Infrared body temperature measurement of mice as an early predictor of death in experimental fungal infections

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Summary

Temperatures of mice were measured using an infrared high performance non-contact thermometer, after the device had been calibrated using implantable microchips containing temperature transponders. Mice were infected with three species of *Candida* (isolates) and the resultant disseminated infections monitored. Mouse temperatures could be reliably measured using the infrared device and this measurement caused little distress to the mice. We were further able to demonstrate that mice rarely recovered if their body temperature dropped below 33.3°C (sensitivity 68%, specificity 97%). Adoption of a 33.3°C endpoint in fungal sepsis experiments measured by infrared non-contact thermometer would significantly reduce the suffering in the terminal stages of this type of infection model.

Keywords Infrared temperature; experimental murine infections; death predictor; sepsis; murine candidiasis

It has been suggested that if the body temperature of mice falls below 32–36°C, after being subjected to infection by bacteria, they are unlikely to recover (Siems 1989, Soothill *et al.* 1992, Wong *et al.* 1997, Kort *et al.* 1998) (the precise endpoint needs to be validated in each experimental procedure). Determination of body temperature by traditional means is stressful to a sick animal and extremely time consuming. Conventionally the core body temperature of mice has been measured by either the insertion of a thermometer into the anus of the mouse or insertion of a thermocouple via the anus into the large intestine (Soothill *et al.* 1992, Wong *et al.* 1997). The measuring device is then left *in situ* until the temperature recorded stabilizes. Each measuring procedure can take up to one minute per animal.

Recently measurement of body temperature has been simplified by the development of implantable microchips containing temperature transponders (Kort *et al.* 1998, Vlach *et al.* 2000). The transponders are small chips (2.2 × 14 mm) encapsulated in biocompatible glass capsules and covered with a polypropylene cap. The transponders are positioned either subcutaneously or intraperitoneally using an inserter provided with the chips. The temperature and chip identifier are then read using a portable reader held approximately 2 cm from the chip location. Using this

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equipment, temperatures can be rapidly determined with an accuracy of ±0.1°C and with little or no distress to the mice.

As previously mentioned, temperature measurement using implantable microchips is simple and rapid but initial insertion of the microchip is stressful to the mice. A further possible disadvantage of this system is the potential risk of localized infection around the device caused either during insertion of the chip or as a device-associated infection established after the mice are infected in experimental protocols.

We have attempted to develop a rapid non-invasive method to determine mouse body temperatures without the need to insert thermometers, thermocouples or implantable microchips, by using an infrared recording device (Krarup *et al.* 1999). The use of infrared temperature measurement has many potential advantages. In particular it is non-invasive and has no consumable elements. Measurement is rapid and causes little stress to sick animals.

In this paper we discuss the development of infrared temperature measurement and its utility in the determination of hypothermia endpoints in fungal LD_{90} experiments.

Animals, methods and materials

All mice included this study were part of ongoing studies performed under UK Home Office project licence PPL/40/1523 entitled Invasive Fungal Infections.

Animals and husbandry

One hundred and sixty male CD1 mice, 4–5 weeks old and weighing between 18–20 g were purchased from Charles River UK Ltd (Margate, Kent, UK). The mice were specific pathogen free and caged in groups of 3–10 mice per cage with sterile wood shaving contact bedding. The mice were acclimatized in our unit for at least 7 days before initiation of experiments. Mice were allowed free access to water (tap water) and food (Beekay Feeds RM1 Diet, Hull, UK). A 12:12 h light/dark cycle was used with a temperature range of 21±2°C and relative humidity of 55% RH ±10%. Each cage was inspected at least four times daily. Entrance to the experimental room was restricted to persons wearing gloves, masks and biological hazard suits.

Test strains

One strain of *Candida albicans* FA6862, one strain of *C. guilliermondii* FA2760 and one strain of *C. glabrata* ATCC 2001 were examined. The strains were maintained on slopes of Oxoid Sabouraud dextrose agar (Oxoid Ltd, Basingstoke, UK) supplemented with 0.5% (wt/vol) chloramphenicol (Sigma, Poole, UK) and at −70°C in nutrient broth (Oxoid) containing 15% glycerol (Sigma) for long-term storage.

