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Cytostatica efficiency enhancement by vitamins C, E and β-carotene under irradiation. State of the art

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Abstract

A review is presented on the enhancement of MMC (mitomycin C) efficiency by the vitamins C, E and β-carotene using E. coli bacteria (AB 1157) and leukemia cells (HL 60) as a model for experiments in vitro. New and previously published spectroscopic and kinetic data of transients obtained by pulse radiolysis of MMC and the above mentioned vitamins were also discussed. Based on all these results a possible cascade electron transfer process from vit. C → vit. E → β-car. → MMC or oxidants in the living cells is presented. By vit. C deficiency a cell mutation might occur, possibly leading to appearance of cancer. The presented radiation chemical and radiation biological characteristic data are of importance as a basis for the development of an improved chemo-radiation therapy of cancer. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Enhancement of cytostatica efficiency by vitamins; Transients of MMC and vitamins C, E and β-carotene

1. Introduction

The study of tumors (oncology) became one of the most important branch in modern medicine. Whereas surgery is still the major effort in cancer therapy, radiation–chemotherapy is more frequently applied. In this respect free radicals, especially oxygen-transients such as OH, $HO_2^{\bullet}/O_2^{\bullet-}$, $ROO^{\bullet}$, $^1O_2$, $HO_3^{\bullet}$, $O_3^{\bullet-}$, etc., may cause oxidative stress in addition to the "solvated electrons" (e_{aq}^-), H-atoms, nitroxide species and others. All these transients can be produced by irradiation and are involved in a great number of processes in the organism as well as with the applied cytostatica. By experiments in vitro coupled with pulse radiolysis studies it has been very recently shown that the efficiency of mitomycine C (MMC; a very effective cytostaticum) can be strongly enhanced in the presence of vitamins C, E (vit. C, vit. E) and β-carotene (β-car.) (Getoff et al., 1999; Kammerer et al., 1999a). In order to get a more complete picture on the subject matter, new results combined with previous data are presented. These studies have been initiated by preceding trials on test persons, which are briefly mentioned below.

2. Testing the preventive action of β-carotene and vitamin E against cancer in smokers and alcohol drinkers

A controlled trial in Finland on 29,133 smokers, based on the expectation that β-car., and vitamin E (α-tocopherol, vitamin E) are acting as cancer-protective substances has been performed (Heinonen et al., 1994). It was found that 18% of the tested persons taking both vitamins showed an increased incidence of lung cancer in comparison with the placebo group. Alcohol and cigarettes consumption enhanced lung and other types of cancer. Within the scope of the second trial performed in the United States on 18,320 male and female smokers, 28% of the participants taking β-car. showed a higher risk of lung cancer (Hennekens et al., 1996). However, in the group of persons consuming both, β-car. and vit. E, 17% more deaths occurred than in the placebo group. Based on these and other similar investigations the WHO — press released on 12th January 1998 the recommendation: "*...that until*

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information becomes available on how β-carotene and other carotenoids influence the process leading to cancer, none of these substances should be promoted to the general population as a tumor preventive treatment''.

It is of interest to state, that vit. C was not involved in the trials mentioned above, although it is well known that its niveau in the organism of smoker and drinker is extremely low. Based on these facts, it was of importance to investigate the influence of vit. C as well as of vit. E and β-car. individually and in mixtures upon the action of cytostatica (antitumor compounds). In order to get a better insight and understanding of the involved mechanisms previously reported as well as new results are presented. The data of two individual lines of investigations are discussed:

(i) Experiments in vitro using bacteria (*E. coli*, AB 1157) and cultered leukemia cells (HL 60) and MMC as cytostaticum (Getoff et al., 1999; Kammerer et al., 1999).
(ii) The spectroscopic and kinetic characteristics of the radiation-induced transients resulting from MMC (Getoff et al., 1997 and references therein) in the presence of oxygen as well as from vits. C, E and β-car. were studied previously by using pulse radiolysis method (Getoff et al., 1999; Getoff, 1999; 2000) and are briefly mentioned for completeness. In addition, new results on the formation of MMC radical anion (semiquinone) are also presented. Further, pulse radiolysis data concerning the absorption spectrum as well as the formation and decay kinetics of vitamin E-acetate (vit. E-ac.) in DMSO measured for the first time are given.

