EFFECT OF ANESTHESIA ON DRUG DISPOSITION IN THE RAT

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The effect of anesthesia induced with pentobarbital and diethyl ether on the drug disposition were investigated in the rat. The disposition rate constants of gentamicin and tobramycin were decreased significantly during the anesthesia with pentobarbital and ether without influence on the volume of distribution. In addition, the decrease in disposition rate constant was correlated significantly with the rectal temperature of the rat. These findings suggest that the decreased disposition rate constant will be responsible for decrease in renal blood flow correlating with the rectal temperature. But, the effect of anesthesia with ether for 30 min persisted for 2 h, even after the rectal temperature recovered to the value for the unanesthetized rats. These facts indicate that factors other than blood flow or rectal temperature might also be considered for the effect of ether anesthesia. Furthermore, the disposition rate constant of sulfanilamide was also decreased under pentobarbital anesthesia, with slight increase in acetylated fraction of sulfanilamide excreted into urine in 24 h. The calculated rate constants of metabolism and excretion were both greatly decreased by the pentobarbital anesthesia. Thus, controlling the anesthetic condition during the experiments will be important to investigate the pharmacokinetics of drugs.

Keywords — effect of anesthesia; drug disposition; rectal temperature; pentobarbital; diethyl ether; gentamicin; tobramycin; sulfanilamide; rats

For the studies employing the experimental animals, such as rats and mice, animals are often anesthetized. With respect to the effect of anesthesia on pharmacokinetics of drugs, few investigation have been presented.\textsuperscript{1} Pessayre et al.\textsuperscript{2} investigated the effect of surgery under general anesthesia on antipyrine clearance in man, and suggested that the general anesthesia caused the induction of hepatic drug-metabolizing enzymes. Johannessen et al.\textsuperscript{3} reported that ether anesthesia decreased the elimination of antipyrine and paracetamol in the rat. They suggested that ether interfered with hepatic conjugation of paracetamol and might interfere with the hepatic oxidation of antipyrine. These drugs will be considered to be eliminated from blood mainly through the metabolism in the liver. There are few studies on the effect of anesthesia on pharmacokinetics of the drugs which are eliminated from blood mainly through the kidney.

Gentamicin and tobramycin, aminoglycoside antibiotics, are excreted into urine by the kidney without metabolism in the body, and little are excreted in the bile.\textsuperscript{4} These antibiotics have little binding capacity to serum protein, but markedly accumulate in the kidney.\textsuperscript{5} Therefore, disposition of these antibiotics are considered to be influenced by changes in renal function or renal blood flow. Many investigators\textsuperscript{6} have revealed that general anesthetics decreased the cardiac output and then blood flow in the various tissues including the kidney. So, the influence of anesthesia on the antibiotics disposition will be predicted.

Presently, the effect of anesthesia induced with pentobarbital and diethyl ether, which are often used as anesthetics for experimental animals, on the disposition of gentamicin and tobramycin were investigated employing unanesthetized and anesthetized rats. Furthermore,
to study the effect of the anesthesia on the hepatic function, disposition of sulfanilamide, which are metabolized in the liver, was also studied under unanesthetized and anesthetic conditions.

EXPERIMENTAL

Materials—Gentamicin sulfate and tobramycin are gifts from Shionogi & Co. Ltd. (Osaka). Commercially available sodium pentobarbital (Abbott Laboratories, Nembutal® Sodium solution) were used. All other chemicals used were of reagent grade and used without further purification.

Animals and Experimental Procedure—Male rats of Wistar strain weighing 200—300 g were used. Left femoral artery of the rat was cannulated with polyethylene tubing (Clay Adams PE-50, 0.58 mm i.d., 0.965 mm o.d.) filled with heparinized saline (200 U/ml). The tubing was anchored securely and then brought subcutaneously to pass through the dorsal skin to the back of the neck. The tubing was terminated with a needle plug. Rats were housed in individual cages and allowed free access to food and water. On 1 or 3 d after cannulation, saline solution of drug was rapidly administered to the rats via the cannula, at a dose of 10 mg/kg for gentamicin and tobramycin or 0.5 μmol/kg for sulfanilamide, and then heparinized saline was flushed to rinse the drug solution remaining in the cannula. For anesthetized rats, drug was administered 15 min after the induction of anesthesia mentioned below. Just before the blood sampling, small amount of blood was discarded to avoid the contamination with injected drug solution or heparinized saline remaining in the cannula. Then, an appropriate volume of blood sample was withdrawn into a small polyethylene centrifuge tube and immediately centrifuged at 12000 rpm for 3 min to obtain the plasma. The cannula was refilled with heparinized saline.

Anesthesia with pentobarbital was performed with Nembutal® 30 mg/kg, i.p. The anesthesia persisted throughout the experiments. Ether anesthesia was performed as follows. Rats were placed for 30 s in a covered glass jar (about 1 l) containing cotton wool (about 3 g) soaked with ether to induce anesthesia, then were held on its back on a board. Ether was administered by covering the nose with a can containing a soaked cotton wool to maintain the anesthesia sufficient to prevent righting reflex. The cotton wool was kept about 5 cm over the nose of rats. Rectal temperature was determined with small thermometer prior to and 30 and 60 min after the administration of drug.

