AD-A235 706

(see title on reprint)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES):
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8. PERFORMING ORGANIZATION REPORT NUMBER:
SR91-3

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES):
Defense Nuclear Agency
Washington, DC 20305

11. SUPPLEMENTARY NOTES:

12a. DISTRIBUTION/AVAILABILITY STATEMENT:
Approved for public release; distribution unlimited.

13. ABSTRACT (Maximum 200 words):

14. SUBJECT TERMS:

15. NUMBER OF PAGES:
4

16. PRICE CODE:

17. SECURITY CLASSIFICATION OF REPORT:
UNCLASSIFIED

18. SECURITY CLASSIFICATION OF THIS PAGE:
UNCLASSIFIED

19. SECURITY CLASSIFICATION OF ABSTRACT:
UNCLASSIFIED

20. LIMITATION OF ABSTRACT:

UNCLASSIFIED
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 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 LD₅₀) 4 days later. When the challenge dose of K. pneumoniae was increased to 5000 LD₅₀ 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 LD₅₀ 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 LD₅₀. 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

zeltne Injektion von TDM (100 μg in 2% ÖI-Emulsion), die intraperitoneal eine Stunde nach Bestrahlung gegeben wurde, erhöhte das 30 Tage-Überleben auf 80% nach der lethalen Infektion mit *Klebsiella pneumoniae* (10 LD₉₀) 4 Tage später. Bei Erhöhung der Infektionsdosis von *Klebsiella pneumoniae* auf 5000 LD₉₀ 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 LD₉₀ 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 LD₉₀ 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 60Co. To determine the effect of TDM against different challenge doses of *K. pneumoniae*, 10, 100, 1000, and 5000 LD₉₀, the bacteria were injected s.c. four days after irradiation, when the mice were neutropenic (1 LD₉₀ = 1.2 x 10⁷ CFU). Another group of mice were given 5000 LD₉₀ *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 x 10⁷ 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.

<table>
<thead>
<tr>
<th>LD₅₀/₃₀</th>
<th>Antibiotic Therapy</th>
<th>% Survival*</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>TDM/o</td>
<td>TDM/s</td>
</tr>
<tr>
<td>5000 ceftriaxone water</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>1000 ceftriaxone water</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>100 nd</td>
<td>69</td>
<td>20</td>
</tr>
<tr>
<td>10 nd</td>
<td>90</td>
<td>60</td>
</tr>
</tbody>
</table>

*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 ELIOTT, 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 in lethally irradiated mice as an indicator of sepsis 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.

ACKNOWLEDGEMENTS

We are grateful to William E. Jackson, III, for analysis of data on survival of mice. This research was supported by Work Unit No. 4420-00129 of the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency. The views presented in this paper are those of the authors. No endorsement by the Defense Nuclear Agency has been given or should be inferred. Research was conducted according to the principles enunciated in the „Guide for the Care and Use of Laboratory Animals“ prepared by the Institute of Laboratory Animal Resources, National Research Council.

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Paper presented as poster by Dr. Elliott at the XIV. International Symposium on Microbial Ecology and Disease, September 21-23, 1989, San Antonio, Texas, USA.

LITERATURE


BROOK, I., ELLIOTT, T. B., and LEDNEY, G. D.: Therapy of *Klebsiella pneumoniae*.


