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COMBINED THERAPY FOR POSTIRRADIATION INFECTION

KOMBINIERTE THERAPIE VON INFEKTIONEN NACH BESTRAHLUNG

THOMAS B. ELLIOTT, GARY S. MADONNA, G. DAVID LEDNEY, and ITZHAK BROOK

SUMMARY

Increased susceptibility to bacterial infection, probably by translocation from the intestinal flora, can be a lethal complication for 2-3 weeks after exposure to ionizing radiation. Antibiotics alone do not provide adequate therapy for induced infections in neutropenic mice. Because some substances that are derived from bacterial cell walls activate macrophages and stimulate nonspecific resistance to infection, such agents might be used to prevent or treat postirradiation infections. In this study, a cell-wall glycolipid, trehalose dimycolate (TDM), was evaluated together with a third-generation cephalosporin, ceftriaxone, for their separate and combined effects on survival of B6D2F1 female mice that were exposed to the sublethal dose of 7.0 Gy ^{60}Co radiation and challenged s.c. with lethal doses of *Klebsiella pneumoniae*. A single injection of TDM (100 μg in 2% oil emulsion) inoculated i. p. 1 hr postirradiation increased 30-day survival to 80% after a lethal challenge by *K. pneumoniae* (10 $LD_{50/30}$) 4 days later. When the challenge dose of *K. pneumoniae* was increased to 5000 $LD_{50/30}$ on Day 4, all mice died. Ceftriaxone (75 mg/kg) injected i. m. from days 5 to 14 postirradiation increased survival to 70% after a lethal challenge by *K. pneumoniae* of 5000 $LD_{50/30}$ on Day 4. However, when TDM and ceftriaxone were combined, survival was enhanced synergistically to 100% even when the dose of *K. pneumoniae* injected on Day 4 was 5000 $LD_{50/30}$. These results indicate that a combination of an immunomodulator and an antimicrobial agent will be more effective for treating postirradiation bacterial infections than either treatment alone in immunocompromised, neutropenic mice.

ZUSAMMENFASSUNG

Die erhöhte Empfindlichkeit gegenüber bakteriellen Infektionen kann 2-3 Wochen nach Aussetzung gegenüber radioaktiver Bestrahlung zu tödlichen Komplikationen führen, möglicherweise beeinflußt durch die Translokation von Mikroorganismen aus der Intestinalflora. Als Therapie für induzierte Infektionen von Mäusen mit einem Mangel an neutrophilen Zellen sind Antibiotika alleine nicht ausreichend. Da bekannt ist, daß einige, in Zellwänden von Bakterien enthaltene Substanzen Makrophagen aktivieren und die unspezifische Resistenz gegenüber Infektionen stimulieren, besteht die Möglichkeit, mit Hilfe solcher Agentien Infektionen nach Bestrahlung zu verhüten oder zu behandeln. Bei dieser Studie wurde Trehalose-Dimycolat (TDM), ein zellwandständiges Glycolipid, zusammen mit einem Cephalosporin der dritten Generation, Ceftriaxon, auf ihre Einzel- und Kombinationswirkung auf das Überleben von B6D2F1 Mäusen weiblichen Geschlechts untersucht, die der sublethalen Dosis von 7,0 Gy Kobalt-60-Bestrahlung ausgesetzt und mit subkutan applizierten, lethalen Dosen von *Klebsiella pneumoniae* infiziert wurden. Eine ein-

zelne Injektion von TDM (100 µg in 2% Öl-Emulsion), die intraperitoneal eine Stunde nach Bestrahlung gegeben wurde, erhöhte das 30 Tage-Überleben auf 80% nach der lethalen Infektion mit *Klebsiella pneumoniae* (10 $LD_{50/30}$) 4 Tage später. Bei Erhöhung der Infektionsdosis von *Klebsiella pneumoniae* auf 5000 $LD_{50/30}$ an Tag 4 starben alle Mäuse. Die intramuskuläre Injektion von Ceftriaxon (25 mg/kg) in den Tagen 5 bis 14 nach Bestrahlung erhöhte das Überleben auf 70% nach lethaler Infektion mit *Klebsiella pneumoniae* von 5000 $LD_{50/30}$ an Tag 4. Durch die synergistische Wirkung kombinierten TDM's und Ceftriaxon's wurde die Überlebensrate jedoch auf 100% gesteigert, selbst bei der Injektion einer Dosis von *Klebsiella pneumoniae* von 5000 $LD_{50/30}$ an Tag 4. Diese Resultate belegen, daß die Kombination eines immunmodulierenden und eines antimikrobiellen Agens in der Behandlung von bakteriellen Infektionen nach Bestrahlung immunkompromittierter Mäuse mit einem Mangel an neutrophilen Zellen effektiver sind, als die Behandlung mit jeweils einem dieser Mittel alleine.

INTRODUCTION

Ionizing radiation causes an hematopoietic syndrome in mice that induces prolonged neutropenia and increased susceptibility to bacterial infections (SCHECHMEISTER, 1954). Antibiotics alone do not assure cure of infections or survival of irradiated animals (BROOK and ELLIOTT, 1989; BROOK et al., 1989; MADONNA et al., 1989a; MADONNA et al., 1989b). Consequently, there is a practical need to develop effective therapeutic modalities for infections following radiation injury.

