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SHORT COMMUNICATION

RADIOPROTECTIVE EFFECTS OF WR-2721, BRONCHO-VAXOM® AND THEIR COMBINATIONS: SURVIVAL, MYELOPOIETIC RESTORATION AND INDUCTION OF COLONY-STIMULATING ACTIVITY IN MICE

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Abstract — The possibilities of combined radioprotection, using preirradiation WR-2721 administration and post- or preirradiation Broncho-Vaxom® administration in lethally whole-body gamma-irradiated mice were investigated. $LD_{50/30}$ dose reduction factors (DRFs) for mice treated with WR-2721 (200 mg/kg i.p. 30 min before irradiation), Broncho-Vaxom® (25 mg/kg i.p. 24 h before irradiation), or both agents were 1.92, 1.17 and 2.07, respectively. These results demonstrated at least additive radioprotective effects of both agents, manifested in increased survival of irradiated mice. Radioprotection from 17 Gy was optimal when WR-2721 in combination with Broncho-Vaxom® was given 30 min before irradiation and Broncho-Vaxom® 24 h before or 4–8 h after irradiation. Combined modality treatments were also more effective than individual treatments alone in accelerating the bone marrow GM-CFC restoration. During the first days after irradiation enhanced colony-stimulating activity (CSA) of the lungs was observed in mice with postirradiation injection of Broncho-Vaxom® alone or in mice injected with WR-2721 and Broncho-Vaxom® (8 h after irradiation), as well as in mice only irradiated.

The most widely studied and effective *in vivo* radiation protector is the thiol compound S-2-(3-aminopropylamino) ethylphosphorothioic acid (WR-2721, ethiofos, gammafos) (Yuhas & Storer, 1969; Brown, Graham, MacKenzie, Pittock & Shaw, 1988; Sverdlov, Bogatyrov, Nikanorova, Timoshenko & Krasotskaya, 1974). Several theories exist regarding the mechanism of cell protection against radiation-induced lethality. Of these, free radical scavenging, hydrogen atom donation, and induction of hypoxia are the most acceptable (Yuhas, 1970). WR-2721 is most effective when relatively high doses are administered shortly before irradiation. A dose reduction factor (DRF) as high as 2.7 against 30-day lethality in mice has been achieved with WR-2721, but high dose-induced protection is accompanied by side-effects that are unacceptable in many situations. WR-2721 is currently undergoing clinical trials as a radioprotector and as a chemoprotector in cancer patients (reviewed by McCulloch, Scheffler & Schein, 1991).

Immunomodulators, either microbial agents or recombinant cytokines, can also enhance survival and hemopoietic and functional cell recovery after irradiation. Their optimal radioprotective effects have usually been observed when administered 20–24 h prior to irradiation. It has been suggested that these agents mediate radioprotective effects by mechanisms such as enhancing the proportion of hemopoietic stem cells in less radiosensitive phases of the cell cycle, increasing the size of the preirradiation stem cell pools, and accelerating restoration of functional hemopoietic cell populations (Ainsworth, 1988; Chirigos & Patchen, 1988). However, review of data on radioprotective immunomodulators indicates that the maximum DRFs achievable are

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approximately 1.2 – 1.3 (Patchen, D'Alesandro, Chirigos & Weiss, 1988; Weiss, Kumar, Walden, Neta, Landauer & Clark, 1990). Broncho-Vaxom® —a lyophilized fraction of bacterial extract (endotoxin-free), is one of such radioprotective immunomodulators (Fedoročko, Brezáni & Macková, 1992; Fedoročko & Brezáni, 1992; Vávrová & Filip, 1992). Broncho-Vaxom® is used as a polyvalent immunobiotherapeutic agent active in the treatment of respiratory tract infections, particularly acute and chronic bronchitis (Maestroni & Losa, 1984; Palma-Carlos, Palma-Carlos, Inacio & Sousa Uva, 1987).

Combined use of substances effective in reducing radiation consequences by combining the different mechanisms of protective action is characteristic of current trends in radioprotection (Walker, 1988; Weiss *et al.*, 1990). The advantages of combined treatments include the possibility of additive or synergistic action from different radioprotective mechanisms, thereby significantly enhancing protection, over that of single agents, and the possibility of decreasing the toxicity of the individual agents used (Patchen *et al.*, 1988; Patchen, MacVittie & Jackson, 1989; Patchen, MacVittie & Weiss, 1990; Pospíšil, Netíková, Pipalová & Jarý, 1991; Kozubík, Pospíšil & Netíková, 1990).

