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COMBINATION OF MISONIDAZOLE AND LIPOIC ACID ON THE RADIATION SENSITIVITY OF BACILLUS MEGATERIUM SPORES

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Abstract

The radiation sensitizing action of a combination of misonidazole and lipoic acid was investigated in Bacillus megaterium spores at various oxygen concentrations. Lipoic acid and misonidazole were applied in two concentrations (0.1 and 1.0 mmol/l) and four combinations were prepared from them. No uniform correlation was found, neither to the combination of the compounds nor to the gas conditions. The combination of two radiation sensitizing compounds with in all probability different action mechanisms does not unequivocally enhance the radiation sensitizing effect under anoxic and hypoxic conditions.

One of the problems in the treatment of cancer with radiation is the presence of cells inside the tumours which are almost totally deprived of oxygen. This means that a given dose of radiation can cause greater damage to the normal, well oxygenated tissues and less to the tumour cells. A large number of radiation sensitizing compounds of the 'electron affinic' class have been developed and tested in vitro and in vivo (1, 2, 3, 14). These compounds have the ability to increase the radiation sensitivity of hypoxic cells without affecting the sensitivity of oxic cells. It appears that the misonidazole (2-nitroimidazole) may be the most promising chemical radiation sensitizer. It has reached a stage of limited clinical trial (4). The main problem in the clinical usefulness of misonidazole is its neurotoxicity. This compound increases the number of treatment complications, causing peripheral neuropathies (5). However, clinical improvement could be obtained in combination with hyperthermia (12), protectors (8), cytotoxic drugs (7, 9, 11) or mixtures of electron affinic and free-radical sensitizers (10).

Lipoic acid (vitamin N) participates widely in the oxidation–reduction processes. It takes part in the oxidative decarboxylation of purivic- and α-ketoglutaric acids and also plays an essential role in the oxidation of fatty acids. It increases the radiation sensitivity of Bacillus megaterium spores under anoxic and hypoxic conditions (6).

A report is now presented on the effect on radiation sensitivity in Bacillus megaterium spores of a combination of misonidazole and lipoic acid.

Material and Method

Bacillus megaterium (ATCC 8245) spores were used. The plating medium was Oxoid Nutrient Broth No. 2 supplemented with 2% Oxoid Agar No. 1. Colonies were counted after overnight incubation at 37°C.

The gamma radiation facility was a PX- γ-30 ^{60}Co apparatus with 105, 89–97, 87 Gy/min dose rate.

Dose-ln curves were constructed from four experimental points, each point being determined from at least four replicate plate counts. All experiments were repeated at least twice. These curves took a linear or near linear form and are described by the expression 1:

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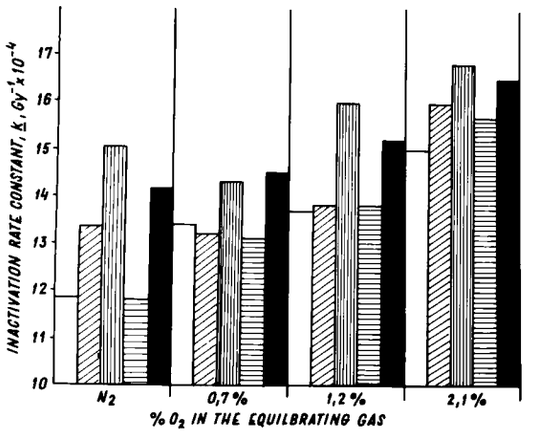

Fig. 1. Relationship between the inactivation constant for Bacillus megaterium spores in the presence of various concentrations of lipoic acid and misonidazole. Gas alone (□). 0.1 mmol misonidazole (▨). 1.0 mmol misonidazole (▥). 0.1 mmol lipoic acid (▤). 1.0 mmol lipoic acid (■).

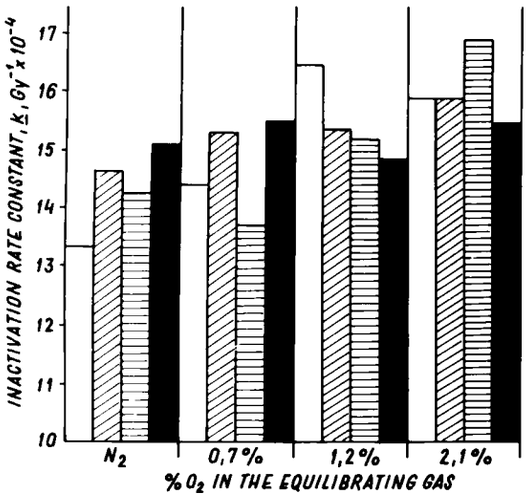

Fig. 2. Radiation sensitivity of Bacillus megaterium spores in the combined application of lipoic acid and misonidazole. 1.0 mmol lipoic acid+0.1 mmol misonidazole (□). 1.0 mmol lipoic acid +1.0 mmol misonidazole (▨). 0.1 mmol lipoic acid+0.1 mmol misonidazole (▤). 0.1 mmol lipoic acid+1.0 mmol misonidazole (■).

