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RESEARCH ARTICLE

Effect of Resveratrol in Acute Hypoxic Pulmonary Vasoconstriction in Isolated Lamb Pulmonary Arteries and Veins

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Summary

Resveratrol (RSV) is a natural phytoalexin with many biological effects, including antioxidant, anti- platelet, anti- atherogenic, anti- inflamatory, estrogenic properties, immunomodulation and chemoprevention. In the cardiovascular system, it has been shown to cause relaxation of vascular smooth muscle, and reports indicate that RSV induces vasodilatory effects in several vascular beds by acting in endothelium dependent and independent manners. The aim of the study was to determine the role of RSV on hypoxia induced vasoconstriction in precontracted isolated pulmonary arteries and veins. Isolated lamb pulmonary vessels were suspended in an organ bath filled with Krebs Heinseleit solution and isolated contractions were recorded continuously via an isometric transducer connected to a computerized polygraph system. The solution was aerated with room air or a gas mixture of 95% N_2 + 5% CO_2 (hypoxic). The oxygen concentration of the bathing medium was measured using an oxygen electrode. Serotonin (5-HT)(10^{-5} M) for arteries and U46619, a thromboxane analog (TXA2) for veins, were used as pre contractile agents. Our results showed that in the presence of RSV (20 µM), hypoxia induced pulmonary vasoconstriction decreased in pulmonary veins pre contracted with U46619, but not in pulmonary arteries pre contracted with 5-HT.

Keywords: *5-HT, Hypoxia, Pulmonary vasoconstriction, Resveratrol, U46619*

Resveratrolün İzole Kuzu Pulmoner Arter ve Venlerinde Akut Hipoksik Pulmoner Vazokonstriksiyona Etkileri

Özet

Resveratrol (RSV), antioksidan, antitrombositik, antiaterojenik, antienflamatuar, immunomodülatör, kimyasallara karşı koruyucu ve östrojenik özellikleri dahil olmak üzere çeşitli biyolojik etkileri olan doğal bir fitoleksindir. Kardiyovasküler sistemdeki etkileri incelendiğinde damar düz kaslarında gevşetici etkisi; çeşitli damar yataklarında endotele bağımlı ve endotelden bağımsız mekanizmalarla vazodilatatör etkilere sebep olduğu bildirilmiştir. Bu çalışmada önkasılım oluşturulmuş izole kuzu pulmoner arter ve venlerinde, hipoksinin damar yanıtlarına etkisi ile hipoksiye bağlı vazokonstriksiyonda resveratrolun etkisi araştırıldı. Krebs-Heinseleit solusyonu ile doldurulmuş organ banyosuna asılmış izole kuzu pulmoner damarları izometrik transduser aracılığı ile bilgisayarlı poligraf sisteminde sürekli kayıt edilmiştir. Çözelti oda havasıyla (normoksik) veya %95 N_2 + %5 CO_2 karışımıyla (hipoksik) havalandırılmıştır. İzole organ banyosundaki solüsyonun oksijen konsantrasyonu oksijen elektrodu ile ölçülmüştür. Ön kasılım oluşturmak için arterlerde Serotonin (5-HT), (10^{-5} M) ve venlerde de bir tromboksan analogu olan U46619 kullanılmıştır. Sonuçlarımız; RSV (20 µM)'ün, U46619 ile önkasılım oluşturulmuş pulmoner venlerde hipoksik pulmoner vazokonstriksiyonu anlamlı şekilde inhibe ettiğini, ancak 5-HT ile önkasılım oluşturulmuş pulmoner arterlerde damar yanıtlarını anlamlı olarak etkilemediğini göstermiştir.