Ceftriaxone is a third-generation semisynthetic cephalosporin that has a broad spectrum of activity against bacteria and a long elimination half-life (6-9 hr), which allows a once-daily administration i.v. or i.m. Trehalose dimycolate (TDM) is a bacterial cell-wall glycolipid (LEMAIRE et al., 1986), which has potentially beneficial properties, including enhanced resistance to bacterial infections (YARKONI and BEKIERKUNST, 1976), activation of macrophages with production of mediators, such as interleukin-1, colony-stimulating factors, and interferons (MADONNA et al., 1986; RIBI, 1986; TENU et al., 1980; YARKONI et al., 1977).

We evaluated the separate and combined effects of TDM and ceftriaxone on survival in mice that were made neutropenic by irradiation and then challenged with *Klebsiella pneumoniae*. The data showed that the combination of TDM and ceftriaxone protected irradiated mice from a fatal infection.

METHODS

The animals, bacteria, radiation dose and dosimetry, therapeutic agents, and statistical evaluation were described (MADONNA et al., 1989; STEWART et al., 1982).

Mice were given TDM in 2% squalene oil-0.2% Tween 80 emulsion (TDM/o), TDM in 0.9% NaCl-0.2% Tween 80 solution (TDM/s), saline solution, or oil emulsion i.p. 1 hr after 7.0 Gy irradiation from ^{60}Co. To determine the effect of TDM against different challenge doses of *K. pneumoniae*, 10, 100, 1000, and 5000 $LD_{50/30}$ of the bacteria were injected s. c. four days after irradiation, when the mice were neutropenic (1 $LD_{50/30}$ = 1.2×10^3 CFU). Another group of mice were given 5000 $LD_{50/30}$ *K. pneumoniae* and treated for ten days with either ceftriaxone or water beginning one day after challenge with bacteria.

RESULTS

Either trehalose dimycolate or ceftriaxone alone enhanced survival of mice that were lethally challenged with *K. pneumoniae* 4 days after sublethal radiation. Combined therapy with TDM and ceftriaxone synergistically protected mice from lethal challenge with *K. pneumoniae* (Table 1).

Mean survival times for all treatments were greater than for saline control for each inoculum ($p < 0.001$) and TDM/o enhanced survival time more than TDM/s ($p < 0.001$), except with 1.2×10^4 CFU/mouse ($p = 0.0835$). Mean survival times were greater for combined therapies than for single therapies: TDM/o-ceftriaxone vs. ceftriaxone, $p = 0.0165$, TDM/o-ceftriaxone vs. TDM/o-water, $p < 0.001$, and TDM/s-ceftriaxone vs. ceftriaxone, $p > 0.05$.

Serum Concentration of Ceftriaxone.

Sera of a separate group of mice that received 10.0 Gy gamma radiation contained an average 142.6 (± 2.2) µg ceftriaxone/ml 1.3 hr after injection and 2.3 (± 0.6) µg/ml 25.9 hr after injection.

TABLE 1

Survival of Mice Challenged with *Klebsiella pneumoniae* and Treated with Combined Therapy of TDM and Ceftriaxone.

$LD_{50/30}$ *K. pneumoniae*	Antibiotic Therapy	% Survival[a]			
		TDM/o	TDM/s	Saline	Oil
5000	ceftriaxone	100	88	69	nd[d]
	water	0	0	0[b]	nd
1000	ceftriaxone	100	94	88	nd
	water	13	0	0[b]	0[c]
100	nd	69	20	0	nd
10	nd	90	60	0	nd

[a]N = 16, except [b] n = 10 and [c] n = 12; [d] nd = not done

DISCUSSION

Our results with the combination of TDM and ceftriaxone lead to a new method to improve the treatment for bacterial infections in irradiated hosts and the prognosis for survival. Other combinations of an immunomodulator and an antibiotic synergistically enhance survival of irradiated mice. Pefloxacin, and other quinolones, given orally, prolonged survival of lethally irradiated mice (BROOK, I., et al.,1989) and was synergistic with glucan F (PATCHEN, M., BROOK, I., and ELLIOTT, T. B., unpublished data). Although the mechanism of action of TDM is unclear, it may involve the release of specific cytokines from macrophages. We are examining the application of interleukin-1 in irradiated mice.

MADONNA and colleagues (1989a) used the number of bacteria in livers of lethally irradiated mice as an indicator of septicemia caused by translocated endogenous bacteria. The number remained low in mice treated with TDM from 7 to 11 days after irradiation, but bacteria increased in mice treated with saline solution. Similarly, both the incidence and the mean numbers of streptomycin-resistant *Escherichia coli* in mesenteric lymph nodes decreased in specific-pathogen-free mice that were treated with killed *Propionibacterium acnes* (FULLER and BERG, 1985).

These observations support the principle that the nonspecific host defences must be enhanced in neutropenic and immunosuppressed animals in addition to use of antibiotics in order to prevent death from bacterial infections.

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