In this paper, we report the effect of WR-2721, Broncho-Vaxom® and their combinations on the survival and myelopoietic regeneration of mice, when WR-2721 is used as a protectant and Broncho-Vaxom® is used as a protectant or a therapeutical agent in irradiated mice.

EXPERIMENTAL PROCEDURES

Mice

Female C57Bl/6 mice, 12 – 14 weeks old, were obtained from VELAZ (Praha, Czechia). Animals were quarantined for a period of two weeks. They were housed in rodent cages, seven to ten animals per cage at about 23°C, and they were given VELAZ/Altromin 1320 St lab chow and tap water acidified to pH 2.4 *ad libitum*. Research was conducted according to principles enunciated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the State Veterinary Office of the Slovak Republic, Bratislava.

Reagents

WR-2721 was obtained from Dr Pavel Kuna (J. E. Purkyně Military Medical Academy, Hradec Králove, Czechia) and was synthesized by C. Krajčovič (Kuna, Volenec, Vodička & Dostál, 1983) according Piper, Stringfellow, Elliot, and Johnston (1969). Approximately 30 min prior to irradiation, mice were intraperitoneally (i.p.) injected with 200 mg/kg WR-2721 in a volume of 0.5 ml. This dose has minimal toxic effects in mice (Landauer, Davis, Dominitz & Weiss, 1987).

Broncho-Vaxom® (Biogal Pharmaceutical Works, Debrecen, Hungary; under the licence of OM Laboratories, Geneva, Switzerland) is a lyophilized fraction of the eight most common bacteria of the upper respiratory tract (*Diplococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella ozaneae*, *Klebsiella pneumoniae*, *Neisseria catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus viridans*), free of endotoxins (less than 0.0002% by Limulus and pyrogenicity tests; Bottex, Cristau, Corrazza, Mougin & Fontanges, 1988). The final dry preparation contains (by weight) 35% proteins, 8% free amino acids, 10% lipids, 8% nucleotides, 2% carbohydrates and approximately 37% salts (Mauel, Pham, Kreis & Bauer, 1989). Immediately before use the drug was resuspended in saline in a volume of 0.4 ml (25 mg/kg) and administered i.p. according the schedule described in the Results. Control animals received 0.4 ml saline at the same time.

Irradiation

Mice were placed in plexiglass containers and exposed to 7.5 – 20 Gy of gamma rays at a dose rate of 0.5 Gy/min. A Chisostat ^{60}Co source (Chirana, Czechia) was used for all irradiations.

Survival

Survival was monitored daily and was reported as percentage of animals surviving 30 days after irradiation. Each experimental group within each experiment consisted of 10 – 11 mice. Moribund animals in this experiment were killed. On day 31, surviving mice were euthanized by cervical dislocation. Experiments were repeated three times. DRF was calculated by dividing treatment $LD_{50/30}$ by control $LD_{50/30}$. Survival rates were compared among groups using the Chi-square test including Yates' correction.

Hemopoietic stem cell assay

Hemopoietic cells (GM-CFC) were cultured *in vitro* on polystyrene Petri dishes (Gama s.p., České Budějovice, Czechia) as described by Vacek,

Michurina, Serova, Rotkovská and Bartoníčková, (1991). Bone marrow cells ($8 \times 10^4 - 1.3 \times 10^5$) were plated in triplicate in a semi-solid environment created by a plasma clot, containing Iscove's modification of Dulbecco's medium (IMDM, TechGen Int. Ltd, U.K.) supplemented with antibiotics (penicilin, 100 U/ml and streptomycin, 1000 μg/ml) and L-glutamine (Calbiochem-Behring, La Jolla, U.S.A.) in a concentration of 1.2 mg/ml plus 15 – 20% newborn bovine calf serum (TechGen Int., Ltd, U.K.), 10% murine lung-conditioned medium (LCM), 10% citrate bovine plasma and 3% $CaCl_2$ (Biotika, Slovenská Ľupča, Slovakia). The cultures were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 in air for seven days. Colonies of at least 50 cells were scored at 30 × magnification. The cell suspension used for these assays represented a pool of tissues from five to seven intact (nonirradiated), irradiated, or treated and irradiated mice at each time. Cells were flushed from femurs with 2 ml IMDM containing 15% heat-inactivated newborn bovine serum. Spleens were homogenized through a 25-gauge needle until a single cell suspension was obtained and the total number of nucleated cells in each suspension was determined using a Bürker chamber. Statistical analysis was performed using Student's *t*-test.