Anahtar sözcükler: *5-HT, Hipoksi, Pulmoner vazokonstriksiyon, Resveratrol, U46619*

INTRODUCTION

Resveratrol (RSV) is a natural phytoalexin with many biological effects, including antioxidant, antiplatelet, anti-atherogenic, anti-inflammatory, estrogenic properties, immunomodulation and chemoprevention [1]. In the cardiovascular system, it has been shown to cause relaxation of vascular smooth muscle [2], and

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reports indicate that RSV induces vasodilatory effects in several vascular beds by acting in endothelium-dependent and independent manners [2-5]. Some of these investigations showed that RSV decreased Ca influx or sensitivity [5]. However, this effect may actually have been due to tyrosine kinase inhibition [6].

Hypoxia-induced pulmonary vasoconstriction (HPV) is an intrapulmonary adaptive mechanism in which circulating blood is diverted away from poorly ventilated to better ventilated regions of the lung. In addition to this physiological role, HPV may also have important pathophysiological consequences [7]. Raj and Cheen showed that, lamb alveolar hypoxia leads to pulmonary arterial and venous constriction and contributing almost equally to the increase in total pulmonary vascular resistance [8]. Although the exact mechanism of HPV remains unclear, vasodilator agents have been shown to reduce the increase in pulmonary vascular resistance [9]. Thus, in the present study, we evaluated the effects of RSV, as a vasodilatory agent, on hypoxia-induced vasoconstriction in isolated lamb pulmonary arteries and veins.

MATERIAL and METHODS

Lungs of freshly slaughtered lamb were obtained from a local abattoir (Duzce) and delivered to the laboratory in cooled, oxygenated, physiological salt solution in first 30 min. One artery and one vein isolated from the same lung, and six spring lamb's (4-5 months, Daglic breed under Bolu province) lungs used. Every lung vessels were cleared of fat and adhering connective tissue and cut into endothelium-intact rings 4-5 mm long. Segments were suspended in a water-jacketed organ bath (10 ml) filled with Krebs-Henseleit solution (119 mM NaCl, 25 mM $NaHCO_3$, 4.6 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 11 mM glucose) at 37°C. The solution was aerated with room air or a gas mixture of 95% N_2 + 5% CO_2 (hypoxic). The rings were suspended on a pair of stainless-steel hooks, one of which was fixed to an L-shaped rod inside the chamber and the other to an isometric transducer (May FDT10-A, Commat Ltd, Ankara, Turkey). Isometric contractions were recorded continuously with a TDA 97 data acquisition system (Commat Ltd). Isolated rings were equilibrated in Krebs-Henseleit solution gassed with room air for an hour at their optimum resting force (3 g for artery rings, 2 g for vein rings) [10]. Isometric contractions were calculated as force developed per cross-sectional area [10]. The oxygen tension of the bathing medium was measured using an oxygen electrode (model 9071, Jenway Ltd, Essex, England). This electrode was calibrated to zero using sodium sulfite (100 mM) in dissolved disodium tetraborate (10 mM).

Experimental protocol

Effects of hypoxia on isolated pre-contracted pulmonary vessels: In pre-contracted pulmonary artery (5-HT at its EC_{50}, 10^{-6} M) and vein rings (U46619 at its EC_{50}, 10^{-6} M) hypoxia was induced by changing to a 95% N_2 + 5% CO_2 gas mixture. After 30 min of hypoxia, oxygenated conditions were reestablished by changing to room air.

Effects of RSV on hypoxic contractions: Following the measurement of a control hypoxic contraction in pre-contracted vessels, RSV (20 µM)[10] was added to the bath (incubated 30 min) and in the continued presence of the drug, a second hypoxic contraction was induced.

Drugs: RSV (dissolved in 50% ethanol), 5-HT (Serotonin hydrochloride, CAS-RN: 153-98-0) (dissolved in water), and U46619 (dissolved in ethanol) were obtained from Sigma (St Louis, MO, USA).

Data analysis: The hypoxic contraction in pre-contracted pulmonary vessels was calculated as the difference between the contraction obtained just prior to hypoxia (i.e., 5 or 8 min of the 5-HT or U46619 contractions, respectively) and that obtained at the peak of and again at the end of the hypoxic response (i.e., 35 or 38 min of the 5-HT or U46619 contraction, respectively).

