Determining body temperature using a microchip implant system

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Summary

Klebsiella pneumoniae was inoculated intratracheally into rats and mice, and the body temperature was monitored twice daily using ELAMS^{TM} temperature transponder system. Transponders were implanted either subcutaneously or intraperitoneally. The microchip temperatures were compared with rectal temperatures taken at the same time. The purpose of the experiments was to compare values given by subcutaneously or intraperitoneally implanted transponders with rectal temperatures, and to determine a 'temperature cut-off point' as an alternative for 'death of the animal'. The temperatures generated by transponders did not differ significantly from rectal temperatures. Furthermore, the results showed that when a 36°C endpoint is used instead of the time of death, the statistical analysis was not altered, however it would spare animals further suffering for approximately 24 h. The argument that measuring body temperature is a laborious job and stressful to the animals is overcome when temperature is monitored by a transponder system.

In rodent protection tests animals suffer greatly and so Acred *et al.* (1994) have described guidelines for the welfare of animals in these types of experiments. Defined and more humane endpoints were suggested based on clinical signs, but because clinical signs are difficult to standardize, independent parameters have to be sought, such as body weight and body temperature. These parameters have the advantage of being more precise, leading to improved statistical power and, in the present study, experiments were carried out determining body temperature of mice and rats that had been challenged intratracheally with bacterial suspensions of *K. pneumoniae*. The progression of illness is accompanied by hypothermia, loss of body weight and clinical signs of discomfort, which can be severe. Using an implanted temperature transponder system we tried to overcome the stress of repeated measurements of body temperature that would be caused by the repeated use of a rectal thermistor probe.

We tried to answer the following questions: What is the accuracy of the system, and is there a preferred site of implantation (subcutaneously or intraperitoneally)? What is the temperature cut-off point, i.e. what is the body temperature at which impending death of the animal can be predicted accurately?

Materials and methods

In this experiment the animals were part of an ongoing study in which new therapeutic regimens were tested using the *K. pneumoniae* as a model infective agent. Thirty SPF female RP rats and 10 male NMRI mice were obtained from Harlan and Iffa-Credo, (Harlan Nederland BV, Austerlitz, The Netherlands;

Iffa-Credo, Belgium). Up to the start of the experiment rats and mice were maintained under standard conventional conditions: acidified tap water (pH 3.0) and a standard diet (Hope Farms AM II, Woerden, The Netherlands) *ad libitum*.

Three experiments each with 10 rats ($n = 10$) and one experiment with 10 mice were performed. In half the animals the transponder was implanted subcutaneously, and the other half intraperitoneally. At the start of each experiment (day 0) the animals were weighed and their rectal temperatures recorded. Animals were then anaesthetized, the infection was administered and the transponders implanted. In the experiments using rats, the temperatures were recorded at times (T) = 0, 24, 48, 72, 78, 96 and 102 h. Whereas in the mice, temperatures were measured exactly every 12 h. All the remaining animals were killed at the end of day 7 (180 h). Rats entered the study at 21–24 weeks weighing 196–230 g. Mean body weight of the animals 2 days after inoculation of the bacterial suspension was 90% of that at the day-0. In the mouse model, the 10 mice weighed 26–30 g at the start of the experiment and most animals did not lose weight (actually most of them gained weight).

Infection model of Klebsiella pneumoniae *pneumonia*

Experimental pneumonia was induced under anaesthesia and the left main bronchus was intubated and the left lung inoculated with *K. pneumoniae*. The infection develops within 24 h and depending on the dose leads to spontaneous death within 5 days.

Measurement of body temperature

Measurement of rectal temperature was done by means of a digital recording with a thermistor using slight restraint, i.e. the animals were held by the tail. All recordings of transponder temperature were carried out while the animals were freely roaming around their cage with the lid removed. To identify animals and to measure the temperature, the scanner of the system had to be directed toward the animals within 5 cm of the transponder. The transponder temperature was measured in duplicate. The system we used, ELAMS™ (Electronic Laboratory Animal Monitoring System by BioMedic Data System, Inc., Seaford, DE, USA) consisted of a notebook (DAS-5002), which is a portable data acquisition system connected to a detachable scanner wand. The implantable programmable temperature transponders (IPTT-100) are encapsulated in biocompatible glass capsules and covered with a polypropylene cap to prevent migration. They measure 2.2×14 mm and weigh 120 mg. According to the manufacturer, the temperature can be read with an accuracy of $\pm 0.5^{\circ}$C, and a resolution of 0.1°C between 32 and 43°C. Each transponder is packed in sterile needles (o.d. 2.2 mm), which can be connected to an 'insertor'. These insertors were used for rats and mice, and for both subcutaneous and intraperitoneal implantations. For the subcutaneous site, the neck region of the animals was chosen.