Assay for CSF activity

Macrophages of the lungs are an important source of CSF in the body, (Burgess, Camakaris & Metcalf, 1977) and production of CSF can be evaluated by the colony-stimulating activity (CSA) of the lung-conditioned medium (LCM) on the growth of *in vitro* bone marrow GM-CFC. At various times after irradiation lungs were aseptically removed and placed in 5 ml IMDM supplemented with antibiotics (penicilin, 100 U/ml and streptomycin, 1000 μg/ml) and LiCl (Merck, Darmstadt, Germany) in a concentration of 1.268 mg/ml. Whole lungs were incubated for 72 h. At the end of incubation, the supernatants were collected, pooled, filtered (0.2 μm Minisart NML units, Sartorius, Germany) and assayed for CSF activity.

RESULTS

The combined use of low (nontoxic) dose of WR-2721 and Broncho-Vaxom® has been investigated. Studies have shown a favorable protective response to gamma irradiation. Compared to saline-treated mice, survival was enhanced by treatment

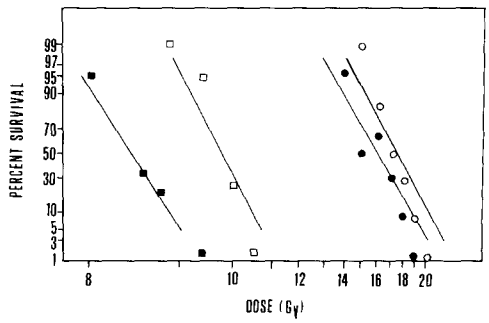

Fig. 1. Animal survival as a function of radiation dose for mice injected with saline (■), Broncho-Vaxom® (25 mg/kg i.p. 24 h before irradiation, (□)), WR-2721 (200 mg/kg i.p. 30 min before irradiation, (●) or a combination of WR-2721 + Broncho-Vaxom® (○). Percentage survival at 30 days was probit-plotted against radiation dose and used to determine $LD_{50/30}$ and dose reduction factors (DRFs). Each data point represents 41 – 60 mice.

either with Broncho-Vaxom® injected at – 24 h, or by WR-2721 administered 30 min before irradiation or by the combination of both agents (Fig. 1). $LD_{50/30}$ values were as follows: saline-treated mice 8.36 (95% CL 8.26, 8.46) Gy, Broncho-Vaxom®-treated mice 9.77 (9.67, 9.86) Gy, WR-2721-treated mice 16.09 (15.90, 16.28) Gy and combination WR-2721 + Broncho-Vaxom®-treated mice 17.27 (17.12, 17.43) Gy. Compared to saline, these values resulted in DRFs of 1.17 for Broncho-Vaxom®, 1.92 for WR-2721, and 2.07 for WR-2721 + Broncho-Vaxom® treatments. For combined WR-2721 + Broncho-Vaxom® treatment, DRFs were 1.07 and 1.77 compared to WR-2721 alone and Broncho-Vaxom® alone, respectively. The DRF values obtained for WR-2721 + Broncho-Vaxom®-treated mice (2.07) compared with saline-treated mice and DRF values for Broncho-Vaxom®-treated mice (1.17) and for WR-2721-treated mice (1.92) suggest the additive effect of both agents in enhancing survival. It can thus be assumed that Broncho-Vaxom® and WR-2721 possess different and mutually independent mechanisms of action.

