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Report

Differential Effects of Anesthetic Regimens on Gentamicin Pharmacokinetics in the Rat: A Comparison with Chronically Catheterized Conscious Animals

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The intravenous disposition of gentamicin was compared in the conscious chronically catheterized rat with that in rats anesthetized using five injectable laboratory anesthetics. Gentamicin plasma clearance in the conscious rat was significantly higher than in animals anesthetized with urethane, fentanyl/fluanisone/midazolam, pentobarbitone, or ketamine/midazolam but similar to that in rats anesthetized with alphaxolone/alphadolone. Urethane anaesthesia resulted in a significantly lower gentamicin clearance than in all other groups. Gentamicin clearance in rats anesthetized with alphaxolone/alphadolone was significantly higher than in rats anesthetized with either fentanyl/fluanisone/midazolam or urethane. No significant differences in the volume of distribution of gentamicin were observed between any of the groups studied, either anesthetized or conscious. Carboxyinulin blood clearance in the conscious group was significantly higher than that with urethane, fentanyl/fluanisone/midazolam, pentobarbitone, or ketamine/midazolam but not significantly different from alphaxolone/alphadolone-anesthetized animals. The differences in carboxyinulin clearance were noted to be proportional to the differences in gentamicin clearance (r^2 = 0.98). These results demonstrate that the choice of anesthetic used in laboratory pharmacokinetic studies is important. Gentamicin clearance was higher in conscious than anesthetized rats, and it may be prudent to use chronically catheterized animals in pharmacokinetic studies.

KEY WORDS: gentamicin; carboxyinulin; pharmacokinetics; anesthesia.

INTRODUCTION

Pharmacokinetic data in the small laboratory animal are often obtained from anesthetized surgically prepared animals. Deleterious effects of general anesthesia on cardiovascular status and regional hemodynamics have been reported (1,2), and changes of blood flow can affect the disposition of xenobiotics (3,4). Therefore, pharmacokinetic data may differ in conscious chronically catheterized animals and in anesthetized surgically prepared animals.

A limited number of studies has been published examining differences in drug disposition between anesthetized and conscious animals. Higashi *et al.* (5) reported decreased elimination rate constants for gentamicin and tobramycin in rats anesthetized with pentobarbitone or ether compared to conscious chronically catheterized animals; the volume of distribution did not change. In chronically catheterized sheep, halothane anesthesia decreased the hepatic clearances of chlormethiazole (6) and pethidine (7) and the renal clearance of cefoxitin (8). The halothane-induced changes in drug disposition paralleled reductions in cardiac output and hepatic and renal blood flow compared to the preanesthetized state. Ether anesthesia similarly lowered the clearance of hexobarbitone from that seen in conscious animals (9).

Few studies are available on possible differences between laboratory anesthetic regimens in their effects on drug disposition. Urethane anesthesia, in comparison with ether inhalation anesthesia, induced dose dependency in the clearance of thiamine (10), and the clearance of carboxyfluorescein was lower in rats anesthetized with urethane than in pentobarbitone-anesthetized rats (11). Gumbleton *et al.* (12,13) reported that urethane anesthesia, in comparison with pentobarbitone or fentanyl/fluanisone/midazolam anesthesia, resulted in reductions of approximately 50% in *p*-aminohippurate clearance and 40% in carboxyinulin clearance. The clearance of these compounds is highly dependent upon renal elimination mechanisms and thus influenced by hemodynamic alterations. Little is known on the effects of other anesthetic agents on renal elimination.

In this study gentamicin was used as a model drug to investigate the influence of five laboratory anesthetic regimens on renal clearance. Gentamicin pharmacokinetics have been extensively studied in man and laboratory animals; it is not metabolized, and its clearance is highly dependent upon renal excretion mechanisms.

Since carboxyinulin clearance is comparable to that of inulin (14), it is commonly used as a measure of the glomer-

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ular filtration rate. In this study, the clearance of carboxyinulin, determined after a single intravenous dose, was used as a measure of glomerular filtration rate, a study design previously validated for inulin by Freestone *et al.* (15).

MATERIALS AND METHODS

Male Wistar rats (259 ± 20 g; $n = 5$ for each regimen) were allowed free access to water and laboratory rat chow (Grain Harvesters Ltd., Canterbury, UK) until the time of experimentation.

