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ORIGINAL RESEARCH

Effects of Resveratrol, Caffeic Acid Phenethyl Ester and Silibinin on Isolated Human Umbilical Artery

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Abstract

Objective: The aim of this study was to compare the vasoactive effects of resveratrol, CAPE and silibinin on the basal tone and serotonin (5-HT)-induced contractile responses of isolated human umbilical arteries.

Material-Method: This study was approved by the Non-Drug and Medical Device Research Ethics Committee (Decision No: 2022/3994). The study used umbilical cord samples separated as medical waste in the Department of Gynaecology and Obstetrics. The umbilical cords taken from the middle 1/3 part were brought to the laboratory in cold Krebs-Henseleit Solution (KHS). The arteries isolated from the umbilical cords were cleaned from the surrounding tissues and spirally cut into 2-3x10 mm strips. The strips were suspended in isolated tissue baths filled with 10 ml KHS continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at 37°C. At the beginning of the experiment, the strips were stretched to an initial tension of 1.5 g and allowed to equilibrate for 60 min in KHS, which was changed every 15 min. At the end of the rest period, responses to the applied agents were recorded isometrically (Commat, Ankara, TURKEY) using a transducer (BIOPAC MP36, California, USA). The first group investigated the effects of resveratrol, CAPE and silibinin on basal tonus in the umbilical artery strips. After control contraction with 10^{-6} M 5-HT, the tissues were washed until resting tone was re-established. Concentration-response curves for resveratrol (10^{-9} M -10^{-4} M), CAPE (10^{-9} M -10^{-4} M) and silibinin (10^{-9} M -10^{-4} M) were obtained by cumulative addition to the organ bath. To evaluate the effects of resveratrol, CAPE and silibinin on 5-HT-induced contraction, the strips were contracted with 10^{-6} M 5-HT. After maximal contractile response was achieved, increasing concentrations of resveratrol (10^{-9} M -10^{-4} M), CAPE (10^{-9} M -10^{-4} M) or silibinin (10^{-9} M -10^{-4} M) were added cumulatively to the bath.

Results: Resveratrol, CAPE and silibinin did not affect the basal tone of umbilical arteries. 5-HT is a potent vasoconstrictor endogenous agent in umbilical arteries. Antioxidants used in the study produced relaxation responses in arteries precontracted with 5-HT. When the sensitivity of tissues to these agents and their maximum relaxant effects were evaluated together, silibinin was found to be more effective than the others.

Conclusion: The use of antioxidants to support or treat complications due to oxidative stress in pregnant women is being investigated. The vasoactive effects of exogenous and endogenous agents are important in regulating umbilical vascular tone. Resveratrol, CAPE and silibinin are polyphenol-derived natural antioxidants that have potential use in pregnant women. Our study investigated the vasoactive effects of these agents on umbilical arteries.

Keywords: CAPE, In Vitro, Resveratrol, Silibinin, Umbilical Artery

INTRODUCTION

Increased placental oxidative stress is an effective factor in the development of complications such as preeclampsia, fetal growth restriction and gestational diabetes in pregnant women. Antioxidant supplementation has been recommended to prevent the development of these complications by reducing oxidative stress in pregnant women. Resveratrol, caffeic acid phenethyl ester and silibinin are polyphenol derivative natural antioxidants. Phenolic compounds have been reported to neutralize reactive oxygen species and have therapeutic effects in diseases associated with oxidative damage, such as inflammatory disorders and neurodegenerative diseases. In clinical studies conducted in pregnant women, the therapeutic effects of resveratrol on inflammation, preeclampsia and gestational diabetes

have been shown.[1] Studies are being conducted on the use of CAPE and silibinin for the treatment or support of oxidative stress-related disorders in pregnant women.

The umbilical cord is the structure that connects the fetus to the placenta. There is no autonomic innervation of the umbilical vessels. In the regulation of umbilical placental circulation, endogenous vasoactive mediators such as 5-HT, histamine and PGF are effective. 5-HT is the most potent vasoconstrictor mediator in umbilical arteries.[2] Furthermore, increased plasma free 5-HT levels and vascular sensitivity to 5-HT have been reported in pregnancy-related complications such as preeclampsia.[3, 4]

Resveratrol, caffeic acid phenethyl ester, and silibinin have been shown to have vasoactive effects. However, there is not enough information about the effects of these drugs, which can be used in pregnant women, on the umbilical vessels. The aim of our study was to compare the vasoactive effects of resveratrol, CAPE and silibinin on the basal tone and serotonin induced contractile responses of isolated human umbilical arteries.