All results are expressed as means±S.E.M. n refers to the number of lungs used in the organ bath assay. The significance of differences between the groups was determined with Student's paired or unpaired t-test, as appropriate. P values of <0.05 were deemed to be statistically significant.

RESULTS

Effects of hypoxia in U46619 pre-contracted endothelium-intact pulmonary vessels

Hypoxia-induced contractions were measured as the difference between the contraction just prior to hypoxia and that obtained at the peak of and again at the end of the hypoxic response. In pulmonary arteries under normoxic conditions, 5-HT (10^{-6} M) caused contraction, which was not sustained; it was

2.16±0.58 mN/mm² (*n*=6). However, introduction of hypoxia produced a further contraction (0.52±0.12 mN/mm², *n*=6) *(Table 1)*.

Similar results were obtained in pulmonary veins pre contracted with U46619. Under normoxic conditions, U46619 (10^{-6} M) induced contraction (4.45±0.90 mN/mm², *n*=6) that was not sustained, but hypoxia caused a further contraction (3.40±1.3 mN/mm², *n*=6; *Table 1*).

Effect of RSV on hypoxic contractions

RSV (20 µM) decreased the 5-HT-induced contraction, from 2.16±0.52 mN/mm², to 1.65±0.45 mN/mm², *n*=6) in pulmonary arteries (not significant; *Fig 1*). RSV

Table 1. *Effect of hypoxia in endothelium-intact pulmonary vessels*
Tablo 1*. Endotelyumu sağlam pulmoner damarlarda hipoksinin etkisi*

Conditions	*n*	Contraction (mN mm^{-2})
Pulmonary arteries		
5 HT (10^{-6} M)	6	2.16±0.58
Normoxia	6	-0.01±0.01
Hypoxia	6	0.52±0.12*
Pulmonary veins		
U46619 (10^{-6} M)	6	4.45±1.30
Normoxia	6	-0.03±0.02
Hypoxia	6	3.40±1.30*

*The hypoxic contraction in pre-contracted pulmonary vessels was calculated as the difference between the contraction obtained just prior to hypoxia (i.e., 5 or 8 min of the 5-HT or U46619 contractions, respectively) and that obtained at the peak of and again at the end of the hypoxic response. * P<0.05, when compared to normoxia values*

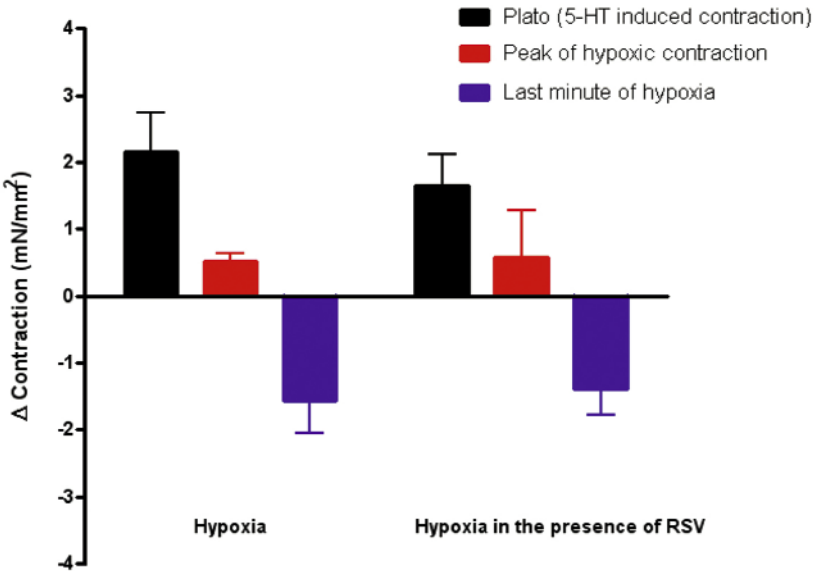

Fig 1. Differences between control and RSV (20 µM) pretreated contractions in pre contracted pulmonary arteries (shown as delta contraction) prior to hypoxia (5 min), at the peak of the hypoxic contraction (8 min), and at the end of the hypoxic response (i.e., at 35 min of the 5-HT contraction; *n*=6)

