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Basic nutritional investigation

Erythritol is a sweet antioxidant

Gertjan J.M. den Hartog, Ph.D.[a,*], Agnes W. Boots, Ph.D.[a], Aline Adam-Perrot, Ph.D.[b], Fred Brouns, Ph.D.[c], Inge W.C.M. Verkooijen, M.Sc.[d], Antje R. Weseler, Ph.D.[a], Guido R.M.M. Haenen, Ph.D.[a], and Aalt Bast, Ph.D.[a]

[a]*Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands*
[b]*Tate & Lyle Ingredients France, Villeneuve d'Ascq, France*
[c]*Department of Human Biology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands*
[d]*Clinical Trial Center Maastricht, Maastricht, The Netherlands*

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Abstract

Objective: Hyperglycemia, oxidative stress, and the onset and progression of diabetic complications are strongly linked. Reduction of oxidative stress could be of utmost importance in the long-term treatment of diabetic patients. The chronic nature of the disease calls for a mode of antioxidant intake that can be sustained easily, e.g., by the diet. Erythritol, a simple polyol, could be such a compound. It is orally available, well tolerated, and its chemical structure resembles that of mannitol, a well-known hydroxyl radical ($HO^{\bullet}$) scavenger.

Methods: We studied the antioxidant properties of erythritol in vitro and subsequently determined its antioxidant activity and its vasoprotective effect in the streptozotocin diabetic rat.

Results: Erythritol was shown to be an excellent $HO^{\bullet}$ radical scavenger and an inhibitor of 2,2′-azo-bis-2-amidinopropane dihydrochloride–induced hemolysis but inert toward superoxide radicals. High-performance liquid chromatographic and electron spin resonance spectroscopy studies showed that the reaction of erythritol with hydroxyl radicals resulted in the formation of erythrose and erythrulose by abstraction of a carbon-bound hydrogen atom. In the streptozotocin diabetic rat, erythritol displayed an endothelium-protective effect and, in accordance with the in vitro experiments, erythrose was found in the urine of erythritol-consuming rats.

Conclusion: Erythritol acts as an antioxidant in vivo and may help protect against hyperglycemia-induced vascular damage.

Keywords: Erythritol; Diabetes; Oxidative stress; Rat

Introduction

Diabetes mellitus (DM) is a chronic disease with a rapidly increasing prevalence characterized by hyperglycemia. It is associated with a high risk of vascular, renal, neuronal, and ocular damage. Patients with DM often develop diabetic complications including myocardial infarction, stroke, blindness, and gangrene [1]. A large body of evidence indicates that these diabetic complications are linked to oxidative stress [2]. There is considerable evidence that hyperglycemia causes formation of oxygen radicals (most notably the hydroxyl radical [$HO^{\bullet}$]) and lowers antioxidant defenses [3]. Reducing hyperglycemia by replacing sucrose with sweeteners will probably reduce the amount of oxidative stress. The remaining oxidative damage can be prevented by supplementing with antioxidants. Preventing hyperglycemia and supplying appropriate levels of antioxidants seems a rational approach to reduce the amount of oxidative damage and thus the onset and development of diabetic complications in patients with DM [4]. Because of the chronic nature of the disease, supplementation with antioxidants also has to be long term. Intake of appropriate levels of antioxidants with food or drink would be an acceptable procedure and easy for the patient. Based on its molecular characteristics, erythritol may be a good antioxidant to incorporate in food and beverage formulations. However, its antioxidant properties have thus far not been studied in detail. Erythritol is a simple polyol (1,2,3,4-butanetetrol), present in small quantities in melons and peaches, and currently produced in large

*Corresponding author. Tel.: +31-43-388-2916; fax: +31-43-388-4149.
E-mail address: gj.denhartog@farmaco.unimaas.nl (G. J. M. den Hartog).

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quantities for use as a low-calorie, tooth-friendly bulk sweetener [5]. Extensive toxicologic testing has shown that erythritol is well tolerated and has no toxic effects, even after consumption of large quantities [6,7]. In addition, it has no impact on blood insulin or glucose levels, which renders it a useful and safe food component for patients with DM. A compound that closely resembles erythritol is the therapeutically applied polyol mannitol, a well-known $HO^{\bullet}$ radical scavenger [8,9]. However, in contrast to mannitol, of which about 75% remains unabsorbed, erythritol is rapidly and virtually completely (up to 90%) absorbed from the gut [10], which makes it easy to reach appropriate systemic concentrations required to neutralize the highly reactive $HO^{\bullet}$ radicals.

The goal of the present study was to evaluate the antioxidant properties of erythritol in vitro and subsequently study its potential protective effect in vivo in the streptozotocin-induced diabetic rat.

Materials and methods

Hydroxyl radical scavenging

The rate constants for the reaction with $HO^{\bullet}$ radicals of erythritol and related polyols were determined according to Halliwell et al. [11]. Erythritol and other polyols (xylitol, sorbitol, and mannitol) were tested in concentrations ranging from 0 to 8 mM.

Superoxide radical scavenging

The superoxide radical scavenging activity of erythritol was determined according to Kirkova et al. [12] using nitroblue tetrazolium (NBT; 50 μM) as detector for superoxide radicals generated by xanthine 0.1 mM and xanthine oxidase 10 mU/mL. Possible interference by inhibition of xanthine oxidase by the test compounds was checked by measuring the rate of uric acid formation spectrophotometrically at 293 nm.

