Mitomycin C-Activity Effected by Vitamins B1, C, E and β-Carotene under Irradiation with γ-Rays

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Vitamin B1 (thiamine) can essentially effect the activity of mitomycin C (MMC), added individually or in combination with antioxidant vitamins (C, E-acetate, β-carotene) as found in experiments *in vitro* (Escherichia coli bacteria, AB 1157) under irradiation with γ-rays. The environment plays a crucial role. In *airfree media* vitamin B1 leads to a 2-fold increase of the MMC-efficiency, but adding vitamin C it decreases. In the presence of all vitamins (B1, C, E-ac., and β-carotene) the MMC-action increases about 1.8-fold. In *aerated media* vitamin B1 causes an about 4-times increase of the MMC-efficiency, but by adding vitamin B1 and C the MMC-activity decreases by a factor of two, whereas in the presence of B1, C, E-ac., and β-carotene it rises again to 2.6-fold. In environment *saturated with N_2O (conversion of e^-_{aq}* into OH radicals) a different picture is observed. The presence of vitamin B1 or vitamin B1 + C causes a strong decrease of the MMC-efficiency, but the addition of all vitamins (B1, C, E-ac., and β-car.) leads to a small increase of the cytostatic action. The results demonstrate the influence of vitamin B1 used individually or in combination with other antioxidants on the MMC-efficiency and the strong effect of the environment. The results are of interest for the application of MMC in radiotherapy.

Key words: Mitomycin C, Vitamins B1, C, E and β-Carotene

Introduction

Previous experiments *in vitro* (Escherichia coli bacteria, AB 1157) under irradiation with γ-rays showed that the efficiency of mitomycin C (MMC) can be strongly effected by the vitamins C, E-acetate and β-carotene (Getoff *et al.*, 1999; Kammerer *et al.*, 1999; Getoff., 2001; 2002). In *aerated media* the cytostatic activity is increased more than 4-fold in the presence of the vitamins C, E-ac., and β-car. (Getoff *et al.*, 1999). Similar observations were also made with sanazole (another cytostaticum). In the presence of vitamin C the sanazole activity in *aerated media* is raised by a factor of two. In *airfree media* the sanazole efficiency increased also twice by adding vitamin C, but in the presence of all vitamins (C, E-ac., and β-carotene) the cytostatic enhancement amounted to more than 2-fold (Heinrich and Getoff., 2000). On the other hand it was recently shown that in *aerated media*, as well as in such *saturated with N_2O* (predominant action of OH radicals) vitamin B1 acts as a radiation protecting agent, but in *airfree* environment it shows a strong cytostatic effect as high as this of sanazole itself (Heinrich and Getoff., 2002). Having in mind the fact, that cancer cells are anaerobic (contain only traces or none oxygen), whereas oxygen is comprised in normal tissue, it is obvious that vitamin B1 leads specifically to the elimination of cancer cells under irradiation, but at the same time protects the normal ones against radiation damage.

All this observations initiated the present investigations *in vitro*, concerning the influence of vitamin B1, applied individually or in combination with the vitamins C, E-acetate and β-carotene on the MMC-efficiency under treatment with γ-rays.

For the sake of completeness a very brief characterisation of the used compounds is given.

Mitomycin C (MMC) is a very efficient cytostaticum applied in radiotherapy. It has been found that its active form is the MMC radical anion ($MMC^{\bullet -}$, semiquinone), which forms metabolic products with the DNA (MMC-adducts) (Pan *et al.*, 1984; Tomasz *et al.*, 1986; Machtalere *et al.*, 1988; Millard *et al.*, 1990). The absorption spectrum and the kinetics of $MMC^{\bullet -}$ have been studied by pulse radiolysis (Getoff., 2001 and ref. therein). Other MMC transients, such as OH-adducts of

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MMC ($MMC^{\bullet}$-OH) and peroxyl radicals (MMC-$OH{\cdot}O_2^{\bullet}$) were also identified by their spectroscopic and kinetic characteristics (Getoff *et al.*, 1997). All these species, including the MMC radical cation ($MMC^{\bullet+}$) were suggested very recently to be able to act similar as $MMC^{\bullet-}$ transients leading to the formation of various MMC-DNA-metabolic products (Getoff., 2002).

