

## In vitro effects of amiodarone on coronary artery bypass grafts

*Amiodaronun koroner arter baypas greftleri üzerine in vitro etkileri*

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### ABSTRACT

**Background:** This study aims to investigate the effects of amiodarone on the most commonly used grafts, internal thoracic artery (ITA), saphenous vein (SV), and radial artery (RA) in organ bath.

**Methods:** Twenty patients (16 males, 4 females; mean ages 58.4±9.9 years; range 38 to 80 years) who underwent isolated coronary artery bypass graft (CABG) surgery between May 2008 and October 2008 were included in this study. Internal thoracic artery, saphenous vein, and radial artery grafts were harvested. Specimens were taken to laboratory in +4 °C Krebs solution. Specimens were suspended in 10 ml organ bath containing Krebs solution.

**Results:** Amiodarone caused relaxation in all grafts (ITA, RA, SV) between 10<sup>-9</sup>-10<sup>-3.5</sup> M concentration in a dose dependent manner (p<0.01). Maximum relaxation rates (mean) induced by amiodarone were 78.9%, 74.9% and 66.5% for ITA, RA and SV, respectively.

**Conclusion:** Although we did not evaluate the endothelium-independent relaxation response in this study, higher rates of relaxation response were observed with ITA grafts comparing to other grafts, and these results were compatible with literature. According to the results of this study, amiodarone-class III antiarrhythmic agent- caused vasodilation in all three grafts *in vitro*. Vasodilator effect of amiodarone on grafts may help to increase patency rates.

**Keywords:** Amiodarone; internal thoracic artery; organ bath; radial artery; saphenous vein.

### ÖZ

**Amaç:** Bu çalışmada amiodaronun en çok kullanılan greftler olan internal torasik arter (İTA), safen ven (SV) ve radial arter (RA) üzerine etkisi organ banyosunda incelendi.

**Çalışma planı:** Mayıs 2008 - Ekim 2008 tarihleri arasında izole koroner arter baypas greft (KABG) ameliyatı olan 20 hasta (16 erkek, 4 kadın; ort. yaş 58.4±9.9 yıl; dağılım 38-80 yıl) çalışmaya dahil edildi. İnternal torasik arter, safen ven ve radial arterler toplandı. Örnekler +4 °C Krebs solüsyonunda laboratuvara taşındı. Örnekler Krebs solüsyonu içeren 10 ml organ banyosunda askıya alındı.

**Bulgular:** Amiodaron 10<sup>-9</sup>-10<sup>-3.5</sup> M konsantrasyon aralığında tüm greftlerde (ITA, RA, SV) doz bağımlı gevşemeye yol açtı (p<0.01). Amiodaron ile indüklenen maksimum gevşeme oranları (ortalama) İTA, RA ve SV'de sırasıyla %78.9, %74.9 ve %66.5 idi.

**Sonuç:** Bu çalışmada endotelden bağımsız gevşeme yanıtı incelenmemiş olsa da İTA grefti ile diğer greftlere göre daha yüksek oranlarda gevşeme yanıtı gözlemlendi ve bu sonuçlar literatür ile uyumlu idi. Bu çalışmanın sonuçlarına göre, sınıf III antiaritmik ajan olan amiodaron *in vitro* olarak her üç greftte de vazodilatasyona neden oldu. Amiodaronun greftler üzerindeki vazodilatör etkisi açık kalma oranlarını artırmaya yardımcı olabilir.

**Anahtar sözcükler:** Aminodaron; internal torasik arter; organ banyosu; radial arter; safen ven.



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The internal thoracic artery (ITA), saphenous vein (SV) and radial artery (RA) are the most common grafts used in coronary artery bypass grafting (CABG) operations.<sup>[1]</sup> However, structural and physiological differences in the grafts affect both short- and long-term patency, which can also be unfavorably affected when late relaxation of the vasospasm develops due to mechanical or pharmacological causes in the perioperative period.<sup>[2]</sup>

Amiodarone (Cordarone, Sanofi Aventis, Turkey) has been accepted as a prototype of the class III anti-arrhythmic drugs for a long time. However, the pharmacological effects of amiodarone are very complex. For instance, with long-term usage, the action potential time and refractory period are extended.<sup>[3,4]</sup> Additionally, this agent inhibits rapid sodium channels and slow calcium channels,<sup>[5,6]</sup> it was known that its effects are anti-sympathetic, thyroid and lipid metabolism-converting and also it has affects on some cytokine's production metabolism.<sup>[7-9]</sup> Amiodarone can be administered intravenously or orally in the preoperative, intraoperative, or postoperative periods, and it is the drug of choice after CABG operations.<sup>[10]</sup> We found several reports in the literature that focused on the effects of several agents on specimens in an organ bath, but none of these analyzed amiodarone.<sup>[11]</sup> Evidently, in spite of its frequent use, the effects of this agent on grafts are not well understood. Therefore, in this study we aimed to analyze the effects of amiodarone on the ITA, SV, and RA in an organ bath.

