Remote monitoring of experimental endpoints in animals using radiotelemetry and bioimpedance technologies

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Summary

Advances in radiotelemetry and bioimpedance technology are providing improved and more humane approaches for monitoring physiological functions in experimental animals. Telemetry systems consist of miniaturized sensors and transmitters which detect and transmit pressures, flows, temperatures, pH, and electrical potentials to remote receivers. Three general types of systems are currently available: (1) fully implantable systems; (2) partially implanted systems (in which animals carry non-implanted components in backpacks); and (3) capsule systems which traverse the gastrointestinal tract. Currently, the technology can be applied in all commonly used laboratory species from mice to monkeys. Non-invasive bioimpedance technologies can also be used to monitor several physiologically important parameters including cardiac output and total body electrical conductivity (TOBEC, an index of lean body mass). The feasibility, validity, and utility of TOBEC to monitor changes in body composition is demonstrated in Sprague-Dawley rats. Telemetry and bioimpedance technologies are replacements for traditional non-survival procedures that can substantially improve animal welfare and reduce animal use in laboratory research.

Comprehensive evaluations of physiological functions in experimental animals have traditionally required invasive techniques. In the last 50 years, acute (non-survival) techniques have given way to increasingly sophisticated, surgically prepared chronic animal models. In these models, catheters, cannulae, electrodes, and electronic probes are implanted and connectors exteriorized so that the animals can be readily connected/disconnected from monitoring equipment (see Gellai & Valtin 1979). Because these sophisticated models afford multiple and prolonged measurements in individual animals over extended periods of time, and potentially allow the re-use of animals in multiple studies, they have resulted in substantial reductions in animal use which have offset, in part, the additional costs associated with the preparations. These models have also afforded quantal improvements in the quality and quantity of experimental data collected. Collectively, the development of sophisticated procedures permitting the chronic monitoring of physiological parameters exemplifies the commitment of modern animal researchers to the principles of reduction and refinement of animal use (Russell & Burch 1959).

A weakness of the early chronic animal models was the vulnerability of the transcutaneous connectors, which introduced avenues for infection and were liable to accidental damage. The presence of externalized cannulae and connectors virtually requires that these animals be single-housed and closely monitored for the duration of their experimental utility. These factors limited their utility in pharmaceutical safety assessment (introduction of ancillary pathology, co-administration of antibiotics, etc.), created potential animal welfare issues and added significantly to overall costs.

The limitations of the early models have been leveraged through refinements, which

left cannulae and connectors in sterile subcutaneous pockets, when not in use. The vascular access port was successfully adapted for subcutaneous arterial, venous, and biliary access, and has also been used for the inflation of vascular cuffs (Mann *et al*. 1987, 1991). For more sophisticated technologies, miniaturized sealed connectors have been positioned in subcutaneous pockets which can be accessed for experimental measurements through a small incision (under local anaesthetic) in a miniaturized sterile field (Kinter *et al*. 1994). Transcutaneous connectors (or studs) are reported to minimize the risk of infection and, when properly placed, are not damaged by the animal. The osmotic infusion pump was the first fully implantable infusion technology widely used in both rodent and non-rodent species (see Webb *et al*. 1998a,b) Without the risk of animal damage to external equipment, animals are generally group-housed, reducing the concerns of some welfarists that reductions in animal use were being achieved at the expense of reductions in animal welfare. However, these models still required substantial human interaction during study procedures to collect physiological data, with encumbant stress factors, model limitations, and research costs.

The removal of most remaining animal–human experimental interactions has been accomplished through the development of 'wireless' reporting technologies, including radiotelemetry and bioimpedance applications. Several of these technologies and their potential impact on animal study designs and animal welfare are briefly discussed in this paper.

