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Metabolic Cage Isolation Reduces Antipyrine Clearance in Rats

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Abstract—Rats are commonly isolated individually in cages during pharmacokinetic studies. However, isolation-induced changes in drug disposition are not commonly examined. Antipyrine is a marker of hepatic oxidative function and total body water. The purpose of the study was to investigate the effect of individual housing on antipyrine pharmacokinetics. Rats were individually housed in either standard polycarbonate boxes (n = 8) or metabolic cages (n = 10). On day 1 and day 9 rats were administered a single intravenous bolus injection of antipyrine 20 mg kg^{-1}. Blood samples (100 μL) were obtained before and at 20, 40, 60, 90, 120, 180, 240, 300 and 360 min following the administration of the dose. Rats remained in their respective cages between evaluations. Serum antipyrine concentrations were determined by capillary electrophoresis. Pharmacokinetic parameters were estimated by model-independent methods. Antipyrine clearance was reduced by 38·4% in rats isolated in metabolic cages for eight days ($P = 0{\cdot}013$) while the volume of distribution remained unchanged in both rat groups. These data suggest that the isolation of rats in metabolic cage systems may markedly alter the pharmacokinetics of xenobiotics, thus possibly masking experimental outcome.

Individual housing of rats, commonly termed isolation, is often used in pharmacokinetic studies. An advantage of using individually housed rats is the ability to monitor accurately food and water intake. In addition, isolation allows for the use of chronic instrumentation without the risk of interference from cage-mates. The use of individual metabolism cages allows measurement of urine and faeces output of single rats, and for the collection of excrement. Rats are often isolated for extended periods of time to facilitate timed measurements of excretory functions, or because of study-design constraints such as chronic cannulation. However, individual housing in metabolic cages may have untoward effects on rat physiology by altering circadian rhythms, limiting coprophagy, or disrupting thermoregulatory mechanisms.

Metabolic cage isolation has been shown to decrease creatinine clearance, urine flow rate, and fractional reabsorption of sodium in rats (Vadiei et al 1990). Interestingly, this effect differed among the same strain of rats from four different breeders. Thus, isolation-induced changes in rat physiology may not be uniform or predictable. To date, studies of the effect of metabolic cage isolation on drug metabolism are not readily available.

Antipyrine disposition has been widely used to investigate the influence of disease (Zysset & Wietholtz 1988), xenobiotics (Anadón et al 1990), and diet on drug metabolism (Kappas et al 1976). Antipyrine has a low hepatic-extraction ratio and total body clearance is independent of hepatic blood flow. Antipyrine is almost exclusively metabolized by hepatic oxidative microsomal enzymes and the determination of antipyrine clearance provides a measure of cytochrome P450 metabolizing activity.

The purpose of the present study was to investigate the effect of individual housing of rats in metabolic cages on the pharmacokinetics of antipyrine.

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Materials and Methods

Animals

Eighteen male Sprague-Dawley rats (300–340 g, Harlan Sprague Dawley, Inc., Indianapolis, IN) were housed in a 12-h light/dark cycle animal facility with controlled temperature and humidity. Access to deionized water and standard laboratory rodent diet (Harlan, Indianapolis, IN) was unrestricted throughout the study unless otherwise noted. Only investigators and animal-care personnel were allowed access to the animal room. All procedures were approved by the Committee for Animal Use for Research and Education at the Medical College of Georgia before the initiation of the study. All procedures were in accordance with the guidelines established by the National Institutes of Health for the humane treatment of animals.

Housing

Two different cage systems were used in the study. One system was a standard clear polycarbonate box (Model 660 Db, Nalge Company, Rochester, NY) with a wire cage lid (Model 665 Db, Nalge Company, Rochester, NY) which incorporated a food dispenser. Dimensions of the polycarbonate boxes were 43 × 27 × 19 cm (length × width × height). Water was dispensed by a polycarbonate bottle with sipper tube. The floor of the standard box cage was covered with 1 cm absorbent bedding (Bed-O'Cobs/Andersons, Maumee, OH). The use of corncob bedding has been shown not to alter microsomal oxidative function (Weichbrod et al 1988). The other cage system was a large rodent metabolism cage (Model 650–0350, Nalge Company) consisting of a clear polycarbonate domicile, excrement collection system, water dispenser, and wire-grid floor. Food was placed onto the wire-grid floor. The domicile of the metabolic cage was circular with an internal diameter of 24 cm and a height of 18 cm.