Anesthetic Regimens

(1) A mixture of alphaxolone (9 mg kg^{-1}) and alphadolone (3 mg kg^{-1}) (Saffan; Glaxovet Ltd., Harefield, UK) was given iv for induction. Maintenance doses (3 and 1 mg kg^{-1}) were subsequently given every 15 min.

(2) A mixture of fentanyl (0.26 mg kg^{-1}) and fluanisone (8.3 mg kg^{-1}) (Hypnorm; Janssen Pharmaceuticals, Wantage, UK) was given ip in combination with midazolam, 4.16 mg kg^{-1} ip (Hypnovel; Roche Pharmaceuticals, Welwyn Garden City, UK) for induction of anesthesia. Anesthesia was maintained by fentanyl and fluanisone (0.08 and 2.5 mg kg^{-1}) given every 30 min.

(3) Ketamine (80 mg kg^{-1}) (Vetalar; Parke Davis, Pontypool, UK) was given ip in combination with midazolam, 5 mg kg^{-1} ip, for induction of anesthesia. Ketamine (20 mg kg^{-1}) was subsequently given every 30 min for maintenance.

(4) Pentobarbitone sodium, 67 mg kg^{-1} ip (Sigma Chemical Co., Poole, UK), was given for induction; subsequent maintenance doses (7 mg kg^{-1}) were given every 60 min.

(5) Urethane, 1.75 g kg^{-1} ip (Sigma Chemical Co., Poole, UK), was given as a single dose.

The anesthetic doses used in this study were the minimum required to produce surgical anesthesia and are within the dose ranges commonly employed for laboratory anesthesia in the rat (16,17). A sufficient depth of anesthesia was judged to have been attained when the corneal reflex test and response to painful stimuli (17) were no longer elicitable.

Surgical Procedures

Once the animals had reached a sufficient depth of anesthesia the left jugular vein was exposed and catheterized (polythene tubing; id, 0.4 mm; od, 1.8 mm; code 800/100/140/100; Portex, Hythe, UK) to allow subsequent iv injection and saline fluid replacement. The right carotid artery was similarly catheterized to enable collection of blood samples. The catheters were kept patent with heparin sodium (25 IU ml^{-1}; Sigma) in saline. All surgical wounds were covered with cotton gauze kept moist with saline to minimize fluid loss. Rectal temperatures were monitored and maintained at 38 ± 1°C using an incandescent lamp and heated surgical tray. Tracheostomies were performed as an aid to respiration throughout the period of anesthesia.

As soon as the surgical procedure was complete the experiment began with the administration of either gentamicin sulfate or ^{14}C-carboxyinulin via the jugular vein.

Chronically Catheterized Conscious Animals

To enable implantation of jugular vein and carotid artery catheters the animals were anesthetized with fentanyl/fluanisone/midazolam (vide supra). The left jugular vein and right carotid artery were catheterized as described above for the anesthetized animals. The distal ends of the catheters were sealed with a removable pin (15 gauge) and exteriorized at the interscapular region of the neck. The wounds were swabbed with chlorhexidine solution and sutured. Throughout the surgical procedure rectal temperature was monitored and maintained at 38 ± 1°C using an incandescent lamp and heated surgical tray. Upon completion of the surgical procedure the animals were allowed to recover, and their progress was monitored. Blood was withdrawn from the catheters prior to all injections to prevent thrombotic emboli entering the circulation.

The experiment began 24 hr after completion of the surgical procedure with the administration of gentamicin sulfate or ^{14}C-carboxyinulin via the jugular vein. Injections and blood sampling were performed with manual restraint, subjecting the animals to the minimum possible stress.

Gentamicin Disposition

Gentamicin sulfate (≡17 mg kg^{-1} gentamicin base; Sigma) dissolved in saline was injected into the left jugular vein. Carotid artery blood samples (250 μl) were collected at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min into 1.5-ml heparinized capped plastic tubes. The blood samples were immediately centrifuged (6000*g* for 5 min) and the plasma was removed and stored at −20°C for up to 30 days until analysis. The plasma samples were thawed at 4°C, and gentamicin concentrations determined by radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). The detection limit of the assay is quoted as 0.1 μg ml^{-1}. The antibody-bound radioactivity was determined using a LKB 1275 Minigamma gamma counter (window setting, 0–80 keV; counting efficiency, 70% for ^{125}I). Unlike some enzyme-multiplied immunoassays, the radioimmunoassay appears not to be affected by the presence of heparin even at concentrations as high as 1000 IU heparin ml^{-1} (18).

