

ESR Investigations on Blood Treated Intravenously with Ascorbic Acid*

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Z. Naturforsch. **36 c**, 1–4 (1981); received September 9/October 20, 1980

ESR, Ascorbic Acid, Blood Count, Copper and Spin Concentration of Blood

The effect of 1 g of ascorbic acid, administered intravenously to healthy male and female volunteers, on blood and its constituents was investigated by means of electron spin resonance (ESR) spectroscopy and by differential hematologic examinations. The native blood ESR spectrum exhibits 2 min after injection of vitamin C a considerable increase in spin concentration and a new signal at about $g = 2.005$ which we previously had found to correlate to the semidehydroascorbate radical. This spectrum is identical to that obtained in acute lymphatic leukemia. While it prevails in untreated leukemic patients, spin concentration and shape of the spectrum obtained return to normal within several hours to one day in healthy individuals. Since neither in erythrocytes nor in plasma modifications could be observed, the ESR changes detected must have their origin in the leukocytes. Hematologic studies of the peripheral blood show that the number of granulocytes, lymphocytes, and thrombocytes was not drastically affected by the vitamin C injection.

Introduction

Recently we have shown that the electron spin resonance (ESR) spectra of lyophilized blood or of its constituents of patients with acute lymphatic leukemia exhibit an increase in spin concentration and a signal at about $g = 2.005$ which is not present in control samples [1]. It could be demonstrated that *in vitro* addition of ascorbic acid to blood constituents of healthy volunteers or to copper-containing proteins produced an identical signal which originates from the semidehydroascorbate (SDA) radical [2–4]. It appears as a singlet due to a change in relaxation time caused by the interaction between vitamin C and its receptor.

In the present study the *in vivo* effect of ascorbic acid, administered intravenously to healthy male and female volunteers, on blood and its constituents was investigated by means of ESR spectroscopy. Concomitantly, differential hematologic examinations were made in each case.

Materials and Methods

16 healthy volunteers of both sexes between 20 and 30 years old were administered intravenously a

5 ml aqueous solution of 1 g of ascorbic acid (Merck, Darmstadt, Germany). Shortly before the administration of the ascorbic acid and 2, 5, 15, 60 min, 3 to 7 h, 24, and 48 h after the injection, 0.5 ml of venous blood and 10 ml of 1:10 ACD-blood (acid-citrate-dextrose anticoagulant solution) were collected. The latter samples were used for the separation of erythrocytes and plasma. All were subsequently lyophilized for the ESR studies. Obtained simultaneously were 2 ml of EDTA-blood for the hematologic examinations.

The ESR spectra were obtained with a Varian E-9, 100-kHz modulation X-band spectrometer. A DPPH (diphenylpicrylhydrazil) standard ($g = 2.0036$) was used as a reference for marking resonance positions. The modulation amplitude was 0.2 mT and the microwave power 5 mW for all samples investigated. The spectra of 50 mg samples each were recorded at different sensitivities marked at the left-hand side of each spectrum. All measurements were done at room temperature. The relative spin concentration was obtained by double integration of the spectra by means of a planimeter.

The concentration of ascorbic acid in erythrocytes and plasma was determined after derivatization with 2,4-dinitrophenylhydrazine according to the method described by Omaye *et al.* [5].

The concentration of Cu in erythrocytes and plasma was determined with a Zeiss atomic absorption spectrometer model FMD 3 using a graphite furnace HGA 72 accessory.

* Dedicated to Professor Dr. W. Hanle on the occasion of his 80th birthday.

** On leave of absence from Stanford University, Stanford, Cal., USA. Recipient of the A. v. Humboldt US Senior Scientist Award.

Reprint requests to Prof. Dr. W. Lohmann.

