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Radioprotective effects of combination broncho-vaxom, a macrophage activator, and indomethacin, an inhibitor of prostaglandin production: relationship to myelopoiesis

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Abstract: The effects of the bacterial extract broncho-vaxom (BV; radioprotective immunomodulator; 500 μg/mouse i.p., – 24 h) and indomethacin (INDO; inhibitor of prostaglandin production; 2 × 40 μg/mouse i.m., – 24 h and – 3 h) on the post-irradiation recovery of hemopoietic functions in mice were investigated. Both agents were administered either alone or in combination. Endogenous spleen colony formation was increased in all treatment groups, with combination-treated mice exhibiting the greatest effects. Similarly, 24 h after combined administration of BV and INDO (i.e. at the time of presumed irradiation) to the non-irradiated mice granulocyte-macrophage colony-forming cell (GM-CFC) numbers were greater in the bone marrow and spleen. Also, as determined by hydroxyurea injection, there was an increase in the number of GM-CFC in the S-phase of the cell cycle in the bone marrow. However, GM-CFC in the spleen of combination pretreated mice was not stimulated to significant proliferation as compared to GM-CFC in the spleen of mice injected with BV alone. Combined modality treatment was also more effective than single agent treatments in accelerating bone marrow cellularity and GM-CFC regeneration, but not in accelerating GM-CFC regeneration in the spleen. Combined administration of BV and INDO to mice prior to lethal irradiation exerted an additional radioprotective effect and protected 95% of the C57B1/6 mice.

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Key words: radioprotection – broncho-vaxom – indomethacin – hemopoiesis

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Introduction

Immunomodulators, either microbial agents or recombinant cytokines, can also enhance survival, hemopoietic and functional cell recovery after 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 pre-irradiation stem cell pools, and accelerating restoration of functional hemopoietic cell populations (1, 2).

Broncho-vaxom (BV), lyophilized fractions of bacterial extract (endotoxin-free) from 8 strains (3), is used as a polyvalent immunotherapeutic agent in the treatment of respiratory tract infections, particularly acute and chronic bronchitis (4–6). The non-specific immunostimulant properties of BV have been demonstrated by several investigators. For example, it is known that it can stimulate the mitogenic response of peripheral blood leukocytes to polyclonal activators (17) and to allogeneic lymphocytes (4). Furthermore, it has been found to stimulate immunoglobulin production in sputum and serum (8), to increase the number of phagocytes, and to enhance the resistance against experimental infection by streptococci and staphylococci in immunosuppressed animals (9). Preincubation of a macrophage cell-line with this extract has been reported to induce interleukin-1 (IL-1) secretion and production of prostaglandins (10).

Our previous studies demonstrated that BV injection before irradiation increased the number of endogenous colony-forming units (endoCFU-S) in

sublethally irradiated mice and increased the number of mice that survived beyond 30 days after lethal irradiation (11, 12). Also, BV-injection accelerated early recovery of hemopoietic stem cells and cells in the peripheral blood (13, 14). BV administered in combination with WR-2721 better enhances survival and also more effectively accelerates post-irradiation hemopoietic recovery than either agent administered alone (15).

Negative feedback control of hemopoietic progenitor cells is thought to be regulated by groups of arachidonic acid derivatives, including prostaglandins, particularly those of the E-series (16), as well as by protein factors including interferon-gamma, TNF-alpha, transforming growth factor-beta and various tetra- and pentapeptides (17). Therefore, another possible mode of treatment to enhance hemopoietic recovery seems to be the use of drugs suppressing prostaglandin production. Administration of indomethacin (INDO), a drug suppressing prostaglandin production, was shown to stimulate hemopoiesis in normal mice (18, 19). Pre-irradiation (20–22) or post-irradiation (23, 24) administration of prostaglandin inhibitors was also found to be effective in enhancing hemopoietic recovery in irradiated mice.