Implantable radiotelemetry systems

Fully implantable miniaturized telemetry systems are the state of the art in radiotelemetry (Brockway & Hassler 1993). These systems consist of one or more sensors (fluid-filled cannulae, catheters, electrodes) connected to hermetically-sealed transducer-radiotransmitters and power supplies. The transmitter broadcasts the signal from the transducer to a remote antenna, generally located within or near the animal's cage or experimental set-up. Following their recovery from surgery to implant the electronics, telemeterized animals can be maintained and even studied while in their 'home' laboratory environment. The range of laboratory species for which telemetric monitoring has been reported spans from mice to monkeys (Schnell & Wood 1993, Brackee *et al*. 1995, Kinter *et al*. 1997, Brockway *et al*. 1998). Physiological parameters routinely monitored using telemetry include blood pressure, heart rate, ventricular pressures, ECG, EMG and body temperature. Respiratory rate and animal activity can be monitored indirectly. Recently, Murphy and colleagues (1998) have reported the telemeterization of respiratory pressures using a novel variant of the oesophageal balloon procedure. The useful lifetime of these systems ranges from several months to over a year depending upon the electrical power requirements and power supply restrictions.

Partially implantable telemetry systems include those in which hard-wire transcutaneous connections are maintained. In these systems, sensors are implanted surgically and are connected to transmitters and/or power supplies which are carried externally in a jacket worn by the subject. These systems permit the use of electronic devices with higher power requirements, greater transmission ranges, and unlimited battery life, than can be accommodated by currently available fully implantable systems. However, they are susceptible to many of the weaknesses of the previous hard-wire technologies.

Capsule telemetry systems

Variants of the implantable radiotelemetry systems include capsule telemetry systems (Anon 1990, Mojaverian 1996). These systems include a miniaturized sensor, a transmitter and a power supply sealed in an enterically compatible capsule. After the capsule is activated and swallowed, it traverses the gastrointestinal tract. Systems

that detect pH can be used to monitor gastric pH, gastric emptying time, intestinal pH and intestinal transit time. Similar systems have been used to monitor core body temperatures in thermally unstable individuals, and in remote situations (e.g. Astronaut J. Glenn in 1998). The primary drawback of these systems today is the low transmission distance such that the subject may need to wear or lie upon the antenna. While the applications for capsule telemetry systems are somewhat limited, compared with implantable systems, their robust and non-invasive nature and the fact that their use requires no surgery, offer advantages in preclinical safety assessment.

Transponder telemetry systems

Transponder telemetry systems are miniature systems powered intermittently using external inductance technologies. While this approach has the theoretical advantage of indefinite use following implantation, transponders are restricted to very short transmission ranges, usually requiring some type of animal/human interaction (e.g. to wand the transponder). The most common of these systems are currently used for individual animal identification (animal number transponders) and for the monitoring of biopotentials (e.g. body temperature, heart rate).

Bioimpedance cardiography

Bioimpedance systems take advantage of biological current flows through endogenous or exogenous magnetic fields. For example, non-invasive bioimpedance cardiography employs a small current flow introduced across the thorax; changes in thoracic impedance are related in part to changes in the volume and velocity of aortic blood flow. The impedance changes during the cardiac cycle are used to determine the stroke volume from which cardiac output can be calculated. The technology was developed to monitor haemodynamic changes during space flight and was subsequently commercialized for both clinical and animal research purposes (see DePasquale & Fossa 1996). The technique currently requires the temporary placement of surface electrodes (similar to ECG electrodes), but potentially replaces the more invasive aortic flow probe preparations, and invasive thermal and dye-dilution techniques. Bioimpedance cardiology may in the future be combined with telemetry technology for completely wireless monitoring.

Total body electrical conductivity (TOBEC)

Total body electrical conductivity provides a non-invasive and non-destructive means for the estimation of lean body mass (Walsberg 1988). Mechanically restrained or lightly anaesthetized animals are temporarily inserted into a low energy electromagnetic field; the magnitude of the current flow induced by the magnetic inductance in the animal's body is related to its lean mass. By measuring the change in the inductance of the current flow in the applied magnetic field, caused by the induced current flow in the animal, the electrical conductivity of the animal's body may be calculated and its lean body mass estimated. The technique potentially replaces current technologies that are labour-intensive, require expensive and specialized equipment, lack sensitivity, and are not suitable for monitoring changes in the body composition of live laboratory animals.