0341-0382/81/0100-0001 \$ 01.00/0



Results and Discussion

The effect of ascorbic acid, administered intravenously, on the ESR spectra of lyophilized native blood and erythrocytes is shown in Fig. 1. There is a characteristic change of the native blood spectrum 2 min after administration of vitamin C consisting of a considerable increase in spin concentration and the formation of a signal at about $g = 2.005$ which we previously had found to correlate to the SDA radical [4]. This spectrum, as shown in the middle of Fig. 1, is identical to that obtained in the cases of acute lymphatic leukemia [1]. While it prevails in untreated leukemic patients, spin concentration and shape of the spectrum return to normal within several hours to one day in healthy volunteers (s. Fig. 2). From these results, one may conclude that a substance (oxidizing compound?) exists in healthy individuals which compensates and regulates the interaction between vitamin C and blood constituents and which is absent, or present only in diminished quantity, in patients with acute lymphatic leukemia. It should be emphasized that lyophilized samples have been used. The results of which seem to agree, however, with those obtained with liquid samples as has been shown by some other authors [6].

The shape of the curve in Fig. 2 suggests that the spin concentration of native blood reaches its peak prior to the obtainment of the first blood sample which is taken two min after injection of vitamin C.

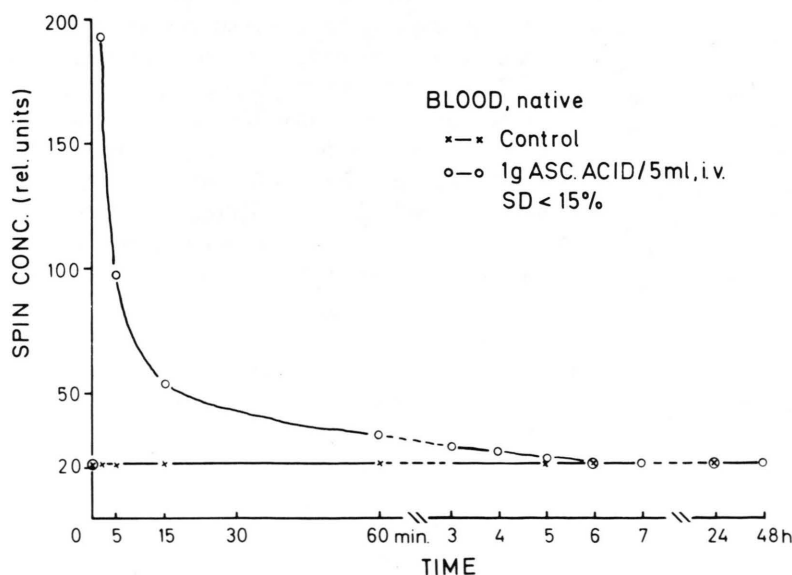


Fig. 2. Change of the rel. spin concentration of native blood after intravenous injection of 1 g of ascorbic acid. SD \cong standard deviation.

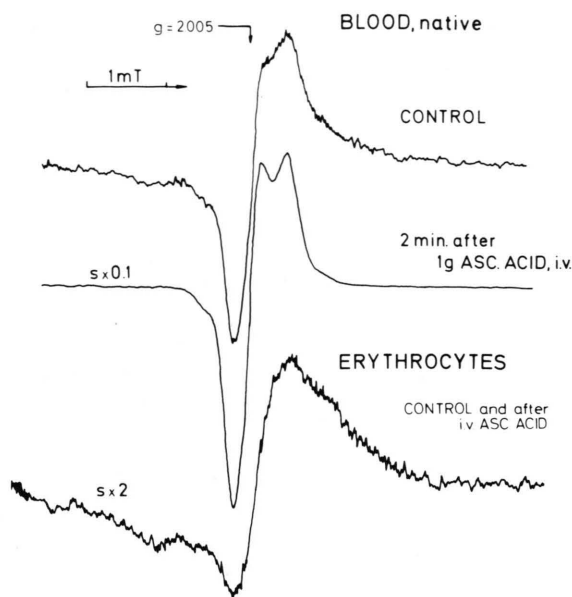
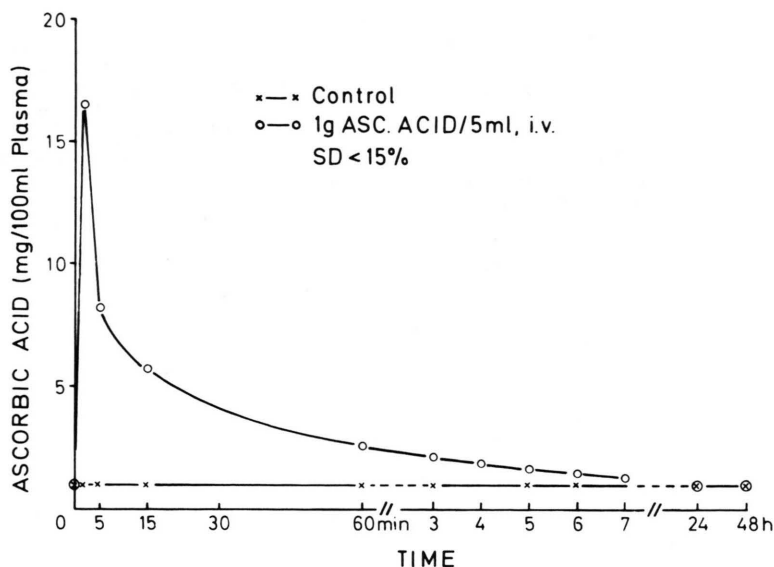