It is established that BV induces the synthesis of both prostaglandin E_2 (PGE_2) and IL-1 (10). IL-1 was included in many experiments as a positive control for elevated myelopoiesis and protection from lethal irradiation (25–27). Therefore, we tested whether the radioprotective effectiveness of BV in mice can be enhanced by concomitant administration of INDO, an inhibitor of prostaglandin synthesis.

The aim of this study was to investigate the numbers and the proportion of GM-CFC in both bone marrow and spleen in the S-phase of the cell cycle of nonirradiated mice that occurred within 24 h of a radioprotective dose of BV and INDO alone or a combined injection. We also studied bone marrow and spleen cellularity and recovery of the number of GM-CFC in sublethally irradiated mice. In addition, we present evidence that joint administration of BV and INDO exert an additional radioprotective effect.

Material and methods

Mice

Female C57B1/6 mice, 8–10 weeks old, were obtained from Velaz (Prague, Czechia). Animals were quarantined for a period of 2 wk. They were housed in rodent cages, 5 to 7 animals per cage at about 22 °C, and were given Velaz/Altromin 1320 St laboratory chow and tap water acidified to pH 2.4 ad libitum. Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals", prepared by the State Veterinary Office of the Slovak Republic, Bratislava.

Treatment with broncho-vaxom^R

BV (Biogal Pharmaceutical Works, Debrecen, Hungary, under licence from OM Laboratoires, Geneva, Switzerland) is a lyophilized extract of the 8 most common bacteria of the upper respiratory tract (11) and free from endotoxins (less than 0.0002% by Limulus and pyrogenicity tests; (10)). Immediately before use, the drug was resuspended in saline in a volume of 0.4 ml and administered intraperitoneally (i.p.) 24 h before irradiation or decapitation (in the case of nonirradiated mice) at a dose of 500 μg per mouse. This time interval has been shown to be an effective administration schedule for most of the immunomodulators (28) and also for BV (11). Control animals received i.p. saline in the same volume and at the same time as the treated group.

Indomethacin treatment

Indomethacin (INDO; Sigma Chemical Co., St. Louis, USA) was prepared by dissolving 10 mg in 1 ml of 95% ethyl alcohol. This solution was then diluted to working concentration with Dulbecco's phosphate-buffered saline (TechGen Int. Ltd., UK) and injected i.m. at 40 μg per mouse, in a volume of 0.2 ml 24 h and 3 h before irradiation or decapitation (in the case of nonirradiated mice). Both drugs were administered either alone or in combination.

Irradiation

Mice were placed in plexiglass containers and whole-body (unilaterally) exposed to 7.0 Gy (hemopoietic recovery) or to 9.5 Gy (survival assay) of gamma rays at a dose rate of 0.4 Gy/min, 24 h after the first injection. 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 d after irradiation. 10 mice per group were used in each experiment. Moribund animals in this experiment were killed. On d 31, surviving mice were euthanized by cervical dislocation. Experiments were repeated three times. Survival rates were compared among groups using the chi-square test including Yates' correction.

Hemopoietic stem cell assays

Two primary assays were used to assess the radioprotective effects of agents on hemopoietic stem

cells. They include the *in vivo* endogenous (endoCFU-S) spleen colony-forming unit assay and the *in vitro* granulocyte-macrophage (GM-CFC) progenitor cell assay.

Endogenous colony forming unit (endoCFU-S) formation was assayed by the method of Till & McCulloch (29). Briefly, mice received 7 Gy of radiation. They were killed by cervical dislocation at 10 d post-irradiation and their spleens were removed and fixed in Bouin's solution. The number of macroscopic colonies per spleen was determined.