3. Studies in vitro using *E.coli* bacteria and cultured cancer cells

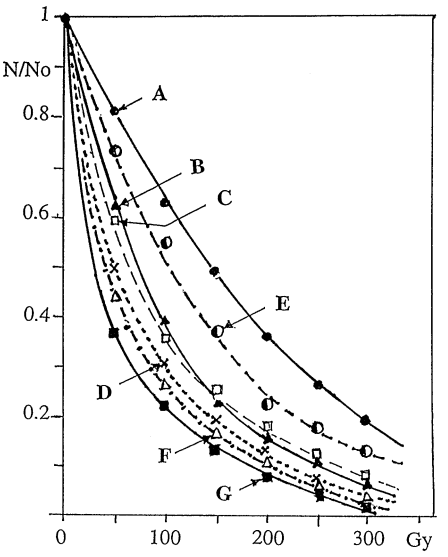
MMC was used as a model substrate since it is a very efficient cytostaticum, applied in radiation–chemotherapy of patients. Also, the MMC–transients studied by pulse radiolysis in the presence of N_2O and oxygen are known (Getoff et al., 1997 and references therein). It has been also shown that its semiquinone ($MMC^{\bullet -}$, radical anion) add to DNA components preventing a multiplication of cancer cells (Pan et al., 1984; Tomasz et al., 1986, 1987; Machtalere et al., 1988; Millard et al., 1990).

The vitamins C, E and β-car. are well known as efficient antioxidants (Machlin, 1990). Moreover, vit. C has been found to act also as a radiation-protecting agent (Platzer and Getoff, 1998). Similar properties were also observed for vit. E and β-car. (Kammerer et al., 2001).

The effect of the three vitamins (C, E and β-car.) on MMC (1×10^{-6} mol dm^{-3}) was studied by following the course of the survival curves of *E. coli* bacteria (AB 1157) used as a model for living systems under various conditions (Getoff et al., 1999 and new results). The bacteria were treated with MMC in the presence of each vitamin individually (1×10^{-4} mol dm^{-3}) and mixtures of them. The survival curves (N/N_0-ratio; N_0 = number of bacteria before; N = after treatment) are given as a function of the absorbed radiation dose (Gy; ^{60}Co-γ-rays) in a very broad range. The obtained results (mean values of at least five series of experiments) are shown in Fig. 1. The calculated D_{37}– and ΔD_{37}–values are presented as an inset Table 1 in Fig. 1. The positive ΔD_{37}- values indicate the radiation-protecting efficiency of a given substrate, whereas the negative values show the efficiency of the bacteria killing under the given conditions (sensitation of MMC).

Based on the course of the survival curves in Fig. 1 and on the ΔD_{37}–results shown in the table, the following can be stated:

- The influence of β-car. on MMC–activity (compare curves B and C) was found to be very weak, whereas that of vit. E-ac. (curve D) is rather pronounced.
- By using a mixture of vit. E and β-car. (curve E) the MMC–activity is essentially diminished. This fact is



Curve	series	D_{37} (Gy)	ΔD_{37} (Gy)
A	buffer	200	0
B	MMC	110	–90
C	MMC +β-Car.	100	–100
D	MMC+ Vit.E.ac.	85	–115
E	MMC+β-Car.+ Vit.E.ac.	150	–50
F	MMC+ Vit C	70	–130
G	MMC+β-Car.+ Vit.E.-ac.+Vit.C	50	–150

[MMC] =1x10^{-5} mol.dm^{-3}; [C] =[Vit.E.-ac.]=[β-Car.] = 1x10^{-4} mol.dm^{-3}

Fig. 1. Survival curves (N/N_0) of *E. coli* bacteria (AB 1157) as a function of absorbed does (Gy) demonstrating the influence of the individual vitamins and of their mixtures on the MMC efficiency in the presence of air. pH = 7.4.

in complete agreement with the observations made within the scope of the trials mentioned above. Namely, the test persons consuming both vitamins (E and β-car.) showed an increased incidence of lung and other types of cancer as well as a higher number of deaths.