Vitamin B1 (thiamine) plays a multiple biological role, *e.g.* it is involved in nerve functions etc. *In vivo* it is converted into thiamine diphosphate, a coenzyme in the decarboxylation of α-keto acids. Its chronic deficiency may lead to neurological impairment (beriberi, Wernicke-Korsakoff syndrome) (Machlin., 1991).

Vitamin C (ascorbic acid) is a well known antioxidant and is contained in various foodstuffs.

Vitamin E (α-tocopherol) used as vitamin E-acetate is not effected by the oxidizing influence of air and UV-light. It is an efficient antioxidant with multi-sided activities *in vivo* (*e.g.* Machlin., 1991).

ß-Carotene is likewise an antioxidizing agent with versatile functions in the organism. It is a precursor of vitamin A for all species, except cats (The Merck Index., 1996).

Finally it may be noted that the mentioned vitamins have a very strong radiation protecting ability (Kammerer *et al.*, 2001).

Experimental

The studies *in vitro* were carried out using Escherichia coli (AB 1157) as a model for living systems. The handling procedure of the bacteria as well as the evaluation of the results have been previously reported (Platzer and Getoff., 1998). "Gammacell 220" (Nordion International Inc., Canada) providing a dose rate of 77 Gy/min was used as irradiation source. The bacteria were added to the buffer and after one hour incubation irradiated with γ-rays. In all other series of experiments the buffer contained individual components or mixtures of them, as given in the Table of the corresponding Figure. The survival curves represent the N/N_0-ratio (N_0 = number of bacteria colonies before and N = after irradiation treatment) as a function of the absorbed radiation dose (Gy). The D_{37}-values represent the N/No-ratio at given dose (Gy) and were taken from the corresponding curves, whereas the ΔD_{37} (Gy)-values were calculated by subtracting the D_{37}-buffer from the individual D_{37} data, *e.g.* D_{37} (sample)-D_{37} (buffer) = ΔD_{37} (sample). The positive ΔD_{37}-values indicate the radiation protecting ability of the system, whereas the negative ones show the corresponding cytostatic efficiency. The presented data are mean values of at least 5 series of experiments.

In the present studies the concentration of the components was kept constant, namely: 10^{-6} M MMC and 10^{-4} M of each individual vitamin. The effect of different applied concentrations of MMC and vitamins is presently under investigation. The obtained results will be published separately. The applied radiation dose was rather high in order to get a better general picture of the radiation induced processes. The high purity vitamins were provided by Hoffmann-La Roche Corp. (Basel, Switzerland). Mitomycin C (2 mg MMC mixed with 48 mg NaCl, Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) was used as obtained. All media were freshly prepared using 4-times distilled water and p. a. chemicals.

Results and Discussion

In order to get a more deeper insight in the involved radiation induced processes a very brief mention of water radiolysis is presented (Equ. 1).

$$H_2O \rightarrow e^-_{aq}\ ,\ H\ ,\ OH\ ,\ H_2\ ,\ H_2O_2\ ,\ H^+_{aq}\ ,\ OH^-_{aq} \quad (1)$$

G-values at pH = 4–9.5: (2.7) (0.6) (2.8) (0.45) (0.72) (3.2) (0.5)

The corresponding yields of the primary radiolytic products, G-values (number of species formed or decomposed per 100 V absorbed energy) in air-free media are given in brackets. The reducing radicals (e^-_{aq} and H) are in this case 54% and the oxidizing ones (OH) are 46%. In the presence of air both, e^-_{aq} and H are converted into peroxyl radicals:

$$H + O_2 \rightarrow HO_2^{\bullet} \quad (k = 2 \times 10^{10}\ l{\cdot}mol^{-1}{\cdot}s^{-1}) \quad (2)$$

$$e^-_{aq} + O_2 \rightarrow O_2^{\bullet-} \quad (k = 1.9 \times 10^{10} l{\cdot}mol^{-1}{\cdot}s^{-1}) \quad (3)$$