Hemopoietic progenitor cells committed to granulocyte/macrophage development were assayed as described by Vacek et al. (28). Bone marrow cells ($8 \times 10^4 - 1.3 \times 10^5$) were plated in triplicate in a semisolid environment created by plasma clot, containing Iscove's modification of Dulbecco's medium (IMDM, TechGen Int. Ltd., UK), supplemented with antibiotics (penicillin, 100 U/ml and streptomycin, 1000 µg/ml) and L-glutamine (Calbiochem-Behring, La Jolla, USA) in a concentration of 1.2 mg/ml plus 15–20% newborn bovine calf serum (TechGen Int. Ltd., UK), 10% murine lung-conditioned medium (LCM), 10% citrate bovine plasma and 3% $CaCl_2$ (Biotika, Slovenská L'upča, Slovakia). The cultures were incubated at 37 °C in a fully humidified atmosphere of 5% CO_2 in air for 7 d. Colonies of at least 50 cells were counted at 30 × magnification. The cell suspension used for these assays represented a pool of tissues from 5 mice at each time.

The percentage of colony-forming cells (CFC; endoCFU-S or GM-CFC) in the S-phase of the cell cycle was determined by i.p. administration of 1000 mg/kg body wt hydroxyurea (Sigma Chemical Co., USA) in saline 1.5 h before irradiation (endoCFU-S). In case of GM-CFC 1.5 h after administration, the bone marrow was assayed for surviving GM-CFC. Control groups of mice received saline without hydroxyurea at the same time. The number of CFC from hydroxyurea-injected mice was compared with the number from saline-injected mice, and the percentage decrease in CFC after hydroxyurea injection was calculated as the percentage of CFC that were in the S-phase of the cell cycle. All hemopoietic experiments were repeated two to six times.

Statistics

The values given in the figures and tables represent the means ± the standard error of the means (SEM). The statistical significance of the differences was evaluated using Peritz's F-test, and the chi-square test, including Yates' correction. A p value (two-sided) of ≤ 0.05 was considered as a statistically significant difference.

Results

First, the effects of the drugs alone or in combination on endogenous spleen colony formation (endoCFU-S) were investigated (Fig. 1). In these studies, treatment with BV, INDO alone or in combination resulted in endoCFU-S numbers significantly greater than those observed in control (saline) mice (2.86 ± 0.70, n = 11). However, the highest sparing of endoCFU-S was found in mice that were injected with a combination of BV + INDO (17.24 ± 1.18 endoCFU-S, n = 17). Combined treatment significantly increased endoCFU-S numbers when compared with mice treated with BV (12.56 ± 1.50 endoCFU-S, n = 16) or INDO alone (6.25 ± 1.15 endoCFU-S, n = 11).

The effect of BV, INDO and combined injection on GM-CFC and cellularity was also determined in bone marrow and spleens from mice prior to irradiation (i.e. pre-irradiated mice). 24 h after saline, BV, INDO or combined injection, there was no significant difference in cellularity in the spleen from saline and BV, INDO alone or combination-injected mice (Table 1). However, total cellularity per femur of mice injected with BV alone or with BV + INDO combination was approximately 76% and 73%, respectively, of bone marrow cellularity from saline-injected mice.

The number of GM-CFC per femur at this point was significantly increased in mice after injection with INDO alone and after combined treatment, when compared with control (saline) mice and when compared with mice injected with BV alone (Table 1). These results also demonstrate that BV injection did not increase the number of GM-CFC in the bone marrow of pre-irradiated mice. However, in the

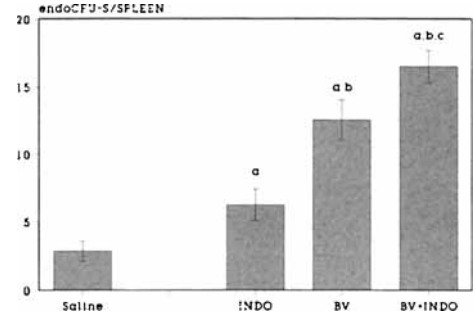

Fig. 1. Numbers of 10-d endogenous macroscopic spleen colonies (endoCFU-S) in control mice (saline) and mice treated with BV (500 µg/mouse, i.p. 24 h before irradiation) and INDO alone (40 µg/mouse, i.m. 24 h and 3 h before irradiation) or their combination and irradiated with a dose of 7 Gy. Data represent the mean ± SEM from two experiments, and each column represents 11–17 mice. Symbols denote statistical significance when compared with controls (a), INDO group (b) and BV group (c). For the sake of simplicity, significance at the 0.05 level was used for all comparisons.