- In the presence of vit. C (curve F) the efficiency of MMC is strongly increased, namely from $\Delta D_{37} = -90$ (MMC) to -130 (MMC + vit. C), indicating that vit. C is a very strong electron donor.
- Using all three vitamins with MMC (curve G) nearly a two-fold increase of MMC–activity is observed. A cascade electron transfer from the vitamins to MMC (formation of $MMC^{\bullet -}$ species) is assumed to take place. This process has been established by pulse radiolysis in DMSO solution by studying the electron transfer from the individual vitamins and of their mixtures to MMC. This process is still under investigation, but preliminary results are mentioned previously (Getoff et al., 1999). It might be mentioned that similar results were obtained by the cooperative action of the vitamins on MMC in the case of human leukemia cell line (HL 60) (Kammerer et al., 1999).

In order to get more this rather complicated mechanisms, pulse radiolysis studies with the same substrates have been performed, which in part are previously reported.

4. Pulse radiolysis studies

4.1. Mitomycin C

Pulse radiolysis data of MMC in aqueous solutions saturated with N_2O and oxygen are previously reported (Getoff et al., 1997 and references therein). The transient absorption spectrum of MMC-semiquinone ($MMC^{\bullet -}$), which is proved to be the reactive form of its antitumor action, is now measured and shown in Fig. 2 (Getoff and Solar, 1994).

It might be mentioned that a similar absorption spectrum results by the OH-attack on MMC, however, the kinetics are different (Getoff et al., 1997). GC/MS analysis in aerated solution showed $G(NH_3) = 0.53$ and in N_2O saturated media $G(NH_3) = 0.85$ as well as several not yet identified products. As noticed above, the one electron-reduced MMC leads to the formation of a