Hemolysis assay

To determine the antioxidant activity of erythritol, its effect on 2,2′-azobis-2-amidinopropane dihydrochloride–induced hemolysis was investigated according to Vosters and Nève [13]. In short, a red blood cell suspension was incubated with erythritol (0–50 mM) for 5 min at 37 °C after which 2,2′-azobis-2-amidinopropane dihydrochloride (50 mM) was added. At regular intervals (0–300 min), 200-μL aliquots were taken and diluted in 2 mL of 0.9% NaCl solution. The extent of hemolysis was determined by treating the sample with Hemoglobina TC (potassium hexacyanoferrate [III] 2.4 mM, potassium cyanide 3 mM) for hemoglobin determination. The absorbance of the mixture was measured at 540 nm. One hundred percent hemolysis was obtained by adding a 200-μL aliquot to 2 mL of demineralized water. The percentage of hemolysis was calculated from the ratio of hemolysis in the test sample to 100% hemolysis. The time needed to obtain 50% hemolysis was determined for each concentration by fitting the data with the three-parameter sigmoid model from SigmaPlot 2001 for Windows (SPSS Inc., Chicago, IL, USA).

Electron paramagnetic resonance spectroscopy

The formation of radical intermediates during scavenging of hydroxyl radicals by erythritol was studied with an electron spin resonance spectrometer (EMX, Bruker GmbH, Karlsruhe, Germany) with 5,5′-dimethyl pyrroline N-oxide (DMPO) as a spin trap. The spectrometer settings were: power, 20 mW; center field, 3488 G; modulation amplitude, 0.5 G; time constant, 20.48 ms.

Identification of oxidation products

Erythritol (100 mM) was treated with a Fenton reagent (10 mM H_2O_2, 1 mM Fe[II]SO_4, and 1.04 mM ethylenediaminetetra-acetic acid) for 30 min. Analysis of oxidation products was carried out according to Nascimento et al. [14]. In short, samples were treated with 2,4-dinitrophenylhydrazine (2.5 mM) in the presence of perchloric acid to form the 2,4-dinitrophenylhydrazones of any present carbonyl compound. After 60 min of shaking at room temperature the solution was analyzed by high-performance liquid chromatography (column Hypersil (Supelco Inc., Bellefonte, PA, USA) 5 μm, eluent 25:75 acetonitrile:0.1% trifluoroacetic acid, detection at 365 nm).

The identity of the reaction products was determined by comparing their retention times with those for pure erythrose (2,3,4-trihydroxybutanal) and erythrulose (1,3,4-trihydroxy-2-butanone) that underwent the same derivation procedure.

Antioxidant activity of erythritol in the streptozotocin-induced diabetic rat

The study protocol was approved by the ethics committee for animals of the Universiteit Maastricht. Twenty rats (Wistar, 10 male, 10 female, 200–250 g of body weight) were supplied by Charles River Nederland BV (Maastricht, Netherlands) and kept under standard conditions. Standard food (Ssniff Spezialdiäten GmbH, Soest, Germany) and acidified drinking were provided ad libitum. The study's protocol was approved by the ethics committee for animals of the Universiteit Maastricht.

Induction of DM

Diabetes mellitus was induced according to the method of Hasselbaink et al. [15]. The rats were anaesthetized with halothane and subsequently treated with 70 mg/kg of streptozotocin in citrate buffer (100 mM, pH 4.5) by intravenous injection in the tail vein. Controls received citrate buffer only. After 7 d, blood glucose was determined with a glucose meter (Lifescan BV, Maastricht, Netherlands).

The 10 diabetic rats were divided in two groups: those that received normal drinking water (group D, $n = 5$) and those that received erythritol-supplemented water (group DE, $n = 5$) for 21 d. The 10 non-diabetic rats were also divided in two groups: normoglycemic rats that received normal drinking water (group N, $n = 5$) and those that received erythritol-supplemented water (group NE, $n = 5$) for 21 d.

The amount of solution consumed by each rat was monitored. The concentration of the erythritol solution was readjusted daily to ensure that each rat had a daily consumption of 1000 mg of erythritol per kilogram of body weight. Weight and blood glucose levels of the rats were determined weekly.

Ex vivo aortic reactivity measurements

After sacrificing the rats, with CO_2/O_2 blood was rapidly collected by venipuncture. The thoracic parts of the aortas were excised. These were immediately placed in Krebs-Ringer buffer (NaCl 118 mM, KCl 4.4 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, glucose 10 mM, and $NaHCO_3$ 25 mM) after which excess fat and connective tissue were removed. Rings approximately 4 mm in length were cut and mounted between stainless steel hooks in a 20-mL organ bath filled with Krebs-Ringer buffer, kept at 37 °C, and bubbled with 95% O_2/5% CO_2. An isometric force transducer (Grass FT03, West Warwick, RI, USA) was attached to each strip, after which the resting tension was constantly readjusted to $1.0 \times g$ for 60 min. Integrity of the tissues was checked with phenylephrine (10 μM) and carbachol (100 μM), followed by a washout period of 30 min. The aortic rings were then submaximally contracted with phenylephrine (10 μM). When a stable contraction was established, a carbachol concentration–response curve (10 nM to 100 μM) was recorded. When a stable relaxation was obtained after the last addition of carbachol, sodium nitroprusside (SNP; 100 μM) was added to the organ bath to induce maximum relaxation.

The pD_2 [negative logarithm of the concentration that produces 50% of the maximal response (EC_{50})] values and maximum effect of carbachol were obtained by fitting the data (four-parameter logistic curve, SigmaPlot 2001 for Windows, SPSS Inc., Chicago, IL, USA). Total relaxation (relaxation by carbachol and SNP) and the SNP fraction (part of the total relaxation caused by SNP) were calculated from the data.

Blood antioxidant and oxidative stress parameters

Erythrocyte glutathione (GSH) and glutathione disulfide (GSSG) were determined according to Baker et al. [16] with the modifications of Vandeputte et al. [17]. The trolox equivalent antioxidant capacity (TEAC) was determined in deproteinized plasma according to Arts et al. [18]. Thiobarbituric acid-reactive substances (TBARS) were measured in plasma according to Lepage et al. [19].