Table 1. Cellularity and number of GM-CFC in tissues of pre-irradiated mice after saline, indomethacin (INDO) and broncho-vaxom (BV)-alone injection or after combined injection

	Injection[a]			
No./tissue[b]	Saline	INDO	BV	BV+INDO
Cellularity				
No./femur ($\times 10^6$)	$26.3 \pm 0.7^{f,g}$	$24.4 \pm 1.3^{f,g}$	20.1 ± 0.7	19.2 ± 0.7
No./spleen ($\times 10^7$)	13.5 ± 0.8	14.8 ± 1.4	11.5 ± 0.6	14.0 ± 0.9
GM-CFC				
No./femur ($\times 10^3$)	18.9 ± 0.9	$26.0 \pm 0.6^{d,f}$	19.0 ± 1.9	$28.0 \pm 1.7^{d,f}$
No./spleen ($\times 10^2$)	24.2 ± 1.6	29.6 ± 2.9	$41.2 \pm 4.4^{d,e}$	$79.1 \pm 5.1^{d,e,f}$

[a] Female C57B1/6 mice were administered 0.5 ml saline, 40 µg/mouse indomethacin by i.m. injections 24 h and 3 h before assay, 500 µg/mouse broncho-vaxom by i.p. injection 24 h before assay or combined injection in three separate experiments.
[b] Cells were pooled from both femurs and the spleens of 5 mice per group in each study. Symbols denote statistical significance when compared to controls (d), indomethacin group (e), broncho-vaxom group (f) and combined group (g). For the sake of simplicity, significance at the 0.05 level was used for all the comparisons.

spleens the number of GM-CFC was significantly greater after the injection with BV alone and after combined injection compared with mice injected with saline or INDO alone (Table 1). This increase in GM-CFC in the spleen may result from the mobilization of cells from the bone marrow to the spleen after BV injection. Simultaneously, GM-CFC in mice treated with a combination of the drugs was significantly increased ($79.1 \pm 5.1 \times 10^2$) with compared with mice treated with BV alone ($41.2 \pm 4.4 \times 10^2$).

The hydroxyurea-induced decrease in endo-CFU-S and GM-CFC was used to determine the percentage of endoCFU-S and GM-CFC that was in the S-phase of the cell cycle (Table 2). BV increased the percentage of GM-CFC in the S-phase

Table 2. Colony-forming cells sensitive to hydroxyurea in preirradiated mice after saline, indomethacin (INDO) and broncho-vaxom (BV)-alone injection or after combined injection

	Injection[a]			
Assay[b]	Saline[c]	INDO	BV	BV+INDO
% Decrease after hydroxyurea[h]				
GM-CFC/femur	25.0 ± 1.0 (4)	33.0 ± 4.5 (4)	44.0 ± 5.7 (4)[d]	60.0 ± 2.2 (6)[d,e,f]
GM-CFC/spleen	12.4 ± 1.5 (3)	27.8 ± 2.4 (3)[d]	56.2 ± 2.9 (3)[d,e]	63.8 ± 7.6 (3)[d,e]
endoCFU-S	33.2 ± 4.5 (2)	54.0 ± 2.0 (2)[d]	84.4 ± 3.5 (2)[d,e]	87.0 ± 5.6 (2)[d,e]

