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ORIGINAL ARTICLE

Free radical monitoring in human blood following therapy interventions with drugs and natural compounds

Évaluation des radicaux libres dans le sang chez l'homme après traitement médicamenteux et absorption de nutriments antiradicalaires

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KEYWORDS
Free radicals;
Reactive oxygen species (ROS);
Chemiluminescence (CL);
Antioxidant activity (AOA);
Venous blood;
Plasma;
Serum;
Atopic eczema;
Chemical sensitivity (MCS);
Vitamin C;
EDTA;
Native juices

Summary Generation of reactive oxygen species (ROS) in venous blood, serum and plasma samples from 19 subjects was investigated before and after various therapies by means of a simplified ultra-weak chemiluminescence (CL) methodology. Nine atopic eczema, five multiple chemical sensitive (MCS) patients and five healthy controls were enrolled in this preliminary study. The CL technique resumes in adding a constant amount of venous blood, plasma or serum to a constant amount of buffered lucigenin, followed by a short preincubation and registration of the photon counts over a time period of 600 s. The in vitro antioxidant activity (AOA) of different drugs and natural compounds was also evaluated at 36.5 °C by inhibiting a lucigenin-perborate ROS generating source over a 5 min time interval. Concentrations (0.05 M) of known water-soluble antioxidants displayed AOAïs ranging between 16.7 ± 0.6 inhibition units (IU) for L-taurine and 187 ± 9 IU for L-ascorbic acid. Teas, natural juices and wines ranged from 1.4 ± 0.1 IU (cystus tea) to 292 ± 12 IU (cherry juice) and 300 ± 13 IU (dry red wine), respectively. Control healthy plasmas showed significantly higher antioxidant activities than MCS plasmas (7.1 ± 1.6 IU vs. 1.2 ± 0.4 IU) whereas different protein solutions ranged between 1.0 ± 0.1 IU (infant formula) and 194 ± 12 IU (bovine colostrum). Oral administration of 500 ml commercial juice mixture (Cellagon) induced a strong increase of photon generation in venous blood and plasma of control subjects, by contrast the same amount of native blueberry juice resulted in an immediate decrease of the ROS generation in both blood and plasma of atopic eczema patients, paralleled by a temporarly increase in the plasma AOA. In vitro addition of

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disodium-ascorbate (pH 7.0) to serum and the in vivo administration of 8.5—20 g ascorbate i.v. induced a dramatic increase of the ROS/photon count generation in both systems at 22 °C associated with a worsening of the clinical course in atopic patients up to 48 h after the infusion. In vitro addition of different drugs to venous blood and plasma showed an excellent correlation to the ''in vivo'' intravenous administration of the same compounds (α-lipoate, MgK_2-EDTA, Na_2-EDTA) in the investigated atopic patients. MgK_2-EDTA and α-lipoate temporarily increased the ROS-activity in whole blood whereas Na_2-EDTA depicted an individual time-related ROS-inhibitory pattern. The described CL assays enable a rapid evaluation of ROS generation and antioxidant activity of whole blood, plasma and serum samples at low operational costs. It further allows the AOA evaluation of different drug and dietary agents and their effects on the total antioxidant status of the body as well as the monitoring of the clinical course and of therapy interventions in various diseases, respectively.

MOTS CLÉS
Radical libre ;
Espèces d'oxygène réactif (ROS) ;
Chemoluminescence (CL) ;
Activité antioxidante (AOA) ;
Sang veineux ;
Plasma ;
Sérum ;
Eczéma atopique ;
Multiple sensibilité chimique (MCS) ;
Vitamine C ;
EDTA ;
Jus naturel

Résumé Les dérivés réactifs de l'oxygène (DRO) (*reactive oxygen species, ROS*) sont pour la plupart des radicaux chimiques dérivés de l'oxygène. La synthèse de DRO dans le sang artériel et veineux de 19 sujets a été étudiée, avant et après absorption de diverses substances médicamenteuses ou naturelles, au moyen d'une méthode de chimiluminescence simplifiée. Neuf patients avec eczéma atopique, cinq patients ayant un syndrome de sensibilité chimique multiple et cinq sujets témoins ont été inclus dans cette étude préliminaire. La technique de chimiluminescence repose sur l'addition d'une quantité constante de sang veineux, plasma ou sérum à une quantité constante de réactif aux DRO, avec enregistrement d'émission de photons sur une période prédéterminée. L'activité antioxydante in vitro (AOA) de ces différentes substances médicamenteuses ou composés naturels a été évaluée à la température constante de 36,5 °C. Les concentrations de 0,05 M d'antioxydants solubles dans l'eau ont montré une activité anti-oxydante in vitro s'étendant de 16,7 ± 0,6 unités d'inhibition (IU) pour la L-taurine à 187 ± 9 IU pour l'acide L-ascorbique. Les thés, les jus de fruits naturels et le vin avaient une activité anti-oxydante in vitro évaluée à 1,4 ± 0,1 IU (thé) à 292 ± 12 IU (jus de cerise) et 300 ± 13 IU (vin rouge) respectivement. L'activité plasmatique anti-oxydante in vitro était significativement plus haute chez les sujets sains que dans le plasma des patients ayant un syndrome de sensibilité chimique multiple (1,2 ± 0,4 IU versus 7,1 ± 1,6 IU). L'activité anti-oxydante de différentes solutions protéiques variaient de 1,0 ± 0,1 IU pour les plus basses, à 194 ± 12 IU (colostrum bovin). L'administration orale de boissons enrichies en antiradicaux libres (type Cellagon) est à l'origine d'une forte augmentation de photons dans le sang de sujets témoins, alors que la même quantité de jus naturel de myrtille entraînait une diminution significative de l'afflux sanguin de DRO chez les patients atteints d'eczéma atopique. L'addition d'ascorbate de sodium (8,5—20 g) a entraîné une augmentation significative de DRO, notamment dans le sérum de patients atopiques avec aggravation de leurs manifestations cliniques 48 heures après l'administration. Ces résultats paradoxaux pourraient être liés à un phénomène d'auto-oxydation de la vitamine C en présence de métaux circulants, comme cela a été rapporté chez les patients atopiques ou présentant un syndrome de sensibilité chimique multiple. Les résultats obtenus par évaluation de l'activité antioxydante in vitro par technique de chimiluminescence étaient parfaitement corrélés, entre l'administration in vitro de différentes substances médicamenteuses (acide ascorbique, mannitol, glutathion, L-cystéine, N-acétyl-cystéine et EDTA) et l'administration intraveineuse « in vivo ». Cette nouvelle technique permet de mieux évaluer l'activité antioxydante dans le sang total, le plasma et le sérum de différentes substances (médicaments ou produits diététiques) et de prévoir leurs effets thérapeutiques dans des affections diverses.

