https://doi.org/10.31925/farmacia.2022.4.21 **ORIGINAL ARTICLE**

ASCORBATE INHIBITS THE ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION MEDIATED RELAXATION IN RESISTANCE ARTERIES

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Abstract

The main path of endothelium-dependent relaxation (EDR) in resistance arteries is independent of nitric oxide synthase and cyclooxygenase and is mediated by endothelium-dependent hyperpolarisation (EDH). We tested the effect of ascorbate upon this path in isolated small mesenteric arteries from the rat. EDR was induced by carbachol 10^{-5} M, in rings contracted by phenylephrine 10^{-5} M or by prostaglandin $F_{2\alpha}$ 10^{-5} M, in the presence of L-N(γ)-Nitro arginine methyl ester (l-NAME) 10^{-4} M and indomethacin 10^{-5} M. EDR proved to be reduced by ascorbate concentrations ranging between 10^{-5} M - 10^{-3} M ($n = 6$; $p < 0.01$). We discuss this new effect in the context of the relations between ascorbate and endothelial function/dysfunction.

Rezumat

Calea principală de relaxare endoteliu-dependentă (RED) în artere de rezistență nu depinde de nitric oxid sintază și ciclo-oxigenază şi este mediată de hiperpolarizarea endoteliu-dependentă (HED). Am testat efectul ascorbatului asupra acestei căi, în artere mezenterice mici izolate de la șobolan. RED a fost indusă de carbacol 10^{-5} M, în inele arteriale contractate cu fenilefrină 10^{-5} M sau cu prostagladină $F_{2\alpha}$ 10^{-5} M, în prezența esterului metilic al metilic L-N(γ)-Nitro-argininei (l-NAME) 10^{-4} M și a indometacinului 10^{-5} M. RED este redusă de ascorbat 10^{-5} M - 10^{-3} M ($n = 6$; $p < 0,01$). Studiul evaluează acest nou efect în contextul relațiilor dintre ascorbat și funcția/disfuncția endotelială.

Keywords: vitamin C, small arteries, EDH-mediated relaxation, oxidative stress

Introduction

Ascorbate (ASC) could be beneficial against endothelial dysfunction [23]. The experimental and clinical data are still controversial, as presented by the most recent reviews [26]. A review has described the benefit of ASC against endothelial dysfunction, but only for high daily doses [3]. Others have found no cardiovascular benefit from ascorbate supplementation [8]. A recent meta-analysis (one main trial with almost 15000 cases) found no cardiovascular benefit from ASC [2]. The essential NOS co-factor tetrahydrobiopterin (BH4) is key to ASC interaction with NO-mediated EDR; ASC benefits *via* BH4 protection with recovery from low NOS activity [4, 10, 11].

Resistance arteries and arterioles together are the main contributor to peripheral resistance. Endothelium-dependent relaxation (EDR) of vascular smooth muscle (VSM) is known to be mediated by nitric oxide (NO). Still, the major EDR mechanism in resistance arteries is independent of NO-synthase (NOS) and cyclooxygenase (COX), and it is mediated by endothelium-dependent hyperpolarisation (EDH) [13]. ASC produces direct VSM relaxation and EDR and modulates NO relaxing potency and guanylate cyclase (GC) [10, 23]. EDH-mediated EDR relies on endothelial Ca^{2+}-activated K^+ channels (K_{Ca}) [5-7, 28], with conductances that are small (sK) or intermediate (iK). EDH-mediated EDR is: a counter-regulator of sympathetic vasoconstriction [18]; important in flow autoregulation, myogenic response and vasomotion [13]; compensatory in endothelial dysfunction, but also involved in its pathogenesis [12, 15-17].

Very few studies on ASC and EDH-mediated EDR have used isolated rabbit iliac artery [14], isolated perfused bovine ciliary bed [24, 25], and isolated perfused rat mesenteric arterial bed [25]. Given the sustained broad interest in EDH-mediated EDR [13] and our sustained interest in endothelial function and dysfunction [9, 19, 30-32] and in various aspects of oxidative stress [20, 22, 34, 35], we decided to test the effect of ASC upon EDH-mediated EDR in isolated small mesenteric arteries. One previous study has examined EDH-mediated EDR in isolated small mesenteric arteries [33], but ours differs in some aspects, and we discuss their relevance.