[a] Female C57B1/6 mice were administered 0.5 ml saline, 40 µg/mouse indomethacin by i.m. injections 24 h and 3 h before assay, 500 µg/mouse broncho-vaxom by i.p. injection 24 h before assay or combined injection.
[b] Cells were pooled from both femurs and the spleens of 5 mice per group in each study; endoCFU-S were evaluated on d 10 after 7 Gy irradiation (10–16 mice per group).
[c] Means ± SEM, with number of separate experiments in parentheses, are given. Symbols denote statistical significance when compared to controls (d), indomethacin group (e) and broncho-vaxom group (f). For the sake of simplicity, significance at the 0.05 level was used for all the comparisons.
[h] Mice were administered DPBS or 1000 mg hydroxyurea/kg body weight by i.p. injection 1.5 h before assay; for endoCFU-S hydroxyurea was given 1 h before irradiation.

in both the bone marrow and spleen and the percentage of endoCFU-S. INDO treatment resulted in an increase in the percentage of splenic GM-CFC and endoCFU-S in the S-phase of the cell cycle as determined by hydroxyurea assay. However, femoral GM-CFC were not stimulated to significant proliferation (Table 2), which corresponds with the findings of Nishiguchi et al. (21). On the other hand, only a fraction of bone marrow GM-CFC in the S-phase was significantly increased in mice treated with a combination of the drugs compared with mice treated with BV or INDO alone.

Measures of GM-CFC are good indications of myeloid hemopoietic activity in animals recovering

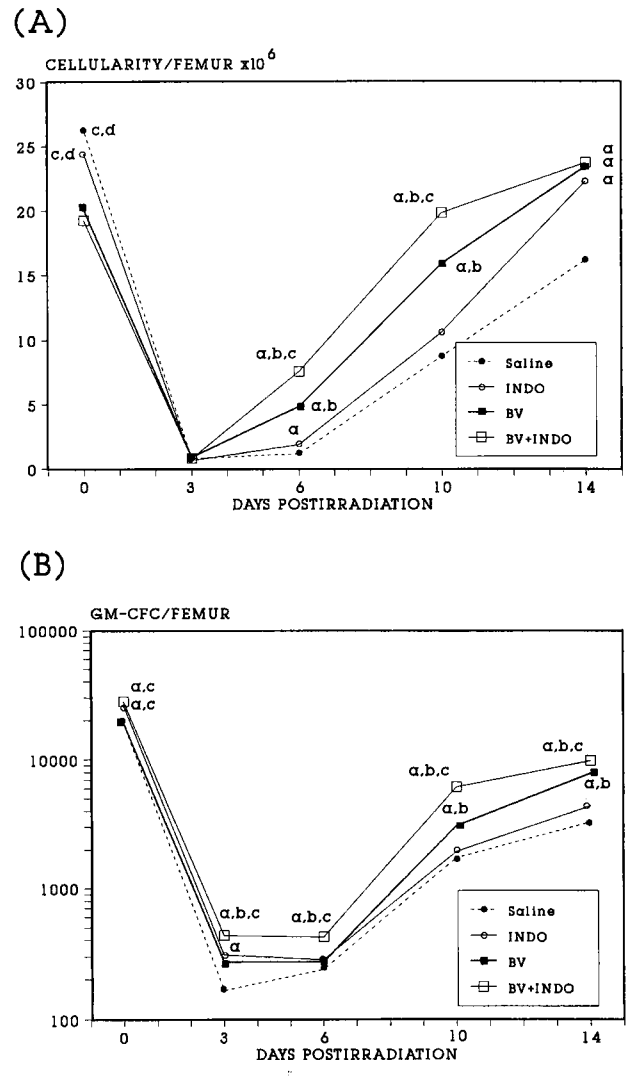

Fig. 2. Numbers of nucleated cells in the femurs (A) and numbers of GM-CFC in femoral marrow (B) on different days after 7 Gy irradiation of control mice (saline) and mice treated with BV and INDO alone or in combination. 10 mice per point were used. Symbols denote statistical significance when compared to controls (a), INDO group (b), BV group (c) and BV + INDO group (d). For the sake of simplicity, significance at the 0.05 level was used for all comparisons.

