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Nitroxyl radicals decrease toxicity of cytostatic agents

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Nitroxyl radicals of a series of piperidineoxyles and pirrolinoxyles increase the tolerance of experimental animals to the injection of otherwise lethal doses of anti-tumor cytostatic agents. Simultaneous injection of nitroxyl radicals in different doses with 6-mercaptopurine, thiophosphamide, cyclophosphamide and the other cytostatic agents results in decreased toxicity and survival of animals. Nitroxyl radicals normalize the level of the oxidized form of P450 cytochrome that is decreased by the injection of lethal doses of cytostatic agents.

Key words: Cytostatic agents, nitroxyl radicals, P450 cytochrome, tolerance.

Introduction

Available anti-tumor agents are generally unsatisfactory in that toxic side effects, often highly organospecific, restrict the therapeutic use and benefit. The degree of drug selectivity is determined by the relation between its toxic and therapeutic doses. In some cases an increase in selectivity can be achieved by changing the molecular structure of the drug, thus reducing the general toxicity while at the same time maintaining anti-tumor potency.[1]

A decrease in the general toxicity of anti-tumor cytostatics of different classes can also be achieved by introducing nitroxyl radicals into their structure.[2–6] In particular, experiments show that nitroxylic modification results in a decrease in general toxicity of thiophosphamide, 5-fluorouracil and daunomycin without cardiotoxicity. In view of these data, we investigated the possibility of using nitroxyl radicals as a means of increasing tolerance of the organism to toxic effect of cytostatics in combined application. Nitroxyl radicals of the structure shown in Figure 1 were used.

Materials and methods

Animals

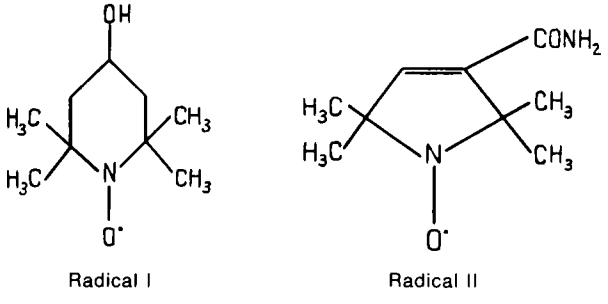
Randomly bred female mice weighing 20–24 g and randomly bred rats weighing 100–120 g were used.

Drugs

Thiophosphamide (ThioTEPA), 6-mercaptopurine (6-MP), cyclophosphamide (CTX), sarcolysine (SL), daunorubicin (DR), vincrystine (VC), nitrosomethylurea (NMR), ftorafur and nitroxyl radicals from the series of piperidineoxyles (Radical I) and pirrolinoxyl (Radical II) were obtained commercially. ThioTEPA, CTX, SL, DR, NMU, Radical I and Radical II were dissolved in water for injection, 6-MP was dissolved in hot water, ftorafur was diluted by water for injection to produce the desired concentration. All drugs were injected intraperitoneally.

Methods

The animals were divided into two groups. One group was injected with one of the cytostatics, listed above, in the absolutely lethal dose LD_{100}, the other



Radical I Radical II

Figure 1. Structure formulae of nitroxyl radicals.

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was injected with the same cytostatic agent in the same dose combined with a radical. Cytostatic agents and nitroxyl radicals were injected in separate syringes. In order to examine the influence of circadian rhythms on drug toxicity, the same drugs were injected in control animals at the same time of day. Mortality of animals and change in their weight were registered during the experiment. The animals which survived were followed up for 60 days. Mann–Whithey's *U*-test was used for statistical analysis.

Determination of cytochrome P450 level

The animals that received LD_{100} of cytostatic agents alone or combined with Radical I were sacrificed daily, starting from the first day after injection of the drugs. Their liver was removed and specimens were prepared by standard methods to produce equal cylindrical samples.[6] Recording of electron spin resonance (ESR) spectra of frozen samples was carried out using a Varian-104 radiospectrometer at the temperature of liquid nitrogen. The level of the oxidized form of P450 cytochrome can be judged by the magnitude of the most intensive component of the ESR spectrum using the '*g*-factor' which characterizes the relationship between the non-paired electron and magnetic field ($g = 2.25$).