Şekil 1. Ön kasılım oluşturulmuş pulmoner arterlerde, hipoksi öncesi (5 dak), hipoksik kasılmanın doruk noktasında (8dak) ve hipoksik yanıtın sonunda (ör. 5-HT kasılmasının 35. dakikası, *n*=6); kontrol ve RSV (20 µM) öntedavi edilmiş gurupların damar yanıtları arasındaki farklılıklar (delta kontraksiyon olarak gösterilmiştir)

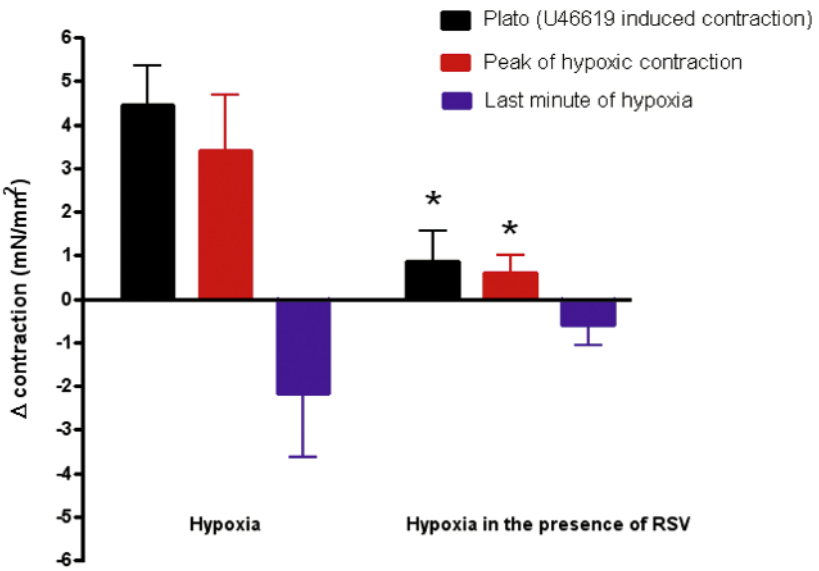

Fig 2. Differences between control and RSV (20 µM) pretreated contractions in pre contracted pulmonary veins (shown as delta contraction) prior to hypoxia (6 min), at the peak of the hypoxic contractions (8 min), and at the end of the hypoxic response (i.e., at 36 min of the U46619 contraction; *n*=6). * P<0.05 compared to peak values.

Şekil 2. Ön kasılım oluşturulmuş pulmoner venlerde, hipoksi öncesi (6 dak), hipoksik kasılmanın doruk noktasında (8 dak) ve hipoksik yanıtın sonunda (ör. U46619 kasılmasının 36. dakikası, n=6); kontrol ve RSV (20 µM) ile inkübe edilmiş grupların damar yanıtları arasındaki farklılıklar (delta kontraksiyon olarak gösterilmiştir). Doruk değerleri karşılaştırıl-dığında * P<0.05.

did not change the hypoxia-induced contraction (0.52±0.12 mN/mm², to 0.57±0.71 mN/mm², *n*=6; *Fig 1*). These contractions were not sustained (-1.57±0.48 mN/mm², to -1.39±0.39 mN/mm2, *n*=6; *Fig 1*).

In contrast to pulmonary arteries, in U46619-pre-contracted pulmonary veins RSV decreased both U46619-induced and hypoxia-induced contractions (from 4.45±0.9 mN/mm² to 0.87±0.7 mN/mm², and from 3.4±1.30 mN/mm² to 0.6±0.4 mN/mm², *n*=6; *Fig 2*). These contractions were not sustained (-2.18± 1.45 mN/mm², to 0.58±0.48 mN/mm², *n*=6; *Fig 1*).