from exposure to radiation. Recovery patterns of both bone marrow and spleen cellularity and GM-CFC numbers in mice irradiated with 7 Gy and pretreated with INDO and BV alone, or INDO and BV in combination, are illustrated in Figs 2a, b and 3a, b. BV alone significantly enhanced the bone marrow cellularity (Fig. 2a) in all the intervals observed starting from d 6, whereas INDO alone manifested its stimulatory effect only on d 6 and d 14 following irradiation. When using the combined treatment, additivity of the stimulatory effects was evident on d 6 and d 10. Pretreatment of mice with BV or with a combination of the drugs significantly enhanced bone marrow GM-CFC number on d 3, 10 and 14 (Fig. 2b). However, the recovery of the number of bone marrow GM-CFC in mice protected with a combination of the drugs was greatly accelerated in all the intervals observed. As shown in Fig. 3a, INDO alone did not markedly modify spleen cellularity. Recovery of spleen cellularity in mice protected with BV alone and with a combination of the drugs was greatly accelerated on d 14. However, we did not find additional effects from combined treatment. 3 days after irradiation (in saline-treated mice also on d 6) splenic GM-CFC were undetectable in all groups of mice (Fig. 3b). In BV, INDO and combination-injected mice GM-CFC numbers decreased to less than 1% of normal levels by d 6 post-irradiation; by d 14 GM-CFC contents in spleens of BV-treated mice had recovered to normal values. At the same point, GM-CFC in spleens of mice treated with both BV and INDO or INDO alone had increased to ≈150% or 220% of normal levels, respectively. Although regeneration began a few days earlier in the femur than in the spleen, the number of GM-CFC in the spleen increased more rapidly than femoral GM-CFC.

Pretreatment of lethally irradiated mice (9.5 Gy) with INDO alone did not promote their survival (Fig. 4). On the other hand, administration of BV alone, at a dose 500 μg/mouse 24 h prior to irradiation, significantly increased survival ($p < 0.001$). Co-treatment of mice with INDO led to a further increase in survival, and a combination of drugs exerted an additional radioprotective effect that was significant when compared to mice treated with BV alone ($p < 0.01$).