Chemicals

Erythritol, xylitol, sorbitol, and mannitol were provided by Cargill (Vilvoorde, Belgium). Dinitrophenylhydrazine (DNPH) and high-performance liquid chromatographic eluents were purchased from Fluka GmbH (Fluka Chemie GmbH, Buchs, Switzerland) and recrystallized from acetonitrile before use. Streptozotocin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade.

Statistical evaluation

The data are expressed as mean ± standard error. Differences between groups were investigated with Student's t test and those with a P value <0.05 were considered statistically significant.

Results

Hydroxyl and superoxide radical scavenging properties of erythritol and related polyols

Figure 1 presents the effects of erythritol, xylitol, sorbitol, and mannitol on hydroxyl radical–induced deoxyribose degradation. Erythritol scavenged $HO^{\bullet}$ radicals with a rate constant of 1.18×10^9 M/s. In comparison, xylitol, sorbitol, and mannitol displayed rate constants of 1.42, 1.56, and 1.62×10^9 M/s, respectively. An interesting correlation was observed between the number of hydroxyl groups in

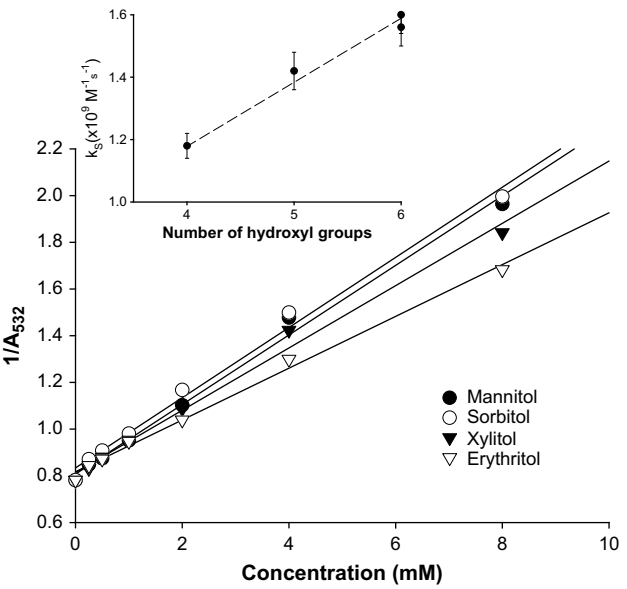

Fig. 1. Effect of test compounds on the $HO^{\bullet}$ radical-induced degradation of 2-deoxyribose. Values are means of three experiments. SEMs for A_{532} were approximately 2% of the measured value; error bars have been omitted for clarity. Fitting using linear regression (least squares method) was performed using SigmaPlot 4.01 for Windows (SPSS Inc., Chicago, IL, USA). (Inset) Correlation between the rate constant for $HO^{\bullet}$ radical scavenging and the number of hydroxyl groups present in the tested polyols. A_{532}; absorbance at a wavelength of 532 nm.

the compound under investigation and its rate constant for the reaction with $HO^{\bullet}$ radicals (Fig. 1, inset).

Figure 2 shows the effect of erythritol on superoxide radical–induced reduction of NBT. Erythritol at concentrations of up to 2 mM did not lower the NBT reduction rate. Catechol, which was used as a reference compound, completely suppressed NBT reduction at 30 μM.

Hemolysis assay

Figure 3 shows the effect of erythritol (0–50 mM) on 2,2′-azobis-2-amidinopropane dihydrochloride–induced hemolysis. Erythritol caused a concentration-dependent increase in lag time (the time it takes before hemolysis starts). The lag time was quantified by determining the time needed to obtain 50% hemolysis. The relation between the increase in lag time and the erythritol concentration is displayed in the inset of Figure 3.

Electron spin resonance

Scavenging of $HO^{\bullet}$ radicals by sugars probably proceeds by abstraction of a hydrogen atom, as shown in Figure 4. This mechanism was verified by spin trapping and analysis using an electron spin resonance spectrometer.

Exposure of erythritol to $HO^{\bullet}$ radicals in the presence of DMPO yielded a 1:1:1:1:1:1 spectrum (Fig. 5) with hyperfine splitting $A_N = 15.8$ G, $A_H = 22.3$ G. This is consistent with generation of an adduct of a carbon-centered radical with DMPO (DMPO-R) [20]. Also visible in this spectrum is a signal from a DMPO-OH adduct (1:2:2:1) with hyperfine splitting $A_N = 15.0$ G, $A_H = 14.8$ G which is the result of scavenging of $HO^{\bullet}$ radicals by DMPO (A_N and A_H are splitting constants due to nitrogen or hydrogen nuclei).

Oxidation products

According to the mechanism proposed in Figure 4, exposure of erythritol to $HO^{\bullet}$ radicals results in the formation of

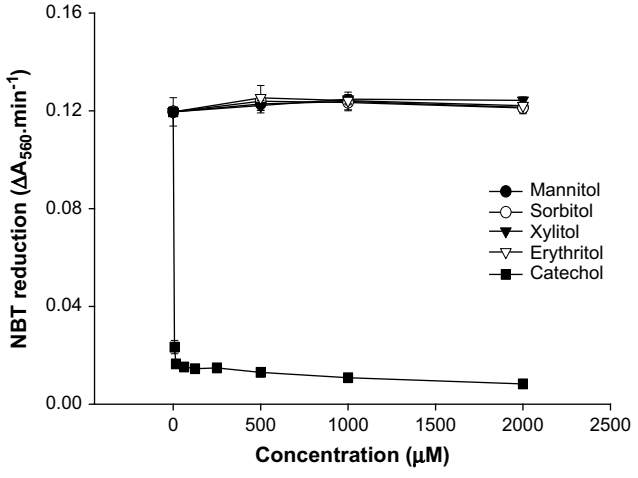

Fig. 2. Effect of test compounds on the rate of NBT reduction in a system consisting of xanthine and xanthine oxidase. Values are means ± SEMs of three experiments. NBT, nitroblue tetrazolium.