Experimental procedure

Animals were acclimatized to the animal care facility for three days before the start of the study. Following the acclimatization period, catheters were surgically implanted under anaesthesia into the right external jugular vein and exteriorized at the base of the skull. Rats were allowed to recover in polycarbonate boxes for three days following catheter placement. Catheters were flushed once daily with 0·2 mL physiological saline containing 25 units mL^{-1} sodium heparin. On the third day, rats were placed into standard clean polycarbonate boxes (STD, n = 8) or metabolic cages (METAB, n = 10), with unlimited access to food and water. Rats from each group were housed separately. On the following day, rats were weighed, returned to their respective cages and administered a single intravenous dose of antipyrine (20 mg kg^{-1}) at a rate of 1 mL min^{-1} via the jugular catheter. The volume of antipyrine solution administered was 1 mL kg^{-1}. The catheter was then immediately flushed with 0·5 mL saline. Serial blood samples (100 μL) were obtained from the jugular catheter before and 20, 40, 60, 90, 120, 180, 240, 300 and 360 min following antipyrine administration. Blood samples were allowed to clot at ambient temperature and serum separated by centrifugation (3000 *g* for 5 min). Samples were stored at −70°C until analysed (within two weeks). Rats remained in their respective cages for eight additional days. On the final day, a similar antipyrine administration with serial blood sampling as described above was performed. Following the last blood sample, rats were administered a single lethal dose of intravenous pentobarbitone (100 mg kg^{-1}).

Sample analysis

Serum antipyrine concentrations were determined by capillary electrophoresis. In brief, analysis was performed using a commercially available capillary electrophoresis apparatus (CES-I, Dionex Corporation, Sunnyvale, CA). Separations were performed in an untreated 70-cm fused silica capillary (Dionex Corporation), with an internal diameter of 75 μm. The exterior of the capillary was coated with polyimide. The total length between injection and detection was 65 cm. Sample introduction was accomplished by gravity injection at 100 mm for 10 s. For all analyses, a pH 8·2 buffer system consisting of 10 mM sodium tetraborate, 50 mM boric acid, and 50 mM sodium dodecyl sulphate was used. All assays were performed at ambient temperature with a detection wavelength of 242 nm. Serum samples were mixed on a vortex, then a 25 μL aliquot of each sample was transferred into a polypropylene microcentrifuge tube. To each aliquot, 100 μL cold acetonitrile containing paracetamol (2·5 μg mL^{-1}) as the internal standard was added. The mixture was vortex-mixed for 15 s, and then centrifuged at 3000 *g* for 5 min. Following centrifugation, the supernatant was removed and placed into another polypropylene microcentrifuge tube. The samples were dried under a filtered air stream at ambient temperature for 2 h. Samples were then reconstituted with 25 μL deionized water and analysed. Peak height data was collected and analysed with the use of a chromatographic computer software system (Baseline 810, Millipore Co., Ventura, CA). The ratio of antipyrine peak height to internal standard peak height was used for the construction of the standard curves and for the determination of antipyrine concentration in the analytical samples. Intra-day and inter-day variability of the assay ranged from 0·7 to 8·1% over the concentration range tested (0·5–25 μg mL^{-1}).

Data analysis

Antipyrine pharmacokinetic parameters were determined by standard noncompartmental methods (Gibaldi & Perrier 1982). Areas under the antipyrine serum concentration-time curve (AUC) and the first moment concentration-time curve (AUMC) were estimated by recursive least-squares analysis with 1/*y* weighting (Rstrip, MicroMath, Salt Lake City, UT). Weighting of 1/*y* was found to improve the model selection criterion (Rstrip, MicroMath). Area estimations were extrapolated to infinity. Mean residence time (MRT) was calculated as the ratio of AUMC to AUC. The theoretical maximum concentration (C_{max}) was estimated as the concentration at the intercept between the line estimating the exponential decline in drug concentration and the dependent variable axis (time = 0). Total body clearance (CL) was determined by the following equation:

$$CL = \frac{D_{i.v.}}{AUC} \quad (1)$$

where $D_{i.v.}$ represents the weight-corrected intravenous dose of antipyrine.

Steady-state volume of distribution (Vd_{ss}) was calculated by:

$$Vd_{ss} = \frac{D_{i.v.} \cdot AUMC}{AUC^2} \quad (2)$$

Model-independent determination of the best-fitting exponential equations describing the concentration-time profiles was defined by the greatest value for the model selection criterion.

Differences between groups were compared using unpaired Student's *t*-test. Differences within groups between baseline and the end of the study were compared using paired Student's *t*-test. Changes in weight between groups over the study period were compared using analysis of variance with repeated measures. Differences were considered significant when the probability of chance explaining the results was reduced to less than 5% ($P < 0·05$).