Although the manufacturer intended the transponders to be used only once, we have re-used them. After death the transponders were removed, gently cleaned with tap water, then 70% alcohol and dried. The transponders were placed in their covering needles and resterilized with a 22.5% glutaraldehyde solution for at least one hour and then rinsed with sterile tap water and dried.

Results

Body temperature change

The ease with which the ELAMS™ generates the temperature as well as a unique animal code cannot be compared with the way the rectal temperature is recorded. Collecting the data was simple and could be established within seconds without any restraint of the animal. Collecting data was easier when transponders were implanted subcutaneously compared with intraperitoneally as the exact location of the transponder was easier to reach. There were no differences in the procedure of collecting data for rats and mice.

Although the injector (a 2.2 mm diameter needle) appeared large for a 20 g mouse, for implanting the transponder intraperitoneally,

no apparent complications (e.g. bleeding, paralysis, etc.) were seen. All 40 transponders were successfully inserted, none came out spontaneously, and all gave accurate data readings during the experiment. The only complication was that two transponders in rats and one in mice, were implanted subcutaneously instead of intraperitoneally. This was because we were very careful not to damage the internal organs and implanted the transponders at an angle of less than 45°. With experience this complication was not repeated.

Repeatability of the ELAMS™ system for temperature recordings was established on the basis of all *duplicate readings*, i.e. values of normal temperatures as well as temperatures during the infection. The results (Tables 1 and 2) in rats and mice showed that the differences between duplicate values (T1–T2) were small, both for subcutaneously and intraperitoneally implanted transponders. Differences between the body temperatures recorded either by ELAMS™ transponders or by rectal measurements (T1–T3) were small as well, and well within the limits of the specifications given by the manufacturer (accuracy = ±0.5°C). However, paired *t*-tests showed that differences between the readings (i.e. first and second measurements; transponder and rectal) were extremely significant with two-tailed *P* values of < 0.001.

In Tables 3–5, 36°C, 35°C and 34°C are taken as cut-off points, and correlated with the number of animals dying during that time period. In Table 3 the results of the mice are plotted, two out of 10 (80% sensitivity) animals recovered from having had temperatures below 36°C. The lowest temperature animals reached with recovery was 35.4°C. This means that with the endpoint of 35°C, 100% sensitivity can be reached.

In Tables 4 and 5 the results of the experiments using rats are given. In the one experiment with rats not given here, the infection was so acute that all the animals died within 4 days without any detectable temperature decrease. In Experiment 1 (Table 4), after reaching a body temperature of 36°C the median survival time of the rats was 24 h, whereas at 34°C the median survival time

Table 1 Mean body temperatures of mice during the course of experiment, either recorded by transponder or rectally

	Transponder ($n = 43$)	Transponder ($n = 55$)
Mice	Subcutaneous	Intraperitoneal
Mean T1[1]	37.2 ± 0.42	36.7 ± 0.19
Mean T2[2]	37.4 ± 0.43	36.9 ± 0.18
Mean T3[3]	36.9 ± 0.46	37.2 ± 0.21

[1]T1 = first recording of the body temperature by transponder
[2]T2 = second recording of the body temperature by transponder
[3]T3 = body temperature established rectally

Table 2 Mean body temperatures of rats during the course of experiment, either recorded by transponder or rectally

	Transponder ($n = 34$)	Transponder ($n = 44$)
Rats	Subcutaneous	Intraperitoneal
Mean T1[1]	36.6 ± 0.22	37.1 ± 0.20
Mean T2[2]	36.7 ± 0.23	37.0 ± 0.20
Mean T3[3]	36.7 ± 0.24	36.8 ± 0.20

[1]T1 = first recording of the body temperature by transponder
[2]T2 = second recording of the body temperature by transponder
[3]T3 = body temperature established rectally