The validity and utility of TOBEC technology has been demonstrated in laboratory (Sprague-Dawley) rats (Kinter *et al.* 1993, Dowling *et al.* 1994). In these studies, TOBEC was determined using a small animal body composition analyser (Model SA-2, EM-SCAN, Springfield, Illinois, USA). Rats were anaesthetized (ketamine/xylazine [40/5 mg/kg, i.p.] or methohexital [50 mg/kg, i.p.]), weighed and measured (nasal–anal length in cm). TOBEC was determined with rats in a supine position, using the 'Fixed' mode of instrument operation, according to the manufacturer's instructions. Five to 10 TOBEC measurements were made in rapid succession on each object or animal at each observation point and the average value used for subsequent calculations. After recovering from anaesthesia, the rats were returned to their cages. All numerical data are expressed

as means ± standard error of the mean (SEM). Body weights and body compositions were analysed using a one-way ANOVA followed by Dunnett's multiple comparisons. Comparisons of TOBEC and reference methodologies were performed using linear regression and Pearson correlation coefficients.

To validate TOBEC for the estimation of body composition, carcasses from 65 male Sprague-Dawley rats weighing from 40 to 450 g (data not shown) and 30 randomly selected male and female rats fed a certified rodent chow either *ad libitum* or approximately 70% of *ad libitum* (diet optimized, DO) (7–8/sex per group, see Fig 1) were analysed for lean body mass and body lipid content by carcass extraction and the results were compared to a pre-necropsy TOBEC measurement (Kinter *et al.* 1993). The correlation coefficients for lean and non-lean body masses estimated by TOBEC and the reference method were >0.99 and >0.90, respectively. The slopes of the relationships were approximately 1.0, and passed very close to the origin. Overall, the correlations between the body composition measurements estimated by the TOBEC and the reference methods in both *ad libitum* and DO rats were highly significant ($P < 0.001$) over a physiological range of body sizes and compositions.

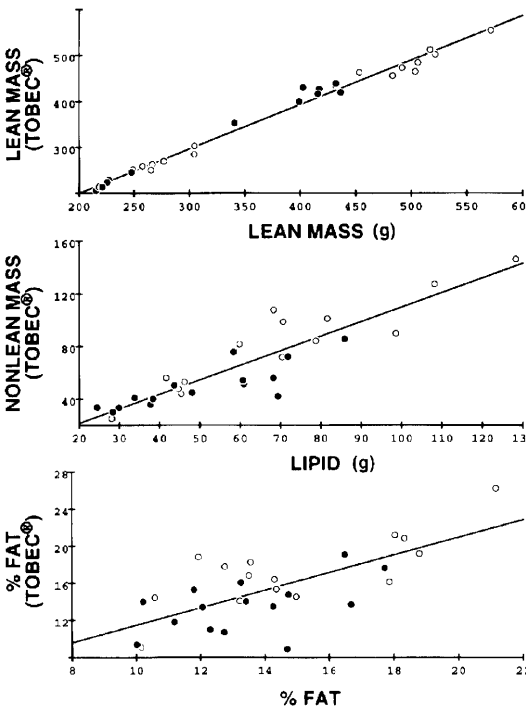

Fig 1 The relationships between lean mass, non-lean mass and % body fat estimated by TOBEC (vertical axes), and lean mass, lipid mass and % lipid estimated by the reference method (horizontal axes) in 23-week old *ad libitum* (open circles) and DO (closed circles) male and female Sprague-Dawley rats after 14 weeks on either regimen. The correlation coefficients are 0.993, 0.903, and 0.702, respectively. Regression analyses show a high degree of correlation for both lean and non-lean mass values estimated using these two methods ($P < 0.0001$). Data from Kinter *et al.* (1993)

Clenbuterol is a β-adrenoceptor agonist that increases body weight and protein deposition and decreases the rate of fat deposition in rats (Carter *et al.* 1991). Male Sprague-Dawley rats (10/group) were lightly anaesthetized and TOBEC was measured on two occasions prior to treatment and four additional occasions during and following treatment (2.0 mg/kg per 24 h clenbuterol or 0.5 µl/h sterile water, i.p., for 14 days using an osmotic minimump; Dowling *et al.* 1994). The effects of clenbuterol on body composition is shown in Fig 2. All the rats gained body weight and lean body mass over the course of the study; however, clenbuterol-treated rats gained approximately 65 g ($\sim$13%) more body weight/lean mass than did the control rats after 2 weeks of treatment ($P < 0.05$). Mean body weight in the clenbuterol-treated rats remained increased, although no longer statistically significantly, 2 weeks following the cessation of drug treatment. The protein-sparing effect of clenbuterol appears to be due to a reduction in protein catabolism resulting from the inhibition of loss of specific mRNAs (Babij & Booth 1988, Rogers & Fagan 1991).