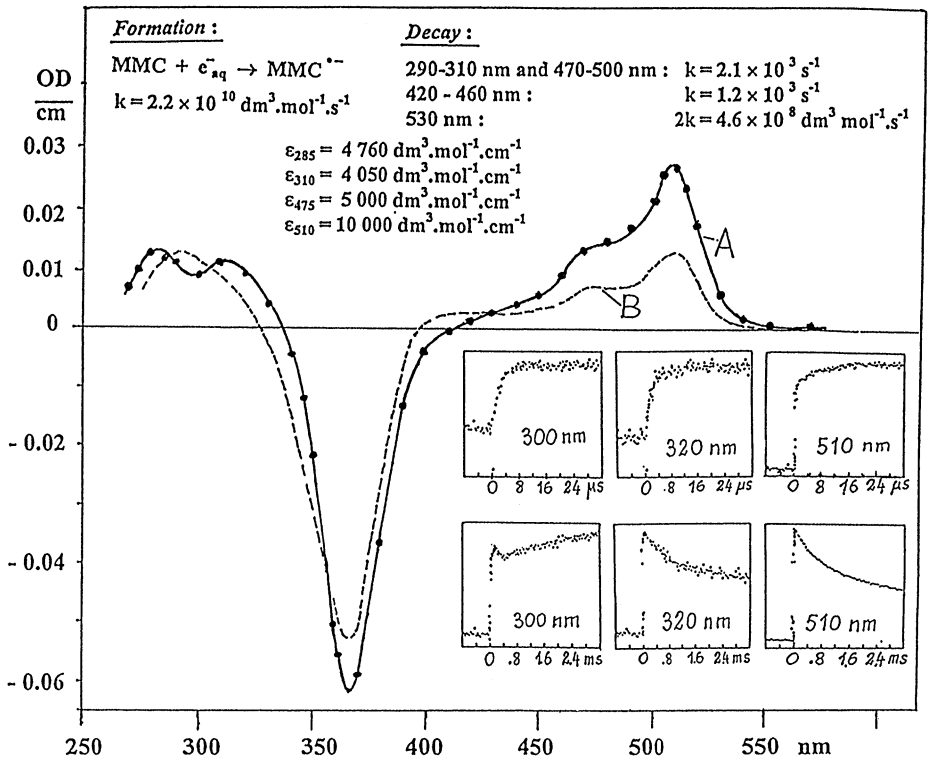
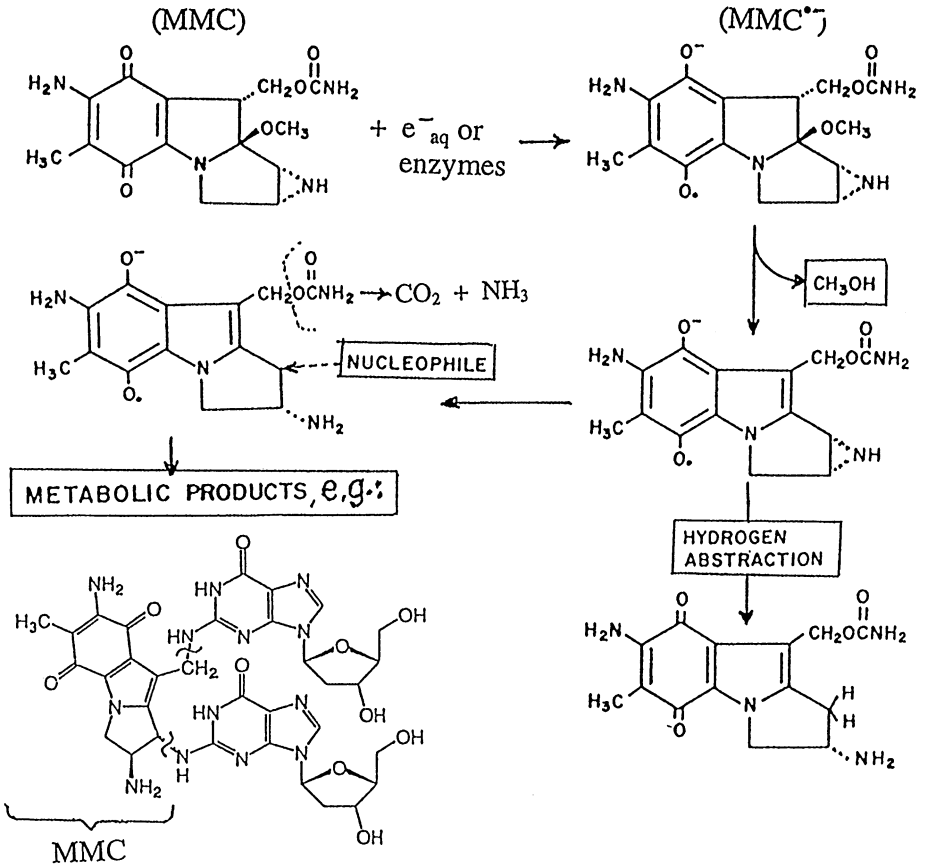


Fig. 2. Transient abosrption spectrum resulting from 3×10^{-5} mol dm^{-3} MMC and 0.1 mol dm^{-3} t-butanol, pH 6.5 in airfree aqueous solution; (A) 15 µs and (B) 3 ms after pule end; OD/cm values normalized to 10 Gy. Insets: Kinetic traces at various wavelenghts, rate constants, ε values of $MMC^{\bullet -}$.



Scheme 1. Formatjion of MMC$^{\bullet-}$ radical anion and its subsequent reactions: elimination of methanol, cleavage of aziridine ring, formation of 2,7-daminomitosene by hydrogen abstraction as well as cleavage of carmate chain (build up of NH_3) and CO_2) and of various metabolic products (MMC–DNA adducts). (Refs. Pan et al., 1984; Tomasz et al., 1986; Machtalere et al., 1988; Millard et al., 1990).

covalent cross-link adduct with DNA, which stops the partition of the tumor cells. This fact has been investigated by several authors (Pan et al., 1984; Tomasz et al., 1986, 1987; Machtalere et al., 1988; Millard et al., 1990). This rather complicated process is presented in Scheme 1 for illustration.