Gentamicin Distribution in Blood

Separate groups ($n = 5$) of male Wistar rats received one of the five anesthetic regimens as described above or received no treatment; none of the animals were catheterized. At 90 min after injection of the anesthetic the animals were sacrificed by decaptitation, and a 5-ml blood sample was collected. Gentamicin sulfate was added to the samples to produce a concentration equivalent to 50 μg ml^{-1} of gentamicin base. The blood samples were then gently inverted for 30 min at 37°C to allow equilibration. The samples were then centrifuged and plasma gentamicin determined as described above. Plasma/blood concentration ratios (C/C_b) were determined from the measured plasma concentration and known spiked blood concentration.

Carboxyinulin Disposition

^{14}C-Carboxyinulin (0.37 μmol kg^{-1}, 4.2 μCi kg^{-1}), radiochemical purity 99% (Amersham International, Amer-

sham, UK), dissolved in saline was injected into the left jugular vein. Carotid artery blood samples (250 μl) were collected at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min into 1.5-ml heparinized capped plastic tubes. The blood samples were immediately solubilized in a 1.5-ml (1:1, v/v) mixture of Soluene 350 (United Technologies, Pangbourne, UK)/propan-2-ol (BDH Chemicals, Poole, UK) for 30 min and then decolorized with 0.5 ml of 30% (v/v) hydrogen peroxide (BDH) added in a dropwise manner. After allowing 30 min for decolorization, a 15-ml (1:9, v/v) mixture of 0.5 *M* HCl (BDH)/Instagel scintillant (United Technologies) was added. The samples were left overnight to reduce chemiluminescence and analyzed (LKB 1217 Rackbeta liquid scintillation counter) against zero-time blood samples. Correction for quenching was achieved using the external standard channels ratio.

Data Analysis

Initial estimates of volume of distribution and elimination rate constant were obtained graphically. These values were then used as initial parameter estimates for a one-compartment model in an extended least squares nonlinear regression program (19) for half-life determination ($t_{1/2}$). Model-independent analysis was also undertaken for the determination of total plasma (gentamicin) or blood (carboxyinulin) clearance (CL or CL_b) and steady-state volume of distribution (V_{ss}). Clearances were determined from CL (or CL_b) = dose/AUC, with AUC (area under the curve to infinity) by linear interpolation and extrapolation to infinity from $C_{180} * t_{1/2}/0.693$ (where C_{180} = concentration at 180 min). V_{ss} was determined from V_{ss} = dose * AUMC/$(AUC)^2$, with AUMC (area under the first moments curve to infinity) determined by linear interpolation and extrapolation to infinity from $C_{180} * 180 * t_{1/2}/0.693 + C_{180} * (t_{1/2}/0.693)^2$. Total blood clearance of gentamicin was estimated from the product of total plasma clearance and plasma/blood ratio (CL_b = CL * C/C_b).

Statistical analysis was performed using one-way analysis of variance and Duncan's multiple-range test (20). The results, where statistically different, are at a significance level of $P < 0.05$.

RESULTS

The calculated pharmacokinetic parameters for gentamicin and carboxyinulin are presented in Tables I and II, respectively. The gentamicin plasma clearance was significantly greater in the conscious chronically catheterized rat than that in all of the anesthetized groups, with the exception of the alphaxolone/alphadolone-treated animals. There was a threefold difference in gentamicin clearance between conscious and urethane-anesthetized rats. The clearance of gentamicin in the urethane rats was significantly lower than in all the other anesthetic groups. As a consequence of the differences in clearance with little variation in volume of distribution, large differences in half-life between the groups were noted (Table I).

