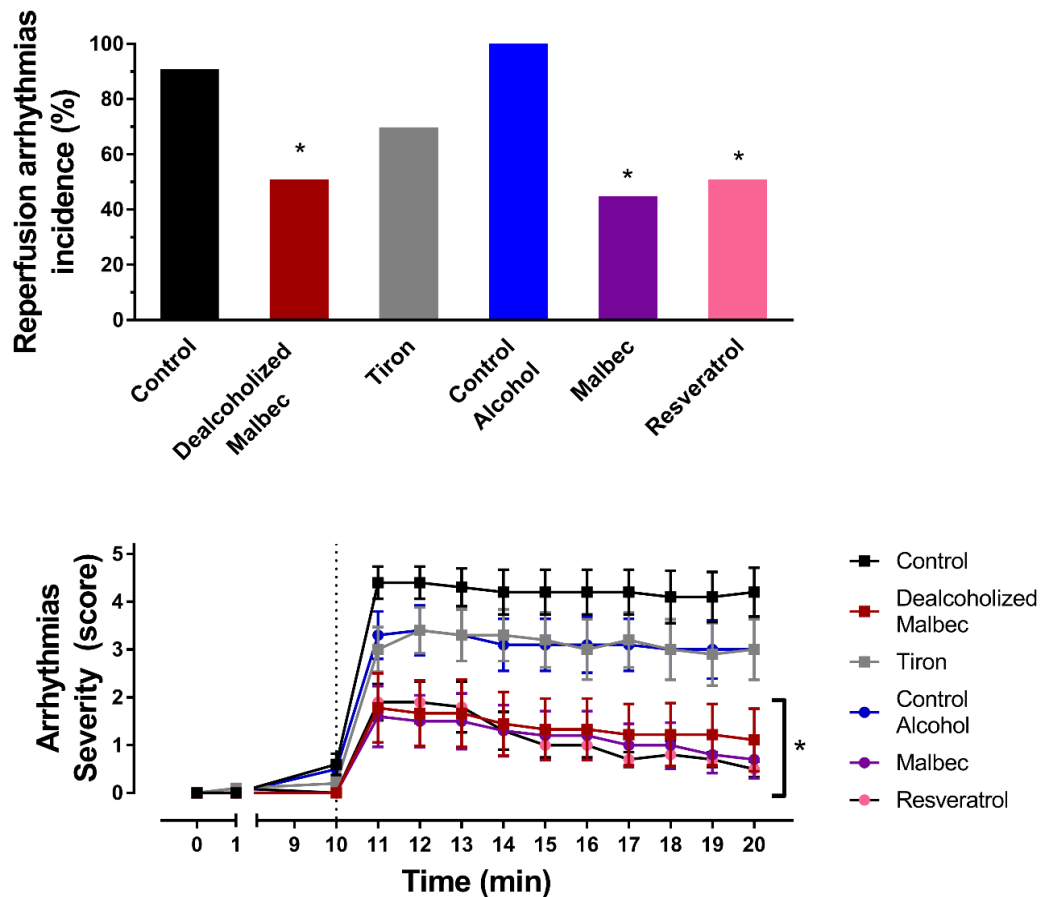


Figure 2. Reperfusion arrhythmias. Above is quantified the combined incidence of ventricular tachycardia and fibrillation. Below is depicted the arrhythmic score observed minute-by-minute during reperfusion. * $P < 0.05$ versus the corresponding control (either with or without alcohol).