Immunosuppression

Cyclophosphamide (Sigma-Aldrich, Poole, Dorset, UK) was administered intravenously via the lateral tail vein to all animals at a dose of 200 mg/kg. A state of profound neutropenia was achieved 3 days after administration, which lasted for 4 days (Denning *et al.* 1995).

For the *C. guilliermondii* and *C. glabrata* models cyclophosphamide was re-administered both after 72 h and 7 days post-infection.

The *C. guilliermondii* infected mice were further immunosuppressed with soluble prednisolone (Sovereign Medical Basildon, Essex, UK) (75 mg/l added to the drinking water). As mice are allowed free access to water this provided approximately 15 mg/kg/day prednisolone (Nugent & Onofrio 1983).

Preparation of inoculum

For each experiment an isolate was thawed then incubated overnight at 37°C on Sabouraud dextrose agar. One colony was transferred into 25 ml of Sabouraud dextrose broth (Oxoid Ltd). The broth was incubated on an orbital mixer for 8–24 h at 37°C, and then centrifuged to pellet the organisms. The cells were washed twice in saline, and then resuspended in physiological saline and the density was adjusted using their optical density (OD) at 490 nm. Organism counts were determined before infection by comparison of the OD with pre-determined curves of OD versus count (data not shown).

Organisms were stored at room temperature for less than 2 h before use.

Infection of animals

All animals were infected 3 days post-immunosuppression by the injection of either 0.15 ml (*C. albicans*) or 0.2 ml (*C. glabrata* or *C. guilliermondii*) into the lateral tail vein. Groups of 10 mice were infected with one dose of a range of inocula to determine the LD_{90} inoculum for each strain of *Candida*. The inoculum concentration was rechecked from the remaining suspension after the animals were infected.

Assessment of animals

All mice were examined at least four times daily with a maximum of 10 h between observations. All animals suffer loss of up to 10% of body mass (due to cyclophosphamide immunosuppression and disseminated infection characterized by lowered responsiveness). Any infected animals with substantially reduced mobility, which were unable to reach the drinker or were otherwise in substantial distress, were culled. Particular attention was paid to postural changes in the mice and the presence of torticollis and staggering, as these were indicators of imminent deterioration; and if these symptoms were severe mice were culled. Further, the anal region was examined for the presence of staining (due to reduced renal function), as this was also a sign of imminent deterioration.

Measurement of body temperature

(a) *Implantable temperature transponders*
Implantable programmable temperature transponders (IPTT-100) and a hand-held reader DAS-5007 (Bio Medic Data Systems, Delaware, USA) were used according to the manufacturer's instructions. In brief, the transponders were pre-programmed with individual number identifiers by the supplier before delivery. The transponders were implanted into the subcutaneous tissue in the neck region of the mice, using the insertion device included with the microchips, 2 days before the mice were immunosuppressed.

Measurement of temperature and identification of the unique identifier code was made by holding the detector approximately 50 mm from the implanted chip (the chips were easy to locate manually through the skin of the neck). Temperatures were displayed on the reader within 2 s. Temperatures were collected using this technique on 20 mice (in conjunction with the infrared technique). These mice were matched with an identical group without implants.

(b) *High performance non-contact thermometer*
The high performance non-contact thermometer Raynger MX4 (Raytek Portable Products Ltd, Santa Cruz, CA, USA supplied by Thames Medical Ltd, Worthing, UK) was set up as per the manufacturer's instructions. In brief, the emissivity (ε) and temperature offset were adjusted by comparison of the reading with that of the IPTT-100, until identical readings were achieved. Temperatures of individual mice were monitored by restraining the mouse then aiming the beam of the reader at the base of the mouse sternum (being very careful to restrain the mouse head and avoid exposing eyes to the beam). Readings were taken over a 3–4 s interval with the unit held 5 cm from the mouse, or until a stable reading was achieved. Care was taken to ensure that the centre of the beam was aimed at the correct site otherwise falsely low readings were achieved.

Statistical analysis

All data analysis was performed using the computer package Arcus QuickStat (Addison Wesley Longman Ltd). To define the diagnostic temperature cut-off, a receiver operator characteristics (ROC) curve was constructed (of sensitivity [probability of death when hypothermia is present] is then plotted against 1-specificity [probability of death without hypothermia]).

