Protective Effect of Prophylactic Penicillin on Splenectomized Mice Exposed to an Aerosolized Suspension of Type III Streptococcus Pneumoniae

By Joseph D. Dickerman, Edwin Bolton, James A. Coil, Bruce J. Chalmer, and George J. Jakab

Prophylactic penicillin has been recommended for use in asplenic patients and postsplenectomy patients. A laboratory model using aerosolized pneumococci has been devised to test the effectiveness of prophylactic penicillin in a manner analogous to human experience. There is increased mortality, over time, in asplenic mice exposed to aerosolized type III Streptococcus pneumoniae. One hundred twenty-one male Swiss mice (mean weight 26 g) were divided into four groups: splenectomized, sham-operated, splenectomized + penicillin, and sham-operated + penicillin. After 2 wk the four groups were exposed for 30 min to an aerosolized atmosphere of $2.4 \times 10^6$ colony-forming units of type III S. pneumoniae using a Tri-R model A-42 airborne infection apparatus. Penicillin was given at a daily intramuscular dosage of 40,000 units procaine penicillin G beginning 2 days prior to exposure and continuing through the third day after exposure. The splenectomized and sham-operated mice given penicillin showed significantly lower mortality ($p < 0.001$) than mice not given penicillin.

Increased awareness of the problem of overwhelming bacterial sepsis as a complication of splenectomy has focused attention on methods of prophylaxis for this condition. More than 50% of the patients who develop this complication die in spite of therapy. The experience gained by several authors who have surveyed this field has led to the recommendation that asplenic patients and postsplenectomy patients receive daily oral penicillin prophylaxis. Although there were no data to contravene their recommendation, we devised an experimental model to test this hypothesis directly. In this study we have demonstrated that prophylactic penicillin protects splenectomized mice who are challenged with an aerosolized suspension of type III Streptococcus pneumoniae.

MATERIALS AND METHODS

Animals

Ninety-one Swiss white male mice, CD-1 strain (purchased from Canadian Breeding Laboratory, a subsidiary of Charles River Farms), weighing 25–29 g were used in these experiments. Prior to aerosol exposure the mice were group-housed in filter-top cages. After exposure, mice were individually housed in separate filter-top cages. Food and water were supplied ad libitum.
**Preparation of Bacterial Suspension**

The organism employed for these experiments was a type III *S. pneumoniae* that had previously been obtained from the sputum of a patient with a respiratory infection. This organism was found to be virulent on passage through mice and was reisolated from infected animals. A stable culture of this organism was maintained in our laboratory by growth in trypticase soy broth (TSB) with 0.25% dextrose; sterile glycerol had been added to the culture to make a concentration of 2%, and multiple vials of the stock culture were frozen in 2-cc aliquots at -70°C and used for each experiment. A 2-cc aliquot of the thawed stock culture was resuspended in 500 cc of fresh TSB with dextrose and incubated at 37.5°C for 15–18 hr prior to the experiment. This broth was then concentrated by centrifugation at 8000 rpm for 45 min at 4°C; the precipitate was resuspended in 10 cc of fresh TSB. The total number of bacteria per cubic centimeter of the resuspended 10 cc was determined by serial dilution.

**Operative Procedure**

Each mouse was anesthetized with ether, the left flank was shaved and cleansed with 95% ETOH, and an ear was punched for identification. Controls were anesthetized only, and they had their ears punched. In the splenectomized group the skin and peritoneum were opened with surgical scissors, and the splenic pedicle was cross-clipped with a small hemostatic clip. The spleen was then removed from the pedicle with a pair of fine scissors, and the splenic pedicle and clip were replaced into the abdomen. The skin and peritoneum were then closed with surgical clips. The complete time for the procedure ranged from 2 to 4 mm. Operative mortality in the first 24 hr was less than 5% and was secondary to bleeding or ether overdosage. There was no long-term mortality. The sham-operated group had their spleens delivered with a pair of forceps, after which their abdomens were closed. Splenectomy was done 14 days prior to pneumococcal exposure.