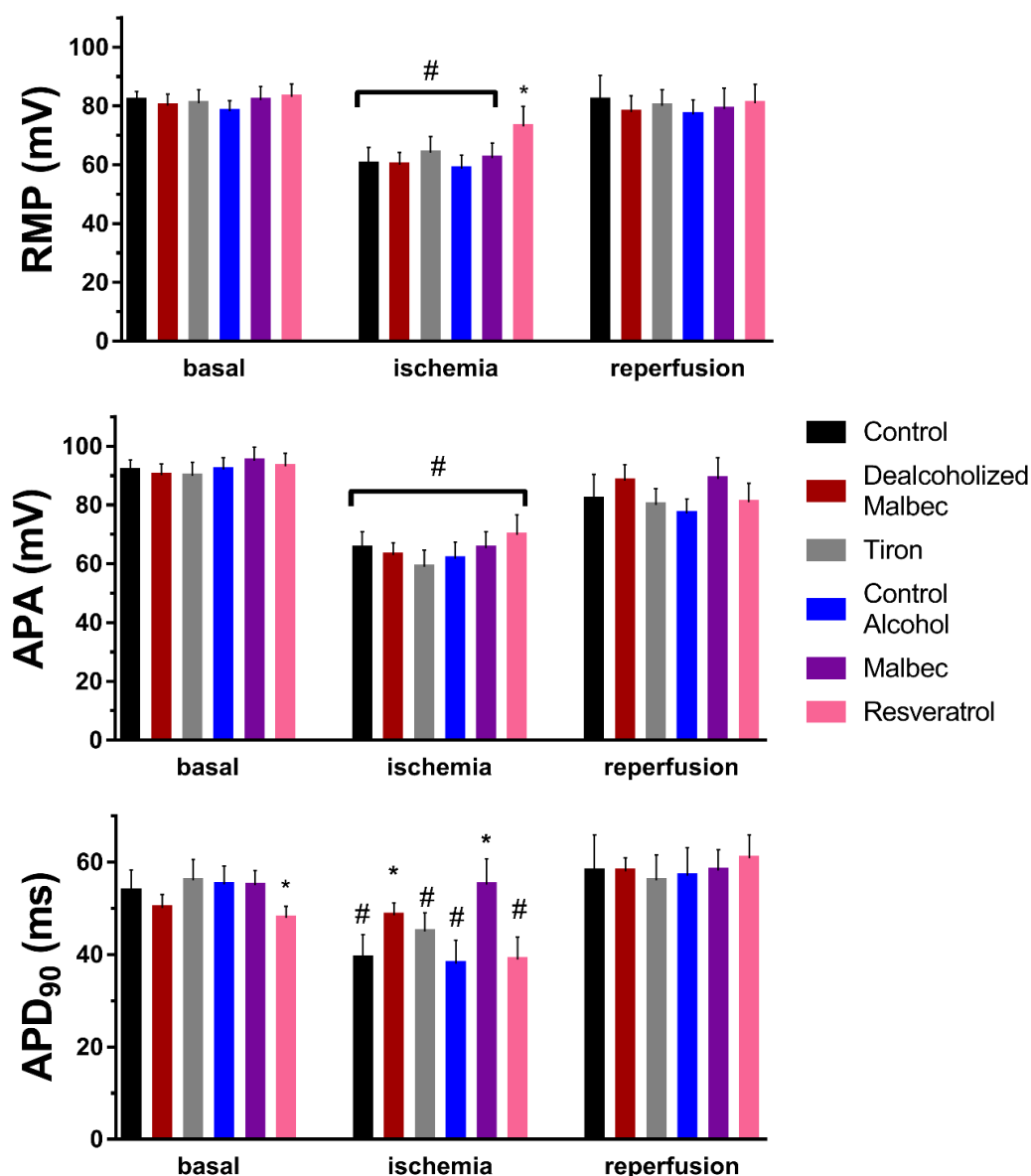


Figure 3. Action potential variables during the three experimental periods. Resting membrane potential (RMP), action potential amplitude (APA) and action potential duration measured at 90% of the repolarization (APD₉₀). * P<0.05 versus the corresponding control (either with or without alcohol), # P<0.05 versus basal state values for the same group.

treated hearts (Figure 1). During the basal state, resveratrol shortened action potential duration during basal state, but resting potential and amplitude remained unchanged concerning Control. Malbec, tiron, and dealcoholized wine did not change the resting membrane potential, action potential amplitude, or duration of basal state recordings.