Introduction

The appropriate choice of oxidative and/or antioxidative therapies in the daily clinical practice remains a permanent challenge for the physician. As the investigation of the internal body status before therapy is essential in this respect, we have reported the possibility to assess it by measuring the redox state and the generation of reactive oxygen species (ROS) in blood and serum in different clinical conditions [1—6].

Using a simplified enhanced chemiluminescence methodology we found that atopic, psoriasis, MCS and cancer patients are exibiting significantly changed light emission patterns in whole blood and serum at room temperature when compared to healthy subjects [3—6]. The free radical quenching, i.e. the antioxidative activity (AOA) of

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patient sera was also different from controls at 22 °C [3–6].

Based on the above results we have prompted the investigation of free radical levels in atopic and MCS patients at 22–36.5 °C before and after oxidative or antioxidative approaches. For the appropriate therapy choice, we have also evaluated in a standardized 5 min test the in vitro antioxidative activity of different drugs and natural compounds before their administration to patients.

Our preliminary data also describe the monitoring of free radical generation in whole blood and plasma samples from patients subjected to different therapy approaches.

Patients and methods

Patients and controls

Five apparently healthy controls (19–35 years) belonging to the clinical staff in Neukirchen, nine atopic eczema (18–53 years) and five multiple chemical sensitive (MCS) patients (23–58 years) belonging to the clinical ward of the Spezialklinik Neukirchen, Germany were enrolled in this study after given informed consent. Patients were selected in accord to standard criteria for these disorders [7,8]. None of them was on steroids, antibiotics or antioxidants when starting the investigations.

Blood sample processing

All blood samples were taken in the morning from fasting patients or healthy controls after 15 min bed rest. Venous blood samples were drawn into heparinized Vacutainer tubes and immediately introduced into the temperated coil of a BJL Ultra-Weak Chemiluminescence Analyzer at 36.5 °C. Fresh plasma samples obtained from heparinized blood after 10 min centrifugation at 4000 rpm were processed similarly. Several measurements in serum (ascorbate tests) were run at room temperature (22 °C).

The same procedure was used before and at different time intervals after individual therapy approaches in selected patients who gave their informed consent for this study. All CL-blood, serum and plasma tests were done in duplicate.

Antioxidative activity (AOA) of drugs and natural compounds

Ascorbic acid, mannitol, reduced glutathione, L-cysteine, N-acetyl-cysteine and were purchased from Fluka AG, Germany and tested as 100 μl aliquots of 0.05 M concentration. L-taurine, kindly donated by American Biologics, USA, was tested in the same concentration. One hundred microlitres of an anti-itching skin lotion supplied by the Spezialklinik Neukirchen, Germany, was also investigated for its AOA. Three hundred microlitres of tea extracts (20 g/l) from cystus, black and green tea as well as 100 μl of freshly pressed fruit and vegetable juices (elder, blueberry, cherry and carrot) were passed through S&S 595 filter papers before being tested for their free radical scavenging activity. One hundred microlitres dry white and red wine samples have also been assessed for their AOA.

Fresh heparinized venous blood (10 μl), plasma (500 μl), human gammaglobulins (500 μl) and 100 μl of hydrolysed whey protein (10%), human serum albumin (5%), and bovine colostrum (4.4%) reported as protein fraction were also tested for their ROS quenching activity.

The inhibition of the ultra-weak photon emission at 36.5 °C by the above compounds was measured by means of an enhanced chemiluminescence (CL) assay using a BJL-Ultra-Weak-Chemiluminescence Analyzer with a high sensitivity detector (3.3×10^{-15} W/cm^2 × count) from American Biologics, USA. Daily calibration of the detector sensitivity was performed with a ^{14}C light source generating an amount of 10,000 photon counts/s at a voltage ranging from 970 to 990 volts. The variability of the ^{14}C light source was < 1%.

The antioxidative activity (AOA) was assessed with a free radical generating mixture (1 ml buffered lucigenin +0.1 ml perborate), using reagents from American Biologics, USA. The reaction mixture produces in the temperated detector coil at 36.5 °C a strong light emission output of $1.195–1.200 \times 10^6$ photons/30 s for at least 15 min. The $^*O_2^-$, H_2O_2, and *OH generation in the mixture was previously demonstrated with standard inhibition tests using SOD, catalase and mannitol [6].

Inhibition of light emission induced by whole blood, plasma, protein solutions, fresh juices, teas, wines, water-soluble antioxidants and anti-itching skin lotion, respectively, was assessed in a 5 min AOA test.

The above-described amounts of test compounds were quickly added to 1.1 ml free radical generating mixture. Total reaction time was 300 s with a photon count reading interval in the CL-analyzer of 30 s.

An inhibition unit (IU) was defined as the 50% reduction of the lucigenin-perborate total light intensity over 5 min incubation at 36.5 °C (11,980,000 c./300 s) through 94 μl 0.05 M L-cysteine. The final AOA result for 100 μl blueberry juice, e.g., was calculated as follows:

$$\text{AOA } 94\mu\text{l L} - \text{cysteine} = \frac{11,980,000\,\text{c}/300\,\text{s}}{2} = 5,990,000\,\text{c}/300\,\text{s} = 1.0\,\text{IU}$$

Photon inhibition by 100 μl native blueberry juice: 21,919 c/300 s.

$$\Rightarrow \text{AOA } 100\mu\text{l native blueberry juice} = \frac{5,990,000\,\text{c}/300\,\text{s}}{21,919\,\text{c}/300\,\text{s}} = 273\,\text{IU}$$

Alternatively, the inhibition units (IU) can be referred to 60 s intervals (IU / min) by dividing the final result by 5.

All AOA tests have been repeated at least four times. Mean values ±SD are reported in the result section. The intra-assay variability in the AOA test was < 10%.

CL tests for blood, plasma and serum

As previously reported the impact of free radical inducing or quenching therapies *in vivo* can be monitored by means of chemiluminescence (CL) determinations [3–5].

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Basal photon counts were recorded after mixing 0.5 ml heparinized blood or plasma with 1 ml of buffered lucigenin to a constant total amount of 1.5 ml in the detector cup. Registration of the basal counts was performed after 5 min preincubation at 36.5 °C or 22 °C in the black detector chamber in the presence of O_2 (air). Total measure time was 600 s, the interval between two countings was 30 s. Preincubation of the venous blood or plasma with the CL substrate for 5 min. allows the sample to reach the reaction temperature in the detector coil and avoids large variations in photon recordings immediately after pipetting and shaking the mixture. Results proved to be reproducible within a 10% margin of error. All tests have been done in duplicate.