Table 3 Number of mice dying and time to death when different temperature endpoints were used (Note: two of the 10 mice survived)

	Time to death (h)				
Temperature cut-off point	< 6	6	12	18	24
< 36°C	1	2	2	2	1
< 35°C	1	3	3	0	1
< 34°C	3	3	2	0	0

Median 36°C = 24 h; 35°C = 12–24 h; 34°C = 12 h

Table 4 Number of rats dying and time to death when different temperature endpoints were used (Experiment 1)

	Time to death (h)				
Temperature cut-off point	< 6	12	24	36	48
< 36°C	0	4	2	2	2
< 35°C	1	7	2	0	0
< 34°C	2	6	2	0	0

Median 36°C = 24 h; 35°C = 12 h; 34°C = 12 h

Table 5 Number of rats dying with time to death when different temperature endpoints were used (Experiment 2)

Temperature cut-off point	Time to death (h)				
	< 6	6	12	18	24
< 36°C	0	1	3	2	4
< 35°C	6	1	2	1	0
< 34°C	7	1	2	0	0

Median 36°C = 18 h; 35°C = 6 h; 34°C = < 6 h

was 12 h. In Experiment 2 (Table 5), the infection seems to be more aggressive with a median survival time of 18 h for 36°C, whereas for 35°C and 34°C, times were 6 and < 6 h respectively.

Discussion

Death as an endpoint has been successfully replaced by other parameters (Olfert 1995), and the benefit was to both science and the animals. However, infection studies in which animals are challenged with bacteria and therapeutic regimens tested, death is commonly used as an endpoint. Disease in many such experiments progresses very rapidly, with a short time between clinical signs of illness and death. Nevertheless death as an endpoint should be avoided. In 'Guidelines for the welfare of animals in rodent protection tests' (Acred *et al.* 1994) it is recommended killing the animals with: (a) hind limb paralysis; (b) hypothermia (not further defined!); (c) nasal discharge; (d) signs of respiratory distress; and (e) loss of righting reflex. Another possible parameter is to take blood samples and to quantitate the bacteraemia. Body weight and temperature are also widely accepted as general clinical signs and for this reason, these parameters may sometimes be taken into account as well. However, none of these parameters is seen as discriminatory enough to decide whether an animal should be euthanized.

We think that for body temperature these arguments are less valid and it should be possible to determine a cut-off point below which the animals are terminally ill. Indeed others have shown this: Soothill *et al.* (1992) challenged mice with different bacterial agents and found that 34°C can be validated as a surrogate endpoint for death. Nevertheless, even if one accepts the value of body temperature as an indicator for euthanasia, recording body temperature per rectum several times a day is not acceptable as it is certainly stressful. But by using temperature transponders this problem is overcome, and our present results as well as those of others (Ball *et al.* 1991) have shown that such a system can be very accurate in determining the body temperature. In the present study we measured the temperature with the same frequency as the animals were routinely observed, twice a day, although we consider this is a rather low level of observation for such experiments.

In the model of *K. pneumoniae* infection, the condition of the animals deteriorated rapidly when temperatures fell below 36°C, and the time between this cut-off point and coma was very short—a matter of hours rather than days. When a lower cut-off point is chosen, on the safe side as proposed by Soothill *et al.* (1992) at 34°C, only 24 h was gained. The results in our model showed that when the infection was even more aggressive and using a 34°C endpoint, only 12 h of suffering could be prevented. Using higher endpoints such as 36°C, decreased sensitivity, again 24 h was gained. However, if the sensitivity is decreased the number of animals per group will have to increase.

Our results show that temperatures taken either subcutaneously or intraperitoneally do not differ significantly, despite the fact that intraperitoneal, rather than subcutaneous would better reflect the core temperature of the animal. Subcutaneously implanted chips were read more easily by the data scanner, and so we consider subcutaneous implantation is preferable.

From the results of this study we conclude that the ELAMS™ data system is practical and accurate in measuring body temperatures and can be of great help in designing humane endpoints in infection experiments. While the temperature transponders are relatively expensive (approximately US $10 each) they can be re-used and so the costs can be reduced, particularly when considering the

gains in accuracy of the experiment and the well-being of the animal. We hope the manufacturer will be willing to deliver separate polypropylene caps for the transponders as these will need replacing with time.

References

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