Further, the effect of various application times of Broncho-Vaxom® with simultaneous WR-2721 treatment on survival of mice irradiated with a 17 Gy dose ($\sim LD_{50/30}$ for combination WR-2721 + Broncho-Vaxom® (– 24 h); see Fig. 1) was tested. Table 1 shows the results of survival studies in which low dose (nontoxic) of WR-2721 alone and combinations of WR-2721 with Broncho-Vaxom® were used. Broncho-Vaxom® alone has no protective effect at the high radiation dose tested. However,

Table 1. Survival of irradiated mice (17 Gy) treated with WR-2721 alone or combinations of WR-2721 with Broncho-Vaxom®

Treatment	30-day survival	*n*	Significance (versus WR-2721 alone)
WR-2721	25.8%	31	–
WR-2721 + Broncho-Vaxom® (−24 h)	56.6%	30	$P<0.05$
WR-2721 + Broncho-Vaxom® (+1 h)	40.0%	30	N.S.
WR-2721 + Broncho-Vaxom® (+4 h)	64.5%	31	$P<0.05$
WR-2721 + Broncho-Vaxom® (+8 h)	63.3%	30	$P<0.05$

WR-2721 (200 mg/kg) was administered i.p. 30 min before irradiation and Broncho-Vaxom® (25 mg/kg) was administered i.p. 24 h before irradiation or 1, 4 or 8 h after irradiation. Data represent the mean of three separate experiments.

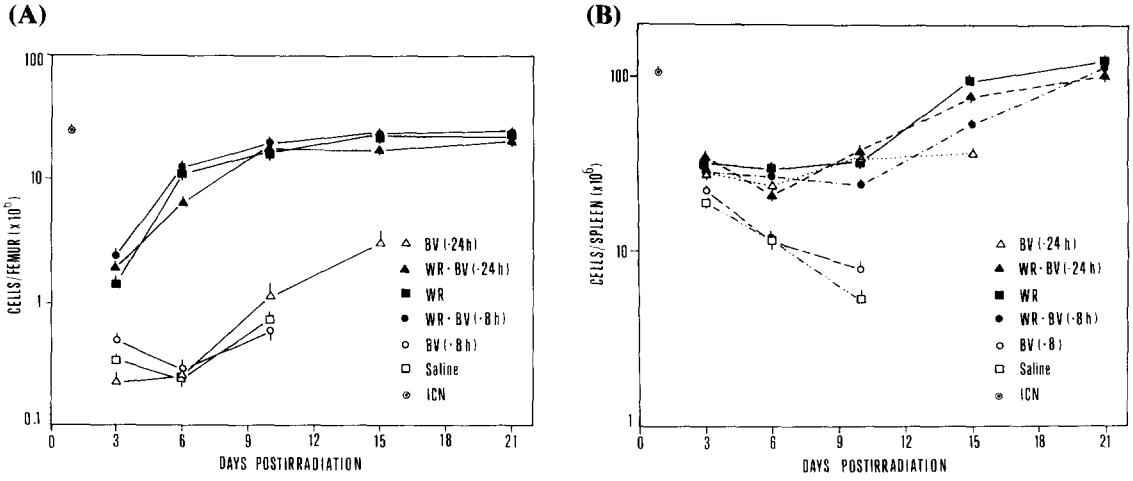

Fig. 2. Effects of Broncho-Vaxom® (BV), WR-2721 (WR) and their combinations on bone marrow cellularity (A) and on splenic cellularity (B) in irradiated mice. Mice were injected with solutions of agents as described above and exposed to a 9.5 Gy dose radiation on day 0. ICN = intact control mice (nonirradiated). (A) WR-2721 and WR-2721 + Broncho-Vaxom® (−24 h; +8 h) values are significantly greater than saline values on days 3 – 10 ($P<0.001$). WR-2721 + Broncho-Vaxom® (−24 h; +8 h) values are significantly greater than WR-2721 values on day 3 ($P<0.01$). WR-2721 values are significantly greater than WR-2721 + Broncho-Vaxom® (−24 h) values on day 6 ($P<0.01$). (B) Broncho-Vaxom® (−24 h) values are significantly greater than saline values on day 3 ($P<0.05$) and on days 6 and 10 ($P<0.001$). WR-2721 values are significantly greater than saline values on days 3 – 10 ($P<0.001$). WR-2721 + Broncho-Vaxom® (−24 h) values are significantly greater than saline values on day 3 ($P<0.05$) and on days 6 and 10 ($P<0.001$). WR-2721 + Broncho-Vaxom® (+8 h) values are significantly greater than saline values on days 3,10 ($P<0.001$) and on day 6 ($P<0.01$). WR-2721 + Broncho-Vaxom® (+8 h) values are significantly lower than Broncho-Vaxom® (−24 h), WR-2721 and WR-2721 + Broncho-Vaxom® (−24 h) values on day 10 ($P<0.05$). Broncho-Vaxom® (−24 h) and WR-2721 + Broncho-Vaxom® (+8 h) values are significantly lower than WR-2721 and WR2721 + Broncho-Vaxom® (−24 h) values on day 15 ($P<0.05$).

when WR-2721 (30 min before irradiation) with Broncho-Vaxom® (24 h before or 4 or 8 h after irradiation) was administered, 30-day survival significantly increased (56, 65 and 63%) as compared with WR-2721 alone (26%, $P<0.05$).