Therapy protocols

Oral intake of native juices

Two fasted atopic eczema patients and five healthy controls aged 23—35 years were orally administered 500 ml native blueberry juice or Cellagon juice mixture diluted 1:5 with deionized water, respectively. The Cellagon concentrate (Berner, Germany) includes carrots, broccoli, artichokes, oranges, acerola, aloe vera and other natural extracts. Serial CL tests were performed in venous blood and plasma before, 45 min and 90 min after intake of Cellagon as well as before, 90 and 180 min after native blueberry juice, respectively.

Intravenous administration of high dose vitamin C

Two of four fasted atopic eczema patients received intravenously 8.5 g the other two 20 g buffered Na-ascorbate (Pascoe, Germany) in 500 ml dextrose 5%, during 2 h infusion time. The free radical generation was monitored in serum and venous blood in vitro and in vivo before, 1, 2, 5 and 24 h after the procedure, respectively.

Intravenous EDTA-chelation therapy

Five MCS patients with metal hypersensitivity (type IV) reactions and three atopic eczema patients gave their informed consent for the i.v. EDTA-chelation procedure. 3 g of Na_2-EDTA or MgK_2-EDTA were diluted in 500 ml 10% mannitol/sodium acetate or Ringer-lactate and slowly administered i.v. during 2.5—3 h. Serial CL investigations were accomplished before and 10 min, 3, 5 and 18 h after the i.v. infusion, respectively. Lead and cadmium release in urine was measured at 1, 2 and 3 h after infusion by means of atomic absorption spectrophotometry (AAS).

Results and discussion

Antioxidative activity (AOA) of various biological samples

The described 5 min test enables a quick and easy assessment of the antioxidative activity in biological samples. This becomes possible by using a strong and stable free radical source as previously reported [6]. One inhibitory unit (IU) induced by 94 μl 0.05 M L-cysteine served as reference when testing the free radical quenching activity of natural compounds, known antioxidants, venous blood, plasma and other protein samples.

Different antioxidative activities of water-soluble compounds have been recorded in the standardized AOA test (Fig. 1).Whereas L-taurine (16.7 ± 0.6 IU), N-Ac-cysteine (30.7 ± 2.7 IU) and mannitol (41.1 ± 3.2 IU) displayed a moderate inhibition of photon emission, the reduced glutathion (170 ± 5.5 IU), the ascorbic acid (187 ± 9 IU) and most of all the anti-itching skin lotion (376 ± 15 IU) demonstrated significantly raised ROS quenching capabilities. The AOA data recorded in Fig. 1 represent mean values of four determinations for each compound.

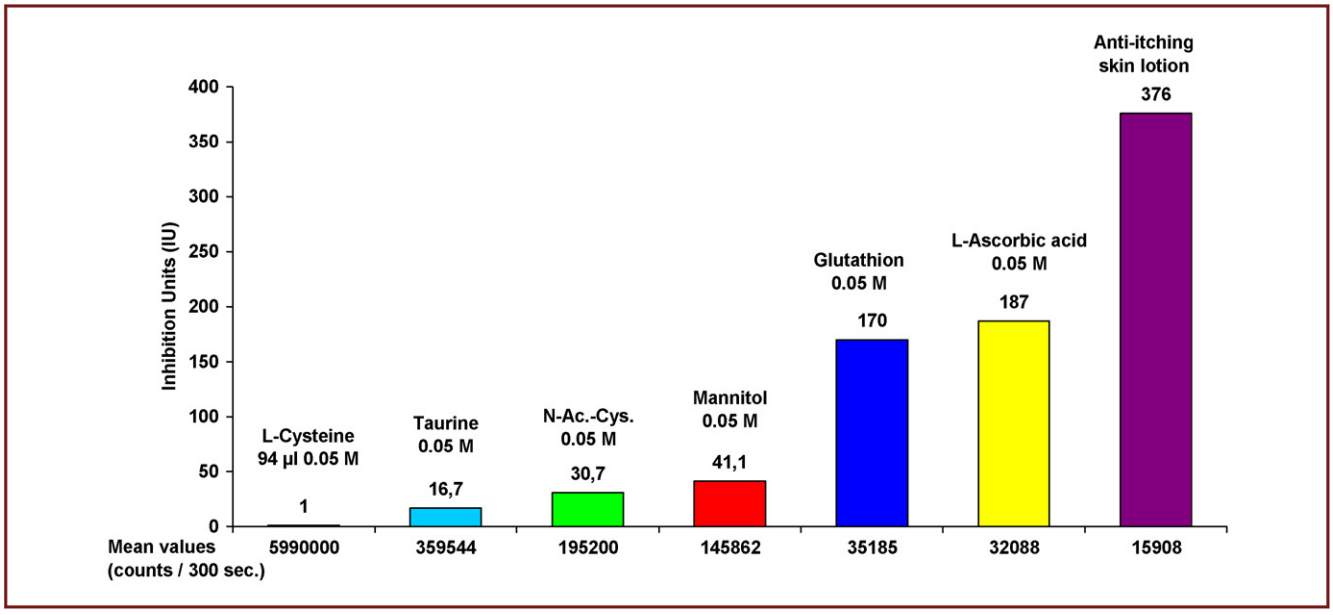

Figure 1. Antioxidative activity (AOA) of 100 μl water-soluble compounds in a standardized 5 min chemiluminescence assay at 36.5 °C.