Results

Total body weight was not significantly different between rat groups at the beginning and at the end of the study period ($P = 0·069$ and $P = 0·541$, respectively). By the end of the study period, STD rats had an 8·1% increase in body weight ($P < 0·001$), and METAB rats had a 3·9% increase in body weight ($P = 0·008$) (Table 1). However, weight gain during the study period was significantly less in METAB as compared with STD rats ($P = 0·049$) (Fig. 1). Pharmacokinetic parameters following the first dose of antipyrine were not significantly different between rat groups ($P = 0·092$–$0·798$, Table 1). At the end of the study period antipyrine AUC in METAB rats had increased by 25·1% ($P = 0·014$) as compared with baseline values, whereas AUC was not significantly different in STD rats ($P = 0·548$) as compared with baseline. Antipyrine AUC in METAB rats was 43·5% greater than STD rats at the end of the study ($P < 0·001$). No

Table 1. Antipyrine pharmacokinetic parameters at baseline and at the end of the study period following a single intravenous dose of antipyrine (20 mg kg^{-1}) in rats isolated in standard polycarbonate boxes (STD) or metabolic cages (METAB). Data are mean ± s.d.

Parameter	Isolation group			
	STD		METAB	
	Baseline	End of study	Baseline	End of study
Weight (g)	326 ± 9	346 ± 8[1]	337 ± 13	349 ± 13*
AUC (μg min mL^{-1})	3388 ± 401	3342 ± 467	3834 ± 1113	4797 ± 686*†
MRT (min)	154 ± 16	147 ± 15	184 ± 47	217 ± 47*†
C_{max} (μg mL^{-1})	26·0 ± 3·9	29·0 ± 6·4	27·4 ± 14·7	37·6 ± 30·3
CL (mL min^{-1} kg^{-1})	5·98 ± 0·71	6·09 ± 0·86	5·63 ± 1·62	4·25 ± 0·60*†
Vd_{ss} (mL kg^{-1})	912 ± 103	888 ± 69	978 ± 120	898 ± 100

* $P < 0·05$ compared with baseline values. † $P < 0·05$ compared with STD group at the end of the study period.

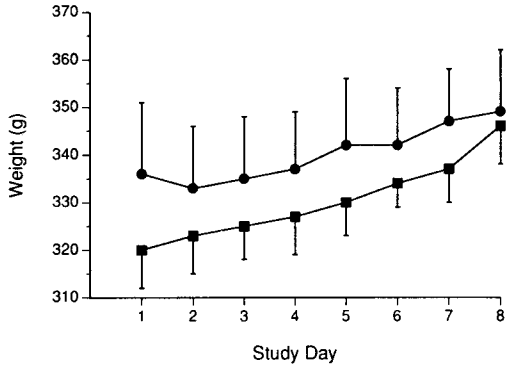

FIG. 1. Daily weight measurements of rats housed in standard polycarbonate boxes (■) or metabolic cages (●) over the study period. Data are mean ± s.d.

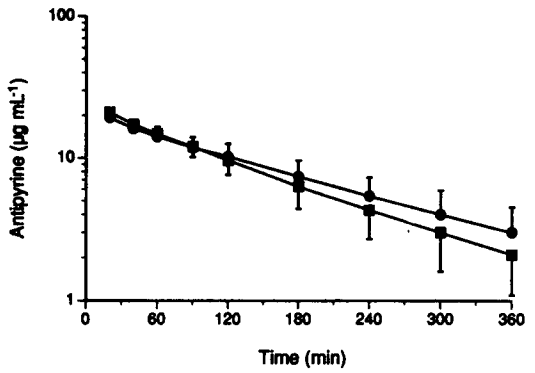

FIG. 2. Serum concentration-time profile of a single intravenous dose of antipyrine (20 mg mL^{-1}) at baseline in rats housed in standard polycarbonate boxes (■) or metabolic cages (●). Data are mean ± s.d.

significant differences in antipyrine MRT were found between groups at baseline ($P = 0·092$). Antipyrine MRT increased by 17·9% in METAB rats by the end of the study ($P = 0·031$); however, MRT was not significantly different in STD rats ($P = 0·268$). Antipyrine MRT was 47·6% greater in METAB rats as compared with the STD rat group at the end of the study period ($P = 0·001$). Antipyrine clearance was 5·8% less in METAB rats as compared with STD rats at baseline ($P = 0·581$). At the end of the study, clearance had decreased by 38·4% in METAB rats ($P = 0·013$), but clearance had not significantly changed in the STD rats

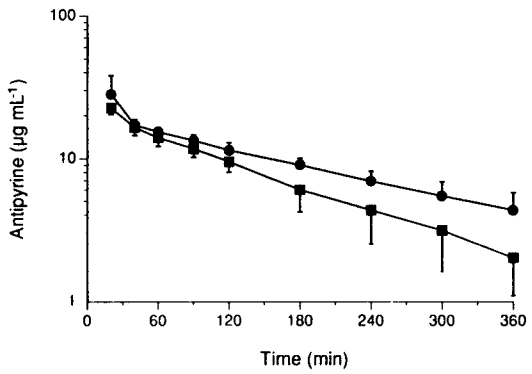

FIG. 3. Serum concentration-time profiles of a single intravenous dose of antipyrine (20 mg kg^{-1}) in rats following eight days of housing in standard polycarbonate boxes (■) or metabolic cages (●). Data are mean ± s.d.