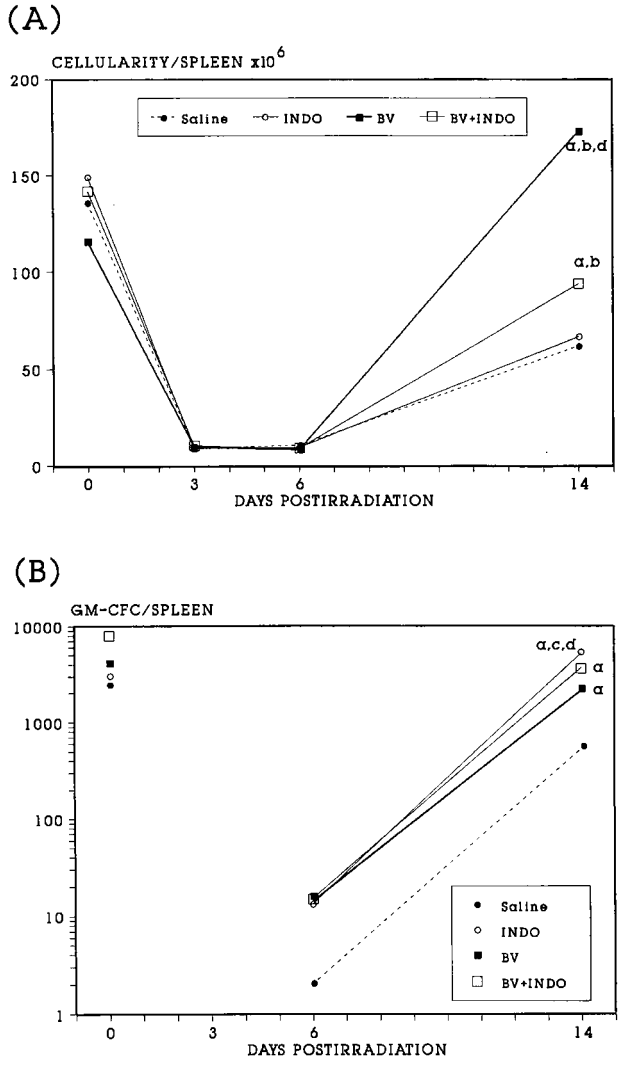

Fig. 3. Numbers of nucleated cells in the spleen (A) and numbers of GM-CFC per spleen (B) on different days after 7 Gy irradiation of control mice (saline) and mice treated with BV and INDO alone or in combination. 10 mice per point were used. Symbols denote statistical significance when compared with controls (a), INDO group (b), BV group (c) and BV + INDO group (d). For the sake of simplicity, significance at the 0.05 level was used for all comparisons.

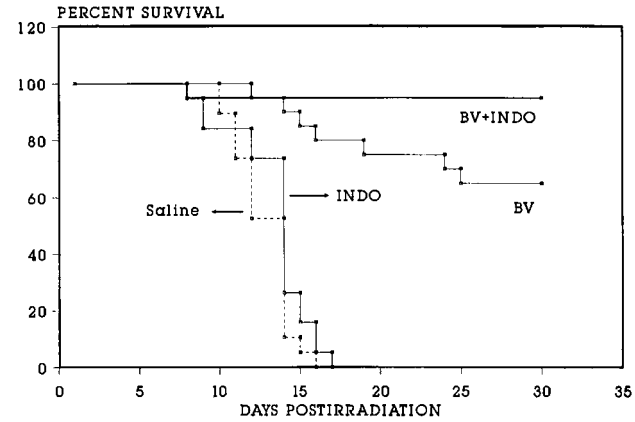

Fig. 4. Survival of different groups of mice irradiated with a dose of 9.5 Gy. The results are a compilation of three separate experiments. Differences in the 30-d survival were calculated by the chi-square test with Yates' correction. The survival of mice treated with the BV and INDO combination was significantly increased when compared to the controls (saline) ($p < 0.001$), to the INDO alone-treated group ($p < 0.001$) and to the BV alone-treated group ($p < 0.01$). The survival of mice treated with BV alone was significantly increased ($p < 0.001$) when compared with the control (saline) and INDO alone-treated groups.

Discussion

In the studies described in this paper we evaluated the ability of BV (hemopoietic stimulants) and INDO (inhibitor of prostaglandin production), two agents that individually can enhance hemopoietic recovery in irradiated mice (11–13, 21, 30), to further accelerate femoral hemopoietic regeneration and enhance survival when administered in combination.

The decrease in radiosensitivity of hemopoietic tissue in immunomodulator pretreated mice has been partly attributed to mobilization of stem cells from bone marrow to spleen, to the release of humoral factors, and to the stimulation and transition of stem cells to the cell cycle in pre-irradiated animals (25).

The hemopoietic effects of BV are presumed to be indirectly mediated through its ability to induce endogenous hemopoietic growth factor production from predominantly radioresistant macrophage cell populations. For example, both interleukin-1 (IL-1) and prostaglandin E_2 (PGE_2) production (10) have been demonstrated following *in vitro* BV administration, which may indicate that BV can accelerate the restoration of functional hemopoietic cells via production of cytokines that stimulate a broad spectrum of progenitor cells. This process may thus be one of the mechanisms leading to earlier hemopoietic recovery after irradiation (26, 27). BV-mediated radioprotection has been demonstrated to accelerate the regeneration of not only peripheral blood cells but also endoCFU-S and femoral CFU-S and GM-CFC (11, 13).