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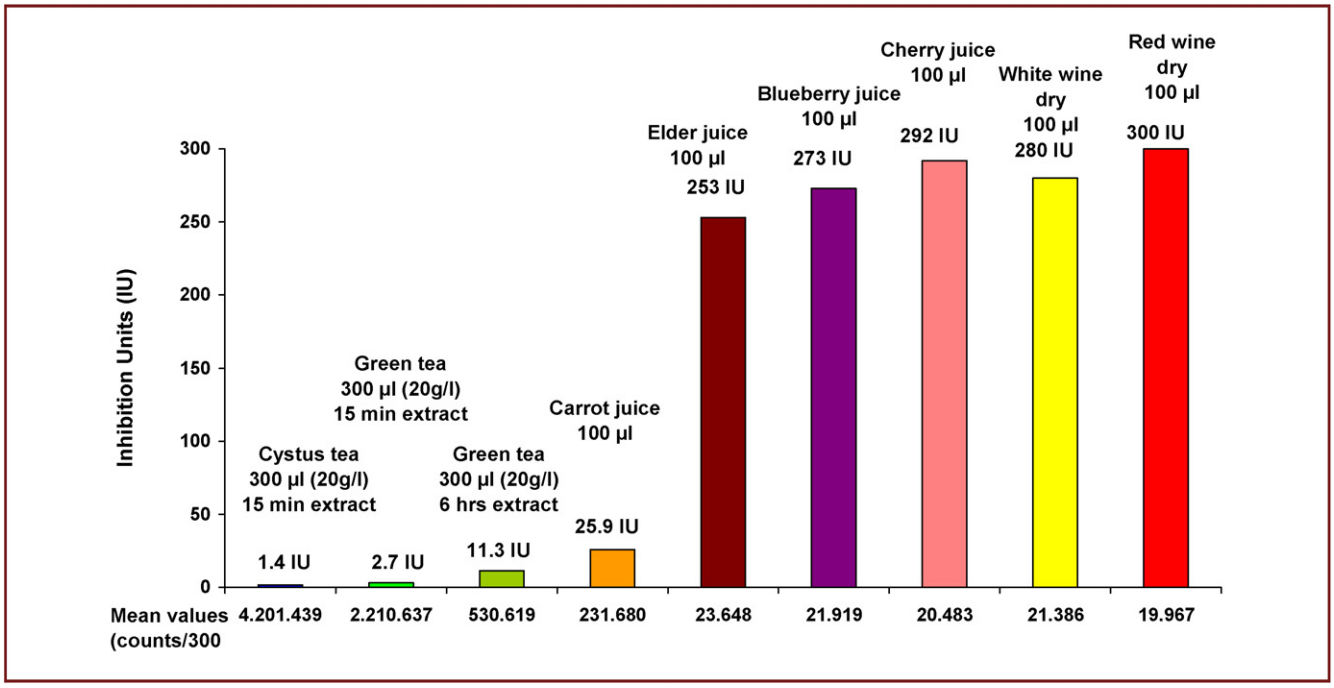

Figure 2. Antioxidative activity (AOA) of teas, native juices and wines in a standardized 5 min chemiluminescence test at 36.5 °C.

The AOA assessment of fat-soluble antioxidants in appropriate solvents is in progress in our lab.

The AOAïs of cystus (1.4 ± 0.1) and green tea (2.7 ± 0.2 IU at 15 min vs. 11.3 ± 0.5 IU at 6 h), native juices as carrot (25.9 ± 0.7 IU) elder (253 ± 6 IU), blueberry (273 ± 18 IU) and cherry juice (292 ± 12 IU), dry white wine (280 ± 7 IU) and dry red wine (300 ± 13 IU) are depicted in Fig. 2. Polyphenols from green tea leaves (epigallocatechins) might be responsible for the inhibition of photon emission especially in the 6 h extract. The 15 min extracts from green and cystus tea were significantly less effective in this respect, and 300 µl black tea showed no inhibtion properties of the free radical source. The high content of polyphenols, bioflavonoids and pigments of the berry juices and wines could account for highly effective AOA, as well as for the recorded increase of total plasma antioxidant activity in vivo after oral intake (see Fig. 4). Interestingly, 6 h of air exposure of the native blueberry juice resulted in a 40% reduction of its antioxidative activity [9].

The AOA of human plasma, whole blood and various protein solutions is described in Fig. 3.

Plasma samples from untreated MCS patients displayed significantly lower AOAïs (mean value: 1.2 ± 0.4 IU/500 µl) when compared to plasma samples from healthy controls (7.1 ± 1.6 IU/500 µl). Concentrated human serum gammaglobulins (16% protein) exhibited higher free radical quenching properties (16.6 ± 0.5 IU/500 µl) when compared to control human plasma (7.6% protein).

Fifty times less amounts of fresh heparinized venous blood demonstrated significantly higher AOAïs than plasma samples (45.4 ± 5.2 IU/10 µl). The presence of antioxidative enzymes and the increased levels of water- and fat-soluble antioxidants in blood cells could explain these phenomena.

A 10% commercial infant formula (Pregomin, Milupa AG, Germany) based on soy protein showed only a slight AOA level comparable to a 10% solution of partially hydrolysed whey protein A (1.0 ± 0.07 IU/100 µl vs. 1.6 ± 0.1 IU/100 µl). By contrast, the same concentration of the 27% hydrolysed whey protein B exhibited obviously increased ROS quenching capabilities (56.3 ± 3.8 IU/100 µl). The addition of sucrose (250 mg/ml) weakened the AOA of hydrolysed whey protein B by 30% suggesting a possible negative effect of additional sugars in infant formulae.

The highest AOA in this series was recorded in the colostrum test (194 ± 18 IU/100 µl) underlining the outstanding value of this product for the newborn in the prelactation phase.

Therapeutical approaches

Oral intake of native juices

The oral administration of 500 ml Cellagon juice mixture (dilution 1:5 with water) to two fasted healthy controls resulted in a continuous increase of the photon generation in the venous blood and plasma samples 45 and 90 min after intake (Fig. 4). The reason for the initially accelerated oxidative processes in whole blood and plasma after Cellagon ingestion is not clear yet but investigations on this topic are in progress. The product is offered in Germany to improve physical performance.

By contrast, oral intake of 500 ml native blueberry juice induced a significant reduction of the ROS generation in whole blood of two atopic eczema patients at 1.5 and 3 h after ingestion (Fig. 4). Plasma ROS levels firstly showing a parallel decrease at 1.5 h, came back to the initial level 3 h after intake suggesting the consumption of the juice antioxidant capacity. This assumption is supported by the time course of the plasma AOA equally showing a first increase after 1.5 h 1.2 to 1.9 IU, followed by a drop, three hours after intake (Fig. 4). The administration of the same blue-