**PATIENTS AND METHODS**

Twenty patients (4 females, 16 males; median age 58.4±9.9; range 38 to 80) who underwent isolated

CABG surgery between May 2008 and October 2008 were included in this study with their permission, and we also obtained the approval of the local ethics committee.

Amiodarone, potassium hydrochloric acid (Sigma-Aldrich, St. Louis, MO, USA), and phenylephrine (Sigma-Aldrich, St. Louis, MO, USA) were used in this study, all of which were dissolved in distilled water.

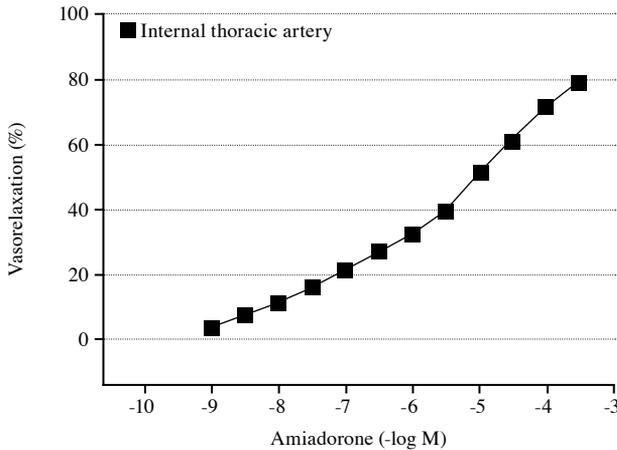
We assessed a total of 48 samples (16 RA, 16 ITA, and 16 SV), and Table 1 shows the perioperative status of the 20 patients. The ITA was harvested along with its peduncle via scissors in order to protect the endothelium from damage, and a section was taken prior to the papaverine administration. The SV was also harvested with its peduncle, and the distal portion was sampled while the RA was harvested in a skeletonized fashion using scissors. All of the specimens were taken to the laboratory in +4 °C Krebs solution.

Next, the specimens were cut into 2 mm rings and suspended in a 10 ml organ bath chamber containing the Krebs solution, which was composed of the following: 122 mmol/L sodium chloride (NaCl), 5 mmol/L potassium chloride (KCl), 1.25 mmol/L calcium chloride (CaCl<sub>2</sub>), 25 mmol/L sodium bicarbonate (NaHCO<sub>3</sub>), 1.2 mmol/L magnesium sulfate (MgSO<sub>4</sub>), 1.0 mmol/L monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>), and 11.5 mmol/L glucose. Then the medium was gassed with a mixture of carbon dioxide (CO<sub>2</sub>) (5%) and oxygen (O<sub>2</sub>) (95%) and maintained at 37 °C (pH 7.4). Next, the specimens were hooked transversely and connected to an FDT10-A forced displacement transducer (COMMAT Ltd., Ankara, Turkey) which was connected to a TDA-97 transducer data acquisition system (COMMAT Ltd.,

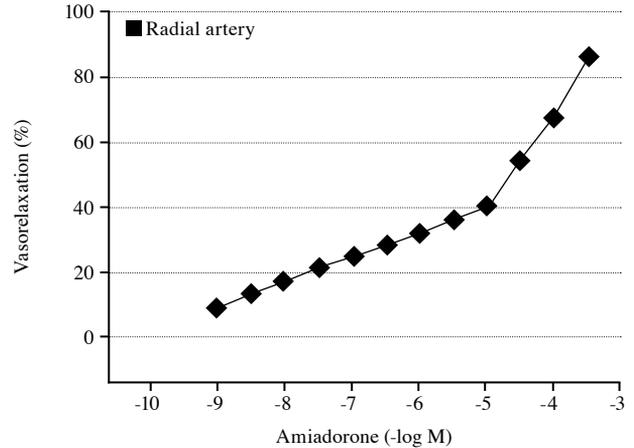
**Table 1. Preoperative and postoperative variables**

Specimens	Internal thoracic artery (n=16)		Radial artery (n=16)		Saphenous vein (n=16)	
	n	Mean±SD	n	Mean±SD	n	Mean±SD
Age (year)		58.4±9.9		50.0±15.2		50.0±15.2
Sex						
Females	4		4		4	
Males	12		12		12	
Body mass index		27.1±3.37		30.3±2.2		30.3±2.2
Ejection fraction (%)		53.0±8.5		54.8±9.0		54.9±9.0
Cross-clamp time (min)		58.3±25.7		54.0±24.1		54.0±24.1
Total bypass time (min)		107.8±28.1		95.0±40.5		95.0±40.5
Diabetes mellitus	8		6		8	
Hypertension	8		8		6	
Hypercholesterolemia	4		6		4	
Preoperative beta blockers	16		16		16	

SD: Standard deviation.



