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Enhancement of Mitomycin C Efficiency by Vitamin C, E-Acetate and ß-Carotene under Irradiation. A Study *in Vitro*

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Abstract. *Using E.coli bacteria (AB 1157) and leukemia cells (HL 60) as a model for in vitro studies it was established that the efficiency of mitomycin C (MMC) can be influenced in the presence of antioxidant vitamins. This synergistic effect of the vitamins C, E-acetate and β-carotene on MMC activity is rather strong for E.coli bacteria under irradiation (15 and 50 Gy) in the presence of air. Vitamin C contributes more efficiently to the MMC - activity in leukemia cells than the other two vitamins. The effect is explained by a cascade electron transfer process from the vitamins to MMC, where vitamin C is acting as a major electron source. These results might be of importance in cancer therapy.*

Controlled trials on smokers (1) as well as smokers and drinkers (2) performed on a great number of participants did not prove the expected cancer prevention effect by ß-carotene (ß- car) consumed alone or in combination with α-tocopherol (vit.E) or retinol (vit.A). Just the opposite observations were made, the test persons taking ß-car only or in combination with vit.E or with vit.A exhibited a strong increase on lung and other types of cancer in comparison to the corresponding placebo group. Additional studies in this respect (3,4) did not bring a satisfying clarification to this unexpected results. Therefore in a WHO press release dated 12th January 1998 it was recommended: "... that until further information becomes available on how ß-carotene and other carotenoides influence the process leading to cancer, none of these substances should be promoted to general population as a tumor preventive treatment". It is remarkable that the effect of vitamin C (vit.C), being a well known efficient antioxidant agent with multiple biological properties (6 and ref. therein), was not considered in all these extensive investigations.

Very recently it has been established by experiments *in vitro* on bacteria that the efficiency of mitomycin C (MMC), a versatile cytostatic agent is very strongly increased in the presence of vit.C or by a mixture of vit.C, vitamin E-acetate (vit.E-ac.) and ß-carotene (7). Applying MMC together with ß-car practically did not lead to any modification of the cytotoxity of MMC, but adding vit.C to the system the MMC-toxicity increased strongly. Obviously vit.C is required for the enhancement of the cytostatic activity. Based on spectroscopic and kinetic data of vit.C, vit.E and ß-car obtained by pulse radiolysis (7-9 and ref. therein) and radiation biological studies *in vitro* on bacteria (E. coli AB1157), a hypothesis explaining the action of the vitamins on cytostatics, involving a cascade electron transfer process (vit.C → vit.E → ß- car → oxidizing radicals) was presented (7).

Concerning the present state of the art in this respect it was of special interest to study the action of the vitamins mentioned above on the MMC-efficiency *in vitro* using *E. coli* bacteria (AB 1157) and for comparison a mammalian cell system (HL- 60 leukemia cells) under similar experimental conditions at a relatively low radiation dose.

Materials and Methods

The preparation and handling of bacteria was previously described (6). The human leukemia cell line HL- 60 (ATCL CCL 240) was maintained in RPMI 1640 containing 10% FCS, antibiotics. Apoptotic cells were visually scored as reported (10). The results presented below are mean values of several experimental series.

Results and Discussion

Figure 1 shows the toxicity of MMC on *E. coli* bacteria (AB 1157) as a function of the concentration range from 10^{-8} to 10^{-5} mol·dm^{-3} MMC for (A) 60 minutes and (B) 90 minutes incubation time (The toxicity effect is expressed as N/No-ratio, where No= number of bacteria or cells before and N= after treatment). Naturally in the second case the toxicity effect is more pronounced, because a higher MMC-amount is accumulated. In both experimental series a remarkable N/No-decrease occurs in the range of $5x10^{-7}$ to $5x10^{-6}$ mol·dm^{-3}

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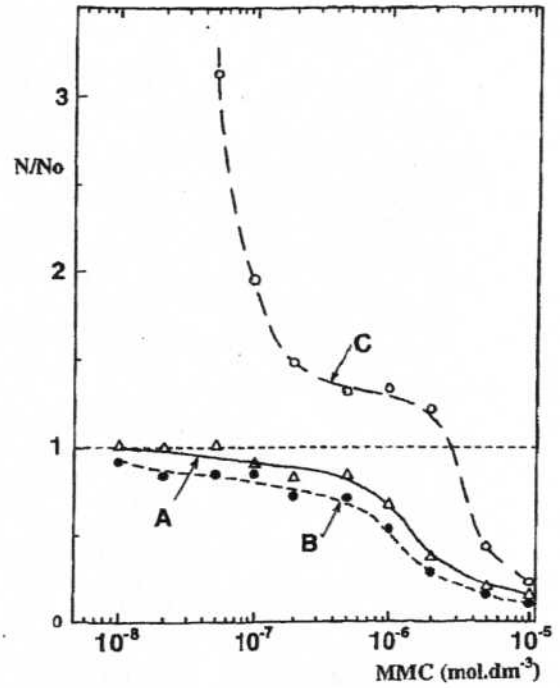