Results

The overall mortality is shown in Fig 1. Individual models demonstrated the following LD_{90}. *C. albicans* 6862 2×10^5/ml

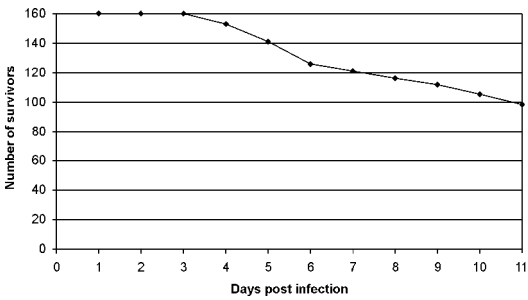

Fig 1 Survival over time of mice after infection with Candida spp

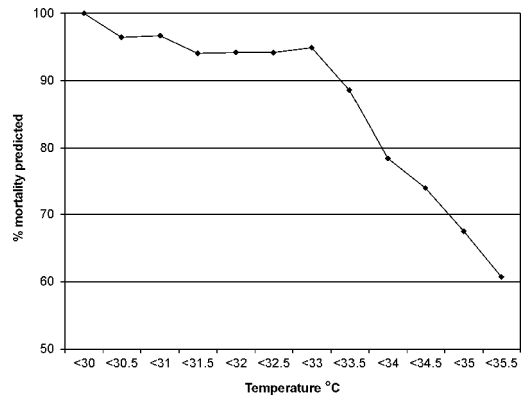

Fig 3 Graph of percentage predicted mortality against temperature

(Fig 2): *C. guilliermondii* 2760 2×10^8/ml: *C. glabrata* ATCC2001 9×10^7/ml.

Mouse body temperatures post-immunosuppression but pre-infection had a mean of 35.5°C (SD 0.9°C).

Temperatures measured were in the range 21.2–38.1°C. Overall mortality in this series of experiments was 61 of 161 (38%) (Fig 1). Of the 61 mice that were either culled or found dead, 49 (80%) had a final temperature recorded below 33.3°C. Thirty-four of the 61 fatalities were humanely culled as they were in substantial distress (see assessment of animals in Methods) but 27 were found dead on cage inspection. Of the 27 mice which were found dead 12 (44%) had a temperature recorded below 33.3°C at an earlier point in the experiment.

The mice that were found dead with temperatures above 33.3°C had a mean final temperature of 35.8°C (SD ±1.1) and a mean lowest recorded temperature of 35.4°C (SD ±0.7). Four of the mice that died with a temperature above 33.5°C had demonstrated mild/moderate torticollis and staggering which probably indicates a cerebral infection.

Hypothermia below 33.3°C was measured in 5 of 100 survivors at least once during the experiment (range 33.3–30.8°C): only one mouse had readings below 33.3°C for more than 12 h (readings below 33.3°C for 5 days, with a lowest temperature measured of 30.8°C).

Temperature measurement with an end-point of 33.3°C as an objective predictor of death would therefore have a positive and negative predictive value of 93% and 97%, respectively (Figs 3 and 4). The sensitivity of a 33.3°C cut-off would be 68% with a specificity of 97%.

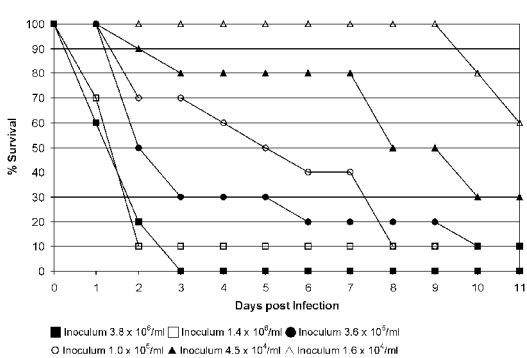

Fig 2 Survival over time of mice post Candida albicans FA6862 infection

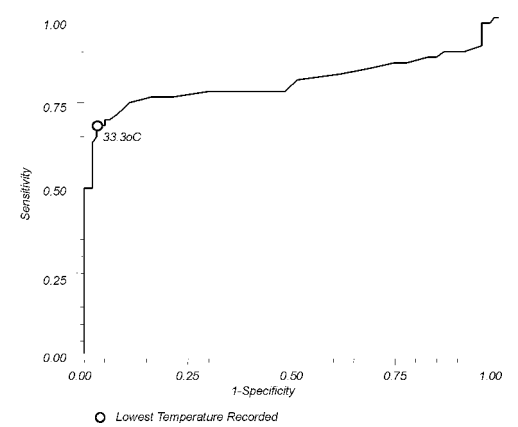

Fig 4 ROC plot of the lowest temperature recorded versus survival

After adjustment of the high performance non-contact thermometer to an emissivity of 0.98 and temperature offset of 5.7, precision of the readings when compared to the readings of the IPTT-100 microchips was excellent with a mean difference of 0.1 and a standard deviation of 1.2°C. The infrared device was slightly less accurate when mouse temperature was outside the range 29.0–39.0°C.

