Vitamin B2 (Riboflavin) and a Mixture of Vitamin B2 and C Affects MMC Efficiency in Aerated Media Under Irradiation

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Abstract. *Vitamin B2 (Riboflavin) acts as a strong radiation protecting agent in Escherichia coli bacteria (AB1157) in aerated media. This ability is reinforced by the addition of vitamin C. Under the influence of gamma-radiation, vitamin B2 completely suppresses the cytostatic activity of mitomycin C (MMC). In the presence of both vitamins, B2 and C, MMC is converted from an efficient cytostatic to a rather strong radiation protecting agent. This effect opens a new pathway for specific protection of normal mammalian cells (with a high O_2-content) under treatment with ionizing radiation.*

It has been established that, in the presence of the vitamins C, E and β-carotene, the cytostatic efficiency of mitomycin C (MMC) (1-4) as well as of sanazole (5) can be strongly increased. The same effect was also observed for vitamin B1 (thiamine) (6,7) and for vitamin B6 (pyridoxine) (8). Similar observations have also been made by the joint application of vitamin B1 and sanazole (9). It is interesting to note that vitamin B1 in air-free media exhibits a rather strong antitumor ability, but in the presence of air it acts as a radiation protecting agent (9). Vitamin B6 also shows strong cytostatic properties, in the absence of air, which are, however, four times reduced in the presence of air (8). On the other hand, it was shown that the antitumor properties of vitamin B3 (nicotinamide) (10) can be increased up to three times in the presence of the antioxidant vitamins (C, E and β-carotene) under irradiation with γ-rays in aerated media (11).

Based on these findings, it was of interest to investigate the influence of vitamin B2 on MMC under irradiation in aqueous media. Vitamin B2 [riboflavin, lactoflavin, 7,8-dimethyl-10 (1'-D-ribityl)isoalloxazine] plays an important biological role, since its isoalloxazin ring acts as a reversible redox-system in enzymes. Riboflavin 5'-phosphate (flavin-mononucleotide: FMN) and flavin adenine dinucleotide (FAD) are the two important derivatives of riboflavin in mammalian cells. It should also be mentioned that the singlet excited states of aqueous riboflavin can eject e-aq (solvated electrons) (12).

The biological role of vitamin B2 is many-sided and its nutritional deficiency has been implicated as a risk factor for various diseases. In a recent review article, Powers (13) reports current evidence on the versatile effect of diets low in vitamin B2. Its influence in respect to cancer is rather complex. It has been reported that deficiency of vitamin B2 increases the risk of cancer, whereas, on the other hand, data are presented on its protective effect (13-16). It has also been found that carcinogen binding to DNA is increased in vitamin B2-deficient rats (17). These findings suggest that, depending on the environment of a given system and in combination with other compounds, enzymes *etc.,* vitamin B2 can act in different ways. Hence, this unique property of vitamin B2 prompted us to investigate its effect on a cytostatic agent in the presence of air.

Experiments *in vitro* were performed using *Escherichia coli* bacteria (AB1157) as a model of a living system. MMC was utilized as a typical cytostatic agent and vitamin C as an electron donor. The fact that MMC can be activated by one electron reduction to $MMC^{\bullet -}$ (radical anion) (3), as well as by one electron oxidation to $MMC^{\bullet +}$ (radical cation) (18), makes the study of the redox properties of vitamin B2 on MMC of special interest. Thereby oxygen plays a very important role in the action of MMC, since a strong competition for the "solvated electrons" (e^-_{aq}) exists between both MMC and O_2 (4,19).

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Materials and Methods

Escherichia coli (AB 1157) was used as an appropriate model of a living system in the present studies. Experimental details have been previously reported (20). The effect of vitamins B2 and C on the MMC activity was investigated by following the course of the survival curves (N/No-ratio) as a function of the absorbed radiation dose (Gy). The D_{37}-value of each curve represents the N/No-ratio