DISCUSSION

We evaluated the effect of RSV, a phytoestrogen on HPV in isolated lamb pulmonary artery and vein preparations. We found that the contraction response to 5-HT in pulmonary arteries was not affected by RSV. RSV did significantly inhibit responses due to U46619, a TXA_2 analog, in pulmonary veins. Hypoxia-induced vasoconstriction in pulmonary arteries was not affected by RSV, while hypoxia-induced pulmonary vasoconstriction in pulmonary veins was inhibited by RSV.

Responses to 5-HT, used as a pre-contraction agent, were not significantly different as compared to 5-HT pre-contraction in the presence of RSV. However, KCl- and 5-HT-induced vasoconstriction was significantly inhibited in 30 µM RSV-incubated pig coronary arteries [12], which may have been due to the preparation, the different species used, and variability in sensitivity to the RSV dose (20 µM).

U46619, a TXA_2 analog, was used as a pre-contractile agent in isolated lamb pulmonary veins. TXA_2, an arachidonic acid by-product, is an important vaso-constrictor in the pulmonary circulation under both physiological and pathological conditions [13]. Ca^{2+} influx and Ca^{2+} release from intracellular storage in pulmonary veins are both increased by U46619. Consequently, increased myofilament sensitivity resulted in vasoconstriction. Signaling pathways involving protein kinase C, tyrosine kinases, and rho kinase have been shown to play a role in contractions due to U46619 [14]. U46619 induced pre-contraction in our experiments was reduced in the presence of RSV. This result was not unexpected because the role of tyrosine kinases in U46619-induced contraction has been previously suggested [14]. Moreover, some studies have reported that RSV inhibites tyrosine kinase activity [6]. Some studies indicated that RSV decreased Ca influx or sensitivity [5].

In the present study, we evaluated the effects of RSV on hypoxia-induced contractions in isolated pulmonary artery and vein preparations. Studies related to the mechanism(s) of HPV have generally been conducted in pulmonary arteries. However, some investigators have shown that the response to hypoxia in large pulmonary arteries and veins was also contraction [10]. It has been demonstrated that pulmonary veins cardiomyocytes are present in the media of pulmonary vein [15]. Since these cardio-myocytes are contractile, they may play a role in pulmonary vein contraction in case of acute hypoxia [16]. In the present work, vasoconstriction was shown in both pulmonary arteries and veins after precontraction, consistent with other reports [7,10].

Contraction due to hypoxia in isolated lamb pulmonary arteries was not significantly affected by RSV. It has been demonstrated that endothelial mediated mechanisms, especially NO, play important roles in HPV in lamb pulmonary arteries [10]. However, in the present study, we showed that NO had no apparent role in the vasodilatation of pulmonary arteries at least under normoxic conditions in case of RSV, (unpublished data). However, we expected that RSV could partially inhibit HPV due to the role of tyrosine kinases in the mechanism of the HPV response in pulmonary arteries [10]. From that point of view, the reason of that result might be due to RSV dose used (20 µM).

In the present study, hypoxia-induced pulmonary vasoconstriction in pulmonary veins was significantly inhibited by RSV. This result can be interpreted in two ways. First, based on knowledge of regional differences in pulmonary vascular beds regarding the response to vasodilator and vasoconstrictor sub-stances [7], pulmonary veins can be concluded to be more sensitive than pulmonary arteries to the vasodilatory effects of RSV. Second, HPV is inhibited by RSV, a phytoestrogen similar to diethylstilbestrol in structure [1], via tyrosine kinase inhibition, like that mediated by genistein [14]. However, to assess that hypothesis requires experiments at least with orthovanadate, which inhibits protein tyrosine phosphatase and leads to increased tyrosine kinase activity because the tyrosine kinase/tyrosine phosphatase balance plays an important role in smooth muscle tension [17].

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