($P = 0·472$). However, Vd_{ss} and C_{max} were not significantly different between groups or from the beginning of the study period ($P = 0·196$–$0·800$ and $P = 0·204$–$0·798$, respectively). Antipyrine concentration-time profiles at baseline and the end of the study period are given for both rat groups in Fig. 2 and Fig. 3, respectively. As determined by in-vitro analysis, antipyrine was not found to bind to jugular catheters (data not shown).

Discussion

Animals are commonly isolated in individual cages to perform animal-specific measurements (Vadiei et al 1990), examine chronobiologic variability (Luke et al 1988), or to prevent contact with other animals (Fagin et al 1983). However, recent investigations have questioned the use of isolation due to the alteration of normal physiologic functions (Everitt et al 1988; Greco et al 1989; Vadiei et al 1990).

In the present study, rats housed in individual metabolism cages showed a decreased growth rate over an eight day period as compared with rats housed in standard polycarbonate boxes. Furthermore, METAB rats had an initial decline in total body weight which was not found with the STD rats. However, these differences in growth rate may only occur during short-term investigations. Welch et al (1974) showed that rats maintained in individual wire-mesh cages for one year had a 10·3% greater increase in total body weight as compared with rats housed individually in plastic solid-

bottom cages. Thus, the decreased growth rate in METAB rats may be due to short-term isolation stress which may not be present during long-term investigations.

The prevention of coprophagy in rats can result in a depletion of vitamin B_{12}, vitamin K, biotin, riboflavin, pyridoxine, thiamine, pantothenic acid, and essential fatty acids (Barnes 1962). Rats which are not nutrient deficient will ingest 30–65% of excreted faeces. The relative percent of faeces consumed increases in the presence of nutrient deficiency (Barnes 1962). The collection of faeces in the metabolic cage system reduces the extent of coprophagy. Thus, chronic housing in individual metabolic cages may predispose the rat to nutrient deficiency, leading to altered drug disposition (Statham et al 1985; Mehta 1990; Bredberg & Paalzow 1990; Cusack et al 1992).

Rodent metabolic cages are constructed with a metal grid floor which allows for the passage of urine and faeces without significant obstruction. However, this floor also allows for the dissipation of body heat from the rat through the grid. This effect is not present in standard polycarbonate boxes with a solid bedding-covered floor. Thus, the type of cage flooring may influence the thermoregulatory activity of the rat.

Hydralazine-induced hypothermia has been shown to decrease the systemic clearance of antipyrine in rats, but when rats were temperature-regulated, hydralazine was not found to change antipyrine clearance (Svensson et al 1987). The alteration in antipyrine clearance appeared to be due to hypothermia rather than the concurrent administration of hydralazine. In dogs, both propranolol clearance and volume of distribution were decreased during hypothermia (McAllister et al 1979). In addition, decreased antipyrine clearance with no change in volume of distribution was found in man following exposure to hypothermia (Shepard et al 1976). Body temperature was not monitored in the present study, thus it is not known whether a state of hypothermia existed.

Antipyrine has been extensively used as a marker compound for hepatic oxidative metabolism and for the estimation of total body water in man and in animals (Vesell 1979; Vickers et al 1989). Following the initial dose, antipyrine pharmacokinetic parameters were not significantly different between rat groups. Following eight days of isolation, rats individually housed in metabolic cages had an increase in the AUC and MRT, and a corresponding decrease in CL as compared with baseline values and with STD rats at the end of the study. However, Vd_{ss} was unchanged in both rat groups over the study period. Thus, the data suggest that there was a decrease in the oxidative metabolism of antipyrine in METAB rats. Antipyrine is not significantly bound to plasma or tissue proteins and is rapidly distributed throughout total body water. There was no change in weight-corrected Vd_{ss} in either rat group over the study period. Therefore, it is likely that no change in percent total body water occurred.

In summary, results from the present study demonstrate that metabolic cage isolation results in a decrease in growth rate and system clearance of antipyrine in rats following eight days of individual housing. Mechanisms of the decreased antipyrine clearance in rats individually housed in metabolic cages may include isolation-induced stress, nutrient depletion, hypothermia, or a combination of these factors. Further investigations are warranted to establish the mechanism of metabolic cage-induced changes in antipyrine clearance.

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