**Figure 1.** The dose-dependent relaxation response curve of amiodarone showing that the *in vitro* ITA contracted  $10^{-6}$  M with phenylephrine.



**Figure 2.** The dose-dependent relaxation response curve of amiodarone showing that the *in vitro* radial artery contracted  $10^{-6}$  M with phenylephrine.

Ankara, Turkey) that sent signals to a computer. The vascular responses were then recorded and analyzed with POLWIN97 acquisition software (COMMAT, Ankara, Turkey). All vascular tissues were stretched to a basal resting tension of 2 g. The preparations were allowed to equilibrate for 80 minutes, and the Krebs solution was refreshed every 20 minutes. Furthermore, smooth muscle cell contractility was measured by the addition of 90 mmol/L KCl to the bath. The vascular endothelium was also tested with epinephrine ( $10^{-6}$  M) for contraction and acetylcholine ( $10^{-6}$  M) for relaxation at the beginning and end of the experiments, and those that did not respond properly were excluded from the study. In order to standardize the assessment, all of the responses were calculated based on the percentage of responses in the control trace obtained at the beginning of the experiment.

### Statistical analysis

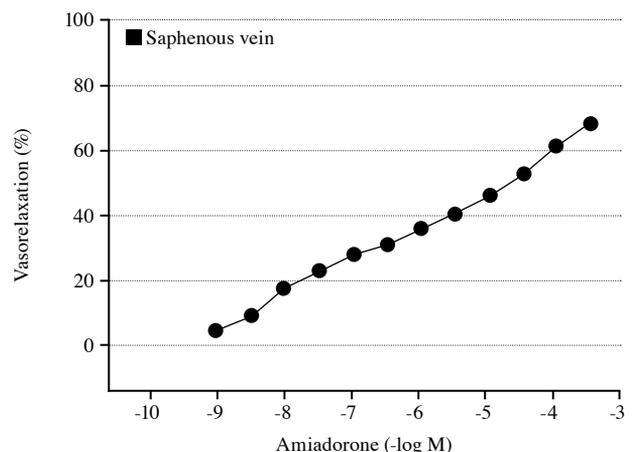
GraphPad Prism 6.0a for Mac OS X 10.9 software (GraphPad Software, Inc., La Jolla, CA, USA) was used to draw concentration-response graphs and analyze the data, and a linear regression (variable slope) analysis was applied to the graphs. A p value of <0.05 was considered to be statistically significant.

## RESULTS

Human ITA, RA and SV were contracted to the submaximal level with  $10^{-6}$  M phenylephrine, and a relaxation response to the amiodarone was obtained using the cumulative method from a concentration of between  $10^{-9}$  M and  $3.10^{-4}$  M. The relaxation induced by the amiodarone was standardized to the contractions induced by the phenylephrine, and the mean minimum

and maximum dose-response values of the ITA grafts were calculated as 3.5%, and 75.9% respectively (Figure 1). In addition, the values for the RA grafts were 7.9%, and 74.9%, respectively (Figure 2), and for the SV grafts, they were 3.9% and 66.5%, respectively (Figure 3).

The amiodarone produced a dose-dependent relaxation in all of the grafts in the concentration range of between  $10^{-9}$  and  $3.10^{-4}$  M, and the average maximum relaxation percentages induced by the amiodarone in the ITA, RA and SV grafts were  $78.9 \pm 1.44$ ,  $74.9 \pm 2.29$ , and  $66.5 \pm 2.85$ , respectively. The amiodarone produced the highest relaxation in the ITA grafts followed by the RA. The SV grafts had the lowest relaxation percentage. Although the maximum relaxation rates in the ITA and RA were



**Figure 3.** The dose-dependent relaxation response curve of amiodarone showing that the *in vitro* saphenous vein contracted  $10^{-6}$  M with phenylephrine.

**Table 2. Comparison of maximum relaxation with amiodarone in the internal thoracic artery, saphenous vein, and radial artery\***

	Mean difference	95% CI of difference	<i>p</i>
Saphenous vein vs. internal thoracic artery	-12.41	-20.39 to -4.439	<0.01
Saphenous vein vs. radial artery	-8.481	-16.45 to -0.5075	<0.05
Internal thoracic artery vs. radial artery	3.931	-4.042 to 11.90	>0.05

CI: Confidence interval; \* As calculated via analysis of variance (ANOVA), post hoc, and Bonferroni tests.

not statistically significantly different, the effect in the SV was statistically significantly lower than that of the ITA ( $p < 0.01$ ) and RA ( $p < 0.05$ ) (Table 2).