Materials and Methods

Chermicals

The salts used for the physiological saline solution (PSS) containing (mM): 119 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 1.2 $MgSO_4$; 25 $NaHCO_3$; 1.18 KH_2PO_4; 5.5 glucose were of analytical grade (Merck, USA), and all drugs were of research grade (Sigma, USA).

Animals and procedures

Male adult Wistar rats (~ 200 g) were euthanised. Jejunal loops were excised and placed in PSS at ~ 5ºC, second-order mesenteric arteries were dissected and rings 1 mm wide were cut out and mounted in wire myographs, then stretched to 1 g passive tension. The PSS was then kept at 37ºC and aerated with 95% O_2 + 5% CO_2 (pH 7.2 - 7.4). After 30 min. equilibration, each ring was checked twice for complete EDR by carbachol 10^{-5} M upon contraction by phenylephrine 10^{-5} M (PHE). Then, after 30 min. re-equilibration, the contraction was induced randomly by either PHE 10^{-5} M or prostaglandin $F_{2\alpha}$ 10^{-5} M (PGF), and global EDR or EDH-mediated EDR was tested; first in the absence of ASC and then in the presence of ASC (10^{-5} M, or 10^{-4} M, or 10^{-3} M; actual tests) or again in its absence (control). EDH-mediated EDR was obtained in the presence of indomethacin 10^{-5} M to inhibit COX and N(γ)-nitro-L-arginine methyl ester (l-NAME) 10^{-4} M to inhibit NOS. Only one of these 16 conditions (12 test and 4 control) was randomly applied to any of the rings tested. Time-match paired rings confirmed the 30 min. stability of contraction (PHE and PGF; without carbachol). These tests in arterial rings in isometric conditions were done with absolutely no PSS flow, even during drug administration (concentrated solution of the drug added to the organ bath; < 1/10 v/v) or removal (repeated replacement of the bath content with fresh PSS with no drugs). This study was approved by the Research Ethics Commission of the host institution. All procedures comply with guidelines from Act on Animal Experimentation and Animal Health and Welfare from Romania; Universities Federation for Animal Welfare; Directive 2010/63/EU of the European Parliament, and of the Council of 22 September 2010.

Statistical analysis

EDR in the presence of ASC was expressed as % of the control value previously obtained in the absence of ASC in each ring. Results are presented as mean ± SD (n = 6 in all series). Data were assessed using two-way ANOVA and t-test. Statistical significance was considered for $p < 0.05$.

Results and Discussion

ASC 10^{-5} - 10^{-3} M inhibits EDH-mediated EDR induced by carbachol 10^{-5} M (without effect on the global EDR) in rat isolated resistance mesenteric arteries when they are precontracted by any of the two agents used (Table I) (dose-dependent ASC effect; IC_{50} 0.078 mM with PHE contraction; IC_{50} 0.114 mM with PGF contraction). This effect is not detectable in the main mesenteric artery (data not shown).

Table I

Ascorbate effects upon endothelium-dependent relaxation (EDR) in rat isolated resistance mesenteric arteries

contraction	**PHE**	**PHE**	**PGF**	**PGF**
relaxation	**EDH-mediated EDR**	**global EDR**	**EDH-mediated EDR**	**global EDR**
No ASC	99.1 ± 1.2	99.8 ± 2.8	98.5 ± 0.6	101.2 ± 1.9
ASC 10^{-5} M	84.1 ± 2.2*	99.7 ± 3.8	90.5 ± 1.4*	104.8 ± 3.9
ASC 10^{-4} M	34.5 ± 3.4*	102.5 ± 2.7	51.7 ± 4.1*	97.7 ± 4.2
ASC 10^{-3} M	7.5 ± 5.2*	98.9 ± 4.1	10.5 ± 3.1*	101.3 ± 3.6