Recovery of bone marrow and splenic cellularity and bone marrow GM-CFC in 9.5 Gy ($\sim LD_{100/30}$ for control irradiated mice; see Fig. 1)-irradiated mice after individual or combined treatments was examined to further evaluate the hemopoietic effects of both agents [Figs 2(A),2(B),3(A)]. Very few saline-, Broncho-Vaxom® (+ 8 h)- and Broncho-Vaxom® (−24 h)-treated mice survived until day 15 and day 21 postirradiation, respectively. Because of this, sufficient data could not be obtained at this time point for these treatment groups. As shown in Fig. 2(a), WR-2721 alone and combinations of either WR-2721 + Broncho-Vaxom® (−24 h) or

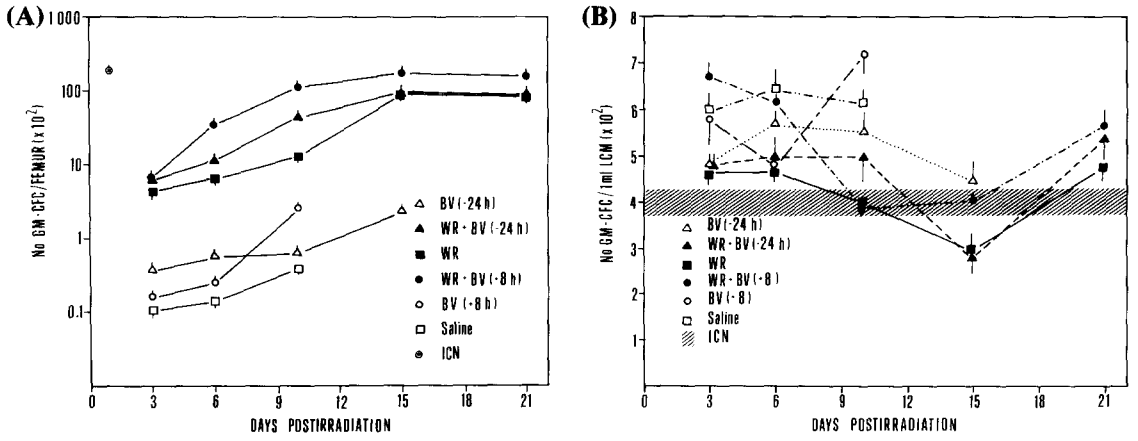

Fig. 3. Effects of Broncho-Vaxom® (BV), WR-2721 (WR) and their combinations on bone marrow GM-CFC numbers (A) and on colony-stimulating activity (CSA) of lungs (B) in irradiated mice. Mice were injected with solutions of agents as described above and exposed to a 9.5 Gy dose radiation on day 0. ICN = intact control mice (nonirradiated). (A) Broncho-Vaxom® (−24 h) values are significantly greater than saline values on days 3, 6 ($P<0.001$), and on day 10 ($P<0.05$). Broncho-Vaxom® (+8 h) values are significantly greater than saline values on days 6 and 10 ($P<0.001$). WR-2721, WR-2721 + Broncho-Vaxom® (−24 h; +8 h) values are significantly greater than saline values on days 3 − 10 ($P<0.001$). WR-2721 + Broncho-Vaxom® (−24 h) values are significantly greater than WR-2721 values on days 3, 6 ($P<0.05$) and on day 10 ($P<0.001$). WR-2721 + Broncho-Vaxom® (+8 h) values are significantly higher than WR-2721 values on days 3 − 21 ($P<0.001$). (B) CSA of saline control is significantly higher than CSA of intact (nonirradiated) control on days 3 − 10 ($P<0.001$). CSA of Broncho-Vaxom® (+8 h) is significantly higher than CSA of intact control on days 3,10 ($P<0.001$), and on day 6 ($P<0.01$). CSA of Broncho-Vaxom® (−24 h) is significantly higher than CSA of intact control on day 3 ($P<0.05$) and on days 6 and 10 ($P<0.01$). CSA of WR-2721 + Broncho-Vaxom® (+8 h) is significantly higher than CSA of intact control on days 3, 6 and 21 ($P<0.01$). CSA of WR-2721 + Broncho-Vaxom® (−24 h) is significantly higher than CSA of intact control on day 21 ($P<0.05$).