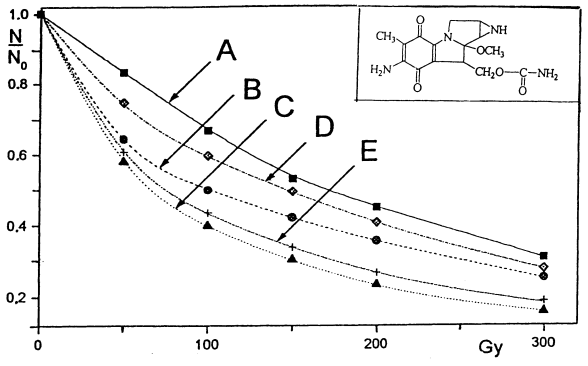
$$HO_2^{\bullet} \leftrightarrow H^+ + O_2^{\bullet-} \quad (pK = 4.8) \quad (4)$$

Hence, in aerated medium 54% $O_2^{\bullet-}$ and 46% OH at pH = 7.4 are involved in the radiation induced biological processes. However, by saturating the media with N_2O the "solvated electrons" (e^-_{aq}) are specifically transformed into OH radicals.

$$e^-_{aq} + N_2O \rightarrow OH + OH^- + N_2$$
$$(k = 0.9 \times 10^{10} l{\cdot}mol^{-}1{\cdot}s^{-1}) \quad (5)$$

Under such conditions about 90% OH and 10% H are involved in the reaction mechanisms.

The effect of vitamin B1 and of the other vitamins (C, E-ac., and β-carotene) on the MMC-activity in *airfree media* is expressed by the course of the survival curves: N/N_0-ratio as a function of the absorbed radiation dose (Gy) in Fig. 1. In the Table the D_{37}-values represent the dose (Gy) taken from the given curve for $N/N_0 = 0.37$, whereas the ΔD_{37}-values (Gy) are obtained by subtracting the D_{37}-value of the buffer from each individual D_{37}-data of the corresponding survival curve as mentioned above. Based on the ΔD_{37}-data it is obvious that the cytostatic activity of MMC is enhanced by a factor of two in the presence of vitamin B1 (Fig. 1, curve C). By adding vitamin C to the mixture of MMC and vitamin B1 the MMC-efficiency is decreased by 50% (Fig. 1, curve D), however in the presence of all studied vitamins (B1, C, E-ac., and β-car.) it increases again about 1.8 times (Fig. 1, curve E). The results indicate a competition between the cytostatic properties of vitamin B1 and the radiation protecting action of the antioxidants in respect to the MMC-activity under irradiation.

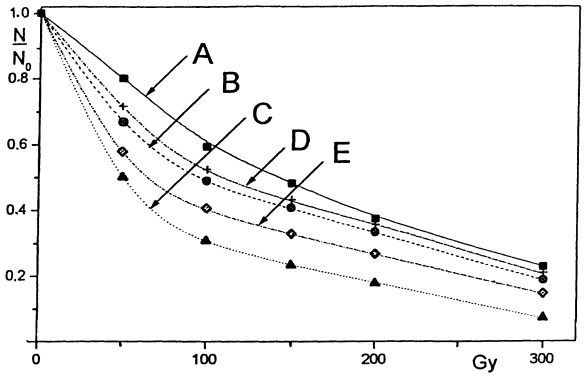


Curve	System	D_{37} (Gy)	ΔD_{37} (Gy)
A	Buffer	260	0
B	MMC	188	– 72
C	MMC + Vit. B1	112	– 148
D	MMC + Vit. B1 + Vit. C	226	– 34
E	MMC + Vit. B1 + Vit. E-Ac. + Vit. C + β-Car.	133	– 127

Fig. 1. Survival curves (N/No-ratio) of *Escherichia coli* (AB 1157) as a function of the absorbed dose (Gy) in *airfree medium.* (**A**) Buffer, (**B**) MMC, (**C**), MMC + vit. B1, (**D**) MMC + vit. B1 + vit. C, (**E**) MMC + vitamins: B1, C, E-ac. + β-car.; pH = 7.4, dose rate: 77 Gy/min; [MMC] = 1×10^{-6}M; [vit. B1] = [vit. C] = [E-ac] = [β-car] = 1×10^{-4}M.