MATERIALS AND METHODS

General

This study was approved by the Non-Drug and Medical Device Research Ethics Committee (Decision No: 2022/ 3994). In the study, umbilical cord samples separated as medical waste in the Department of Gynaecology and Obstetrics were used. The umbilical cords taken from the middle 1/3 part were brought to the laboratory in cold KHS. The arteries isolated from the umbilical cords were cleaned from the surrounding tissues and spirally cut into 2-3x10 mm strips. The strips were suspended in isolated tissue baths filled with 10 ml KHS continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at 37°C. At the beginning of the experiment, the strips were stretched to an initial tension of 1.5 g and allowed to equilibrate for 60 min in KHS, which was changed every 15 min. At the end of the rest period, responses to the applied agents were recorded isometrically (Commat, Ankara, TURKEY) using a transducer (BIOPAC MP36, California USA).

Experimental procedure

Effects of resveratrol, CAPE and silibinin on basal tonus in the umbilical artery strips were investigated in first group. After control contraction with 10^{-6} M 5-HT, the tissues were washed until resting tone was re-established. Concentration-response curves for resveratrol (10^{-9} M -10^{-4} M), CAPE (10^{-9} M -10^{-4} M) and silibinin (10^{-9} M -10^{-4} M) were obtained by cumulative addition to the organ bath. To evaluate the effects of resveratrol, CAPE and silibinin on 5-HT-induced contraction, the strips were contracted with 10^{-6} M 5-HT. After maximal contractile response was achieved, increasing concentrations of resveratrol (10^{-9} M -10^{-4} M), CAPE (10^{-9} M -10^{-4} M) or silibinin (10^{-9} M -10^{-4} M) were added cumulatively to the bath.

Drugs and solutions

5-HT (Sigma); Resveratrol (Sigma); CAPE (Sigma); Silibinin (Sigma). Krebs-Henseleit Solution [mM]: NaCl 118.3; KCl 4.69; KH2PO4 1.18; CaCl2 1.25; MgSO4 1.17; NaHCO3 25.0; Glucose 11.1. Serotonin was dissolved in distilled water, CAPE in 70% alcohol, resveratrol in DMSO and silibinin in a mixture of DMSO and distilled water. The solvent mixtures used in preliminary experiments were found to be ineffective.

Statistical analysis

Responses to the agents applied in the study were evaluated as the percentage (%) of the maximum contraction response obtained with 10^{-6} M 5-HT. The data were expressed as mean±standard deviation (SD). Analyses were conducted on a computer using the SPSS 29.0 (Armonk, NY: IBM Corp.) package program. Maximum relaxation (E_{max}) and pD_2 (negative log value of molar concentration producing contraction by 50%) values calculated from the concentration-response curves obtained with resveratrol, CAPE and silibinin were compared. The Shapiro-Wilk's test was applied to the E_{max} and pD_2 values obtained from all groups to check that assumptions of normality were met for continuous numerical data. According to outcomes of the Shapiro-Wilk's test, it was seen that all the data, which were obtained from groups, were normally distributed. So, one-way ANOVA test was used to compare the E_{max} and pD_2 values for the three groups (resveratrol, CAPE, silibinin) followed by the post hoc procedure Tukey HSD. Results were considered statistically significant if $p<0.05$ in all analyzes.

RESULTS

5-HT (10^{-6} M) produced sustained contraction in umbilical artery strips. These contractions were reproducible and time-dependent changes were not observed. Resveratrol (10^{-9} M -10^{-4} M), CAPE (10^{-9} M -10^{-4} M) and silibinin (10^{-9} M -10^{-4} M) added cumulatively to the bath did not affect the basal tone of the tissues. Relaxing effects of resveratrol, CAPE

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and silibinin on the umbilical cord strips were studied in the other group. The supplements used in the study produced dose-dependent relaxation in 5-HT-contracted umbilical artery strips. The maximum relaxant effect of silibinin was higher than the other two drugs in Figure 1 ($p<0.05$). The E_{max} value of resveratrol was also higher than that of CAPE in Figure 1($p<0.05$).