4.2. Vitamin C

The absorption spectrum resulting from the OH-attack on various positions of the ascorbate molecule is previously reported (Schöneshofer, 1972; Bielski, 1982), showing absorptions maxima at 360 nm ($\varepsilon = 3240$ dm^3 mol^{-1} cm^{-1}) and 300 nm ($\varepsilon = 2000$ dm^3 mol^{-1} cm^{-1}) as well as a broad absorption band from 450 to 550 nm. In order to monitor the transient originating from ascorbate (AH^-) acting as antioxidant (electron donor), azide radicals ($N_3^{\bullet}$) were used as specific electron acceptors in N_2O saturated solution (Getoff et al., 1999).

$$N_2O + e^-_{aq} \rightarrow OH + OH^- + N_2$$
$$(k = 9.1\times10^{10}\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}), \quad (1)$$

$$N_3^- + OH \rightarrow N_3^{\bullet} + OH^-$$
$$(k = 1.1\times10^{10}\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}), \quad (2)$$

$$AH^- + N_3^{\bullet} \rightarrow AH^{\bullet} + N_3^-$$
$$(k = 3.2\times10^{9}\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}). \quad (3)$$

$$AH^{\bullet} + AH^{\bullet} \rightarrow \underset{\text{(ascorbic acid)}}{AH_2} + \underset{\text{(dehydroascorbic acid)}}{DHA}$$
$$(k = 1.7\times10^{7}\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}) \quad (4)$$

The absorption spectrum of AH$^{\bullet}$ (vit. C$^{\bullet}$) radical has two maxima at 300 nm ($\varepsilon = 2060$ dm^3 mol^{-1} cm^{-1}) and at 360 nm ($\varepsilon = 3400$ dm^3 mol^{-1} cm^{-1}), however, a broad

absorption band at 400–600 nm observed previously (Schöneshofer, 1972; Bielski, 1982) does not appear (see Fig. 3). The dehydroascorbic acid (DHA) is unstable in aqueous solution and decomposes to about 50 products (Washko et al., 1992; Sawamura et al., 1994). In living systems, however, DHA is enzymatically converted to ascorbic acid (May et al., 1996).

4.3. α-Tocopherol (vit. E)

Similar pulse radiolysis experiments have also been performed with α–tocopherol (vit. E) and with α–tocopherol acetate. Both forms of vit. E are insoluble in water, therefore DMSO (dimethylsulfoxide, $(CH_3)_2SO$) was used as an appropriate polar solvent. The primary major radiolytic products of DMSO are solvated electrons (e_s^-) and the $DMSO^{\bullet+}$ radical cation with $G(e_s^-) = G(DMSO^{\bullet+}) = 1.3$ (Getoff et al., 1999 and references therein). In the presence of oxygen, e_s^- is scavanged forming the very slowly reacting peroxyl radicals ($O_2^{\bullet-}$), hence the $DMSO^{\bullet+}$ transients remain to react with the substrate. In the case of α–tocopherol (vit. E), the corresponding radical cation (vit. $E^{\bullet+}$) is formed as major transient. A neutral radical (vit. $E^{\bullet}$) can also result in small amount (Getoff et al., 1999).