Several groups of authors have shown repeatedly that the administration of INDO, a drug inhibiting prostaglandin (PG) synthesis, can enhance both murine hemopoiesis (18, 19) and post-irradiation recovery of hemopoiesis (21, 30, 31). PGs may influence hemopoiesis by modulating the production of IL-1, which acts synergistically with other hemopoietic growth factors to increase colony formation *in vitro*. Kunkel et al. (32) and Rasmussen et al. (33) have shown that PGE_2 inhibits the production of IL-1. The beneficial effects of INDO on hemopoiesis under radiation conditions can be explained by the fact that PGs of the E series are inhibitors of myelopoiesis at the level of progenitor cells and play a role as negative hemopoietic control (34, 35). The pharmacological removal of the PG action could thus lead to preponderance of positive control and enhancement of hemopoietic recovery. Recent murine data demonstrate that *in vivo* administration of INDO also enhances the regeneration of peripheral blood cells as well as endoCFU-S and femoral and splenic CFU-S and GM-CFC (21, 30, 31).

According to Pospíšil et al. (22) the radioprotective effects of immunomodulator and inhibitor of prostaglandin production combination could be explained by the additive action of both drugs, i.e. by the increased cell proliferation in hemopoietic compartments induced by immunomodulator and the suppression of negatively acting prostaglandin production. Such enhancement of cell proliferation in the hemopoietic tissue might be radioprotective either by increasing the number of hemopoietic stem or progenitor cells at risk at the time of irradiation or by inducing the hemopoietic cells into the relatively radioresistant S phase of the cycle. Probably, this additive action of both drugs may also be valid for the combination of BV with INDO. In the present report, 24 h after BV + INDO injection (i.e. at the time of presumed irradiation), increased cell proliferation in the bone marrow and a higher portion of femoral GM-CFC in the S-phase were found. An increased number of GM-CFC in the S-phase, the most radiation-resistant phase of the cell cycle of hemopoietic cells (36–38), at the time of irradiation may indicate the cause of the increased survival observed following lethal irradiation after administration of these compounds. Also, differences in cellular radiosensitivity that have recently been documented for various stem cell subsets (39–41) should also be considered. It remains possible that combination of drugs causes a redistribution of hemopoietic subsets with increased numbers of more radioresistant cell types. For example, administration of INDO resulted in an increase in the numbers of GM-CFC the bone marrow but not in the spleen of animals 24 h following injection. In contrast, BV treatment resulted in a significant increase in the numbers of GM-CFC in the spleen but not in the bone marrow after 24 h. Interestingly, administration of IL-1 has also been associated with a significant increase in splenic GM-CFC, but did not result in any change in GM-CFC number within the bone marrow 24 h after injection (42). However, 24 h after combined administration of BV and INDO both bone marrow and splenic GM-CFC numbers were greater. It is of interest that, at the time of irradiation, the radioresistant bone marrow GM-CFC population was increased and bone marrow cellularity reduced. These results, along with an increase in endoCFU-S, demonstrate stem cell mobilization and redistribution of hemopoiesis over the bone marrow, blood and spleen. In addition, recent results of Pelus (35) have shown that the increase of splenic myelopoiesis induced by IL-1 can be greatly amplified by pretreatment of mice with INDO, suggesting a regulatory relationship between IL-1 and prostaglandins. Similarly, in the present report splenic myelopoiesis induced by BV was augmented by pretreatment with INDO. Nishiguchi et al. (21) suggest that removal of the immunosuppressive activity of PGs would augment the functions of the immune system which, through the release of IL-1

and other cytokines, could stimulate hemopoietic cell proliferation.

When used in combination, BV + INDO not only resulted in better survival than treatment with either agent alone, but it also resulted in better femoral hemopoietic regeneration than either agent used individually. However, additivity of both of the stimulatory effects was not evident in splenic hemopoietic repopulation. The lower recovery of hemopoiesis in the spleen after combined administration of BV and INDO can possibly be explained by the fact that this function is taken over by the bone marrow where intensive increase in the numbers of GM-CFC and cellularity could be observed.

In conclusion, our studies suggest that different hemostimulatory mechanisms – through which BV and INDO appear to mediate their hemopoietic-enhancing effects – might contribute to further enhancement of survival and accelerate hemopoietic regeneration if used in combination in irradiated mice.

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