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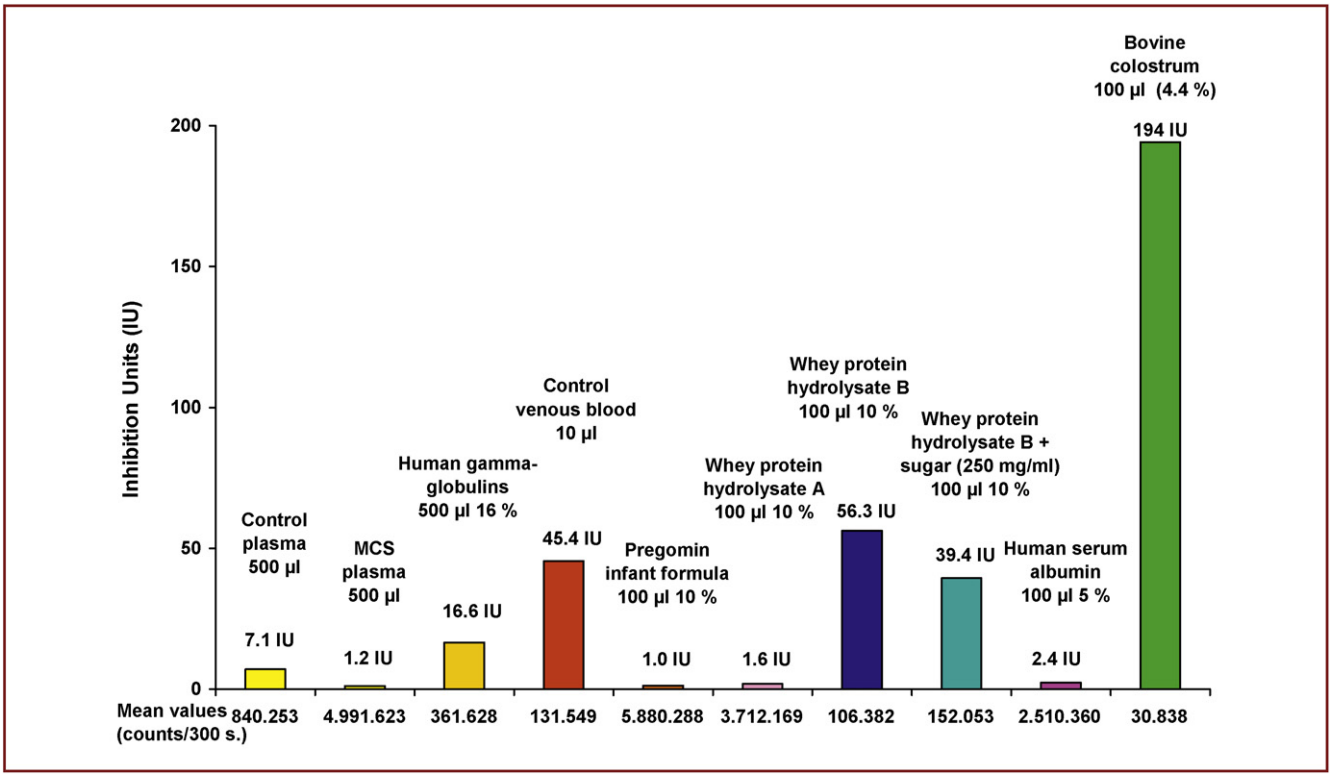

Figure 3. Antioxidative activity (AOA) of human plasma, whole blood and different protein solutions in a standardized 5 min chemiluminescence test at 36.5 °C.

berry juice to three healthy subjects with normal AOA levels in plasma resulted in a continuous and stronger increase of the antioxidative capacity over the same time intervals (not shown here).

The above data demonstrate the possibility of in vivo serial studies with different natural compounds for the evaluation of their oxidative or antioxidative activity as previously reported with this technique [5,9].

Intravenous administration of high doses vitamin C

In spite of the known antioxidant character of vitamin C, the i.v. administration of large doses of Na-ascorbate induced in both atopic serum and plasma a significant ROS generation. This phenomenon may be explained through the autoxidation of the vitamin C in the presence of circulating transitional metals, as already reported in atopic or MCS patients [5,9] and in other experiments [10—13].

An in vitro ROS raising pattern after addition of 0.5 ml 0.01 M Na-ascorbate to the serum/lucigenin mixture at 22 °C is shown in Fig. 5 a. Addition of 500 U of superoxide dismutase (SOD) resulted in a strong inhibition of the light intensity indicating the participation of the $*O_2^-$ radical in this process. Catalase (500 U) and 50 mg mannitol also reduced by 50 and 30% the free radical generation in vitro suggesting the presence of H_2O_2 and *OH radical in the ascorbate induced ROS mixture (not shown here).

The intravenous infusion of 8.5 g Na_2-ascorbate in 500 ml dextrose in two atopic eczema patients resulted in a dramatic increase of the ROS counts at 22 °C in serum (e.g. from 16,335 to 278,280 counts/600 s) as monitored at 1, 2, 5 and 24 h after infusion (Fig. 5b). However, as long as the antioxidative systems of the blood cells are intact (SOD, catalase, glutathione peroxidase and non-enzymatic antioxidants), this increase in plasma was not paralleled by a detectable ROS increase in the whole venous blood at 22 °C.

However, the i.v. administration of 20 g ascorbate in two atopic eczema patients with type IV metal sensitivity resulted in an even higher free radical generation in serum (e.g. from 18,448 to 614,720 counts/600 s) associated with a constant ROS increase in the venous blood, up to 62% at 24 h after the infusion (Fig. 6a and b).

All four atopic patients receiving the i.v. ascorbate exhibited a 24—48 h worsening period of the clinical symptoms with increased erythema and itching. Similar effects were noticed after simultaneous oral administration of iron or copper preparations.

Based on the above data, we suggest that the prescription of large doses ascorbate alone or in association with iron or copper should be avoided in atopic or MCS patients with metal sensitivities.

By contrast, cancer patients with increased heavy metal levels in their tumours may take advantage when receiving higher amounts of ascorbate, because of defective antioxidative enzymes in cancer cells which renders them more sensitive to the free radical attack [14,15]. In the same time the activation of the phagocytes and natural killer cells through ascorbate is also supportive for an anti-cancer therapy [16,17].