Figure 1. *Toxicity of MMC on E.coli bacteria (AB 1157). (A) N/No as a function of MMC-concentration for 60 min and (B) for 90 min incubation time. (C) as above, but in the presence of 1x10^{-4} mol·dm^{-3} ß-car, 60 min incubation. The experiments were performed in the presence of air. No = starting number of bacteria, N =number after treatment.*

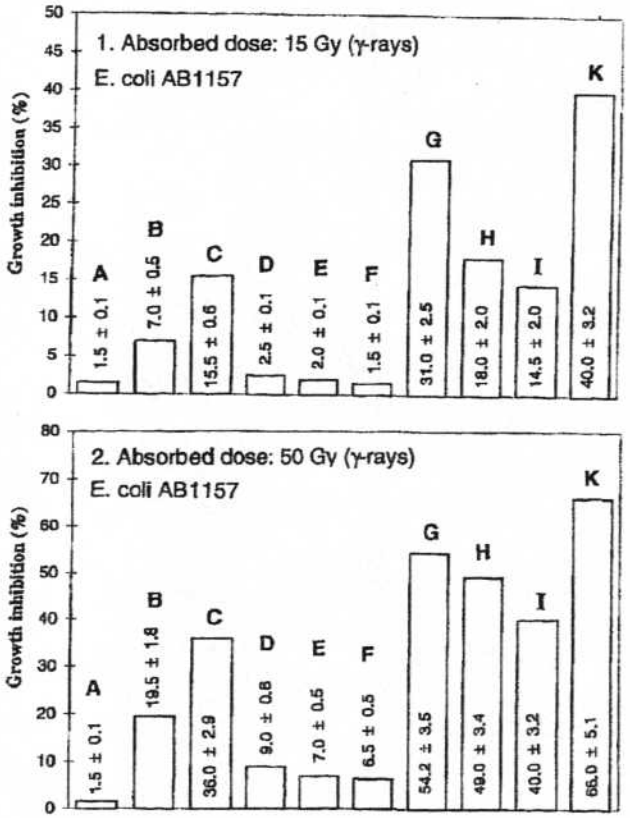

Figure 2. *Growth inhibition (%) showing the effect of vitamin C, E-acetate and ß-carotene on MMC-efficiency under γ-irradiation using E.coli bacteria (AB 1157) as a model for experiments in vitro in the presence of air. Absorbed dose: (1) 15 Gy and (2) 50 Gy. (A) control (unirradiated), (B) bacteria suspension in buffer (pH= 7,4), (C) MMC, (D) vit.C, (E) vit.E-ac., (F) ß-car, (G) MMC + vit.C, (H) MMC + vit.E-ac., (I) MMC + ß-car., (K) MMC + vit.C + vit.E-ac. + ß-car. [MMC] = 1x10^{-5} mol·dm^{-3}. [vit.C]= [vit.E-ac.]= [ß-car.] = 1x10^{-4} mol·dm^{-3}.*

MMC followed by a plateau. Curve C, Figure 1, represents the antioxidant effect of ß-car in the system and shows a stronger toxicity decrease in the range of about 10^{-6} to 10^{-5} mol·dm^{-3} MMC.

Using again *E. coli* bacteria as a model for living systems the growth inhibition (%) caused by MMC was investigated in respect to the action of vitamin C, E-acetate and ß-carotene individually and in mixtures for two absorbed doses of γ-rays: 15 and 50 Gy (see Figure 2). In the first case the data expressed by columns (B) and (C) demonstrate that by applying a radiation dose of 15 Gy the MMC-efficiency (C) is more than twofold higher in comparison to the bacteria suspension in buffer (B). In the presence of vit.C (D), vit.E-ac (E) and ß-car (F) the growth inhibition (%) is rather low, because the vitamins are acting as radiation protecting agents (6,7). A much stronger effect is observed in combinations of MMC and vit.C (G) and a smaller increase is found with vit.E-ac. (H). The application of MMC and ß-car (see column 1) showed even a smaller decrease in the growth inhibition, confirming previous observations (7). The strongest effect, however, was observed by the synergistic effect of MMC with all three vitamins (K). This can be explained by the cascade electron transfer process mentioned above (7).