**Animal Exposure**

The model A-42 Tri-R airborne infection apparatus (Tri-R Instruments, Rockville Centre, N.Y.) was used for aerosol inoculation. This apparatus uses a Venturi nebulizer to create the aerosolized suspension of bacteria. A prototype of this machine has been described previously. A 7-cc aliquot of the final suspension was placed in the nebulizer. A 30-min nebulizing period resulted in the utilization of 6 cc. Antifoam A spray (Dow Chemical) reduced foaming of the broth. The particle size analysis of the pneumococcal aerosol, as determined with an Anderson air sampler, showed 88% infective particles less than 2 μ in diameter. Each animal was placed in a separate compartment, thereby eliminating the effects of huddling, which have been shown to decrease inhalation of bacteria and increase variability. All animals were exposed at the same time.

**Penicillin Treatment**

Forty thousand units of procaine penicillin G were given intramuscularly each day for 5 days, beginning 2 days prior to bacterial exposure and continuing for 3 days after exposure.

**Statistical Analysis**

The effects of splenectomy and prophylactic penicillin treatment on mortality over time were estimated through the use of the proportional hazards model. This model has been previously used in analyzing survival data in cancer patients. For a given group of animals, the hazard function at time t is the conditional probability of death in the next unit of time for those animals still alive at time t. Under the proportional hazards model, the hazard functions for various groups are assumed to be proportional to one another. Thus the ratio of the hazard functions for any two groups gives a measure of the relative risks experienced by the animals in the two groups. A ratio significantly different from unity means that at any particular time point the animals in one group tend to be at greater risk than the animals in the other. These ratios can be estimated from the data.

A computer program based on the theoretical work of Cox and Breslow was used to estimate the ratios (and associated standard errors) of the hazard functions between various pairs of groups. Table I

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*Kindly furnished by Dr. N. Breslow of the University of Washington, Seattle.*
Table 1.

<table>
<thead>
<tr>
<th>Effect of penicillin:</th>
<th>Ratio of Hazard Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Splenectomized, no penicillin vs. penicillin</td>
<td>27.78*</td>
</tr>
<tr>
<td>2. Sham-operated, no penicillin vs. penicillin</td>
<td>25.64*</td>
</tr>
<tr>
<td>B. Effect of splenectomy:</td>
<td></td>
</tr>
<tr>
<td>1. Penicillin-treated, splenectomized vs. sham-operated</td>
<td>2.90†</td>
</tr>
<tr>
<td>2. Not penicillin-treated, splenectomized vs. sham-operated</td>
<td>3.113*</td>
</tr>
<tr>
<td>C. Interaction of penicillin treatment and splenectomy:</td>
<td></td>
</tr>
<tr>
<td>1. Ratio of effect of penicillin treatment on splenectomized group to effect of penicillin treatment on sham-operated group</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*Ratios significantly different from 1 at p < 0.0001.
†Ratio significantly different from 1 at p < 0.10.

shows the results. Part A of this table shows that penicillin has a striking effect for both the splenectomized and sham-operated animals: the hazard functions in both cases were over 25 times greater for the animals not treated with penicillin than for the animals that had been treated. In Fig. 1 this is reflected in the lower mortality curves for the two penicillin-treated groups as compared with the two groups not treated with penicillin. Part B of Table 1 shows that splenectomy had a more moderate (but still significant) effect: the ratio of hazard functions for splenectomized versus sham-operated animals was about 3, whether or not the animals had been treated with penicillin. The significance levels for the two ratios given in part B are different because the greater mortality experienced by the untreated groups allowed for more reliable hazard function estimates. Figure 1 shows that each of the mortality curves for the two splenectomy groups was higher than that for the corresponding sham-operated groups. It can be concluded from parts A and B of Table 1 that penicillin treatment and splenectomy acted independently of each other. This is confirmed explicitly in part C of Table 1.

In summary, both penicillin treatment and splenectomy showed significant effects on mortality experience. Specifically, the penicillin treatment greatly decreased the risk of death, over time, whereas splenectomy tended to increase that risk.

RESULTS

Figure 1 demonstrates that there is a marked increase in survival when splenectomized or sham-operated mice given prophylactic penicillin are compared to splenectomized or sham-operated mice without penicillin. Furthermore, although the mortality at 20 days is approximately the same for the splenectomized
group and the sham controls, there is a significant increase in mortality, over time, for the splenectomy group. This last observation confirms an earlier study that demonstrated the protective effect of the spleen to an aerosolized challenge of pneumococci. The dose of pneumococci used in this experiment is such that all animals unprotected by penicillin eventually die. When lower doses of bacteria are used \(10^8\) and \(10^7\), there is a decrease in mortality such that a significant difference between the splenectomized group and the sham group is not obtained.

