Reviewer's report

Title: Depolymerase improves gentamicin efficacy during Klebsiella pneumoniae induced murine infection

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Reviewer: Shih-Hsiung Hsiung Wu

Reviewer's report:

The resubmitted manuscript entitled as “depolymerase improves gentamicin efficacy during Klebsiella pneumoniae induced murine infection” which was revised by the authors to add more detail information responding to the comments from both reviewers. From their answers to my comments, some information might add in the Methods and Discussion sections of this manuscript in order for readers to understand the detail animal studies and antibiotic effects.

Add in the Discussion section:

1. Under in vivo conditions (as in our study), antibiotic action against the bacteria depends on the dose of bacteria used for infection rather than the growth phase. Similarly, in the ex vivo macrophage experiment also (Method 2.9.2), we tried using similar counts of overnight culture as well as log phase culture and incubated them with macrophages. The results of phagocytic killing were similar in both cases, indicating that it actually depends on the bacterial number rather than the growth phase.

2. After establishment of acute lung infection model, it was treated with gentamicin (1.5 mg/kg) at 0 h, 6 h, 12 h, 24h, 48 h, 24 h + 48 h post infection. In the first 3 groups, significant reduction in bacterial count was observed on the peak day (day 3) whereas in the other groups, gentamicin could not control the infection alone. These results indicated that the antibiotic was effective during the initial time since the bacteria has not completely established themselves or started to proliferate in the lungs. Once they colonized, proliferated in the lung and CPS production is maximal, gentamicin is no longer effective. Moreover, in clinical situations also, it took some time to initiate antibiotic treatment. Thus, depolymerase was injected 24 h post infection, to check whether it could remove the CPS and render the bacteria susceptible to gentamicin. The bacterial load, 24 h post infection, was 4.2 logs (Figure 1, day 1). It was similar to the intranasal dose administered to mice. Depolymerase injected at 24 h was not directly bactericidal rather it degraded the CPS matrix encasing the bacteria thus rendering them susceptible to gentamicin and components of immune system.

3. After intranasal instillation of bacteria in mice, bacteria overcome the innate immune response operating in the respiratory tract and establishes in the lungs by day 1. Histopathological analysis of the lung tissue showed that on day 1 mice develop mild pneumonia (Indian J Med Res 118, July 2003, pp 47-52). Thereafter, bacteria proliferate in the lungs, express virulence factors resulting in
a corresponding increase in bacterial number. Lungs of mice sacrificed on day 2 post infection reveal moderate changes. The bacteria multiply and reach a peak by day 3. Thus, the animals showed well-developed pneumonia with abscess formation and destruction of alveoli (Indian J Med Res 118, July 2003, pp 47-52). At day 3, tissue injury was characterized by increased levels of nitric oxide and free radicals, and pro-inflammatory cytokines is also maximal. Beyond Day 3, bacteria were tackled by the activated host immune response resulting in decrease in bacterial number. Histopathology of mice sacrificed on day 7 PI showed resolving pneumonia and macrophages dominating in the affected areas (Indian J Med Res 118, July 2003, pp 47-52). Depolymerase and gentamicin were comparatively less effective on the 3rd day because the bacterial infection and tissue injury were at its peak and they were unable to tackle it alone. Since the infection was confined in the lung, therefore, as the bacterial number decreased by day 4/5, the agents became more effective.

Add in the Methods part:
1. Different doses 102-108 have been tried for inducing acute lung infection after intranasal administration and septicemia after intraperitoneal administration. The dose which gave 100% infection without causing any mortality was chosen for this work. (i.e. 104cfu in 50µl for i.n infection and 102cfu in 100 µl for systemic infection).

2. Lane 229: may add these sentences: For phagocytosis, the killing efficacy depends on the MOI i.e ratio of bacteria and macrophages. In our study, we tried different MOIs i.e 1, 10, 100, 1000. But the best results were obtained with MOI 100.

3. The lethal dose of Klebsiella pneumoniae B5055 used by us is different after intranasal or intraperitoneal administration. The dose causing 100% infection and no mortality i.e 104 cfu by intranasal route and 102cfu by intraperitoneal route, used in our study is also different.

Minor essential revision:

5. In Line 164, 166, 168, 170 and other places, ........6h....... should change to ......6 h....... It should have a space between number and unit. Please check and correct all the manuscript.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:

I declare that I have no competing interests.