## DISCUSSION

Amiodarone is a well known, effective anti-arrhythmic agent that is also beneficial for preventing sudden cardiac death in hypertrophic cardiomyopathy.<sup>[12]</sup> Following CABG operations, atrial fibrillation (AF) occurs in 5-65% of patients,<sup>[13,14]</sup> and amiodarone, which is highly tolerated, can be used before, during or after CABG operations to prevent and treat arrhythmias via peri-oral or intravenous administration.<sup>[15]</sup> Preoperative amiodarone prophylaxis reduces by 25% the incidence of postoperative AF, but postoperative adverse events, such as bradyarrhythmia, acute lung injury, and infusion-related hypotension, have been reported with this agent.<sup>[16]</sup>

Using ITA, RA, and SV grafts during CABG operations may affect the short- and long-term patency rates because of their different characteristics with regard to contraction and endothelial function,<sup>[17]</sup> and these differences depend on the physiological and anatomical properties of the grafts. Thus, it is accepted that postoperative graft function and patency are affected by the susceptibility to vasospasm, which can occur due to either physical factors, such as surgical manipulations, or biochemical, molecular and pharmacological factors, for example an increase in smooth muscle hyperactivity.<sup>[17]</sup> As a result, increased perioperative morbidity and postoperative myocardial failure, which can lead to death, may be seen.

Initially, the endothelial layer was thought to be made up of a simple array of cells that lined up between the vessel lumen and intima. However, with the discovery of endothelium-derived vasoactive substances, the complex functions of the vascular endothelium are now better understood. The endothelium-dependent hyperpolarization factor (EDHF) plays an important role in ensuring the appropriate vascular tone. In addition to variations in the components of EDHF, there may be dissimilarities because it includes at least three components [endothelium-dependent nitric

oxide (NO), prostaglandin I<sub>2</sub> (PGI<sub>2</sub>), and the EDHF itself].<sup>[18]</sup> Endothelium-dependent relaxation occurs as a result of the effect of various factors caused by the vasodilatation of different mechanisms. Endothelium NO causes vasodilatation by using cGMP pathway and PGI<sub>2</sub> uses the cAMP pathway. The structure of EDHF is not fully understood, however it is through that it creates hyperpolarization by affecting the potassium channels of smooth muscle cells.<sup>[18]</sup> Endothelium-dependent relaxation also plays an important role in providing appropriate vascular tonus. In this study, the arterial graft samples had an increase in basal production as well as a higher NO release than the SV grafts. Furthermore, Luscher et al.<sup>[19]</sup> found a significant difference in favor of ITA in terms of the NO release versus the SV, and Sellke et al.<sup>[20]</sup> attributed the higher patency rate of ITA to the higher basal NO release.

In clinical studies, the most common side effect of intravenous amiodarone administration was hypotension, with incidence rates ranging between 15 and 26%.<sup>[12]</sup> Additionally, in experimental studies that involved that use of dog hearts, the vasodilatory effects of amiodarone were only partially endothelium-dependent.<sup>[21]</sup> Moreover, according to Gessner et al.,<sup>[22]</sup> the amiodarone derivate KB130015 induced endothelium-independent relaxation in porcine pulmonary arteries, and Traupe et al.<sup>[23]</sup> reported that amiodarone acutely increased endothelium-dependent vasodilation and phasic contractions in atherosclerotic arteries in a gender-dependent fashion.

Despite the frequent use of commercial amiodarone after CABG operations, to our knowledge, no one has addressed the impact that this agent has on graft function in the literature. In this in vitro study, amiodarone resulted in relaxation for all three graft samples, with the relaxation responses in both arterial grafts being higher than in the SV. Furthermore, although this study did not evaluate the endothelium-independent relaxation response, the higher rates in the ITA grafts were compatible with the literature; thus, we concluded that these were associated with the anatomical and physiological properties of the graft.

At this point, we think that the differences in the NO synthesis and release rates from the ITA grafts and the differences in the level of the receptors on ITA grafts may affect this condition. Furthermore, the variations in the relaxation response between the arterial grafts and the SV may be explain with the same reasons. However, further studies are needed to investigate our suppositions.

### Conclusion

According to the results of this study, amiodarone, a class III anti-arrhythmic agent, dilated the ITA, RA, and SV grafts used in CABG in vitro, and based on the vasodilator effect of amiodarone on these grafts, the use of this agent may help reduce the short-term complications associated with this type of surgery.

### Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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