The group of mice implanted with microchips had an overall mortality rate of 95% in comparison to the rate of an identical group without implants of 70%. At post-mortem examination a focus of infection could be clearly identified around the implant site.

Discussion

An essential element of all laboratory experimentation with live animals should be the aim of refining the procedure to minimize suffering (Russel & Burch 1959). In many experiments death or imminent death is accepted as a suitable endpoint, but this has long been considered an unsuitable 'humane endpoint' (Olfert 1995). Replacement of a death endpoint by another surrogate marker is therefore not simply a desirable alternative but an urgent necessity.

Hypothermia has been recognized as an alternative and relatively precise surrogate marker for nearly a decade (Soothill *et al.* 1992) but difficulty in determining temperature and the time-consuming nature of the examination have discouraged researchers from adopting this protocol. A further complication of hypothermia as a surrogate marker of imminent death is that it is necessary to establish the temperature cut-off in each experimental set-up; widely different endpoints have been published, reflecting different host/pathogen interactions (Kort *et al.* 1998 37°C, Wong *et al.* 1997 32°C, Vlach *et al.* 2000 21.1°C after Staphylococcal endotoxin). Recently, implantable programmable temperature transponders have been developed (Kort *et al.* 1998, Vlach *et al.* 2000) that simplify temperature measurement. Body temperature measured further by subcutaneous transponders is not significantly different from core body temperature (Vlach *et al.* 2000). This technique has many advantages as measurement is rapid and causes little stress to experimental animals. Unfortunately the technique has the disadvantages of requiring expensive implantable transponders as well as the distress from the initial insertion of the microchip. There is also a potential problem of increased mortality in those mice in which the microchip is inserted (95% mortality in a group of mice with implants *vs* 70% in an identical group without implants). This could be due to device-associated sepsis (as indicated by post-mortem examination) or additional handling and discomfort.

High performance non-contact thermometers (HPNT) demonstrate most of the advantages of the IPTT-100 but, as the measurement is non-invasive, there is no distress from chip insertion and no possibility of device-associated sepsis. Slight distress is obviously caused during measurement of temperature, as it is necessary to restrain the mice (temperatures read by aiming at the back of a moving mouse were much less reliable and in general 3–4°C below the core temperature). In our hands after an initial calibration the instrument proved very reliable and simple to use. Although the initial cost of the equipment is fairly high there are no consumable costs. This equipment is also likely to be suitable for use in a wide range of animals after an initial calibration exercise.

Initial calibration of HPNT is essential, as changes in the emissivity and temperature offset will determine the exact temperature recorded. Calibration is possible without sophisticated equipment if recently culled animals are available. Temperatures can be compared to conventional thermometers inserted into the anus as the body cools and adjustments are made.

It is important to note a further technical point on the determination of mouse temperatures. It was clear during the study that the core temperature of mice rises rapidly by up to 2°C during handling and these changes start within 10 s of the mouse being restrained (Clement *et al.* 1989, Krarup *et al.* 1999). It is therefore essential that body temperature measurements are

made as soon as possible after the mice are restrained (minimal disturbance is essential to reduce stress in cage mates in group-housed situations).

Adoption of a hypothermia endpoint is not an easy decision as the exact temperature at which animals are to be culled can have a significant effect on the experiment (an increase to 34°C would increase the sensitivity to 75% but would reduce the specificity to 89%). In this paper we have presented data on an endpoint of 33.3°C. Using this cut-off we would have been able to predict the mortality of 67% of the mice. If a single temperature measurement below 33.3°C were accepted, 5% of the mice would have been culled unnecessarily but if hypothermia was detected for >12 h then only 1% of mice would have recovered.

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Contributors TP and MB conceived the idea; PAW, TP and DWD designed the study. The work was conducted and results analysed by PAW and AS, with contributions from GM and NS. PAW and TP act as guarantors for the content of the paper.

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