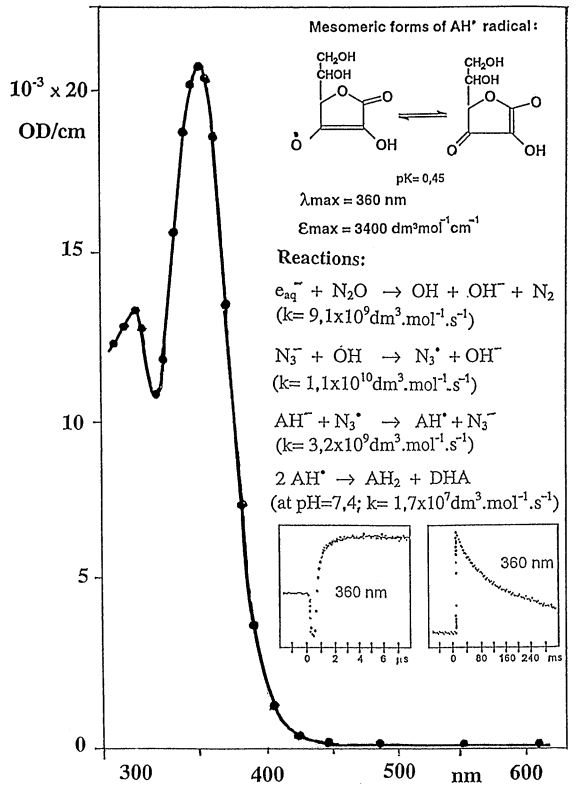


Fig. 3. Transient abosrption spectrum resulting from the N_3^- attack on aqueous ascorbate staturated with N_2O at pH = 8.4. OD/cm values normalized to 10 Gy. Insets: mesomeric form of $AH^{\bullet}$ species, spectroscopic data as well as formation and decay kinetic traces at 360 nm. (Permission for reproduction in part is granted by Elsevier Science Ltd; Getoff, 1999.)

Using vit. E-acetate as a substrate under the same conditions the corresponding radical cation, vit. E-ac.$^{\bullet+}$, is obtained. Its spectrum, which is entirely different from that of vit. E, as well as the kinetic data are presented in Fig. 4. It is interesting to note that the radical cations disappear by first-order reaction, very likely forming dimers (vit. E-ac.)$_2^{\bullet+}$.

4.4. β-carotene

β-car. like α-tocopherol belongs to group of the "fat–soluble" vitamins. As well known β-car. quenches singlet oxygen ($^1O_2$) and is also acting as a rather efficient antioxidant in tissues especially at low-oxygen tension and is able to stimulate the immune response. The spectroscopic and kinetic characteristics of β-car. transients have been studied by pulse radiolysis as well as by flash photolysis in different non-polar solvents. An excellent review on this topic is presented by Benasson et al. (1983). For more recent studies in this respect see, e.g. Lafferty et al. (1977); Almgren and Thomas (1980).

Very recently it was shown (Getoff, 1999) that β-car. in alcohol–water mixtures reacts with e_{sol}^- forming *radical anions* (β-car.$^{\bullet-}$) with a main absorption band at 850 nm ($\varepsilon \sim 1.3 \times 10^4\ dm^3\ mol^{-1}\ cm^{-1}$) and smaller absorptions in the UV range ($\lambda < 350$ nm) (Fig. 5).

The β-car.$^{\bullet-}$ transients disappear by a first-order reaction, very likely after formation of a dimer radical anion (β-car.)$_2^{\bullet-}$. This is in contradiction with the previous pulse radiolysis studies in unpolar media (see e.g. Bensasson et al., 1983). Obviously, the polarity of the solvent can strongly affect the chemical behavior of the transients.

As a result of the antioxidant action of β-car. the *radical cation* produced is of great biological interest. It has been studied by pulse radiolysis in *n*-hexane ($\lambda_{max} = 1040$ nm; $k = 2.5 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$) and disappears by the second reaction (in *n*-hexane: $2k = 4.7 \times 10^{11}\ dm^3\ mol^{-1}\ s^{-1}$) (Bensasson et al., 1983). Similar data were reported also by other authors (e.g. Lafferty et al., 1977; Almgren and Thomas, 1980). In order to get a more deeper insight on the formation and decay of β-car.$^{\bullet+}$ transients in polar media pulse radiolysis studies using DMSO also as an appropriate polar solvent were recently performed (Getoff, 2000). The transient absorption spectrum, observed in the presence of oxygen (0.1 μs pulse, 10 MeV electrons) showed a broad and a very strong maximum at 942 nm. It is completely developed at 200 μs after pulse, whereas the absorption band of β-car. triplet state at 520–690 nm is quenched at the same time by O_2. It is important to