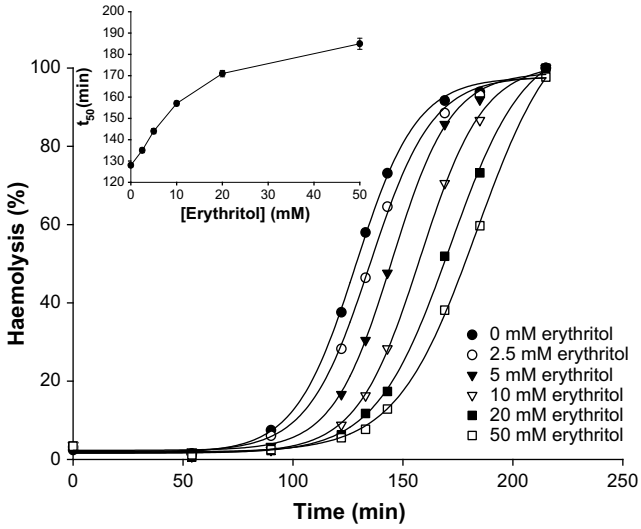

Fig. 3. Effect of erythritol (0–50 mM) on 2,2′-azobis-2-amidinopropane dihydrochloride–induced hemolysis. Data points represent the average of three separate experiments. Curves were fitted using SigmaPlot (three-parameter sigmoidal). Erythritol concentration-dependently shifted the curve to the right. (Inset) Relation between erythritol concentration and t_{50}. t_{50}, time needed to obtain 50% hemolysis.

a carbonyl group containing the products erythrose or erythrulose. Figure 6 shows chromatograms of erythritol solution before (Fig. 6B) and after (Fig. 6A) exposure to $HO^{\bullet}$ radicals followed by treatment with DNPH. Exposure of erythritol to $HO^{\bullet}$ radicals resulted in the formation of an at least 5-carbonyl group containing DNPH reactive oxidation products. The two most prominent products had chromatographic properties identical to those of the hydrazones of erythrose [retention time (RT) 3.99 min] and erythrulose (double peak at RT 5.09 min and RT 5.41 min).

Erythritol in the streptozotocin-induced diabetic rat

General characteristics

Blood glucose levels were increased 7 d after treatment with streptozotocin (25.0 ± 1.4 mM) compared with control rats (6.7 ± 0.2 mM).

Blood antioxidant and oxidative stress parameters

Antioxidant levels and status were determined at the end of the study in the animals by measuring erythrocyte GSH and GSSG concentrations and the TEAC value. As presented in Table 1, no differences among the different experimental groups were observed in GSH and GSSG levels. In diabetic rats, the erythritol-supplemented rats (DE group) tended to have slightly higher GSH (172 versus 162 μM) and slightly lower GSSG (15 versus 14 μM) levels than normoglycemic rats that drank water (D group). The same effect of erythritol supplementation was observed in the normoglycemic rats. The TEAC did not differ among experimental groups. However, a trend was observed that the diabetic rats (D and DE groups) displayed somewhat lower TEAC values than their

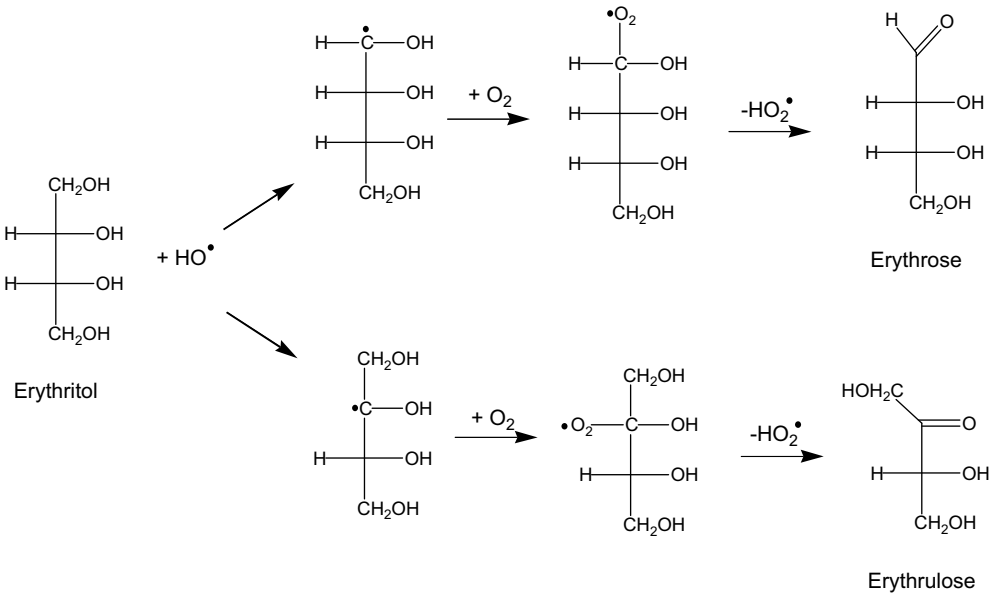

Fig. 4. Proposed mechanism for the scavenging of HO• radicals by erythritol. HO• radicals attack erythritol by abstracting carbon-bound hydrogen atoms, generating two different primary erythrityl radicals. In the presence of oxygen, these carbon-centered radicals are converted into the corresponding peroxyl radicals, which after expulsion of a protonated superoxide radical ($HO_2^•$), yield erythrose or erythrulose.

normoglycemic control counterparts (N and NE groups). In addition to the antioxidant parameters, we assessed oxidative damage in the rats by measuring TBARS levels in plasma. Similar TBARS levels were found in all four experimental groups (Table 1).

Urine production and analysis

Urine from all four groups was collected for 24 h. Samples were treated with DNPH and the amount of erythrose hydrazones was determined by high-performance liquid chromatography. The total amount of erythrose excreted (urinary erythrose concentration × urine production in 24 h) was higher in the DE group (262 ± 37 nmol) compared with the NE group (28 ± 6 nmol, $P < 0.05$; Fig. 7).