Carboxyinulin blood clearances in the conscious and alphaxolone/alphadolone-anesthetized rats were similar, but both were significantly greater than in the fentanyl/fluanisone/midazolam and urethane groups. Carboxyinulin clearance in the urethane-anesthetized group was only one-third of that in the conscious group and was significantly lower than in the other anesthetic groups. No significant differences were observed in the volume of distribution of carboxyinulin between any of the anesthetized and the conscious groups (Table II).

The gentamicin clearances between the groups correlated (r^2 = 0.98) with the carboxyinulin clearances (Fig. 1).

DISCUSSION

This investigation demonstrates differential effects of anaesthetics on the clearance of gentamicin and carboxyinulin in the acute surgically prepared rat. The clearances in alphaxolone/alphadolone-anesthetized animals more closely reflected those in the conscious chronically catheterized rats than did any of the other anesthetic regimens. The gentamicin plasma clearance values obtained for conscious rats in this study are in close agreement with recently reported values ranging from 9 to 12 ml min^{-1} kg^{-1} in groups of conscious chronically catheterized rats (21). The volumes of distribution of gentamicin noted in this study are comparable to those reported previously in healthy experimental animals (21,22). The effects of pentobarbitone anesthesia on gentamicin clearance and volume of distribution noted in this study are in agreement with a previous report (5).

The renal clearance of gentamicin is a function of glomerular filtration and tubular reabsorption, with reported clearances between 80 and 100% of the glomerular filtration

Table I. Gentamicin Pharmacokinetic Parameters in Conscious and Anesthetized Rats[a]

	CONSC [C]	F/F/M [F]	PENTO [P]	URETH [U]	K/M [K]	A/A [A]	Multiple comparisons[b]
CL (ml min^{-1} kg^{-1})	9.8 ± 0.9	6.2 ± 1.8	7.9 ± 0.6	3.2 ± 0.3	8.0 ± 0.9	8.9 ± 0.6	C A K P F U
V_{ss} (ml kg^{-1})	473 ± 56	423 ± 75	372 ± 68	433 ± 33	411 ± 54	390 ± 64	C U F K A P
$t_{1/2}$ (min)	34 ± 8	51 ± 4	34 ± 7	109 ± 22	38 ± 3	30 ± 3	U F K C P A
C/C_b	1.69 ± 0.04	1.74 ± 0.07	1.73 ± 0.06	1.72 ± 0.02	1.70 ± 0.04	1.74 ± 0.05	F A P U K C
CL_b (ml min^{-1} kg^{-1})	16.5 ± 2.6	10.9 ± 3.2	13.7 ± 1.2	5.5 ± 0.5	13.7 ± 1.7	15.5 ± 1.1	C A K P F U

[a] CONSC, conscious; F/F/M, fentanyl/fluanisone/midazolam; PENTO, pentobarbitone; URETH, urethane; K/M, ketamine/midazolam; A/A, alphaxolone/alphadolone. The data are presented as mean ± SD; n = 5.

[b] Groups jointly underlined are not significantly different ($P > 0.05$) from each other (one-way analysis of variance and Duncan's multiple-range test).

Table II. Carboxyinulin Pharmacokinetic Parameters in Conscious and Anesthetized Rats[a]

	CONSC [C]	F/F/M [F]	PENTO [P]	URETH [U]	K/M [K]	A/A [A]	Multiple comparisons[b]
Cl_b (ml min^{-1} kg^{-1})	22.9 ± 3.1	15.7 ± 2.7	17.2 ± 2.1	7.7 ± 0.9	18.6 ± 1.9	20.3 ± 2.4	C A K P F U
V_{ss} (ml kg^{-1})	549 ± 87	486 ± 72	469 ± 79	567 ± 82	525 ± 92	454 ± 67	U C K F P A
$t_{1/2}$ (min)	18 ± 2	22 ± 3	20 ± 3	55 ± 9	20 ± 3	17 ± 2	U F K P C A

[a] CONSC, conscious; F/F/M, fentanyl/fluanisone/midazolam; PENTO, pentobarbitone; URETH, urethane; K/M, ketamine/midazolam; A/A, alphaxolone/alphadolone. The data are presented as mean ± SD; $n = 5$.