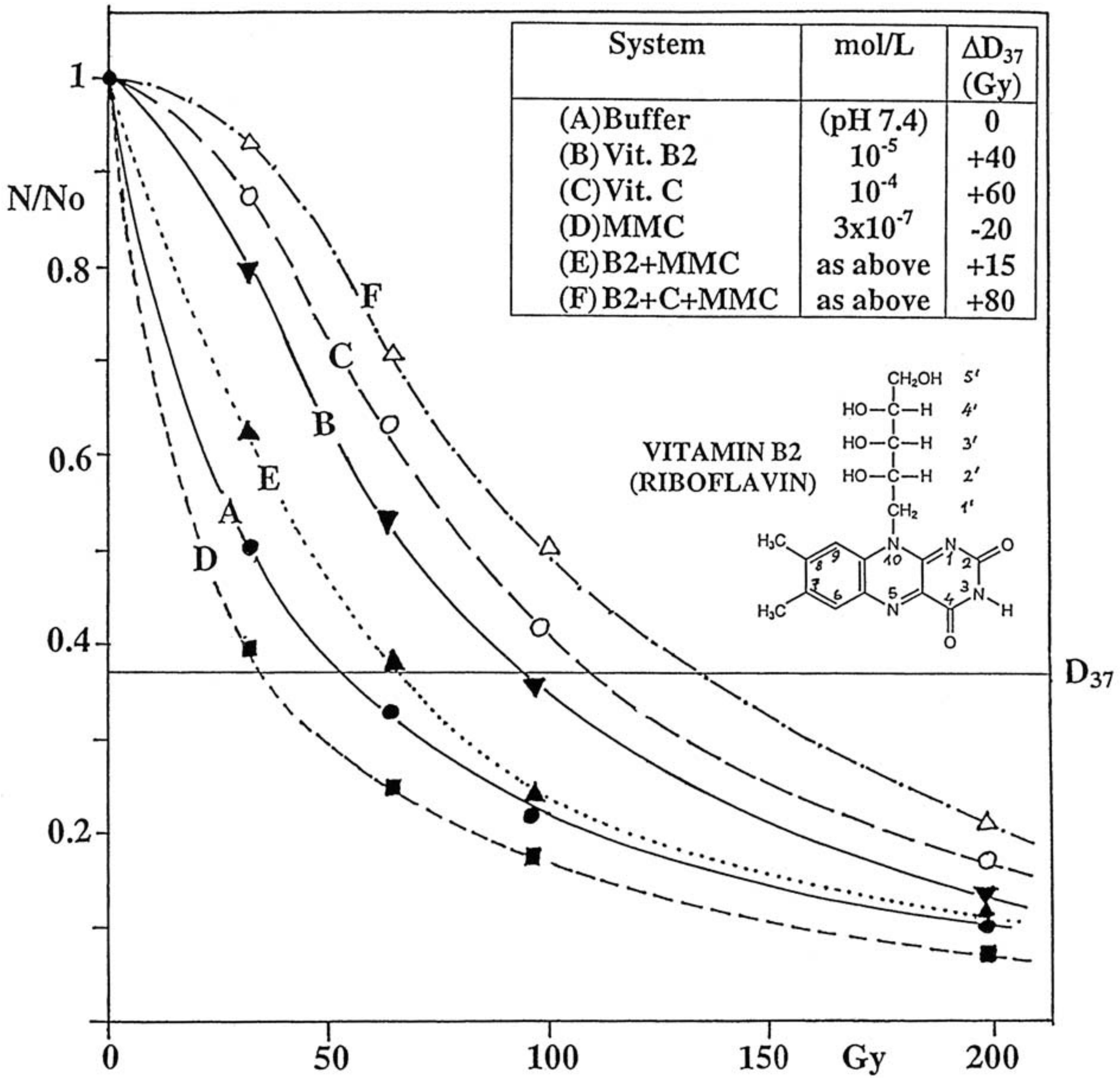


Figure 1. *Survival curves (N/No-ratio) of E. coli bacteria (AB 1157) as a function of absorbed radiation dose (Gy) in media saturated with air in the presence of various agents (pH=7.4). Insert: concentration (mol/L) and determined ΔD_{37}-value of each system.*

of a given dose (Gy), and is obtained from the corresponding curve, whereas the ΔD_{37}-values (Gy) are calculated by subtracting the D_{37}-buffer value 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 property of the given system, whereas the negative ones show the cytostatic efficiency. The concentrations of the solutions were kept constant in all series of experiments and are given in Figure 1, insert.

For the irradiation treatment with γ-rays, a "Gammacell 220" (Nordion International Inc., Canada) was used. By means of a lead attenuator, a dose rate of 19 Gy/min was achieved. All applied chemicals were of analytical grade. The high purity (>99%) vitamins B2 and C were provided by Hoffmann-La Roche Corp. (Basel, Switzerland). Mitomycin C (2 mg MMC mixed with 48 mg NaCl) was obtained from Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan). All media were freshly prepared using 4 times distilled water at pH=7.4 (phosphate buffer). Laboratory utensils were radiation-sterilized and provided by Greiner Holding GmbH, KG & AG, Austria.

Results and Discussion

Figure 1 shows the results of the present study.