Similar results were obtained with E.coli bacteria using a higher radiation dose of 50 Gy, under otherwise the same experimental conditions as discussed above (see Figure 2). In this case the synergistic effect of the vitamins on MMC is even more pronounced.

All three vitamins applied individually or in mixture with MMC lead to an enhancement of the growth inhibition (Figure 2, column G, H and 1). As expected a very strong increase up to 66% inhibition effect was obtained using the mixture of MMC and all three vitamins. This fact demonstrates that the effectiveness of the synergistic effect is even increasing with the absorbed radiation dose.

The results originating from the experiments with human leukemia cells (HL 60) used as an *in vitro* model to study the effect of vit.C, vit.E-ac. and ß-car on the MMC-efficiency are presented in Figure 3. The comparison between the data from cells in buffer (B; 20.25%) and those in the presence of MMC (C; 50.25%) illustrates the MMC-activity on these cells. The application of the individual vitamins (Figure 3, columns D, E

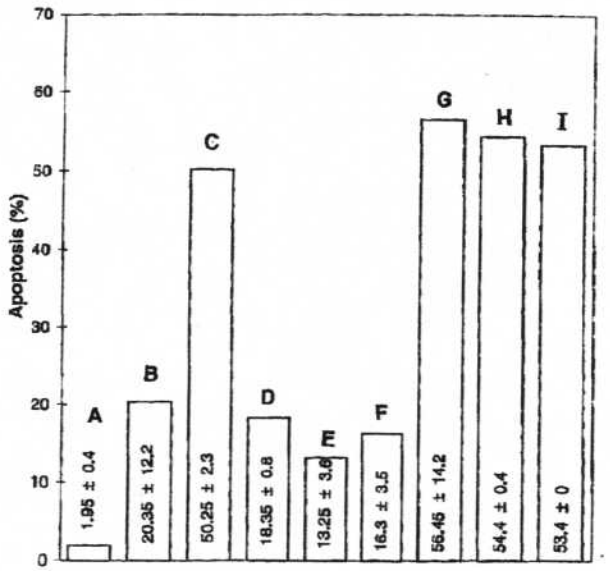

Figure 3. *Apoptosis (%) showing the effect of vitamin C, E-acetate and ß-carotene on MMC-efficiency under x-rays irradiation using cancer cells (HL 60) as a model for studies in vitro. Absorbed dose: 15 Gy. (A) control (unirradiated), (B) no additivies, (C) MMC, (D) vit.C, (E) vit.E-ac., (F) ß-car, (G) MMC + vit.C, (H) MMC + vit.E-ac., (I) MMC + vit.C + vit.E-ac. + ß-car. [MMC] = $1x10^{-6}$ mol·dm^{-3}, [vit.C]= [vit.E-ac.] = [ß-car.]= $1x10^{-5}$ mol·dm^{-3}.*

and F) shows once again their radiation protection behaviour as in the case of bacteria (see also Figure 2). It is obvious that apoptosis using MMC in combination with vit.C (G; 56.45%) is higher than in the case of MMC (C). The results obtained by applying the mixture of MMC and vit.E-ac. (H; 54.4%) as well as MMC in the presence of all three vitamins (I, 53.4%) is somewhat higher towards the experimental series containing MMC only (C; 50.25%), but slightly lower than MMC in the presence of vitamin C only (G; 56.45%).

Conclusion

Based on the obtained results using *E.coli* bacteria (AB 1157) and human leukemia cells (HL- 60) as an *in vitro* model it can be concluded:

- There is a synergistic effect of the vitamins C, E-acetate and ß-car on the MMC-efficiency against *E. coli* under, irradiation in the presence of air at pH=7,4.
- This effect is enhanced in *E. coli* by increasing the absorbed radiation dose from 15 to 50 Gy.
- Vit.C contributes more efficiently to the MMC-activity in leukemia cells than vit.E-ac. and ß-car.
- Vit.E-ac. and ß-car seem to compete with MMC for electrons donated by vit.C (see cascade electron transfer mentioned above and ref. 6, 7).
- The present findings are in agreement with previously reported data (1,2) carried out on a large number of persons, where ß-car alone or in combination with retinol (vitA) or vit.E-ac. were applied: these vitamins do not act as cancer preventive agents in the absence of vit. C.
- Exclusively the presence of vit.C, which acts as an electron source, alone or in combination with vit.E-ac and ß-car, causes a strong enhancement of the MMC-activity.
- The observed synergistic effect on MMC by the three vitamins could be of importance for the combined radiation-chemo therapy of cancer patients.

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