[b] Groups jointly underlined are not significantly different ($P > 0.05$) from each other (one-way analysis of variance and Duncan's multiple-range test).

rate (23–25). The estimated blood clearance of gentamicin in this study was lower than that of carboxyinulin, suggesting some tubular reabsorption. Gentamicin half-life is often closely correlated with glomerular filtration rate. In this investigation, the anesthetic-induced changes in gentamicin half-life correlated with the changes in carboxyinulin clearance ($r^2 = 0.86$), although the correlation was weaker than that between the clearances of the two compounds ($r^2 = 0.98$). The clearance of carboxyinulin is an indicator of glomerular filtration rate, of which renal blood flow is an important determinant (26). Renal haemodynamic alterations may have a profound influence upon the clearance of compounds whose elimination is dependent upon renal excretion. The rank order of anesthetic-induced changes in gentamicin clearance in this study is in close agreement with previously reported anesthetic-induced changes in renal blood flow (27). In addition to effects on glomerular filtration, alterations in renal blood flow may influence the clearance of renally secreted compounds, as previously reported for *p*-aminohippurate (12). The anesthetics used in this study have effects on cardiac output as previously reported (27), however, the changes in cardiac output do not correlate with those in renal blood flow.

The results of this study demonstrate that alphaxolone/alphadolone anesthesia results in a minimal perturbation of glomerular filtration rate and would appear to be the most appropriate regimen to employ in studies where renal clearance is of importance. In most drug disposition studies the use of conscious chronically catheterized animals may be preferable; however, there are some inherent methodological problems associated with this technique. An inevitable component of the technique is the use of anesthesia to enable catheterization. Anesthetics have been reported to influence drug dispostion for a considerable time after anesthesia. For example, halothane anesthesia in rats has been shown to influence intrinsic drug metabolizing capacity for at least 24 hr following anesthesia (28).

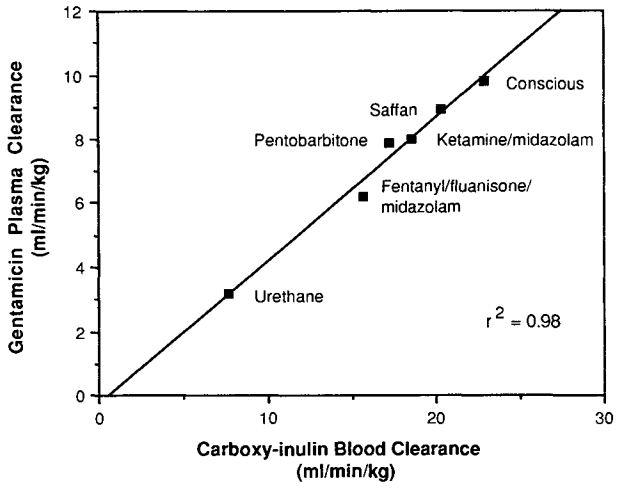

Fig. 1. Correlation of the clearances of gentamicin and carboxyinulin in conscious and anesthetized rats.

The presence of an indwelling catheter in the rat has been noted to increase serum α_1-acid glycoprotein and γ-globulins and slightly decrease albumin (29). Plasma protein binding of gentamicin is low (<20%) (30); thus any anesthetic-induced alterations of protein binding will not influence gentamicin clearance.

The use of conscious animals in pharmacokinetic studies necessitates the use of restraint for drug administration and blood sampling. The stress associated with restraint of conscious animals may be of importance in drug disposition studies as evidenced by a 50% reduction in the clearance of amikacin in rats subjected to handling-induced stress (31). The rats used in the present study were accustomed to manual restraint prior to the study.

Of importance in the interpretation of the results from this investigation is that the experimental design employed does not allow the effects of anesthesia upon drug disposition to be investigated in isolation since, by necessity, the animals also received surgery. The objective of the investigation was to use conditions that are commonly adopted in pharmacokinetic studies and thus compare the differences between six experimental protocols, viz., five different anesthestic regimens in the acute surgically prepared rat and the conscious chronically catheterized rat.

To conclude, in the acute surgically prepared rat, there exists a differential influence of some laboratory anesthetic regimens upon gentamicin clearance. Gentamicin clearance was higher in the chronically catheterized rat than in the anesthetized groups, and use of the conscious animal should be considered in laboratory pharmacokinetic studies.

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