Vitamin B2 (Figure 1, curve B) exhibited a strong radiation protecting ability, similar to that of vitamin C (curve C). Unexpectedly, the cytostatic property of MMC (curve D) was eliminated in the presence of vitamin B2 (compare curve D with curve E). Moreover, by addition of both vitamins B2 and C to MMC, a profound strong radiation protecting effect was observed (curve F). An antioxidant behavior, expressed as radiation protection, has also been observed for vitamin B1 (thiamine) in aerated media, as already mentioned (9). However, under similar conditions the MMC activity was up to 4 times increased in the presence of vitamin B1 (6). As mentioned above, the cytostatic property of vitamin B3 (nicotinamide) (10) is very strongly enhanced by adding antioxidant vitamins (C, E and β-carotene) in aerated media (11). It should also be noted again that vitamin B6 (pyridoxine) in combination with MMC showed a very intense synergistic effect under similar conditions (more than 3 times increase of MMC-efficiency) (8).

Radiation-induced processes in biological systems are rather complicated and, hence, difficult to completely elucidate and understand. In order to explain, at least partly, the particular biological behavior of vitamin B2 in the presence of air, first the radiolysis of water and then the reactivity of the primary products of water radiolysis (eq.1) with the substrate should be briefly mentioned. The yields of the primary products are given as G-values*) in brackets:

$$H_2O \rightsquigarrow e^-_{aq}, H, OH, H_2, H_2O_2 \qquad [1]$$

G at pH: 6.5- 8.5: (2.7) (0.6) (2.8) (0.45) (0.72)

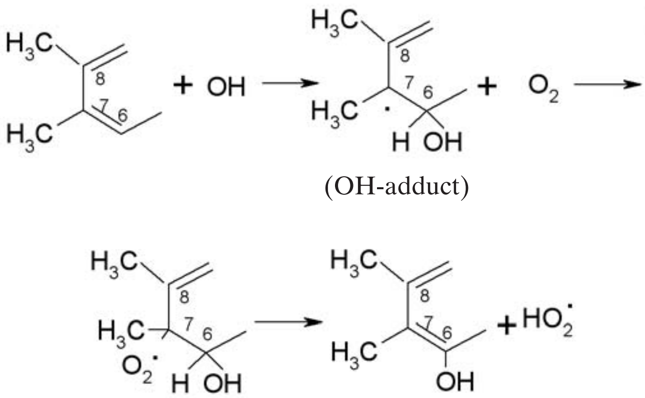
In the presence of air, the H-atoms and e-aq ("solvated electrons") are converted into peroxyl radicals:

$$H + O_2 \rightarrow HO2^{\bullet} \quad (k = 2.0\text{x}10^{10}\ L.mol^{-1}.s^{-1}) \qquad [2]$$

$$e^-_{aq} + O_2 \rightarrow O_2^{\bullet -} \quad (k = 1.9\text{x}10^{10}\ L.mol^{-1}.s^{-1}) \qquad [3]$$

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

Under the present experimental conditions (see eqs. **1-4**) it is expected that only oxidizing transients are operative (46% OH and 54% $O_2^{\bullet -}$). The OH radicals having the strongest oxidizing ability will preferentially attack the double bonds of the vitamin B2 molecule (formation of OH-adducts) and to some extent by splitting off an H-atom with a total rate constant, $k = 1.2 \times 10^{10}$ $L.mol^{-1}.s^{-1}$ (21). The rate constant for the reaction of OH-radicals with the ribose chain of the molecule (see formula in Figure 1) amounts to $k = 1.5 \times 10^9$ $L.mol^{-1}.s^{-1}$, which makes about 12.5% of the total reactivity. Particulary reactive on the vitamin B2-molecule towards the OH-radicals are positions 1, 5, 6 and 9 (Figure 1, insert). The produced OH-adducts on these positions can subsequently add O_2 forming very unstable peroxyl radicals ($ROO^{\bullet}$), which can result in a number of final products, *e.g.*:



(peroxyl radical) (hydroxylated B2)

[5]

In addition to reactions **[5],** also a break of the C6-C7 bond can occur, followed by deposition of oxygen and finally resulting into aldehydes and carboxylic acids. Although $HO_2^{\bullet}/O_2^{\bullet -}$ are very slowly reacting species, their properties are similar to those of OH. Therefore, they can initiate similar reactions and are of biological interest. The resulting vitamin B2-transients can naturally interact with MMC, finally consuming the produced oxidizing transients (*e.g.* OH, $HO_2^{\bullet}/O_2^{\bullet -}$). Such processes might explain, to some extent, the strong effect of vitamin B2 in mixture with vitamin C and MMC, in irradiated bacteria. Under the given experimental conditions, a number of transients are formed and implicated in various processes. Hence, the radiation mechanisms are very complicated and, as yet, not clear.