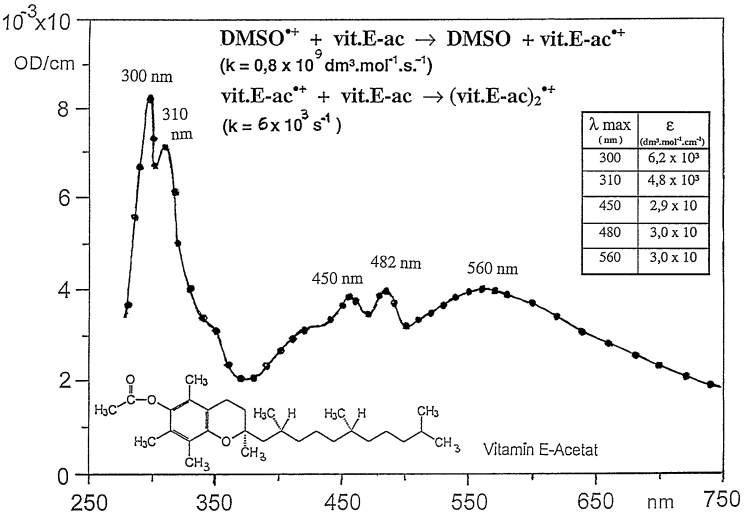


Fig. 4. Absorption spectrum of vitamin E-acetate radical cation (vit. E-ac.$^{\bullet+}$) resulting from 1.25×10^{-2} mol dm^{-3} vitamin E-acetate in DMSO saturated with oxygen. OD/cm values normalized to 10 Gy/0.4 μs electron pulse.

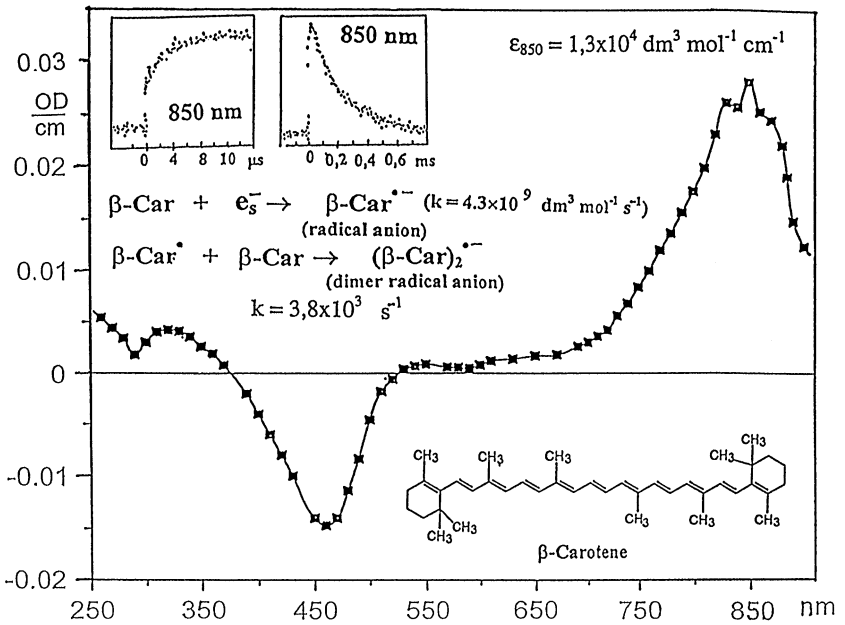
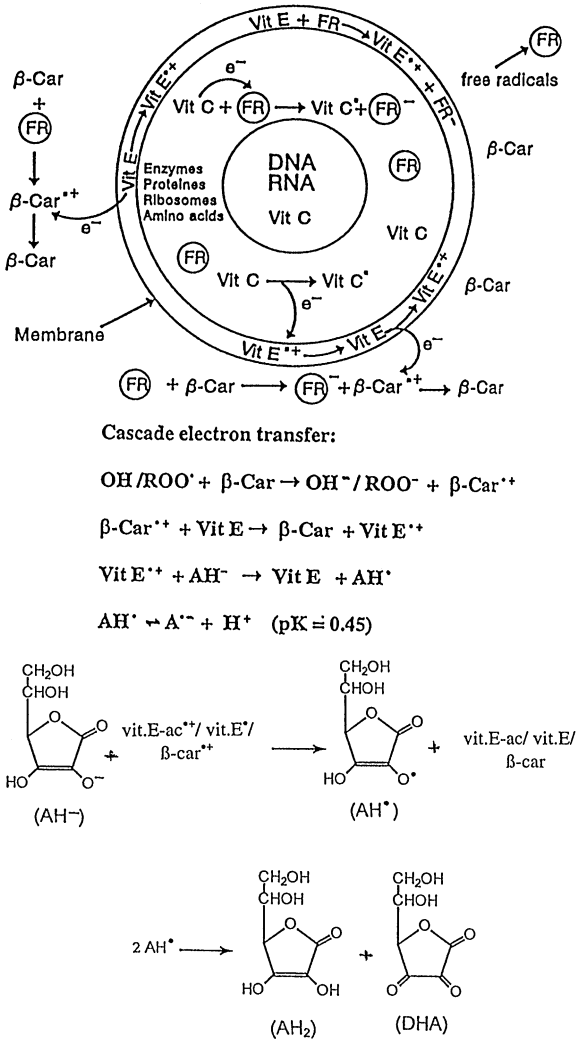