Endothelial function

Endothelial function was determined by measuring the response of isolated aortic rings to phenylephrine, carbachol, and SNP. As presented in Table 2, phenylephrine 100 μM caused an identical contraction in the aortic rings from all four groups. Figure 8 shows the carbachol concentration–response curves recorded with rings from diabetic rats (D group) and normoglycemic rats (N and NE groups). The carbachol concentration–response curve from aortic rings from diabetic rats differed markedly from the curves obtained with rings from the normoglycemic rats. The pD_2 value for carbachol was lower in the aortic rings obtained from the diabetic animals (Table 2). The curve was shifted to the right and total relaxation was lowered (Fig. 8, Table 2). Figure 9 presents the carbachol concentration–response curves from rats in the D and DE groups. Total relaxation in the aortic segments from group DE was greater, although not significantly. The pD_2 value for carbachol in the aortic rings from group DE was higher and approached the value found in the aortic rings of groups N and NE (Table 2).

Discussion

Erythritol is intended for use as a bulk sweetener in, e.g., confectionery, chewing gum, beverages, and bakery products. Because it does not affect glucose and insulin levels, it is safe for diabetics. Its structural properties resemble those of mannitol, a well-known antioxidant. Because oxidative damage has been implicated in the pathogenesis and development of diabetic complications, the antioxidant activity of erythritol was investigated in vitro and in vivo.

The hydroxyl radical scavenging activity of erythritol and other polyols such as xylitol, sorbitol, and mannitol were evaluated in the deoxyribose assay (Fig.1). The rate constant for the reaction was found to be close to that of mannitol. In fact, a good correlation between the number of hydroxyl groups in a polyol and its rate of HO• radical scavenging was found (Fig. 1, inset). Erythritol is therefore an appealing HO• radical scavenger that combines a high scavenging rate constant with good bioavailability. Erythritol did not affect superoxide-induced reduction of NBT at concentrations of up to 2 mM. This did not come as a surprise, because erythritol does not possess a hydroxyl group attached to an aromatic moiety, which has been shown to be a major structural requirement for superoxide scavengers [21].

Because of their susceptibility to peroxidation, red blood cells were used as a model to investigate the ability of erythritol to prevent oxidative damage in biological membranes.

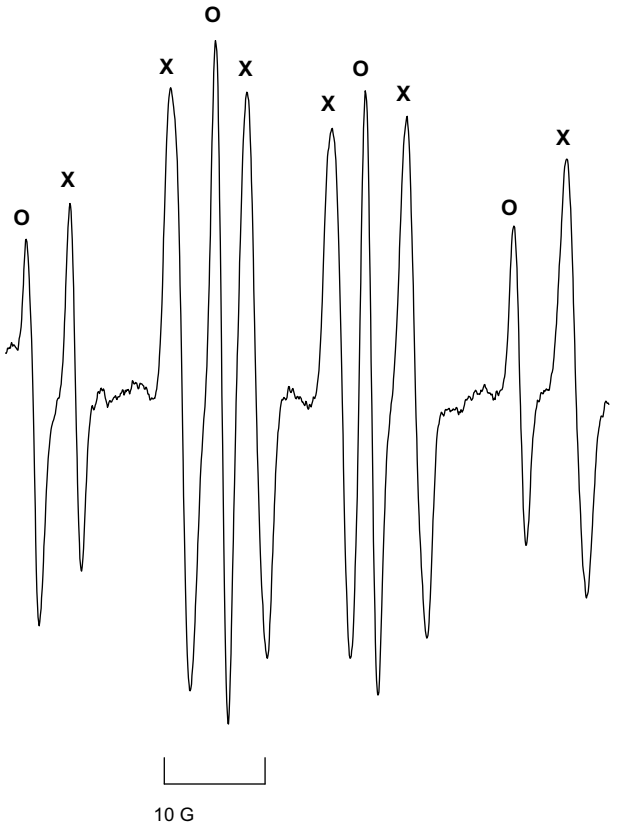

Fig. 5. Electron spin resonance spectrum of the mixture containing H_2O_2 (2 mM), 5,5′-dimethyl pyrroline N-oxide (50 mM), erythritol (500 mM), and $FeSO_4$ (1 mM) 75 s after mixing the components of the incubation. X, peaks belonging to the carbon-centered radical adduct; O, peaks belonging to the 5,5′-dimethyl pyrroline N-oxide OH adduct.

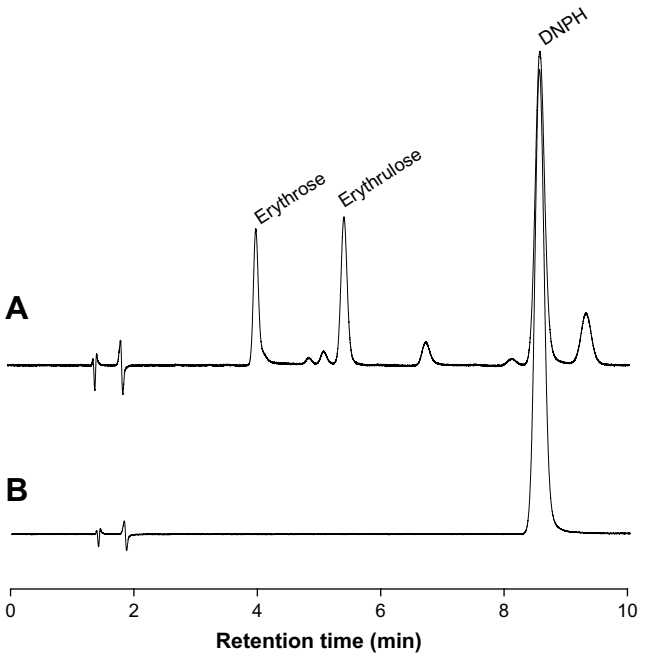

Fig. 6. Chromatograms A (erythritol plus Fenton reagent treated with DNPH) and B (blank, erythritol treated with DNPH). Oxidation products observed in chromatogram A are erythrose (3.99 min), unknown 1 (4.85 min), erythrulose, double peak (5.09 and 5.41 min), unknown 2 (6.73 min), unknown 3 (8.11 min), and unknown 4 (9.31 min). The large peak visible in both chromatograms at time 8.56 min is unreacted DNPH. DNPH, dinitrophenylhydrazine.