Results and discussion

In order to define the optimal doses of nitroxyl radicals and the schedule of combined injection with a cytostatic agent we varied the dose of Radical I from 0.04 to 200 mg/kg, with simultaneous or subsequent injection with the anti-tumor drug at its absolutely lethal dose. For mice LD_{50} of Radical I is 400 mg/kg, Radical II is 425 mg/kg; for rats it is 300 and 350 mg/kg, respectively. LD_{100} of cytostatic drugs was independent of diurnal rhythm.

Figure 2 shows that simultaneous injection of 0.32 mg/kg Radical I is the optimal dose for 6-MP. In the case of ThioTEPA the best results were obtained when Radical I was injected 5 h before administration of the cytostatic agent. In this case the optimal dose of Radical I was 5 mg/kg (Figure 3).

Similar experiments were conducted for all other drugs studied (Table 1). The data presented in Table 1 show that in all cases the addition of Radical I resulted in increased survival of animals that

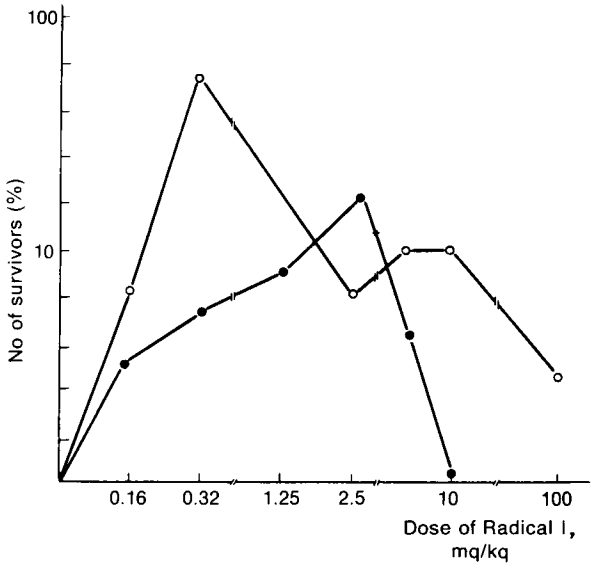

Figure 2. Effect of interval between injection of 6-MP and Radical I on toxicity of 6-MP for mice: (○) Simultaneous injection of 6-MP and Radical I; (●) injection of 6-MP 5 h after Radical I.

received the absolute lethal doses of cytostatic agents irrespective of structure and mode of action of these agents. However, the degree of the detoxifying effect of Radical I differed; the best results were obtained with combined injection of Radical I with 6-MP and ThioTEPA.

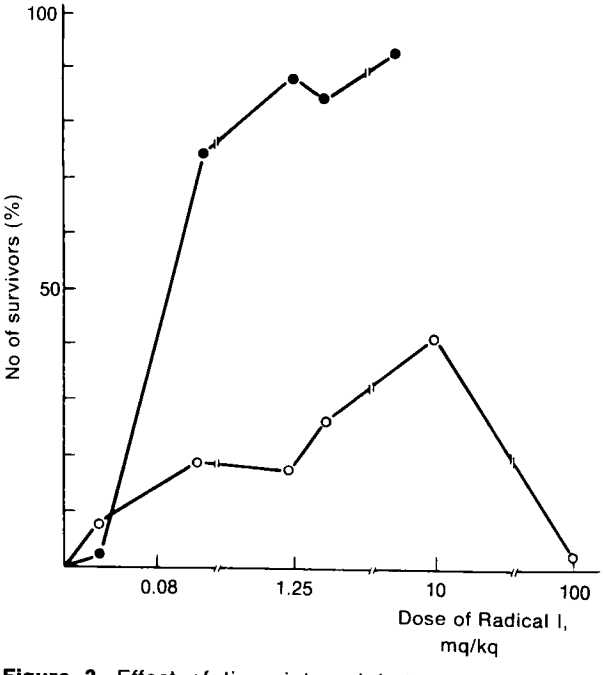

Figure 3. Effect of time interval between injection of ThioTEPA and Radical I on toxicity of ThioTEPA for rats: (●) ThioTEPA injected 5 h after Radical I; (○) ThioTEPA and Radical I injected simultaneously.

