SKIN-TEMPERATURE CHANGE AS AN ENDPOINT IN A MURINE MODEL OF PNEUMONIA

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REVISED ABSTRACT

Background and Objectives

Temperature change and weight loss can serve as predictors of both the severity & outcome of an infectious disease. A ≥4°C decrease in body temperature and a ≥10% reduction in weight have been shown to correlate with death in several rodent models. Nevertheless, these measures are excluded from studies that evaluate antibiotic efficacy, since the procedures used to obtain them are regarded as problematic or expensive. In this study, skin-temperature change and weight loss were assessed for their value in predicting imminent death in a murine pneumonia model used specifically to evaluate antibiotic efficacy. In addition, the use of an infrared temperature-scanning thermometer (ITST) was evaluated as an affordable and practical way to measure temperature.

Materials and Methods

Immunocompetent Swiss mice were infected by peroral tracheal instillation of 10^5 colony-forming units of a *Streptococcus pneumoniae* serotype 3 strain. Skin temperature was measured every 12 hours until death using the Raynger MX4 Series High Performance ITST. Body weight was measured at the same time points using an electronic balance. Colony-forming units in the lungs of infected mice were determined at specific time points and temperature measurements.

Results

All infected mice died within 84 hours of inoculation (median survival time, 60 h) and showed marked reductions in weight (median weight change, 3.8 g) and lowered skin-temperature (median temperature change, 5.8°C) 12 h prior to death. By comparison, all uninfected mice survived past 84 h and showed no significant changes in temperature or weight during the course of the experiment. We fit a Cox proportional hazards model to infected mice data with temperature and weight as time-dependent covariates and determined that the relative risk (RR) was significant for temperature but not for weight (weight, 95% CI for RR = 0.58 - 1.23, *P*-value = 0.37; temperature, 95% CI for RR = 0.57 – 0.87, *P*-value = 0.001). Viable counts were not significantly different between infected mice allowed to die and those that were sacrificed when their skin-temperature dropped at least 3°C.

Conclusion

We conclude that skin-temperature change is a suitable parameter for monitoring animal health and a potential predictor of death in our model. An ITST is a novel, affordable, effortless and humane way of measuring temperature that will improve the quality of the data collected during drug efficacy studies.

INTRODUCTION

Clinical signs, including changes in body temperature and weight loss can serve as predictors of the severity of an infectious disease in experimental animal models (Kort et al. 1998; CCAC 1998). However, these signs are under-utilized in studies to test the efficacy of novel antimicrobial compounds, despite their usefulness in assessing the treatment outcome. In the past, death has served as the experimental endpoint in therapeutic trials in animals, but has since been discouraged and / or disallowed by many research facilities. Therefore, earlier, more humane endpoints must be established in order to reduce and eliminate the pain and distress as a result of disease progression (Morton and Townsend 1995). Unfortunately, the procedures used to measure biological changes such as body temperature, which could be used to monitor disease progression, are either problematic (rectal probes) or expensive (microchip implants).

OBJECTIVES

- To assess the utility of an infrared temperature-scanning thermometer as an affordable and practical means by which to measure temperature in animal models.
- To evaluate changes in skin-temperature and weight as predictors of imminent death in a murine model of pneumonia which can be used to assess efficacy of antimicrobial treatment.

MATERIALS AND METHODS

PNEUMOCOCCAL PNEUMONIA MODEL UTILIZED IN THIS STUDY

- Immunocompetent female Swiss mice (weight 20 – 22 g) were inoculated by peroral tracheal delivery with 10^5 log-phase colony-forming units of the *Streptococcus pneumoniae* 6303 serotype 3 strain. If left untreated, mice develop acute pneumonia & typically die within 3 days. At death, the bacterial counts in the lungs generally range from 10^7 – 10^8 colony-forming units.

TEMPERATURE CHANGE AND WEIGHT LOSS AS INDICATORS OF DISEASE AND PREDICTORS OF IMMINENT DEATH

- Twenty-one mice were infected as described above. Ten mice were used as uninfected controls. Skin-temperature was measured 3 times every 12 hours until death using the Raynger MX4 Series High Performance Infrared Temperature Scanning Thermometer (Raytek, Santa Cruz, CA). This portable device measures the temperature of a subject by detecting and quantifying the subject's emitted infrared radiation without physical contact. Body weight was measured at the same time points using an electronic balance. This experiment was repeated.

USING TEMPERATURE CHANGE TO PREDICT PULMONARY BACTERIAL LOAD

- In an effort to correlate skin-temperature change with the bacterial load in the lungs, viable counts of 40 infected mice were determined of which 20 were sacrificed by cervical dislocation when their skin-temperature dropped at least 3°C[1] and 20 that were allowed to die without intervention. This experiment was repeated.

[1]Chosen as the most appropriate predictor of death within 24 h [see Results and Discussion]

RESULTS AND DISCUSSION

TEMPERATURE CHANGE AND WEIGHT LOSS AS INDICATORS OF DISEASE AND PREDICTORS OF IMMINENT DEATH

- All infected mice died within 84 hours of inoculation (median survival time, 60 h) and showed marked reductions in weight (median weight change, 3.8 g) as well as lowered temperature (median temperature change, 5.8°C) 12 h prior to death.
- By comparison, all uninfected mice survived past 84 h and showed no significant changes in skin-temperature or weight during the course of the experiment

RESULTS AND DISCUSSION (continued)

- We fit a Cox proportional hazards model to infected mice data with temperature and weight as time-dependent covariates and determined that the relative risk (RR) was significant for temperature but not for weight (weight, 95% CI for RR = 0.58 – 1.23, *P*- value = 0.37; temperature, 95% CI for RR = 0.57 – 0.87, *P*- value = 0.001).
- Although most mice lost up to 20% of their body weight during the course of the experiment, it was not a reliable predictor of death, and was therefore excluded from further analyses.
- A temperature change of ≥ 3°C was most appropriate for predicting death within 24 h (test efficiency, 84%; positive predictive value, 97%; negative predictive value, 68%).

USING TEMPERATURE CHANGE TO PREDICT PULMONARY BACTERIAL LOAD

- Viable counts of infected mice allowed to die and those that were sacrificed were not significantly different, thereby demonstrating that our chosen endpoint does not compromise the pre-death bacterial count in the lung (allowed to die: mean CFU pf 1.2 x 10^8; sacrificed: mean CFU of 8.8 x 10^7).

CONCLUSIONS

- Infrared temperature scanners are an effective, affordable and practical means by which to monitor temperature and animal health.
- Changes in skin-temperature are predictive of mortality in a murine model of pneumonia used for evaluating drug efficacy and could likely be used as an earlier, more humane endpoint than death in other infectious disease models without compromising scientific outcomes.

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