Two days after exposure, 1 sham-operated + penicillin animal died, and the lung culture was sterile. Two splenectomized + penicillin animals also died, and the lung cultures were negative for pneumococci but positive for a Gram-negative rod. Three days after exposure, 1 splenectomized + penicillin animal died, and their lung cultures were sterile. One splenectomized + penicillin animal also died, and the lung culture was negative for pneumococci but positive for a Gram-negative rod. In three of these six instances we considered that death was related to the penicillin, since the lung cultures were sterile. On day 8, 1 splenectomized + penicillin animal died, and the lung culture was positive for pneumococci and Proteus. In addition, 1 sham-operated + penicillin animal died, and the lung culture was positive for pneumococci. All the treated animals who died and had positive lung cultures showed gross and microscopic evidence of pneumonia. Treated animals with negative lung cultures had no macroscopic changes suggesting pneumonia in their lungs.

All animals who died and who did not receive penicillin grew pneumococci from the lung and showed gross and microscopic evidence of pneumonia. In addition, 10 from the splenectomized group and 3 from the sham-operated group grew Proteus from the lung, whereas 5 from the splenectomized group and 3 from the sham-operated group grew staphylococci from the lung. No infected animal showed evidence of adrenal hemorrhage or disseminated intravascular coagulation.

DISCUSSION

Recent review articles have focused attention on the role of the spleen in the host’s defense against bacterial infection. The asplenic individual is at risk for developing bacterial sepsis, usually pneumococcal, and this risk increases as the age of the patient decreases and/or as the severity of the underlying systemic condition for which splenectomy was done increases. In spite of early therapy, more than 50% of patients who develop postsplenectomy sepsis (PSS) die, and in the majority of these patients the pneumococcus is the responsible organism.

Polyvalent pneumococcal polysaccharide vaccines have been shown to be effective for at least 2 yr in patients susceptible to pneumonia, children with sickle cell disease, and a small number of asplenic children. The major drawback to the vaccine is the variable response rate seen in children under 2 yr of age, an important consideration when dealing with congenital asplenia. Furthermore, the pneumococcal serotypes in PSS possibly are not the same as those known to occur in patients with sickle cell disease or in the upper respiratory tract of normal children. Finally, patients with absence of the spleen may not be immunologically comparable to those with functional asplenia.

We have demonstrated a significant protective effect of prophylactic penicillin in asplenic mice. Only 2 animals (1 splenectomized and 1 not) died of pneumococcal
disease after receiving penicillin. Significantly, all animals who died and did not receive penicillin demonstrated pneumococcal pneumonia. The Gram-negative rods seen in some of these animals may well be attributed to a secondary infection once the pneumococcal disease has been established. We were not able to perform blood cultures prior to death because of technical problems, and therefore we do not know if pneumococcal septicemia was present, although preliminary studies in our laboratory have demonstrated that some animals not receiving penicillin did have positive blood cultures for pneumococci.

Extrapolation of information obtained from animal studies to human beings is quite difficult. The dose of penicillin employed was very high, and no attempt was made to equate it with the amount currently being recommended for human prophylaxis. Preliminary results from work currently in progress indicate that 4000 units of penicillin are just as effective as 40,000 units. The study presented here suggests that prophylactic penicillin is effective in protecting asplenic mice from the lethal effects of inhaled pneumococci. Therefore, even though strains of pneumococci that are resistant to penicillin are being reported, prophylactic penicillin may have a role, along with pneumococcal vaccine, in the protection of asplenic individuals. This would certainly seem to be the case in children under 2 yr of age, a population whose protection by the vaccine is less than satisfactory. In addition, impaired antibody response to pneumococcal vaccine has been reported in patients who have previously been treated for Hodgkin disease.

REFERENCES

10. Likhite VV: Immunological impairment and susceptibility to infection after splenectomy. JAMA 236:1376–1377, 1976


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