Action potentials from control hearts during ischemia showed resting membrane depolarization, an activation delay before the steepened depolarization (indicated by the arrow), a reduction of the amplitude and a shortening in action potential duration (Figure 1). Malbec wine with and without alcohol prevented the action potential shortening induced by ischemia (Figures 1 and 3). Resveratrol was the only treatment that preserved the resting potential during ischemia. Tiron did not modify the action potentials during ischemia.

During reperfusion, the number of action potentials to evaluated was reduced due to the presence of irregular rhythms, as can be seen in

Figure 1. The membrane oscillation could be named afterdepolarizations, but the resting potential was not clear in many cases. Therefore, the values in Figure 3 only corresponds to action potentials of beats in sinus rhythm confirmed in the ECG signal.

Interestingly, resveratrol showed delay after depolarization during the first minutes of reperfusion (see arrows in Figure 1), but the lower resting potential probably prevented the membrane from reaching the threshold to start a new action potential.

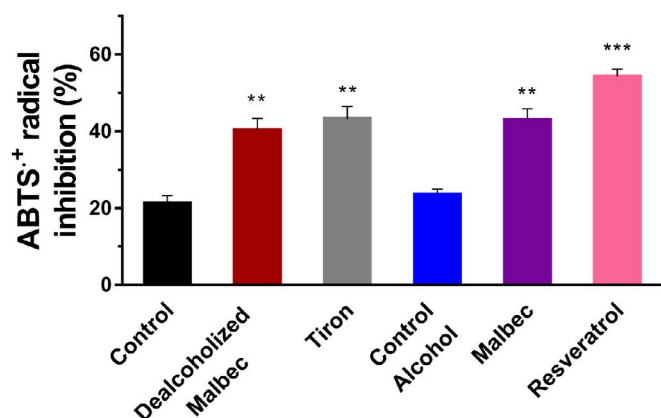
In tiron hearts that were in sinus rhythm during reperfusion, the action potentials recovered to the values observed during the basal state.

Antioxidant

The addition of alcohol to the KH solution did not increase the antioxidant capacity of the ventricles (Figure 4). Tiron and dealcoholized

Table 1. Heart rate and coronary flow in the isolated rat heart subjected to regional ischemia and reperfusion.

Group	Heart rate (beats/min)		Coronary flow (ml/min)		
	Before ligation	Before reperfusion	Before ligation	During ligation	Reperfusion
Control	275.7 ± 12.5	239.7 ± 10.4 #	15.0 ± 1.4	7.4 ± 1.4 #	14.3 ± 1.8
Dealcoholized Malbec	290.7 ± 13.0	223.5 ± 10.2 #	13.6 ± 0.8	6.9 ± 0.9 #	10.3 ± 1.6
Tiron	279.2 ± 14.4	236.7 ± 11.9 #	14.9 ± 1.5	7.5 ± 0.8 #	13.2 ± 1.9
Control Alcohol	270.7 ± 12.0	256.7 ± 11.9 #	17.6 ± 1.6	9.4 ± 0.9 #	14.1 ± 1.1
Malbec	291.2 ± 12.8	256.7 ± 11.9 #	13.4 ± 1.5	7.0 ± 1.2 #	14.2 ± 1.5
Resveratrol	298.1 ± 12.1	231.8 ± 12.4 #	15.2 ± 1.4	9.1 ± 1.0 #	15.4 ± 1.3

**Figure 4.** Antioxidant effect of ventricular homogenates measured as percentage of inhibition of the ABTS•+ radical. ** P<0.01 and *** P<0.001 versus the corresponding control (either with or without alcohol).

Malbec increased the antioxidant power in the myocardial tissue when compared to the control group. Malbec also increased the antioxidant capacity respect to both control and control with alcohol groups. The most effective antioxidant in this study was resveratrol.

Heart rate and coronary flow

Heart rate was the same in all groups before ischemia (Table 1). During ischemia, heart rate decreases around 20 to 50 beats/min in all hearts. Values of heart rate from reperfusion are not shown due to big disparities caused by arrhythmias.