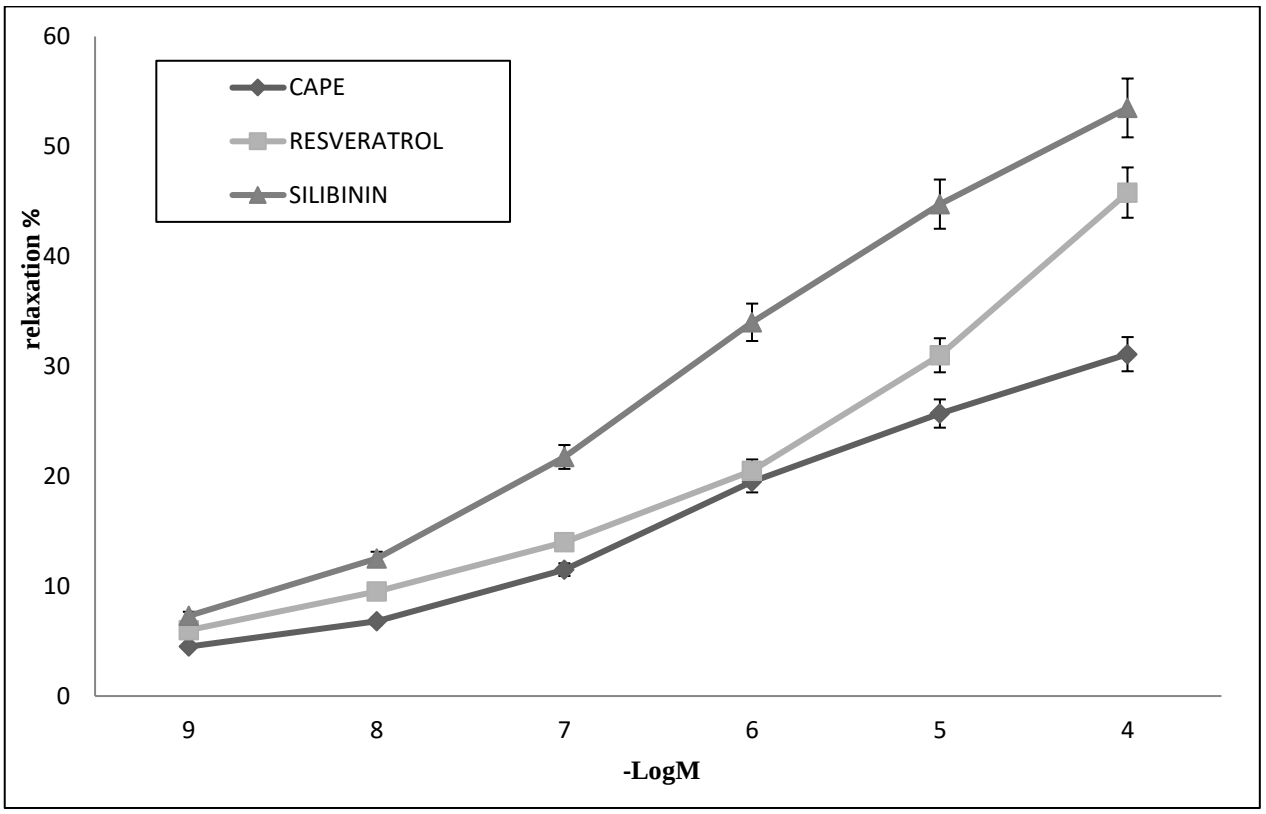

Figure 1. Concentration-response curves of CAPE, resveratrol and silibinin in human umbilical artery contracted with 5-HT (10^{-6} M)

: Relaxation responses to resveratrol were calculated as % of contraction responses obtained with 5-HT (10^{-6} M) and the results were expressed as mean±standard deviation (n=8).

: Relaxation responses to CAPE were calculated as % of contraction responses obtained with 5-HT (10^{-6} M) and the results were expressed as mean±standard deviation (n=8).

: Relaxation responses to silibinin were calculated as % of contraction responses obtained with 5-HT (10^{-6} M) and the results were expressed as mean±standard deviation (n=8).

Our study evaluated the potency of these drugs in the umbilical artery. The pD_2 value of resveratrol was higher than other drugs ($p<0.05$). No significant difference was found between the potencies of CAPE and silibinin ($p>0.05$). E_{max} and pD_2 values calculated for resveratrol, CAPE and silibinin are summarised in Table 1.

Current study showed that the antioxidants, resveratrol, CAPE and silibinin produced significant relaxation in umbilical arteries precontracted with serotonin.

Table 1. Maximum relaxation (E_{max}) found for resveratrol, CAPE and silibinin in human umbilical artery -$logEC_{50}$ (pD_2) values

	E_{max}	pD_2
Resveratrol	45.88±4.12*	5.68±0.256**
CAPE	31.13±3.13	6.56±0.230
Silibinin	54.75±3.69#	6.46±0.087

*$p<0.05$ according to the E_{max} value obtained with CAPE
#$p<0.05$ compared to the E_{max} value obtained with CAPE and resveratrol
**$p<0.05$ according to pD_2 value obtained with CAPE and silibinin
E_{max} values were calculated as % of 10^{-6} M 5-HT contractions. Expressed as mean±standard deviation (n=8).

DISCUSSION

In this study, resveratrol, CAPE and silibinin did not affect the basal tone of the umbilical arteries, but significantly reduced 5-HT-dependent contractile responses. The maximum relaxant effect of silibinin was significantly higher than that of the other two agents. Resveratrol produced more relaxation than CAPE. The potencies of silibinin and CAPE were found to be higher than resveratrol.