Fig. 1. ESR spectra of native blood and of erythrocytes of controls and after intravenous injection of 1 g of ascorbic acid. $s \cong$ rel. sensitivities.

It should be pointed out, that the spectrum of the erythrocytes (s. Fig. 1, lower curve), which is not affected by ascorbic acid, remained unchanged throughout the period of time the measurements were made. Whether higher concentrations of ascorbic acid will alter the spectrum has yet to be investigated. Clinical experience and physiological findings

Fig. 3. Ascorbic acid concentration of the plasma after intravenous injection of 1 g of ascorbic acid. SD \cong standard deviation.



have shown that injection of doses of more than 10 g of vitamin C are contraindicated.

The spin concentration of plasma is increased 1.7 fold in the 2 minute-sample, however, it returns to normal within 15 min after injection. Thus, the ESR changes observed in the native blood must have their origin in the leukocytes. Unfortunately, it was not possible to obtain large enough quantities of this cell type in order to determine their response to ascorbic acid.

The change in ascorbic acid concentration in the plasma after *i.v.* administration of vitamin C is shown in Fig. 3. After a steep increase within the first 2 min, the concentration gradually decreases to near normal levels within several hours to one day. It is interesting to note that ascorbic acid is distributed uniformly all over the body within the first 2 min. Assuming a total blood volume of 5000 ml, one should expect an ascorbic acid concentration of about 20 mg/100 ml after injection of 1 g. The average value obtained 2 min after injection is about 17 mg/100 ml (s. Fig. 3).

In the erythrocytes, only a slight increase in ascorbic acid concentration takes place within the first few minutes after injection (1.4 fold). After 5 to 15 min, however, the values have returned to normal.

The copper concentration of the plasma decreases slightly after the first 5 min after ascorbic acid treatment (about 14%), but returns to normal after

60 min. In the case of erythrocytes, no major change in Cu contents could be observed.

Hematologic studies of the peripheral blood show that the number of granulocytes, lymphocytes, and thrombocytes was not drastically affected by the vitamin C injection. However, it should be pointed out, that in two individuals with an increased leukocyte count (11.5 and 16×10^3 cells/ μ l), *i.v.* administration of 1 g of ascorbic acid resulted in a steady decrease (8.5 and 10.5×10^3 cells/ μ l after 24 h).

Another interesting observation should be mentioned. Several of the volunteers used as controls received 5 ml of a physiological saline solution. ESR examination of lyophilized samples of this solution shows a strong Mn^{2+} spectrum. The shape of the Mn^{2+} spectrum suggests the existence of two different Mn-complexes. The Mn-concentration of the physiological saline solution is about 15 ppb, as determined by atomic absorption analysis.

Acknowledgements

We kindly appreciate the generous help of several colleagues of the Medizinische Universitäts-Poliklinik. The excellent technical assistance of Miss Lieselotte Giloy, Zentrum für Klinische Chemie und Pathobiochemie, and Mrs. Gundula Will, Institut für Ernährungswissenschaft, is greatly appreciated. This work was supported in part by Euratom grant EUR no. 213-76-7 BIO D, by the Bundesministerium des Innern, and by the Fonds der Chemischen Industrie.

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