The obtained results demonstrate that vitamin B2 acts as a radiation protecting agent. This ability of vitamin B2 becomes even stronger by adding vitamin C. This property of both vitamins is strong enough, to completely suppress the cytostatic activity of MMC. The mixture of MMC, vitamins B2 and C acts as an extremely efficient radiation protecting agent (see Figure 1, curve F and insert). Considering that normal mammalian cells contain oxygen, the behavior of vitamin B2 (or mixture of B2 and C) offers a new pathway in radiation oncology implementing MMC.

*) G-value = number of species produced or decomposed by 100 eV absorbed energy. For conversion in SI-units: multiply the G-value by 0.10364 to obtain G(x) in μmol/J. Radiation absorbed energy: $6.24\text{x}10^{15}$ eV/g = 100 rad = 1 Gy (Gray) = 1 J/kg.

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References

1 Getoff N, Platzer I and Winkelbauer C: Transients and co-operative action of β-carotene, vitamin E and C in biological systems *in vitro* under irradiation. Radiat Phys Chem *55*: 699-704, 1999.
2 Kammerer C, Czermak I, Getoff N and Kodym R: Enhancement of MMC- efficiency by vitamin C, E-acetate and β-carotene under irradiation. A study *in vitro*. Anticancer Res *19*: 5319-5322, 1999.
3 Getoff N: Cytostatica efficiency enhancement by vitamins C, E and β-carotene under irradiation. State of art. Radiat Phys Chem *60*: 351-358, 2001.
4 Getoff N: Effect of antioxidants and oxygen in radiotherapy. *In*: Progress in Radio-Oncology VII, eds. Kogelnik HD, Lukas P and Sedelmayer F; pp 99-105, 2002. Monduzzi Editore Intern. Publ. Division, Bologna, Italy.
5 Heinrich E and Getoff N: Radiation induced effect of the vitamins C,E and β-carotene on sanazole efficiency. A study *in vitro*. Anticancer Res *20*: 3615-3618, 2000.
6 Heinrich E and Getoff N: Mitomycin C-activity effected by vitamins B1, C, E and β-carotene under irradiation with γ-rays. Z Naturforsch *58C*: 244-248, 2003a.
7 Heinrich E and Getoff N: Effect of vitamin B1 and mixtures of B1 with other vitamins on cytostatica efficiency of sanazole under irradiation. A study *in vitro*. Radiat Phys Chem *67*: 487-492, 2003b.
8 Svoboda B and Getoff N: Vitamins B6 and C-effect mitomycin C efficiency under irradiation. Anticancer Res *22*: 949-952, 2002.
9 Heinrich E and Getoff N: Influence of vitamin B1 on sanazole activity under irradiation. A study *in vitro*. Anticancer Res *22*: 927-930, 2002.
10 Kjellen E, Joiner M C, Collier J M, Johns H and Rojas A: A therapeutic benefit from combining normobaric carbogen or oxygen with nicotinamide in fractionated x-ray treatments. Radiother Oncol *22*: 81-91, 1991.
11 Getoff N: Effect of vitamin C, E-acetate and β-carotene on the cytostatic activity of nicotinamide (vitamin B3). In Vivo *17*: 169-172, 2003a.
12 Getoff N, Solar S and Mc Cormick DB: Photoejection of electrons from flavins in polar media. Science *201*: 616-618, 1978.
13 Powers H J: Riboflavin (vitamin B2) and health. Amer J Clin Nutr *77*: 1352-1360, 2003.
14 Rivlin R S: Riboflavin and cancer. A review. Cancer Res *3*: 1977-1986, 1978.
15 Qiao CH: Mechanisms of riboflavin deficiency facilitating carinogenesis of N- nitrosamine –effect on carinogen-metabolising enzymes. Chin J Oncol *11*: 322-325, 1989.
16 Webster RP, Gawde MD and Bhattacharya RK: Modulation of carcinogen-induced damage and repair enzyme activity by riboflavin. Cancer Lett *98*: 129-135, 1996.
17 Pangekar J, Krishnaswamy K and Jagadezdan V: Effects of riboflavin-deficiency and riboflavin administration on carcinogen-DNA binding. Food Chem Toxicol *31*: 745-750, 1993.
18 Getoff N: Mitomycin C radical cation studied by pulse radiolysis. In Vivo *17*: 173-176, 2003b.
19 Getoff N, Solar S and Quint R-M: One-electron oxidation of mitomycin C and its corresponding peroxyl radicals. A steady-state and pulse radiolysis study. Radiat Phys Chem *50*: 575-583, 1997.
20 Platzer I and Getoff N: Vitamin C acts as radiation protecting agent. Radiat Phys Chem *51*: 73-76, 1998.
21 Buxton GV, Greenstock CL, Helman WR and Ross AB: Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals in aqueous solutions. J Phys Chem Ref Data *17*: 513-886, 1988.

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