The coronary flow remained stable in all the hearts without difference between the treatments (Table 1). After coronary ligation, the flow decreased 30 to 50 % in all cases. Reversibility of this reduction confirmed that reperfusion was successfully achieved.

Discussion

This study describes antiarrhythmic properties of Malbec wine and confirms resveratrol ones [25–30]. The electrophysiological mechanisms were different between them. During ischemia, Malbec prevented action potential shortening, and resveratrol prevented resting depolarization. Our results with dealcoholized wine refute any contribution or interference of alcohol to wine electrophysiological effects. The lack of effect of antioxidant tiron questions the role of free radicals as the primary determinant of reperfusion arrhythmias.

To our knowledge, this is the first report of the direct antiarrhythmic effect of Malbec wine (both with and without alcohol). Epidemiological studies suggested that red wine consumption is associated with a reduction of mortality and morbidity from ischemic heart disease, but there is no direct evidence to support wine as antiarrhythmic [6,11,15,16,18]. The antiarrhythmic mechanisms of natural drugs are usually deductions based on physiopathological reasoning, *in vitro*

determinations and theoretical conclusions [38]. Here we demonstrate action potential modification by wine as a plausible explanation for its antiarrhythmic action after ischemia/reperfusion in rat hearts. Since the changes observed with wine occurred during ischemia, further electrophysiological characterization by techniques like patch clamp will be difficult. Reproducibility is a potential problem because wine composition changes over time due to the varieties and vinification [39–41]. Our results interpretation should be taken with caution because they were obtained in isolated hearts, without autonomous nervous system or hemodynamic stress. The translation to human arrhythmias is unknown, but rat and human electrophysiology are markedly different [42–44].

The electrophysiological characteristics of Malbec and resveratrol in ventricular myocytes may play an important role in its antiarrhythmic effects beyond its antioxidant and free radical scavenging actions. Tiron was as antioxidant as Malbec but failed to protect from arrhythmias or modified action potentials. The preventive effect of Malbec against action potential shortening during ischemia could homogenize repolarization and reduce the substrate for reentrance [45–47]. We have previous results that support the protective role of antiischemic modification with melatonin (also a wine constituent) [37,48]. Besides, the activation was not delayed (compare the delay indicated by the arrows in control hearts from Figure 1) during ischemia suggesting that conduction could be preserved. Resveratrol also preserved conduction during ischemia, but the main change observed was relative hyperpolarization. This could be attributed to the reported activation of $I_{K_{ATP}}$ channels [26,27,31,49–51]. During ischemia, this channel open do to ATP/ADP imbalance, oxidative stress and acidosis [46,52–54]. Indeed, hyperpolarization could counteract triggered arrhythmias originated from delay after depolarizations, because the membrane threshold is more difficult to reach.

Despite the beneficial effects of doses of red wine, it is a health risk due to liver damage and physical addiction [11,15]. The antiarrhythmic effect of dealcoholized wine could overcome the problems mentioned above. We have previously reported protective effects of dealcoholized red wine against vascular remodeling and metabolic syndrome [34]. Our results support the nutraceutical properties of dealcoholized wine [11]. Another non-alcoholic derivative from grapes, like pomace, also possesses cardioprotective potential [55].

We could not attribute our results to any specific component of wine. For example, resveratrol content of Malbec was one thousand times lower than the one in Resveratrol group [12,20,21,56]. Also, most of the electrophysiological properties describe until now were obtained above the micromolar concentration range of resveratrol [25–30]. These concentrations are impossible to be achieved by Malbec or any red wine consumption alone [12,33]. Catechin, melatonin, and quercetin are also wine components, but the effective concentrations are at least two orders of magnitude below the active electrophysiological levels [7,12,36,57–59].

We conclude that Malbec wine is an antiarrhythmic as effective as resveratrol, beyond its antioxidant properties and unrelated to alcohol content. Protection from ischemic action potential shortening could be a relevant contributor to the antiarrhythmic effect of wine.

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