Table 1. Decrease in general toxicity of cytostatics by combination with Radical I

No.	Drug	LD_{100} (mg/kg body weight)	Optimal dose of NR-I[a]	Interval between injections (h)	Body weight change from day 7 to day 0 (g)	Survivors on day 30 compared to total number of animals	Increase of life span (%)	Significance[b]
1	ThioTEPA	12			−30.0	0/36	0	
2	ThioTEPA + NR-I		10.0	sim[c]	−15.0	15/36	18	$p < 0.001$
3	NR-I + ThioTEPA		5.0	5	−13.0	13/14	10	$p < 0.001$
4	6-MP	500			−2.6	0/28	0	
5	6-MP + NR-I		0.32	sim	0	15/18	0	< 0.001
6	NR-I + 6-MP		1.25	5	0	6/11	130	$p < 0.001$
7	CTX	600			−7.2	0/60	0	
8	CTX + NR-I		2.5	sim	−4.2	6/37	100	$p < 0.05$
9	NR-I + CTX		2.5	5	−3.0	3/10	520	$p < 0.05$
10	DR	9			−1.6	0/60		
11	DR + NR-I		10.0	sim	−0.8	10/40	22	$p < 0.005$
12	NMU	120			−8.0	0/45		
13	NMU + NR-I		5	sim	−4.0	4/20	0	$p < 0.001$
14	ftorafur	1200			−3.6	0/26		
15	ftorafur + NR-I		10	sim	0	2/15	130	$p < 0.005$
16	SL	40			−2.0	0/70		
17	SL + NR-I		10	sim	0	3/30	2	NS
18	VC	6.5			−2.0	0/35		
19	VC + NR-I		10	sim	0	7/25	72	$p < 0.005$

[a] Radical I.
[b] Mann–Whitney's *U*-test ($p < 0.05$); NS, not significant.
[c] Simultaneous injection.
Rats were used in experiments 1, 2 and 3; mice were used in experiments 4–19.

Figure 4 illustrates differences in the mortality kinetics of animals after injection of the drug in the absolute lethal dose alone or combined with Radical I.

After combined injection of Radical I with CTX, the number of animals that survived was small; however, the life span of the remaining animals increased by 520% as compared with the animals that received CTX alone. It should be noted that such an index as weight reduction of animals also testifies to a lower toxicity of the combined effect (Table 1).

Similar experiments were conducted with Radical II (Table 2). In this case the results were less significant but again some animals survived. In order to elucidate the mechanism of these effects, we investigated whether there were any changes in the level of the oxidized form of P450 cytochrome in the liver of mice after administration of cytostatic agent alone or in combination with Radical I.

The functional role of P450 cytochrome, which belongs to the system of non-specific multipurpose oxidases, is mainly associated with its participation

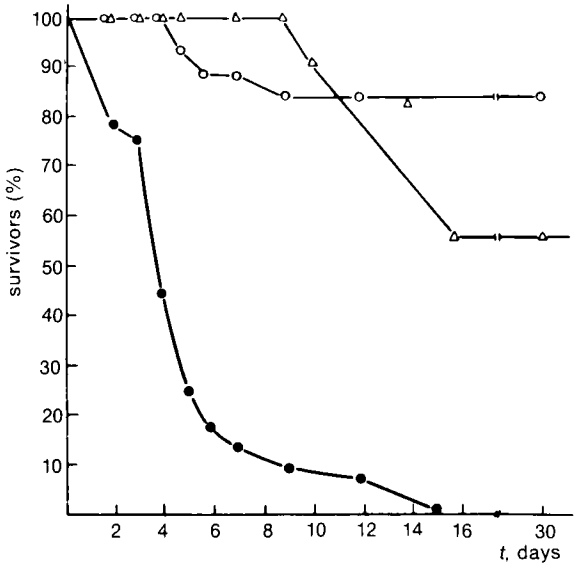

Figure 4. Survival mice of after injection of 6-MP at absolute lethal dose level (●); after combined simultaneous injection of 6-MP and Radical I (○) and injection of 6-MP 5 h after Radical I (△). Radical I was used in optimal dose: 0.32 and 1.25 mg/kg, respectively.