Analytical Methods—Gentamicin and tobramycin were assayed by the paper disk method with Bacillus subtilis ATCC 6633 as the test organism. Sulfanilamide was assayed with a modified Bratton-Marshall method.7

RESULTS

Effect of Anesthesia on the Disposition of Gentamicin and Tobramycin

Logarithmic plots of plasma concentration of gentamicin against time after intra-arterial ad-

![Figure 1. Logarithmic Plots of Gentamicin Plasma Concentration against Time after Intra-arterial Administration](image)

**FIG. 1. Logarithmic Plots of Gentamicin Plasma Concentration against Time after Intra-arterial Administration**

Dose: 10 mg/kg, i.a.
- ● unanesthetized rat,
- ▲ pentobarbital anesthetized rat,
- ■ ether anesthetized rat.

Each value represents the mean ± S.E. for ten rats.
FIG. 2. Disposition Rate Constant (A) and Volume of Distribution (B) of Gentamicin and Rectal Temperature (C) of the Rat
Dose: 10 mg/kg, i.a.

- unanesthetized rat,
- pentobarbital anesthetized rat,
- ether anesthetized rat.

Each value represents the mean ± S.E. for ten rats.
** significantly different from unanesthetized rats at p < 0.01.

FIG. 3. Disposition Rate Constant (A) and Volume of Distribution (B) of Tobramycin and Rectal Temperature (C) of the Rat
Dose: 10 mg/kg, i.a.

- unanesthetized rat,
- pentobarbital anesthetized rat.

Each value represents the mean ± S.E. for ten rats.
** significantly different from unanesthetized rats at p < 0.01.
ministration are presented in Fig. 1. Linear correlations were obtained for unanesthetized and anesthetized rats with ether and pentobarbital. Plasma concentration for rats anesthetized with pentobarbital and ether were higher than those for unanesthetized rats at all time studied.

**FIG. 4. Correlations between Disposition Rate Constant and Rectal Temperature of Gentamicin (A) and Tobramycin (B)**
Dose: 10 mg/kg, i.a.
- •, unanesthetized rat,
- △, pentobarbital anesthetized rat,
- ■, ether anesthetized rat.

**FIG. 5. Effect of Artificially Elevated Rectal Temperature (C) on Disposition Rate Constant (A) and Volume of Distribution (B)**
Dose: 10 mg/kg, i.a.
- , unanesthetized rat,
- △, pentobarbital anesthetized rat,
- ■, rat forcing to elevate the rectal temperature artificially.
Each value represents the mean ± S.E. for six rats.
** significantly different from unanesthetized rat at p < 0.01.
Within 15 min after the administration, distribution phase were diminished for unanesthetized and anesthetized rats. Therefore, disposition of drugs was analysed with one-compartment open model, although some investigators analysed with two-compartment open model. As parameters of disposition, disposition rate constant, \( k_p \), and volume of distribution, \( V_d \), were calculated (Fig. 2(A) and (B)). Disposition rate constants of gentamicin for anesthetized rats with pentobarbital and ether were both decreased significantly, compared with that for unanesthetized rats. Whereas, volume of distribution of gentamicin were not affected by anesthesia. The decreased disposition rate constant by ether anesthesia was much greater than that by pentobarbital anesthesia. As shown in Fig. 3(A) and (B), disposition rate constant of tobramycin was decreased similarly for gentamicin by pentobarbital anesthesia, without influence on the volume of distribution.

**Correlation between the Disposition Rate Constant and Rectal Temperature**

The rectal temperature of the rat was decreased by anesthesia (Fig. 2(C) and 3(C)). The decreased temperature was kept almost constant during experiment. The rectal temperatures shown in figures are the mean of the three determinations. Correlations between disposition rate constants and rectal temperatures were studied. As shown in Fig. 4(A) and (B), for gentamicin and tobramycin, respectively, disposition rate constants and rectal temperatures were significantly correlated (correlation coefficient, \( r = 0.851, n = 52, p < 0.01 \) for gentamicin and \( r = 0.728, n = 33, p < 0.01 \) for tobramycin). Further investigation was made on the effect of rectal temperature on the disposition rate constant of gentamicin by elevating rectal temperature artificially. The pentobarbital anesthetized rats were placed on the water-bed which was perfused with hot water (39°C) and forced to elevate the rectal temperature. As shown in Fig. 5, rectal temperature of pentobarbital anesthetized rats was elevated to the value for unanesthetized rats, and disposition rate constant also recovered near to that for unanesthetized rats.