WR-2721 + Broncho-Vaxom® (+8 h) induced the most, statistically significant elevation of bone marrow cellularity as compared to saline controls and mice treated with Broncho-Vaxom® alone (given either +8 or −24 h) at all time points evaluated. Bone marrow cellularity in mice treated with WR-2721 + Broncho-Vaxom® (+8 h), except for day 3 postexposure, was not significantly different from that observed in mice treated only with WR-2721. On the other hand, bone marrow cellularity in mice treated with WR-2721 + Broncho-Vaxom® (−24 h) was significantly lower than in WR-2721-treated mice on days 6 and 15 postexposure. On day 15 postexposure bone marrow cellularity in mice treated with WR-2721 alone and WR-2721 + Broncho-Vaxom® (+8 h) reached the level of intact nonirradiated mice.

As shown in Fig. 2(b), splenic cellularity in all treated groups severely decreased following irradiation. However, Broncho-Vaxom® (−24 h), WR-2721, and combinations of WR-2721 + Broncho-Vaxom® (−24 h; +8 h) induced a significant elevation in splenic cellularity compared with saline control and Broncho-Vaxom® (+8 h)-treated mice at all times assayed. Splenic cellularity in mice treated with WR-2721 + Broncho-Vaxom® (−24 h) was not significantly different from that observed in mice treated only with WR-2721, both reaching the levels of intact nonirradiated mice on day 15. Though the rate of spleen cell recovery in mice treated with WR-2721 + Broncho-Vaxom® (+8 h) was slower than in mice treated with WR-2721 and WR-2721 + Broncho-Vaxom® (−24 h), this group also reached the level of intact nonirradiated mice 21 days after irradiation.

Bone marrow GM-CFC content in all treated groups was severely reduced following irradiation [Fig. 3(A)]. In saline and Broncho-Vaxom® (−24 h; +8 h)-treated mice, bone marrow GM-CFC content decreased to less than 0.5% of intact nonirradiated mice by day 3 postirradiation, and recovery beyond 1.5% of intact nonirradiated mice was not observed, even at day 10 and 15, respectively. However, the number of these cells isolated from WR-2721 alone and combinations of WR-2721 + Broncho-Vaxom® (−24 h; +8 h)-treated mice significantly increased at all times assayed. In addition, the content of

GM-CFC in mice treated with WR-2721 + Broncho-Vaxom® (+8 h) was significantly ($P<0.001$) greater within days 3–21 postexposure. Also, WR-2721 + Broncho-Vaxom® (−24 h)-treated mice exhibited significantly ($P<0.05$) more GM-CFC than WR-2721-treated mice on days 3–10. On day 15 postexposure the content of GM-CFC in WR-2721 + Broncho-Vaxom® (+8 h)-treated mice reached the level of intact nonirradiated mice.

Since macrophages in the lungs are an important source of colony-stimulating factor (CSF) (Burgess *et al.*, 1977) the colony-stimulating activity (CSA) of lung-conditioned medium (LCM) on the growth of bone marrow GM-CFC was examined. As can be seen in Fig. 3(B), there was increased CSA of lungs within the first 3–10 days after irradiation in all groups except from WR-27271 and WR-27271 + Broncho-Vaxom® (−24 h). Enhanced CSA in lungs may relate to the extent of radiation-induced damage of the organism (saline control) and to the injection of Broncho-Vaxom® as a macrophage activator.