Table 2. Decrease in general toxicity of cytostatics by combination with Radical II

No.	Drug	LD_{100} (mg/kg body weight)	Optimal dose of NR-2[a]	Interval between injections (h)	Body weight change from day 7 to day 0 (g)	Survivors on day 30 compared to total number of animals	Increase of life span (%)	Significance[b]
1	CTX	600			−6.8	0/20		
2	CTX + NR-II		20	sim[c]	−5.0	2/20	0	NS
3	DR	9			−2.0	0/16		
4	DR + NR-II		2.5	sim	−1.0	2/16	6	NS
5	SL	40			−2.3	0/20		
6	SL + NR-II		0.32	sim	0	3/20	56	NS

[a] Radical II.
[b] Mann–Whitney's *U*-test; NS, not significant.
[c] Simultaneous injection.
The mice were used in all experiments.

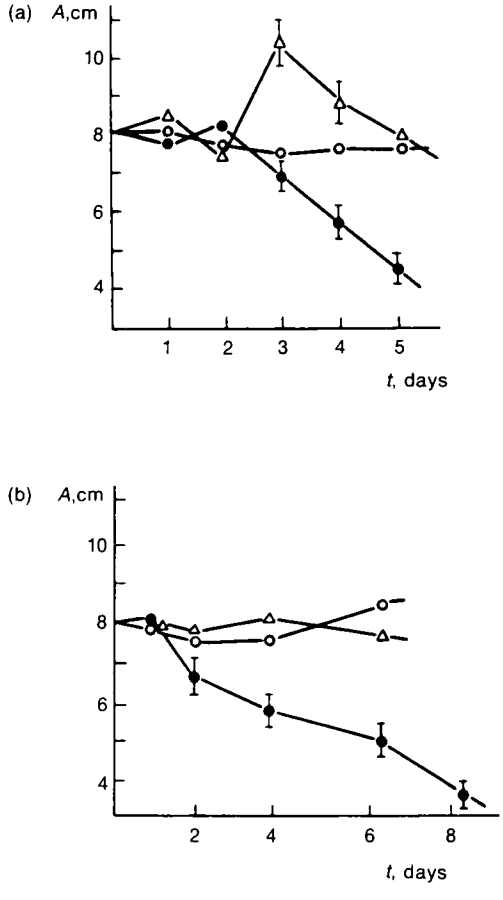

Figure 5. Change in the activity of cytochrome P450 in the mouse liver after injection: (a) SL at lethal dose 40 mg/kg (●), Radical I at 10 mg/kg (○), SL with Radical I(△); (b) DR at lethal dose 9 mg/kg (●), Radical I at 10 mg/kg (○), DR with Radical I (△). Ordinate: ESR signal amplitude (cm). Abscissa: time (days).

in metabolic processes which detoxify exogenous compounds.[7] The oxidized active form of this enzyme is characterized by paramagnetic properties, caused by low- and high-spin states of iron in hemoprotein; these can be registered using ESR techniques at low temperatures. Injection of cytostatic agents at the absolute lethal dose results in a sharp fall of the level of the oxidized form of P450 cytochrome (Figure 5). Addition of nitroxyl radicals normalized the level of the oxidized form of P450 cytochrome. Nitroxyl radicals alone did not change the level of the oxidized form of P450 cytochrome.

It can be assumed that elevation of the organisms tolerance to the toxic effect of cytostatic agents is associated with activation of anabolic processes; this is confirmed by the fact that DNA synthesis is increased in the liver after injection of nitroxyl radical.[8,9] Biological response modifiers affecting different regulatory processes in the cell and the whole organism are currently of interest to oncologists.[10,11] As defined, chemical agents which increase the ability of the host to tolerate damage by chemotherapy should therefore also be classified as biological response modifiers. Therefore, nitroxyl radicals can be considered as biological response modifiers. The data concerning the anti-cancer potency of cytostatic agents in combined use with nitroxyl radicals will be published in another paper.

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