Erythritol inhibited radical-induced hemolysis in a concentration-dependent manner, which indicates that erythritol is also able to exert its antioxidant activity in a cellular system.

Schuchmann and von Sonntag [22] thoroughly investigated the reaction between glucose and $HO^•$ radicals. If their findings are applicable to the reaction of erythritol with $HO^•$ radicals, erythrose and erythrulose would be formed as shown in Figure 4. This scheme is supported by the findings of Cherqaoui et al. [23] who detected these compounds after electrolysis of an aqueous erythritol solution with platinum electrodes. Our study provides strong evidence that exposition of erythritol to $HO^•$ radicals indeed results in the formation of erythrose and erythrulose, among two or three other, as yet unidentified, minor oxidation products. The mechanism by which erythrose and erythrulose are formed out of erythritol appears to be analogous to the findings of Schuchmann and von Sonntag, as shown by our spin trapping experiment in which we confirmed the formation of a carbon-centered radical after abstraction of a carbon-bound hydrogen atom by a $HO^•$ radical.

The antioxidant properties of erythritol in the in vitro experiments prompted us to test its antioxidant activity in diabetic rats. Yokozawa et al. [24] studied the effect of erythritol on glucose metabolism and oxidative stress in diabetic rats. Erythritol (100, 200, or 400 mg/kg of body weight per day) showed a beneficial effect through lowering glucose levels of serum (by 15% at the highest dose) and hepatic and renal tissues. Our in vivo study focused on the $HO^•$ radical scavenging activity of erythritol and the possible prevention of diabetic complications. $HO^•$ radicals are extremely reactive and because of that almost instantaneously react with any biomolecule in their vicinity. It is therefore crucial that plasma concentrations in the millimolar range are reached. To obtain these levels rats were supplemented with erythritol at the highest well-studied dose, i.e.,

Table 1
Antioxidant and oxidative stress parameters in blood*

Experimental group	GSH (μmol/L)	GSSG (μmol/L)	TEAC (mmol/L)	TBARS (μmol/L)
N	139 ± 12	11.9 ± 0.7	589 ± 34	3.5 ± 0.2
NE	153 ± 13	10.7 ± 1.1	638 ± 34	3.8 ± 0.2
D	162 ± 7	14.8 ± 1.8	561 ± 53	3.7 ± 0.2
DE	172 ± 16	14.2 ± 0.6	517 ± 34	3.9 ± 0.5

D, diabetic rats that received normal drinking water; DE, diabetic rats that received erythritol-supplemented water; GSH, glutathione; GSSG, glutathione disulfide; N, normoglycemic rats that received normal drinking water; NE, normoglycemic rats that received erythritol-supplemented water; TBARS, thiobarbituric acid-reactive substances; TEAC, trolox equivalent antioxidant capacity

* Values are means ± SEMs, $n = 5$ for groups N and NE and $n = 4$ for groups D and DE.

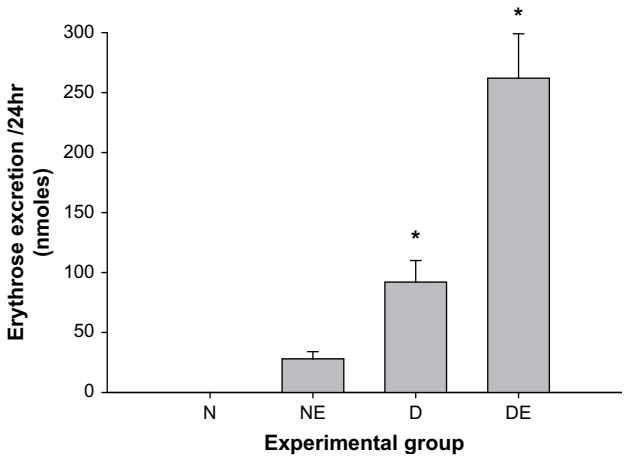

Fig. 7. Urinary erythrose excretion per 24 h by the four experimental groups. * $P < 0.05$ compared with NE group. D, diabetic rats that received normal drinking water; DE, diabetic rats that received erythritol-supplemented water; N, normoglycemic rats that received normal drinking water; NE, normoglycemic rats that received erythritol-supplemented water.

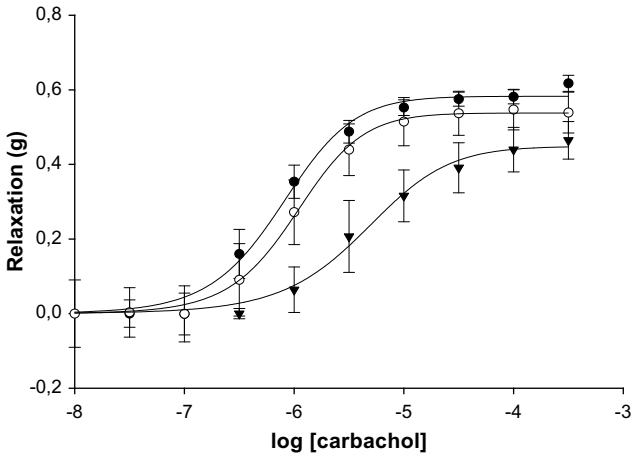

Fig. 8. Carbachol concentration–response curves recorded with aortic rings from normoglycemic rats (filled circles), normoglycemic rats that had consumed erythritol (1000 mg · kg^{-1} · d^{-1}, open circles), and diabetic rats (triangles). Consumption of erythritol did not have an effect on the concentration–response curve. The curve recorded with the diabetic rat aortic rings is shifted to the right with a lower maximum effect compared with curves from the normoglycemic rats.