Table. Composition of the solutions and the corresponding D_{37-} and ΔD_{37-}values. Insert: MMC formula.

In *aerated media* this tendency appears even more strongly, namely vitamin B1 causes a MMC-enhancement by a factor of about 4 (Fig. 2, curve C). By adding vitamin C (a strong antioxidant), however, even in the presence of vitamin B1, the MMC-activity is reduced to about 50% (Fig. 2, curve D). Finally, the MMC-efficiency is increased 2.5-fold in the presence of all vitamins (Fig. 2, curve E).

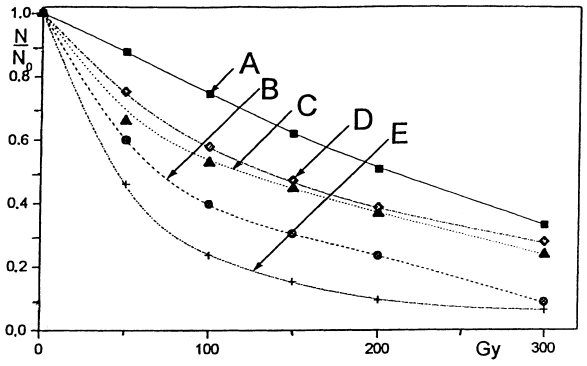


Curve	System	D_{37} (Gy)	ΔD_{37} (Gy)
A	Buffer	206	0
B	MMC	172	– 34
C	MMC + Vit. B1	74	– 132
D	MMC + Vit. B1 + Vit. C	190	– 16
E	MMC + Vit. B1 + Vit. E-Ac. + Vit. C + β-Car.	119	– 87

Fig. 2. Survival curves (N/No-ratio) of *Escherichia coli* (AB 1157) as a function of the absorbed dose (Gy) in *aerated medium.* (**A**) Buffer, (**B**) MMC, (**C**), MMC + vit. B1, (**D**) MMC + vit. B1 + vit. C, (**E**) MMC + vitamins: B1, C, E-ac. + β-car.; pH = 7.4, dose rate: 77 Gy/min; [MMC] = 1×10^{-6}M; [vit. B1] = [vit. C] = [E-ac] = [β-car] = 1×10^{-4}M.

Table. Composition of the solutions and the corresponding D_{37-} and ΔD_{37-}values.

A completely different picture is observed in *media saturated with N_2O* (90% OH radicals involved). The presence of vitamin B1 causes a 50% decrease of the MMC-efficiency (Fig. 3, curve C). By adding vitamin C to the MMC-vitamin B1 mixture a further 2.5-fold cytostatic decrease is observed (Fig. 3, curve D), but in the presence of all studied vitamins a moderate enhancement of the MMC-activity is observed (Fig. 3, curve E). In this context the role of the very strongly oxidizing OH radicals is a determining factor for the observed effects.



Curve	System	D_{37} (Gy)	ΔD_{37} (Gy)
A	Buffer	280	0
B	MMC	112	– 168
C	MMC + Vit. B1	200	– 80
D	MMC + Vit. B1 + Vit. C	213	– 67
E	MMC + Vit. B1 + Vit. E-Ac. + Vit. C + β-Car.	63	– 217

Fig. 3. Survival curves (N/No-ratio) of *Escherichia coli* (AB 1157) as a function of the absorbed dose (Gy) in *medium saturated with N_2O.* (**A**) Buffer, (**B**) MMC, (**C**), MMC + vit. B1, (**D**) MMC + vit. B1 + vit. C, (**E**) MMC + vitamins: B1, C, E-ac. + β-car.; pH = 7.4, dose rate: 77 Gy/min; [MMC] = 1×10^{-6}M; [vit. B1] = [vit. C] = [E-ac] = [β-car] = 1×10^{-4}M.

Table. Composition of the solutions and the corresponding D_{37-} and ΔD_{37-}values.