The principal pitfalls of TOBEC measurements are that they are sensitive to the size, geometry, and positioning of the subject. The present studies show that these variables are controlled when lightly anaesthetized rats are positioned consistently in the instrument. Anaesthesia can be avoided if the rats are mechanically restrained in a simple disposable plastic cone (Decapi Cone, Braintree

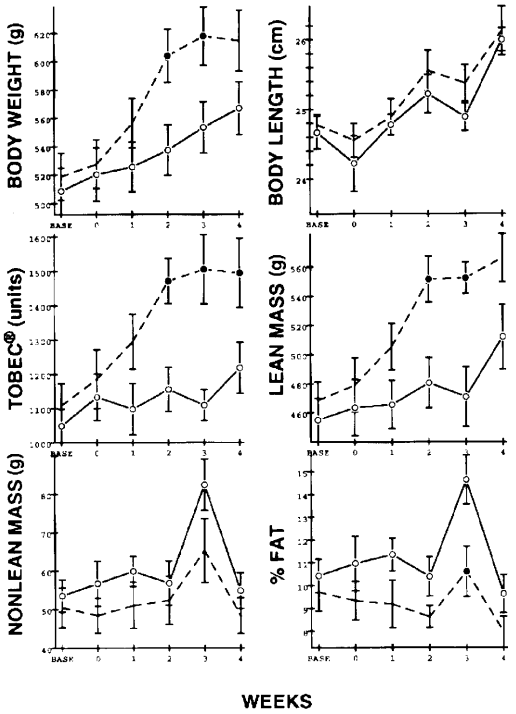

Fig 2 Effects of 2-week clenbuterol treatment on body composition of male Sprague-Dawley rats. TOBEC measured on two occasions prior to dosing (pre-treatment; weeks BASE and 0). Rats were then given clenbuterol (2.0 mg/kg per day; broken line) or vehicle (sterile water; 0.5 μl/h; open symbols/single line) for 2 weeks (weeks 1 and 2). An additional 2 weeks of recovery (weeks 3 and 4) was allowed. Body weight, body length, TOBEC (conductivity index units), lean mass, non-lean mass, and % body fat were determined weekly. Values are means; closed symbols represent statistically significant differences from the vehicle control ($P < 0.05$; $n = 8$/group). The results show that TOBEC analysis is able to detect the pharmacological effect of clenbuterol to selectively increase lean body mass in Sprague-Dawley rats. Data from Dowling *et al*. (1994)

Scientific, Braintree, Massachusetts, USA) to preserve a constant orientation and geometry (unpublished findings). Finally, lean body mass detected by TOBEC may offer an alternative denominator (instead of body weight) for the normalization of the organ weight data without the potential confounding factors of differences in body fat content. Lean body mass reflects a more homogeneous body component than does terminal body weight and is not susceptible to differences in body fat content. Lean body mass is also a relatively large mass, and tissue/lean body mass ratios are less susceptible to errors associated with ratios with very small denominators (e.g. tissue/brain weight ratios).

Discussion

Modern telemetry and bioimpedance technologies offer opportunities substantially to reduce and refine animal use and to reduce research costs. Consider the savings when telemetry or bioimpedance technologies are used to replace destructive techniques (e.g. carcass analysis), or are used in conjunction with traditional study endpoints (e.g. a single dose, dose-ranging, or repeat-dose toxicology study). The study illustrated in Fig 2 represents more than an 80% reduction in animal use using TOBEC, compared with the same study design using traditional carcass analysis. When physiological and toxicological data are gained from one set of animals, the data complement one another, and the need for separate studies (e.g. safety pharmacology studies) is eliminated. A decrease in blood pressure, increase in heart rate, electrocardiogram change, or other physiological response may provide direct evidence of a dose-limiting effect (Morgan *et al*. 1994, Kinter *et al*. 1997), eliminating the need to evaluate higher doses for toxicity, which saves animals, time, and other resources.