1000 mg · kg^{-1} · d^{-1} for a period of 3 wk after diabetes was induced. $HO^{\bullet}$ radical scavenging by erythritol in the diabetic rat was investigated by studying the presence of erythrose and/or erythrulose in the urine of diabetic rats. Under normal circumstances, erythritol is excreted unchanged in the urine [7]. In the hyperglycemic state, high levels of systemic oxidative stress are known to be present [3,25]. If $HO^{\bullet}$ radicals are among the reactive species formed and sufficient erythritol is present, it would be expected that the oxidative metabolites erythrose and erythrulose would be formed. Their hydrophilicity and structural similarity to erythritol make it likely that these compounds are excreted by the urine. Therefore, the presence of erythrose and erythrulose, two of the oxidative metabolites of erythritol, in the urine of diabetic rats that were supplemented with erythritol was investigated. Interestingly, erythrose was indeed found in the urine of diabetic rats, indicating that $HO^{\bullet}$ radicals are produced systemically during hyperglycemia and that these have been scavenged by erythritol. However, it should be realized that the kinetic consequences of the extremely high reactivity of the $HO^{\bullet}$ radical make it questionable that it is only scavenged by erythritol. Furthermore, it cannot be ruled out that the erythrose also originates from alcohol dehydrogenases, which might also explain the presence of erythrose in the normoglycemic rats.

The effect of erythritol on endothelial function was studied in rings prepared from the thoracic aorta. Contraction was elicited by stimulating with phenylephrine an α-adrenergic receptor agonist. Subsequently, the endothelium-dependent relaxation was studied by constructing concentration–response curves with the muscarinic receptor agonist carbachol. In rat aorta the relaxant response to carbachol is mediated by nitric oxide (NO) produced in the endothelium. NO diffuses to the vascular smooth muscle cells, where it activates cyclic

Table 2
Effect of erythritol on reactivity in aortas from normoglycemic and diabetic rats*

	Normoglycemic		Hyperglycemic	
	Control	Erythritol	Control	Erythritol
PE (g)	0.66 ± 0.09	0.70 ± 0.06	0.62 ± 0.02	0.60 ± 0.07
CAR (g)	0.63 ± 0.07	0.60 ± 0.07	0.48 ± 0.03	0.61 ± 0.07
CAR pD_2	6.12 ± 0.05	5.98 ± 0.02	5.35 ± 0.07	5.65 ± 0.05
SNP (g)	0.13 ± 0.03	0.21 ± 0.06	0.30† ± 0.07	0.14 ± 0.03
TR (g)	0.76 ± 0.09	0.81 ± 0.09	0.78 ± 0.04	0.75 ± 0.09
SNP fraction (%)	17.4 ± 3.1	24.7 ± 5.5	36.8 ± 7.0†	17.8 ± 3.1‡

CAR, relaxation induced by carbachol 100 μM; pD_2, negative logarithm of the concentration that produces 50% of the maximal response (E_{50}); PE, contraction induced by phenylephrine 10 μM; SNP fraction, contribution of sodium nitroprusside 100 μM to total relaxation; SNP, relaxation induced by sodium nitroprusside 100 μM after stable response to carbachol 100 μM was obtained; TR, total relaxation induced by carbachol 100 μM followed by sodium nitroprusside 100 μM

* Values are means ± SEMs, $n = 5$ for normoglycemic rats that received normal drinking water and normoglycemic rats that received erythritol-supplemented water and $n = 4$ for diabetic rats that received normal drinking water and diabetic rats that received erythritol-supplemented water.

† $P < 0.05$ compared with normoglycemic rats that received normal drinking water.

‡ $P < 0.05$ compared with diabetic rats that received normal drinking water.

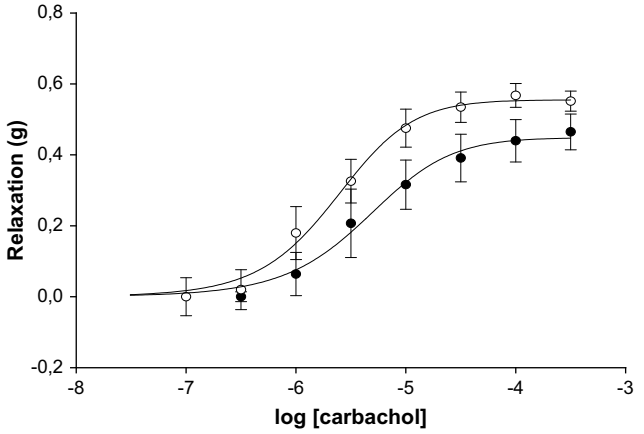

Fig. 9. Carbachol concentration–response curves recorded with aortic rings from diabetic rats (closed circles) and diabetic rats that had consumed erythritol (1000 mg · kg^{-1} · d^{-1}, open circles). Consumption of erythritol caused a leftward shift of the concentration–response curve and an increased maximum effect.