Fig. 5. Transient absorption spectrum of β-car.$^{\bullet+}$ radical anion resulting form 5×10^{-5} mol dm^{-3} β-car. in a mixture of 86% ethanol and 14% four times destilled water. OD/cm-values are normalized to 10 Gy. Inset: Kinetic traces, reactions as well as rate constants for formation and decay of β-car.$^{\bullet+}$ species. (Permission for reproduction is granted by Elsevier Science Ltd; Getoff, 1999.)

stress that in polar media (DMSO) the radical cation, β-car.$^{\bullet+}$, decays by first-order reaction after the formation of dimer radical cation, $(\beta\text{-car.})_2^{\bullet+}$. This is also in contradiction to the previously reported second-order decay in non-polar solvents (Bensasson et al., 1983). Obviously, the polarity of the solvent influences the reactivity of the radical cations (β-car.$^{\bullet+}$, vit. E-ac$^{\bullet+}$) and radical anions (β-car.$^{\bullet-}$) as mentioned above. This fact is strongly supported by the findings that chemical analysis of tumor tissue showed 26.3% β-car. more than the adjacent normal tissue (Fischer, 1999). Very likely the $(\beta\text{-car.})_2^{\bullet+}$ species are subsequently leading to the formation of oligomers of β-car. and to an enrichment of this vitamin in the tumor issue.



Scheme 2. Cascade electron tranfer form vit. C → vit. E → β-car. (for details see text).

5. Cascade electron transfer process

As mentioned above, the cascade electron transfer process with the sequence: vit. C → vit. $E^{\bullet+}$/vit. E → β-car.$^{\bullet+}$/β-car.$^{\bullet+}$ → MMC (or other antitumor agent, OH, peroxyl radicals, etc.) is based on kinetic studies in DMSO solution using pulse radiolysis method. This reaction mechanism is not yet well known and investigations in this respect are still in progress. However, based on the present data the process can be visualized by Scheme 2.

Similar observations were made using sanazole (a known sensitizer) instead of MMC (Heinrich and Getoff, 2000). The precise reaction mechanisms are presently under study.

6. Conclusion

As a consequence of the present and previous results mentioned above the following can be stated:

- The vitamins C, E and β-car., well known as efficient antioxidants, can also initiate a synergistic effect on a given cytostatica (e.g. MMC) by a strong increase of its activity.
- Vitamin C seems to act as a "primary" electron source, alone or in combination with vit. E/vit. E-ac. and β-car., causing a very strong enhancement on the cytostatic efficiency.
- This synergistic effect is very likely based on a cascade electron transfer with the sequence vit. C → vit. $E^{\bullet+}$-ac./vit. $E^{\bullet+}$-ac. → β-car.$^{\bullet+}$/β-car. → cytostatica (e.g. MMC) or peroxyl radicals, studied by pulse radiolysis in DMSO solutions.
- Vit. C seems to be an indispensable electron source for the regeneration of the produced radical cations (vit. $E^{\bullet+}$-ac., β-car.$^{\bullet+}$), which otherwise as strong oxidizing species may cause the formation of tumor cells by attacking DNA and/or other cell components.
- The presented results can explain the negative effect previously observed in the frame of trials, performed on a large number of smokers and drinkers using β-car., alone or in combination with vit. E-ac. or retinol (vit. A) as preventive cancer agents in the absence of vit. C.
- Based on the reported findings it is expected that the application of the three vitamins (C, E and β-car.) in combination with radiation–chemotherapy, the required radiation dose can be strongly reduced. This is of great advantage for patients with weak immuno–system.

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