Intravenous EDTA-chelation therapy

Repeated metal chelation by EDTA salts is advocated to improve atherosclerosis, cerebral vascular insufficiency,

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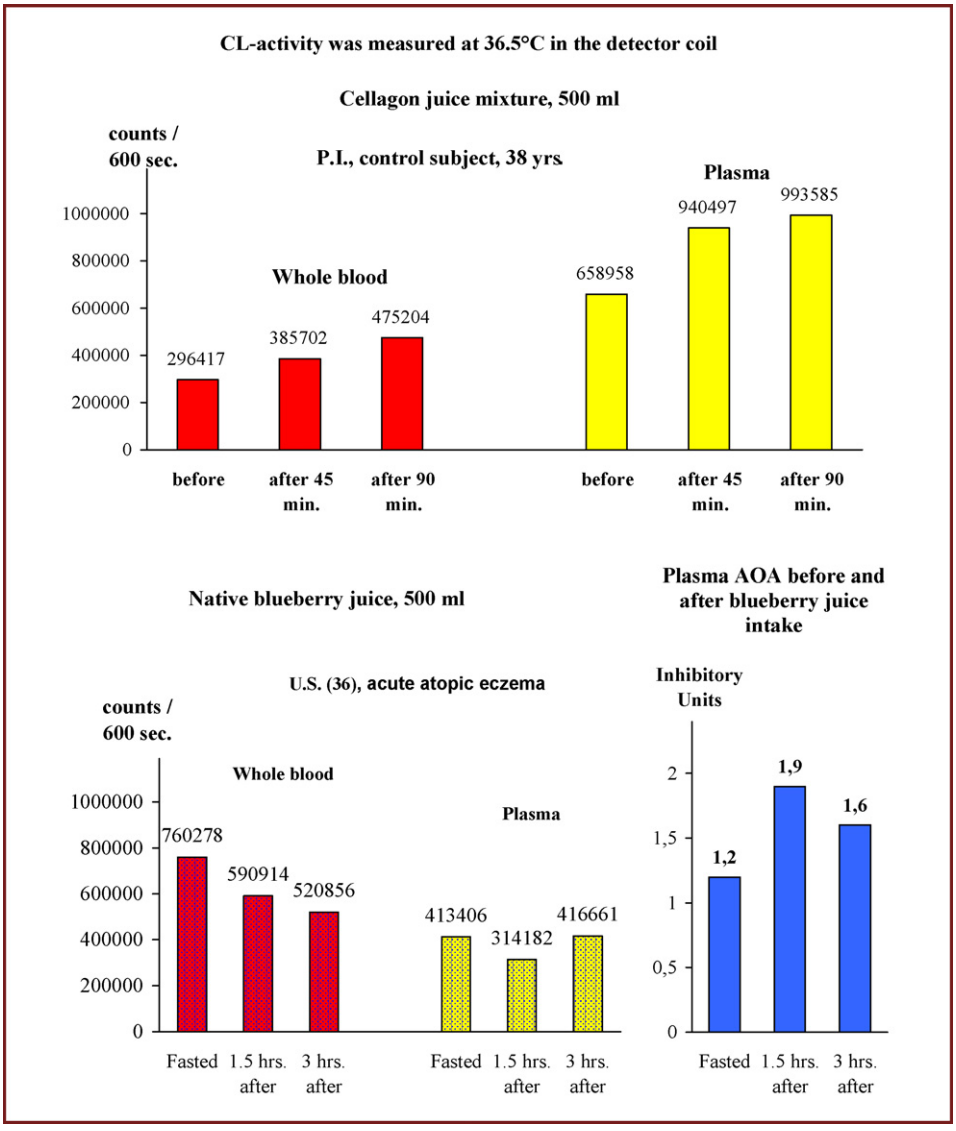

Figure 4. Effects of oral intake of Cellagon mixture and native blueberry juice on free radical generation in whoole blood and plasma.

peripheral artery disease, scleroderma, porphyria and other clinical conditions [18—21]. We therefore tested the effects of intravenous MgK_2-EDTA and Na_2-EDTA on free radical generation in five MCS and three atopic eczema patients in parallel with in vitro tests for the same compounds.

Administration of 3 g MgK_2-EDTA i.v. over 2.5 h infusion time resulted in an initial increase of the ROS production in whole blood and plasma followed by a significant reduction of the photon counts at 5 and 18 h after the infusion (Fig. 7). The free radical peak at 3 h was associated with a strong metal elimination in urine. The overall effect in all five MCS patients 18 h after MgK_2-EDTA was a general reduction of blood ROS associated with a temporary relief of the clinical condition. No side effects were observed during the i.v, administration.

We also studied the free radical profiles in three atopic eczema patients subjected to three different intravenous treatments at 1-week intervals. A typical pattern of the in vitro and in vivo ROS time course in one patient is depicted in Fig. 8.

There was en excellent correlation between the in vitro and in vivo venous blood ROS profiles for all three tested drugs. Whereas the alpha-lipoate and the MgK_2-EDTA addition induced a short-term increase of free radicals in venous blood, the administration of Na_2-EDTA resulted in an immediate ROS decrease in both test systems. Plasma ROS levels showed a repeated decrease in vivo 15 min after all three infusions. Clinically, there was no change after the alpha-lipoate and MgK_2-EDTA infusions, by contrast the Na_2-EDTA administration temporarily alleviated the symptoms in this particular case.

These and additional clinical observations suggest that blood and plasma ROS reactions to different compounds show an individual pattern related to the patientŭs pathobiochemistry. Further serial investigations in a large number of patients are required to confirm the clinical relevance of the above data.

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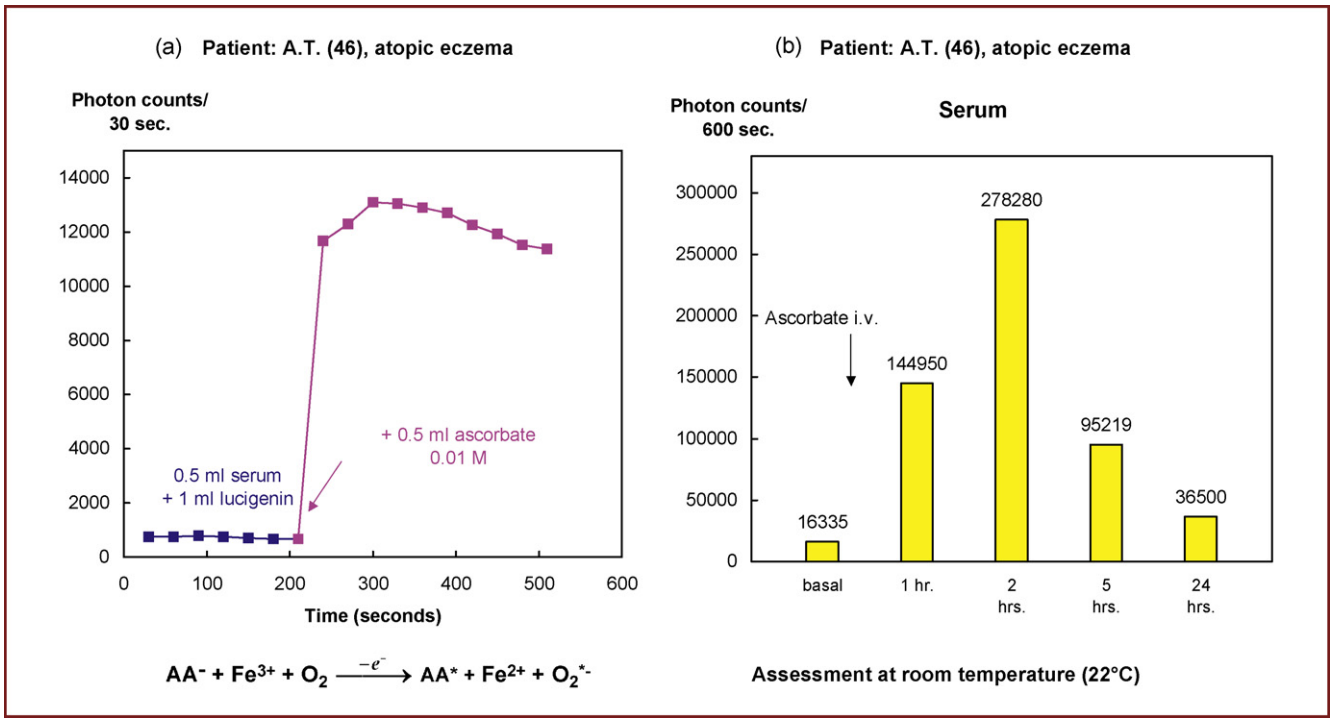