Table

The effect of combined treatment of lipoic acid (L) and misonidazole (M). Values based on eq. (2)

Per cent O_2 in N_2	0.1 mmol L+ 0.1 mmol M	0.1 mmol L+ 1.0 mmol M	1.0 mmol L+ 0.1 mmol M	1.0 mmol L+ 1.0 mmol M
N_2	0.20>0.12	0.27=0.27	0.12<0.31	0.23<0.36
0.7	0.02>−0.03	0.18>0.05	0.08=0.08	0.15<0.16
1.2	0.13>0.02	0.10<0.21	0.24>0.14	0.14<0.33
2.1	0.14<0.15	0.04<0.21	0.08<0.18	0.08<0.24

$$\ln S/S_0 = \ln n - kD \qquad (1)$$

where S is the number of spores surviving a dose D, S_0 is the original number of spores, k is the slope of the curve and is referred to as the inactivation constant and n is known as the extrapolation number. Suspended spores were irradiated in distilled water. The test suspension containing about 5×10^7 viable spores were equilibrated with nitrogen and oxygen in nitrogen gas mixture over the bubbled suspension for 30 min before the commencement of irradiation. Gas bubbling continued throughout irradiation. Experiments were made by equilibrating suspension with gas mixture of 0.7, 1.2 and 2.1% O_2 in N_2 and pure nitrogen at 20°C. Misonidazole was purchased from Agvar Chem. Inc. (New York), lipoic acid was kindly supplied by Medexpert (Moscow). The concentration range of misonidazole and lipoic acid was extended from 0.1 to 1.0 mmol/l.

Result and Discussion

Lipoic acid and misonidazole were applied in two concentrations (0.1 and 1.0 mmol/l) and four combinations were prepared from them. Treating separately, it was observed that the highest inactivation constant values were obtained with each kind of gas at 1.0 mmol concentration of misonidazole (Fig. 1). The radiation sensitizing effect of lipoic acid in both concentrations remained below the response of misonidazole. Increase of the concentration of both compounds led to a rise in *k* values. In some cases no radiation sensitizing effect was detectable in 0.1 mmol concentration (lipoic acid at N_2, lipoic acid and misonidazole at 0.7% O_2 in N_2, lipoic acid and misonidazole at 1.2% O_2 in N_2). The results of four kinds of combination of both compounds are illustrated in Fig. 2. When using nitrogen or 0.7% O_2 in N_2 gas mixture the highest radiation sensitizing ef-

fect was observed in the combination of 0.1 mmol lipoic acid and 1.0 mmol misonidazole. At 1.2% O_2 in N_2 the 1.0 mmol lipoic acid and the 0.1 mmol misonidazole, at 2.1% O_2 in N_2, the 0.1 mmol lipoic acid and 0.1 mmol misonidazole combination resulted in the best radiation sensitizing effect.

The presentation of the results, however, gives a qualitative picture only. The isobole used in pharmacology is necessary for the correct quantitative analysis. Only in the light of this can it be defined whether a combined treatment is synergic, antagonist or additive (13).

When planning the experiment it was assumed that the two compounds in all probability have different effects. The action mechanism of electron affinic radiation sensitizing compounds is more or less clear (1) but the explanation of the radiation sensitizing effect of lipoic acid can only be assumed (6). In the present experiments the radiation sensitizing effect obtained from the combination of both compounds remained below the expected value. For this reason the experiment necessary for the complete quantitative analysis (isobole determination) has not been performed because it could not give essentially more information. The following formula was introduced for the characterization of the combined effect:

$$SER_{LM}-OER=(SER_L-OER)+(SER_M-OER) \quad (2)$$

where OER is the oxygen enhancement ratio at a given oxygen concentration, SER_M is the sensitizer enhancement ratio of misonidazole, SER_L is the sensitizer enhancement ratio of lipoic acid, SER_{LM} is the common sensitizer enhancement ratio of both compounds. According to the conventional explanation equality is considered an additive (=) effect, in case of inequality synergic (>) and antagonistic (<) effects are observed. The Table has been prepared accordingly.

The combination of 1 mmol lipoic acid and 1 mmol misonidazole with any kind of gas shows that the sum of the separate effect is higher than the response of combined application. A similar relationship is seen when examining the effect of all four combinations at nitrogen and at 2.1% O_2 in N_2. With the other treatments no uniform correlation was found, neither to the combination of the compounds nor to the gas conditions. It can be stated that in any case the radiation sensitizing effect does not cease, since if that were so, zero value would be presented on either side of Equation 2. The only exception was seen with the application of 0.1 mmol lipoic acid+0.1 mmol misonidazole at 0.7% O_2 in N_2, although in this case, individual values remained below the gas control.

Summing up, it can be said that the combination of two radiation sensitizing compounds with in all probability different action mechanisms does not unequivocally enhance the radiation sensitizing effect under annoxic and hypoxic conditions. Radiation sensitization is a significantly complicated oxido-reduction process which cannot be explained by simple electron affinity or the primary and secondary processes of water radiolysis.

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