The umbilical vessels mediate the transport of oxygen and nutrients between the mother and the foetus. Umbilical blood flow is important for fetal development and health. These vessels do not have autonomic innervation. Therefore, endogenous or exogenous substances affecting umbilical artery tone are essential for regulating umbilical-placental circulation and the development of the fetus. Resveratrol, CAPE, and silibinin, which are known to have vasoactive effects, are widely used by the public due to their antioxidant effects. Clinical and experimental studies are being conducted on the use of these substances in oxidative stress-related complications in pregnant women.

Resveratrol supplementation has been suggested to have potential in preventing and/or treating oxidative stress-related complications in pregnant women.[1] In one study, it was found that supplementation of the maternal diet with resveratrol in hypoxic pregnancies prevented fetal death.[5] A rodent study found that resveratrol prevented embryonic oxidative stress and apoptosis and improved glucose and lipid profiles in diabetic mothers. Researchers have suggested that resveratrol may be helpful in diabetic pregnant women.[6] In a randomised, double-blind, placebo-controlled trial of 400 patients with pre-eclampsia carrying a singleton pregnancy, aged 21 to 32 years, patients were divided into two groups: the first group received nifedipine and resveratrol, and the second group received nifedipine and placebo. Compared to the nifedipine and placebo group, the time to control blood pressure was significantly reduced in the nifedipine and resveratrol group. In addition, the time between hypertensive crises was longer in the nifedipine-resveratrol group than in the control group. No side effects were observed when the mothers and babies were examined after delivery. It was suggested that resveratrol could be complementary to nifedipine treatment.[7] According to the European Food Safety Authority EFSA data in 2016, the safe dose of resveratrol is 150 mg per day.[8] Relaxant effects of resveratrol have been demonstrated in human saphenous vein and mammary artery.[9] Similarly, in this study, resveratrol induced relaxation in umbilical artery strips. The maximum relaxant effect and potency of resveratrol was lower than that of silibinin.

CAPE is the active component of propolis. In the study by Usman et al.[10], propolis showed a protective effect against oxidative stress in diabetic pregnant rats. In another study, propolis was found to have a maternal protective effect in pregnant mice.[11] When compared with the other agents used in the study, the maximal relaxant effect of CAPE on the umbilical artery was found to be significantly less than that of resveratrol and silibinin. However, its potency was higher than that of resveratrol and similar to the potency of silibinin. In our previous study, CAPE relaxed umbilical arteries contracted by endothelin-1 and prostaglandin F2α more than in this study.[12] In a study conducted in 5-HT-contracted uterine tissue, CAPE was found to cause almost complete relaxation.[13] These results show that the vasodilator effect of CAPE varies depending on the contracting agent and the tissue used.

Silimarin from the plant Silybum marianum and its active component silibinin are known to have antioxidant and hepatoprotective effects. Silibinin is included in the antidote list of the National Poison Advisory Centre as an antidote for mushroom poisoning.[14] The effects of treatment with silimarin and silibinin have been studied in pregnant women and experimental animals. In a randomised clinical trial, silimarin treatment was reported to improve liver function in women with pre-eclampsia.[15] In another clinical trial, silimarin treatment of 150 mg drug twice daily in pregnant women was shown to cause no abnormalities or other adverse effects.[16] In blood mononuclear cells from women with pre-eclampsia, silibinin was found to have a potent anti-inflammatory effect by reducing inflammatory cytokines.[17] In a study in rats with a pre-eclampsia model, silibinin treatment reduced blood pressure and proteinuria and had a protective effect on liver damage.[18] There are few studies on the effects of silibinin on vascular preparations in vitro. In one study, the relaxant effect of silibinin was observed in rat aortic rings contracted with phenylephrine.[19] In our study, silibinin produced greater relaxation than CAPE and resveratrol in the umbilical artery contracted with 5-HT. In addition to causing more relaxation in the umbilical artery than other agents, the sensitivity of tissues to silibinin was found to be high.

CONCLUSION

The use of antioxidants for support or treatment of complications due to oxidative stress in pregnant women is being investigated. The vasoactive effects of exogenous and endogenous agents are essential in regulating umbilical vascular tone. Resveratrol, CAPE and silibinin are polyphenol-derived natural antioxidants that have potential use in pregnant women. In our study, the vasoactive effects of these agents on umbilical arteries were investigated. Resveratrol, CAPE and silibinin did not affect the basal tone of umbilical arteries. 5-HT is a potent vasoconstrictor endogenous agent in umbilical arteries. Antioxidants used in the study produced relaxation responses in arteries precontracted with 5-HT. When the sensitivity of tissues to these agents and their maximum relaxant effects were evaluated together, silibinin was found to be more effective than the others.

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