Figure 5. a: ascorbate induced free radical activity in atopic serum, in vitro; b: free radical activity in serum before and after 8.5 g i.v. ascorbate, in vivo.

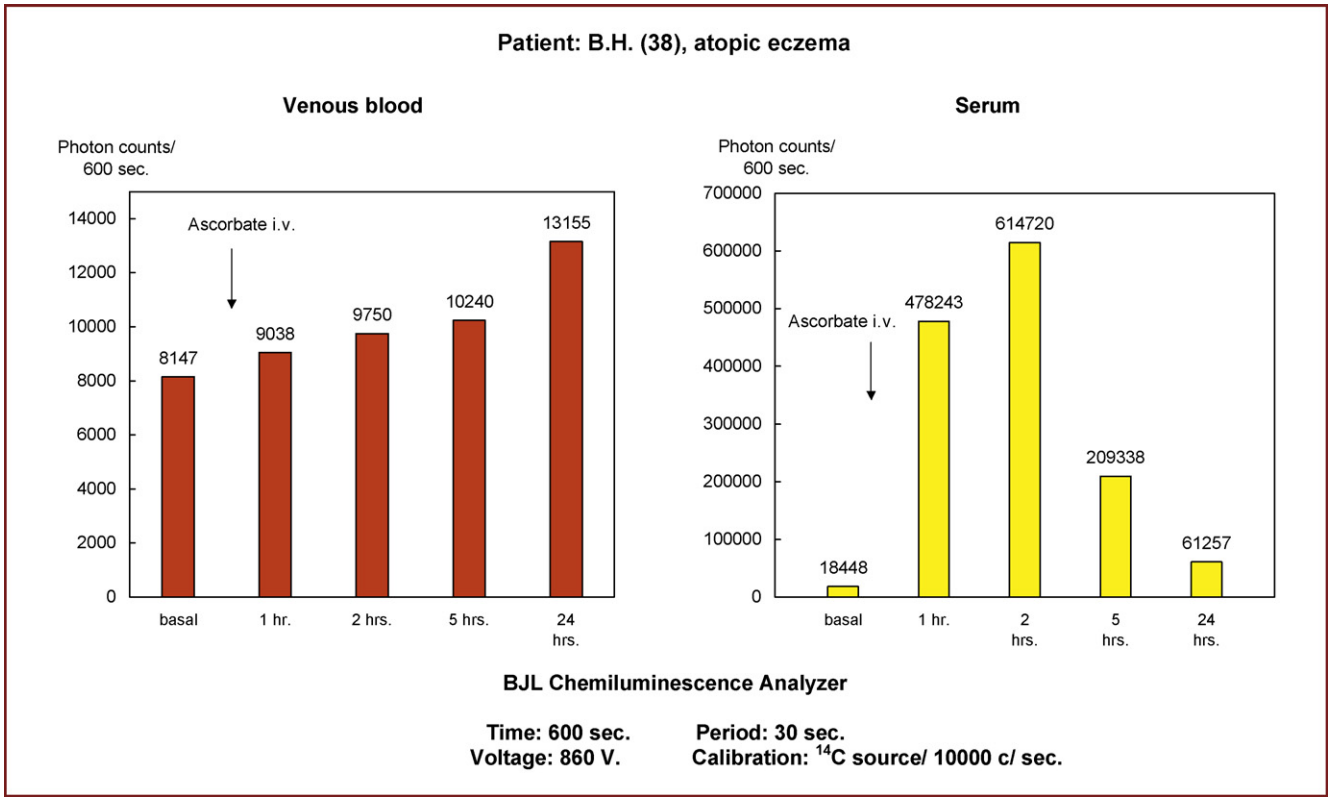

Figure 6. Free radical activity in (a) venous blood and (b) serum before and after 20 g ascorbate i.v.