guanosine monophosphate formation by soluble guanylate cyclase, which eventually causes relaxation. Maximum relaxation was induced by the addition of SNP, a compound that releases NO and thus bypasses endothelial NO production. Our in vitro determination of the endothelium-dependent relaxation of aortic rings showed that the carbachol response in aortic segments from diabetic rats was markedly attenuated after 3 wk of hyperglycemia. The pD_2 value and the maximum effect were lowered compared with those in normoglycemic rats. Total relaxation of the diabetic rings was not less compared with the other groups but a larger part had to be provoked by SNP, indicating that the relaxant capacity of vascular smooth muscle was not affected in the diabetic rats. The attenuated response to carbachol in diabetic rats combined with the unaltered total relaxation provides strong evidence that NO production is hampered in diabetic animals. It is known that hyperglycemia is associated with attenuation or loss of endothelium-dependent relaxation [26] and that reactive oxygen species mediate this loss [25]. This reduced endothelium-dependent vasodilator capacity of the coronary and peripheral circulation to acetylcholine or other M_3 receptor agonists is commonly referred to as *endothelial dysfunction* [27]. Endothelial dysfunction is thought to play an important role in vascular diabetic complications or diabetic vasculopathy [28] and appears to be a stepping stone for atherosclerosis [29]. In diabetic rats (group D) we found an attenuated response to the vasorelaxant compound carbachol. The total relaxation and the pD_2 value were lower in aortic rings from group D, whereas the total relaxant response (carbachol plus the NO-donating compound SNP) was unchanged. This indicates that the endothelium of the diabetic rats was damaged and therefore not capable of generating sufficient NO to induce maximum relaxation. The smooth muscle that lies underneath the endothelium, however, appears to be intact as evidenced by the unaltered contractile response to phenylephrine and the total relaxant response to the carbachol–SNP combination. In the diabetic rats that were consuming erythritol, the endothelium appeared intact, and the response to carbachol was almost identical to that observed in the normoglycemic rats. Reflecting this uncompromised response to carbachol is the smaller SNP contribution to the total relaxation of aortic segments in the erythritol-supplemented diabetic rats. Our results are in good agreement with those obtained by Dhein et al. [30] who studied the effect of vitamin E supplementation in the streptozotocin-induced diabetic rat. After 7 mo endothelium-dependent relaxation was found to be decreased by 50% in diabetic rats on a medium or high vitamin E diet, whereas in vitamin E–deprived rats, a complete loss of endothelium-dependent relaxation was found [30]. From these results and the results of our study it can be concluded that dietary antioxidants can play an important role in preventing vascular damage associated with diabetes. It is nevertheless intriguing that two different compounds, vitamin E and erythritol, which differ greatly in terms of lipophilicity and structural characteristics such as the presence of an aromatic function, demonstrate a similar vasoprotective effect in vivo. The lipid-soluble vitamin E owes its antioxidant activity to trapping lipid peroxyl radicals in membranes [31]. This contrasts sharply with the highly hydrophilic erythritol, which will almost exclusively act in the aqueous compartments. Further mechanistic research into the vasoprotective effect of erythritol is therefore indicated. In addition to its potential vasoprotective action, we investigated the effect of erythritol supplementation on antioxidant levels and markers of oxidative stress in blood. Surprisingly, the hyperglycemia to which the diabetic rats were subjected did not cause detectable changes in GSH, GSSG, and total antioxidant capacity. These findings contrast with the larger part of data on antioxidant levels and oxidative stress markers in diabetic patients and laboratory animals [1,2,30,32]. This discrepancy may result from the relatively short period (21 d) that our diabetic rats were hyperglycemic. Their antioxidant reserves may have been sufficient to prevent systemic oxidative damage for that period. The observed endothelial dysfunction may be the result of locally generated $HO^{\bullet}$ radicals, which fits perfectly with the observation of Pennathur et al. [33] who found that glycoxidation reactions in the arterial microenvironment form the first step toward heart disease in diabetics. Thus, not the generalized oxidative stress but rather the very local overproduction of $HO^{\bullet}$ radicals could be responsible for the effects we observed. Pennathur et al. [33] also demonstrated that a $HO^{\bullet}$ radical-like species is implicated in diabetic vasculopathy by comparing oxidation products of amino acids oxidized in vitro with those obtained from human arteries.

The protective effects of erythritol need not be restricted to DM. Its unique free radical scavenging properties could be beneficial in other chronic disorders in which oxygen radicals are responsible for tissue damage. Efficient scavengers of $HO^{\bullet}$ radicals, namely mannitol and dimethylsulfoxide, are used in the treatment of complex regional pain syndrome I [34]. In a rat model of cisplatin-induced acute renal failure, dimethylthiourea attenuated the increase in serum creatinine,

accumulation of malonaldehyde, and reduced the amount of tubular damage [35]. Li et al. [9] showed that polyols protect proteins from oxidation, indicating that a significant demand for compounds that reduce $HO^{\bullet}$ radical-induced damage exist. For biomedical research purposes, erythritol could be employed as a marker for endogenous $HO^{\bullet}$ radical formation during inflammatory diseases in the same fashion as antipyrine is currently employed [36].

In summary, the present results demonstrate that in vitro erythritol is an excellent $HO^{\bullet}$ scavenger with membrane-protecting properties. Scavenging of $HO^{\bullet}$ radicals by erythritol results in the formation of erythrose and erythrulose. In vivo, erythritol prevented endothelial dysfunction in diabetic rats, although it remains difficult to attribute this to its $HO^{\bullet}$ radical scavenging properties. In addition, analytical data are presented that provide strong, although not conclusive, evidence that erythrose and erythrulose are among the oxidative metabolites of erythritol and that erythrose was found in the urine of erythritol-consuming diabetic rats. Safety studies have indicated that erythritol is well tolerated and shows no signs of toxicity [7]. It is therefore an excellent sugar substitute for individuals with DM. In this respect erythritol may help reduce the glycemic impact of a food or beverage, thereby reducing the effects of hyperglycemia-induced free radical formation. Both are expected to reduce the onset and progression of painful and life-threatening diabetic complications.

Conclusion

Erythritol proved to be a good $HO^{\bullet}$ radical scavenger and inhibitor of diazocompound-induced erythrocyte damage in vitro. In the diabetic rat, erythritol consumption resulted in unaffected endothelial function that was accompanied by the presence of oxidative erythritol metabolites in the urine.

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