Conclusion

Based on the obtained results it can be stated that vitamin B1 can essentially contribute to an enhancement of the MMC-efficiency under irradiation in *airfree* (2-fold) as well as in *aerated media* (about 4-fold). In both cases about 46% OH radicals are involved in the processes, but in *aerated environment* about 54% $O_2^{\bullet -}$ species (instead of e^-_{aq} and H atoms) are available. Since $O_2^{\bullet -}$ transients are rather unreactive, it seems that the components in the media are less radiolysed and vitamin B1 can contribute to the cytostatic properties of MMC (synergistic effect).

It is also evident, that the basic properties of the used vitamins are strongly influenced by the given environment, which naturally governs the kind and concentration of the involved primary products of the water radiolysis. The involved reaction mechanisms are very complicated and not yet well understood.

The observed results are of interest for the radiotherapy of cancer.

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Getoff N., Solar S., and Quint R. M. (1997), One-electron oxidation of mitomycin C and its corresponding peroxyl-radical. A steady state and pulse radiolysis study. Radiat. Phys. Chem. **50**, 575–583.

Getoff N., Platzer I., and Winkelbauer C. (1999), Transients and cooperative action of β-carotene, vitamin E and C in biological systems *in vitro* under irradiation. Rad. Phys. Chem. **55**, 1469–1484.

Getoff N. (2001), Cytostatica efficiency enhancement by vitamins C, E and β-carotene under irradiation. State of the art. Radiat. Phys. Chem. **60**, 351–358.

Getoff N. (2002), Effect of antioxidants and oxygen in radiotherapy. In: Progress in Radio-Oncoklogy VII. (Kogelnik H. D., Lukas P., and Sedelmayer F., eds.). Monduzzi Editore, Bologna, Italy, pp. 99–105.

Heinrich E., and Getoff N. (2000), Radiation induced effect of the vitamins C, E and β-carotene on sanazole efficiency. A study *in vitro*, Anticancer Res. **20**, 3615–3618.

Heinrich E., and Getoff N. (2002), Influence of vitamin B1 on sanazole activity under irradiation. A study *in vitro.* Anticancer Res. **22**, 927–930.

Kammerer C., Czermak I., Getoff N., and Kodym R. (1999), Enhancement of MMC Efficiency by vitamin C, E-acetate and β-carotene under irradiation. A study *in vitro.* Anticancer Res. **19**, 5319–5322.

Kammerer C., Czermak I., and Getoff N. (2001), Radiation protecting properties of vitamin E-acetate and β-carotene. Radiat. Phys. Chem. **60**, 71–72.

Machlin L. J. (ed. 1990), Handbook of Vitamins; 2nd edition, Marcel Dekker, Inc., New York and Basel.

Machtalere G., Honee-Levin Ch., Gardes A. M., Ferradini Ch., and Hickel B. (1988), Pulse radiolysisstudy of the reaction mechanism of an anti-tumor antibiotic mitomycin C. C. R. Acad. Sci. Paris **307**, 17–22.

Millard J. T., Weidner M. F., Raucher S., and Hopkins P. B. (1990), Determination of DNA cross-linking sequence specifity of reductively activated mitomycin C at single nucleotide resolution oxyguanosine residues at cpG are cross-linked preferentially. J. Am. Chem. Soc. **112**, 3637–3664.

Pan S. S., Andrews P. A., Glover C. J., and Bachur N. R. (1984), Reductive activation of mitomycin C and mitomycin C-metabolites catalyzed by NADPH-cytochrome p-450 reductase and xantine oxidase. J. Biol. Chem. **259**, 959–966.

Platzer I., and Getoff N. (1998), Vitamin C acts as radiation-protecting agent. Radiat. Phys. Chem. **51**, 73–76.

The MERCK Index, An Encyclopedia of chemicals, Drugs and Biologicals (1996), 20th Ed., Budavari S., Editor, publ. by Merck Research Laboratories, N. Y., USA.

Thomasz M., Chowdary D., Lipman R., Shimotakahara S., Veiro D., Walker V., and Verdine G. L. (1986), Reaction of DNA with chemically or enzymatically activated mitomycin C: Isolation and structure of the major covalent adduct. Proc. Natl. Acad. Sci. (USA), **83**, 6702–6706.