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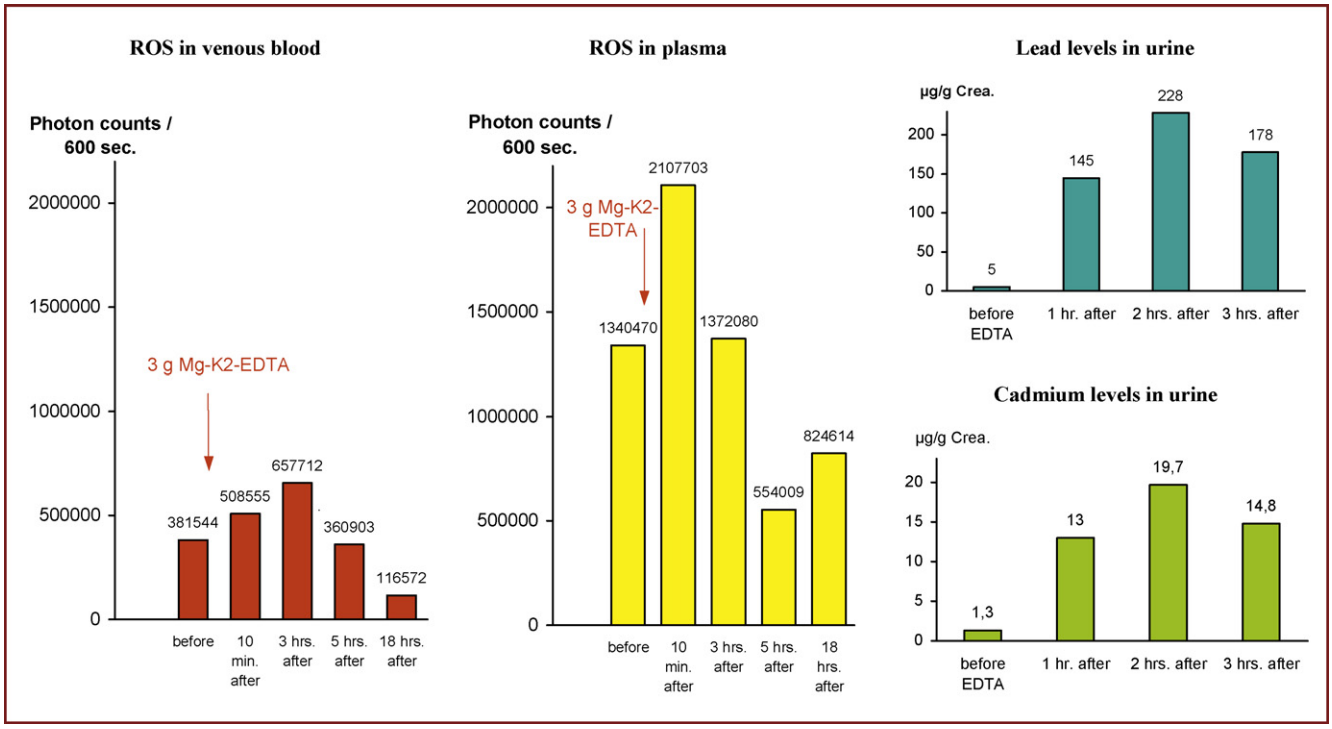

Figure 7. Effects of 3 g MgK_2EDTA i.v. on free radical generation in blood and plasma at 36.5 °C.

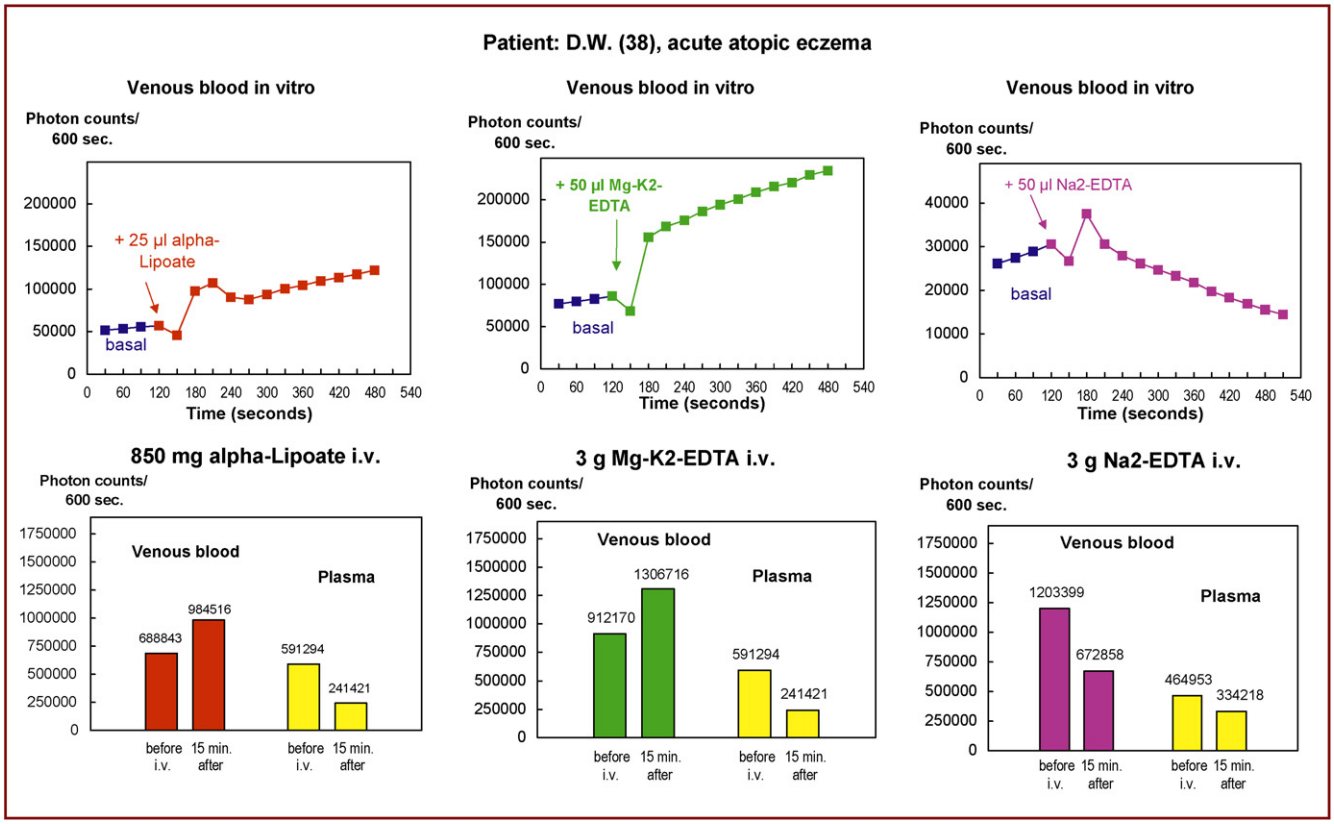

Figure 8. Effects of alpha-lipoate and EDTA salts on free radical activity in venous blood and plasma. In vivo and in vitro investigations.

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Conclusions

Assessment of photon/ROS counts in venous blood, plasma and serum by enhanced CL allows a clear-cut evaluation of the free radical activities in different clinical conditions as well as the serial monitoring of the therapy effects. In addition, the direct functional analysis of the circulating phagocyte activity in whole blood offers the advantage to minimize artifacts induced by cell purification, reduces the time required for each test and maintains the in vivo cellular environment.

The described 5 min AOA test enables a rapid and inexpensive investigation of the antioxidative potential of various compounds in a two-step procedure.

Modulation of free radical levels in chronic skin disorders, MCS and other clinical conditions together with the restoration of a normal redox potential [1,4,5,16,22], antioxidative status and cellular energy supply leads to a significant improvement of the symptoms and to major reduction of the therapy length.

Conflict of interest statement

There is no conflict of interest in this work.

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