DISCUSSION

Broncho-Vaxom® is one of the radioprotective immunomodulators that, like polysaccharides and interleukin-1 (Patchen *et al.*, 1989, 1990; Maisin, Kondi-Tamba & Mattelin, 1986; Weiss *et al.*, 1990), enhances the radioprotective effects of thiol compounds. WR-2721 is a representative of thiol radioprotective compounds acting via mechanisms such as free radical scavenging and hypoxia, thus decreasing radiosensitivity of hemopoietic stem cells and inducing their higher postirradiation survival (Harris & Phillips, 1971). However, high doses of WR-2721 have undesirable toxic side-effects and cause problems with toxicity due to its low therapeutic indices. Therefore, the concept of combined radioprotection proposes the joint use of agents that would potentiate protective action without causing toxic effects.

The reasons why we decided to use Broncho-Vaxom® as the second radioprotective agent in combined radioprotection were as follows: Broncho-Vaxom® is a stimulator of the body's natural defense mechanisms and strengthens the resistance to bacterial and viral infections of the respiratory tract (Palma-Carlos *et al.*, 1987), and to various experimental bacterial infections (Bosch, Lucena, Pares & Jofre, 1984). Broncho-Vaxom® administration induces an increase in the numbers of macrophages in the peritoneal cavity, proliferation of macrophages, and release of various mediator factors (Podleski, 1985; Bottex *et al.*, 1988; Mauel *et al.*, 1989), which bear radioprotective effects (Fedoročko *et al.*, 1992).

Our results show that protection using a combination of low nontoxic doses of WR-2721 (−30 min) with Broncho-Vaxom® (−24 h) was at least additive as compared to single WR-2721 or Broncho-Vaxom® treatments. Maximal radioprotection was obtained when Broncho-Vaxom® in combinaton with WR-2721 was administered 24 h before or 4–8 h after irradiation. Comparing experiments in which thiol compounds were combined with radioprotective immunomodulators administered 1–2 h after irradiation (Patchen *et al.*, 1989; Weiss *et al.*, 1990), we found that in our case, the longer the time interval between irradiation and Broncho-Vaxom® application (4–8 h), the better the survival enhancing effect that was achieved. However, this result does not hold for Broncho-Vaxom® application alone because with increasing time intervals between lethal irradiation and application, the number of surviving animals decreases (P. Fedoročko, unpublished data). The studies presented in this paper clearly indicate that tolerable doses of WR-2721 and Broncho-Vaxom® can be used in combination to obtain better survival rates compared with treatment by either agent alone.

Also, hemopoietic regeneration is significantly accelerated in mice treated with WR-2721 + Broncho-Vaxom® when compared to mice receiving a single agent. Patchen *et al.* (1989) suggest that low doses of WR-2721 which protect hemopoietic cell populations and subsequent application of immunomodulators which stimulate hemopoietic regeneration, result in a higher number of surviving animals as compared to using the same agents singly. The same mechanisms could operate with combinations of WR-2721 and Broncho-Vaxom®, as suggested by accelerated myelopoietic recovery and increased CSA of lungs. Higher CSA of lungs within the first days after irradiation was observed in saline controls (related to the extent of radiation-induced injury to the organism; results of Morley, Rickard, Howard & Stohlman (1971) and Kaspar & Seed (1984) showed that colony-enhancing activity of sera increased with increasing radiation dose) as well as in mice with postradiation application of macrophage activator Broncho-Vaxom®. The role of granulopoiesis stimulation in increased survival of animals exposed to ionizing radiation is well documented. The results suggest that in animals in which hemopoietic stem cell pool was protected partly at least from radiation effects by chemical

radioprotector (WR-2721), an important role in acceleration hemopoietic regeneration and/or function could play a different ability in producing CSF (WR-2721 + Broncho-Vaxom® (+8 h) versus WR-2721), thereby resulting in a different survival of animals after supralethal irradiation. On the other hand, a relatively high CSF production (probably as the defence response of the organism to its substantial injury) was also not capable of ensuring the reconstitution and/or function of hemopoietic cell populations in those groups, in which the hemopoietic stem cell pool might be reduced below critical values [Broncho-Vaxom® (−24 h, +8 h), saline]. This finding seems to be more generally valid since a similar course of CSA in the lungs was found in sublethally irradiated mice after endotoxin application (Vacek, 1992). These results indicate the possibility of inducing GM-CSF production by lungs, which can act favorably on the differentiation of hemopoietic cells toward the granulocyte pool and more rapid recovery of this hemopoietic series damaged by irradiation, thereby leading to an increased survival rate.

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