*$p < 0.05$

EDR was induced by 10^{-5} M carbachol during contraction (first row in Table I) by PHE 10^{-5} M or PGF 10^{-5} M. EDR (second row in Table I) was examined either as global EDR or as its major part, EDH-mediated EDR. The influence of ascorbate (ASC, 10^{-5} M - 10^{-3} M) is shown in the last three rows (Table I). All ASC data are here normalised: EDR expressed as % of the respective EDR previously obtained in the same ring in the absence of ASC (mean ± SEM; n = 6 in all cases; *$p < 0.01$). The third row (ASC none) shows the reproducibility of that EDR in the same ring in the absence of ASC; values are used as the control for statistics. Our preliminary data showed carbachol 10^{-5} M induces complete global EDR and nearly maximal EDH-mediated EDR.

Within extensive research on EDR, studies also tested the relation between oxidative stress and NO-mediated EDR, including the possible benefit from ASC. But here, we show that in isolated resistance arteries, ASC inhibits EDH-mediated EDR, the major EDR path in there. EDH-mediated EDR was inhibited by ASC in small mesenteric arteries (Table I). Still, the global EDR was not, probably because the reduced EDH-mediated EDR is compensated by the increased NO release by ASC shown by others [10]. Others showed ASC inhibits EDH-mediated EDR but used only perfused vascular beds [24, 25], then isolated larger arteries [14]. In the bovine ciliary bed and rat mesenteric bed, EDH-mediated EDR was blocked by physiological [ASC] [25]. They explored the same effect in isolated resistance mesenteric arteries, too [33]. Such studies focused on the relation between H_2O_2 and ASC as oxidative stress modulators, but then the EDHF role of H_2O_2 was not so well known.

Still, they did find that, in iliac artery rings, ASC can increase EDH-mediated EDR, by higher H_2O_2 [14], with its subsequent actions [27]. There they approached [14] ASC effect on the main path of EDH-mediated EDR, relying on hyperpolarisation propagating (via gap junctions) in both the endothelium and the VSM [13]. Typical resistance arteries, isolated, were tested in one study on ASC effects upon EDH-mediated EDR; in the model, we used here, they found no effect of ASC on EDH-mediated EDR [33]. Maybe because we used other contracting agents; see Table I *vs.* [33], while precontraction affects EDH-mediated EDR [36]. We used another way to compare test and control effects: separate tests for ASC 0.01, 0.1, and 100 mM; effects as % of the control in the same ring, limiting bias from other ASC effects. The flow was required for inhibition of EDH-mediated EDR by ASC [29, 33], but there was no flow in our tests and ASC still was able to inhibit EDH-mediated EDR (Table I).

We confirmed that ASC inhibits EDH-mediated EDR in rat mesentery [25], showing the effect is intrinsic to the vascular wall and resides distally, in accord with the increasing importance of EDH-mediated EDR along the arterial tree [36]. This ASC effect could be related to NOS facilitation by increased BH4 and/or a change in NO/EDHF balance (known ASC effects on NOS-NO-cGMP path). ASC can improve defective EDR by scavenging O^{2-}, NO release from S-nitrosothiols, nitrite reduction to NO, and NOS activation [23]. ASC-induced endothelial recovery in atherosclerosis involves anti-oxidative NO protection, direct BH4 stabilisation, and better BH4-NOS operation; ASC endothelial saturation is required for BH4 protection and optimal NO synthesis [11]. VSM has large-conductance K_{Ca} (BK), an ascorbate target to protect against ischemic brain stroke [21]. The iK in endothelial projections are in a feedback loop with Ca_L in VSM [13, 28]; impaired NO-EDH cross-talk may be a therapeutic target in early endothelial dysfunction [1].

Conclusions

ASC reduces EDH-mediated EDR in resistance arteries. But ASC does not change the global EDR therein, probably due to the known effect of ASC on preserving and promoting NO-mediated EDR by opposing oxidative stress, but not only. EDH-mediated EDR should be considered in vascular studies on ASC. ASC addition to PSS for *in vitro* tests could be indicated to avoid discrepancies with *in vivo* studies, especially in studies regarding EDH-mediated EDR and oxidative stress.

Conflict of interest

The authors declare no conflict of interest.

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