Because telemetry and bioimpedance technologies permit continuous or periodic data collection over prolonged periods of time, they are compatible with the use of randomized block study designs in place of conventional completely randomized study designs. Blocking works to filter out the inter-animal variation, reducing the number of animals needed to obtain the same level of statistical power for evaluating dose/treatment responses (Snedecor & Corcoran 1980, Festing 1994). In a conventional completely randomized study design, there are four groups of animals, with each animal in each group receiving one treatment. In a randomized block design, there is one group of animals with each animal receiving each treatment in a randomized fashion. The

assumptions necessary to use the randomized block designs are that a sufficient washout period can be incorporated between treatments to minimize or prevent carryover. A variant of the randomized block design is one in which all animals receive all treatments, but in an ascending order (e.g. vehicle, then low, mid, and high dose). This design has the same improved statistical power as the randomized block design, but may require an additional time control to separate treatment effects from time effects. For comparable levels of power and error, the use of the randomized block design provides for up to a 75% reduction in the numbers of animals needed.

Because of the prolonged lifetime of telemetry and bioimpedance systems, investigators may consider reusing study animals to study additional doses, different treatments, or alternate routes of administration, or to verify individual response differences. The principle in the reuse of animal preparations is to define objective criteria with which to re-qualify and schedule animals for additional studies in a proactive fashion. Requalification criteria should include the following considerations:

(1) A veterinary examination.
(2) Haematology, serum biochemistry, urinalysis.
(3) Blood pressure, heart rate, ECG, and other endpoints.
(4) Clinical history from prior studies.
(5) Sufficient time for the washout of previously administered test substances.

Items 1, 2, and 3 are used to verify the health and welfare of the test animal and the proper function of the telemetry or bioimpedance system. Item 4 requires the investigator to review all experimental records to ensure that critical physiological systems were not inadvertently compromised in preceding studies. This is particularly important when telemeterized preparations are to be reused in safety studies. Items 3 and 4 may be facilitated through the periodic evaluation of pharmacological responses to standard agents (Table 1). Item 5 is to permit sufficient time for the recovery from previous procedures and the clearance of previously administered drugs. As a general rule, individual telemeterized animals should be left to recover for at least 7 days between studies.

In conclusion, telemetry and bioimpedance technologies are replacements for traditional non-survival procedures which permit the intermittent repeated or continuous monitoring of laboratory animals maintained (for the most part) in their home environments. In addition to improvements in animal welfare, the overall reductions in animal use that may be achieved using telemetry and bioimpedance technologies are as follows. Reductions achieved by eliminating an extra study or study group are at least 50%. Reductions achieved by using randomized block study designs in place of completely randomized designs are 75%, depending upon the inherent pure error variation (σ) and the power required. Further reductions achieved by requalifying and re-using instrumented animals in additional studies are a factor of

Table 1 Standard agents and doses for assessments of stability of pharmacological responses in instrumented dogs

Agent (route)	Dose (mg/kg)	Anticipated response
Chromokalim (p.o.)	0.1	Hypotension with tachycardia
Hydralazine (p.o.)	10	Hypotension with tachycardia
Nitrendipine (p.o.)	3	Hypotension with tachycardia
Haloperidol (p.o.)	3	Sedative, with little or no CV effects
Clonidine (p.o.)	0.03	Hypotension with tachycardia
Epinephrine (i.v.)	0.003	Hypertension with bradycardia
Isoproterenol (i.v.)	0.003	Hypotension with tachycardia
Fenoldopam	0.003	Hypotension with venal vasodilation

Values are unreported data from our laboratories, and those of Dr S. Pettinger. The doses are used to establish responses in individual animals, to monitor those responses for changes over time and exposure to multiple studies

two per additional study. In our laboratories, we have achieved total reductions through eliminating extra studies and study groups, using randomized blocking, and re-using animals in multiple studies in excess of 90%, compared with past practices.

Acknowledgments The authors acknowledge the technical assistance of Keith Dowling, William A. Mann, W. Keller, A. Silver, and J. Ventre (SmithKline Beecham Pharmaceuticals), Dr R. Martin (University of Georgia), C. Metz (EM-SCAN Inc., 3420 Constitution Drive, Springfield, Illinois, USA) and support from Drs T. Leonard, D. J. Meyer, W. D. Kerns, and D. G. Morgan. Clenbuterol was prepared by SmithKline Beecham chemists. We thank B. Clemmer for technical assistance in preparing the manuscript.

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