**Persistency of the Effect of Ether Anesthesia**

Rats were anesthetized with ether for 30 min, followed by standing for 1, 2 and 3 h without applying ether. At designated time after anesthesia, gentamicin was administered and disposition rate constant was determined. As shown in Fig. 6, rectal temperature already recovered to the level of unanesthetized rats at 1 h after removing ether. However, disposition rate constant of gentamicin was still decreasing even after 2 h. At 3 h after removing ether, disposition rate constant recovered to the value for unanesthetized rats.

**Effect of Anesthesia on the Disposition of Sulfanilamide**

It is already known that sulfanilamide is metabolized to acetylated metabolite in rats, \(^9\).
and during 24 h after the administration, over 90% of sulfanilamide administered was excreted into the urine. As shown in Fig. 7, per cent acetylated of total sulfanilamide excreted into urine in 24 h was slightly but significantly increased by pentobarbital anesthesia, compared with that for unanesthetized rats. But, disposition rate constant was greatly decreased by anesthesia. Elimination of sulfanilamide from blood is performed by processes of excretion into the urine and metabolism in the living body. Thus, the disposition rate constant, $k_p$, for unmetabolized sulfanilamide is the sum of the rate constant of excretion and the rate constant of metabolism. Rate constant of excretion, $k_e$, and rate constant of metabolism, $k_m$, were calculated as reported previously. The rate constants of excretion and metabolism were both greatly decreased under the pentobarbital anesthesia.

DISCUSSION

The disposition rate constants of gentamicin and tobramycin were decreased significantly during the anesthesia with both pentobarbital and diethyl ether (Fig. 2 and 3). Aminoglycoside antibiotics are excreted mainly through the kidney without metabolism and remarkably accumulated to the kidney, therefore, the change in renal function is thought to affect the disposition of the antibiotics. Various anesthetics were known to decrease the cardiac output and organ blood flow. And renal plasma flow and glomerular filtration rate were also reported to be decreased by various anesthetics including barbiturates and ether. Therefore, the decrease in the disposition rate constants of aminoglycoside antibiotics by anesthesia with pentobarbital or ether are considered to be attributable to the decrease in the renal blood flow. In addition, as shown in Fig. 4, the decrease in disposition rate constant was correlated significantly with the rectal temperature of the rat. These findings suggest that the decrease in the rectal temperature is responsible for the decrease in blood flow. During progressive hypothermia, the glomerular

FIG. 7. Per cent Acetylated (A) and Rate Constant of Disposition (B), Metabolism (C) and Excretion (D) of Sulfanilamide

Dose: 0.3 $\mu$mol/kg, i.a.

- Unanesthetized rat,
- Pentobarbital anesthetized rat.

Each value represents the mean $\pm$ S.E. for eight rats.

* significantly different from unanesthetized rat at $p<0.05$.

** significantly different from unanesthetized rat at $p<0.01$. 
filtration rate and effective renal plasma flow was reported to decrease in a linear manner with decreasing rectal temperature.\textsuperscript{10} Besides, Ham \textit{et al.}\textsuperscript{11} demonstrated that the elimination of \textit{d}-tubocurarine was decreased by hypothermia. Thus, the rectal temperature may be considered as a measure for the effect of anesthesia on pharmacokinetics of drugs. In the present study, artificial elevation of the rectal temperature of the pentobarbital anesthetized rats led to recovery of the disposition rate constant near to the value obtained from unanesthetized rats. Thus, controlling the rectal or body temperature is thought to be important for the study employing the experimental animals under anesthesia.

It is reported that light ether anesthesia for a short time has little effect on the distribution or elimination of drugs.\textsuperscript{8,12} But, as shown in Fig. 6, the effect of anesthesia with ether for 30 min persisted for 2 h, even after rectal temperature recovered to the value for the unanesthetized rats. Since anesthesia with ether was reported to cause acute intrinsic renal failure,\textsuperscript{13} delay of recovery for disposition rate is thought to be responsible for the renal acute toxicity of ether. Therefore, other factor rather than blood flow or rectal temperature might also be considered for the effect of ether anesthesia.

Hepatic blood flow was also reported to be decreased by anesthesia, same as renal blood flow. In the present study, hepatic participation to the pharmacokinetics of drugs under anesthesia was investigated using sulfanilamide. Sulfanilamide\textsuperscript{8,14} is eliminated through enzymatic metabolism and renal excretion. As shown in Fig. 7(A) and (B), for sulfanilamide, rate constants of metabolism and excretion were both decreased significantly, which were considered to be attributable to the decrease in hepatic and renal blood flow, respectively. In the present study, acetylation of sulfanilamide was increased by pentobarbital anesthesia. The increase will not be attributable to enzyme induction by pentobarbital since rate constant of metabolism was decreased. The increase will be explained as follows. Decrease in excretion rate constant will result in persistency of blood level of sulfanilamide. Persistency of the blood level will also allow to increase enzymatic attack in the liver even under the decreased blood flow by anesthesia.

In conclusion, pharmacokinetics of drugs were affected by the anesthesia induced with pentobarbital and ether, through the excretion and metabolism. Therefore, controlling the anesthetic condition during the experiments will be important to investigate the pharmacokinetics of drugs.

REFERENCES


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