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Kinetic characteristics and toxic effects of benzalkonium chloride following intravascular and oral administration in rats

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Abstract

Kinetic characteristics and toxic effects of benzalkonium chloride (BZK) following injection via jugular vein (JV), femoral artery (FA) and oral administration (PO) were experimentally investigated using rats. The BZK concentrations in blood and tissues (lung, liver and kidney) were determined by high-performance liquid chromatography with solid phase extraction. Toxic doses of 15 and 250 mg/kg of BZK were used for intravascular (JV and FA) and PO administration, respectively. The fatal effects appeared soon after the dose in JV-rats, while delayed in FA- or PO-rats. The blood BZK concentrations and the elimination half-lives were similar between JV- and FA-rats, while the distribution of BZK in tissues was slightly different. In PO administration, the rats that aspirated BZK into their lungs had some symptoms, while the rats that did not aspirate BZK appeared to be normal. The BZK concentrations in blood and tissues were significantly higher in the aspirated PO-rats. The toxic degree of BZK was correlated with the BZK concentration in orally dosed rats. Lung and kidney had higher BZK concentrations compared to blood or liver, and they could be the target organs of BZK.

Keyword: Benzalkonium chloride

1. Introduction

A cationic surfactant, benzalkonium chloride (BZK) is a mixture of alkylbenzyldimethylammonium chlorides [$C_6H_5CH_2N(CH_3)_2RCl$] in which the alkyl groups have a chain length from C_8 to C_{18} [1], with C_{12} and C_{14} predominating in pharmaceutical products [2,3]. BZK has various uses, i.e. preoperative topical disinfection, sterilization of surgical instruments and gloves, antisepsis of contact lenses, and pharmaceutical preservation. BZK is widely used in hospitals, homes and other places as a disinfectant. These products are sold in solution and look identical to water. Most over-the-counter drugs available in Japan contain about 10% BZK [4] and have to be diluted for use. Some products sold for hospital use contain as much as 50% of BZK [5] and even 80% in some countries [6]. Because of its easy accessibility, accidental or intentional ingestion have occurred relatively often, which results in serious poisoning symptoms [7–9] and sometimes results in death [10–13].

Quantification of drug concentrations in patient's biological samples is generally considered useful to determine the degree of acute toxicity, especially for the drug that is poorly metabolized such as BZK [14]. However, no data regarding systematic kinetics and distribution of BZK are available by pharmaceutical companies since BZK was not developed as a medicine to be administered. One of the other reasons for the limited analytic data of BZK is the difficulty of BZK to be analyzed in biological materials. There have been various methods reported to determine BZK, i.e. extraction by complexed

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BZK with dyes [15], titration of quaternary ammonium compounds [3], pyrolysis and subsequent gas chromatography [16], direct inlet mass spectrometry [17] and various high-performance liquid chromatography (HPLC) techniques for non-biological aqueous solution [18] or pharmaceutical formulations [19–21]. Blood or tissue BZK concentrations have been rarely measured [11,22,23]. Even if the blood BZK concentrations in patients or victims are measured in cases of poisoning, the quantified values are hard to interpret without reference data.

We have recently developed a sensitive method to determine BZK concentrations in blood and tissue samples, in which we selected the dominant component C_{12} as an indicator to quantify BZK, and then adjusted the concentration based on the proportion of C_{12} in the BZK product [23]. The purpose of this study was to investigate the kinetics and toxicity of BZK in rats treated with a single toxic dose of intravascular injection via jugular vein (JV) or femoral artery (FA), and by oral administration (PO) utilizing our recently developed HPLC method utilizing solid phase extraction.

2. Experimental

2.1. Materials

Standard BZK was purchased from Sigma (St. Louis, MO, USA), which is the mixture of 60–70% of C_{12} homologue (C_{12}), 30–40% of C_{14} homologue (C_{14}) and <5% of C_{16} homologue (C_{16}) (data from Sigma). C_{16} was purchased from Sigma. Osvan®, one of the most common disinfectants in Japan, which contains 10% of BZK was purchased from Nihon Seiyaku Ltd. (Tokyo, Japan), which consists of 80-85% of C_{12} and more than 98% of total of C_{12} and C_{14} (data from Nihon Seiyaku). Domiphen bromide used as an internal standard (IS) was purchased from Aldrich (Milwaukee, WI, USA). The reagents for mobile phase were of HPLC grade and all other reagents were of analytical grade. BZK and IS solution were prepared at 0.1–45.0 and at 30 μg/ml dissolved in HPLC grade water, respectively.

2.2. HPLC conditions

The HPLC apparatus consisted of SCL-10 system equipped with a controller, a pump, and an auto injector (Shimadzu, Kyoto, Japan), a UV detector 2487 (Waters, Milford, MA, USA) and an integrator D-7500 (Hitachi, Tokyo, Japan). Chromatography was performed using a reversed-phase column YMC-Pack CN (250 mm × 4.6 mm i.d., 5 μm) (Waters) eluted in isocratic mode with a mixture of acetonitrile–sodium acetate buffer (0.1 M, pH 5.0) (48:52, v/v) at a flow-rate of 1 ml/min. The UV signal was monitored at 254 nm.

2.3. Extraction procedure for biological samples

Whole blood or serum (0.1–2.8 ml), or tissue sample (0.1–3.4 g) cut into small pieces, was added with 8–15 ml water, and homogenized with a sonicator (Waken GE 100 Ultrasonic Processor, Japan). The homogenate spiked with IS (50 μl) was centrifuged at 18,000 rpm for 60 min at 4 °C, and then the supernatant was applied to a disposable solid phase extraction column, BAKERBOND spe Octadecyl C_{18} (3 ml, 500 mg) (J.T. Baker, Phillipsburg, NJ, USA). The column was pre-conditioned with methanol (6 ml), followed by HPLC grade water (6 ml). Column was rinsed sequentially with water (3 × 3 ml), methanol (3 × 3 ml) and ethyl acetate (3 × 3 ml). After flushing with air for 5 min, BZK and IS were eluted with 4 ml of methanol–ethyl acetate (1:1) containing 0.01% ammonium chloride. The eluate was evaporated to dryness under a stream of nitrogen. The residue was reconstituted with ethyl acetate (1 ml) and 2% sodium carbonate (1 ml) containing 0.1% bromophenol blue (50 μl) to eliminate ammonium chloride. The organic phase was dried and the residue was dissolved in 2/3 diluted mobile phase (150 μl). This dilution-ratio was obtained in preliminary study to achieve the highest solubility of the residue, otherwise the thick residue was not completely dissolved in either 100% of mobile phase or water. The 100 μl was injected into the HPLC.

2.4. Animal experiments

The experimental protocols were approved by the Animal Experimental Committee at Shimane University. Male Sprague–Dawley rats (Charles River Breeding Labs, Yokohama, Japan) (B.W. 310–470 gm) were used in this study and randomly assigned to receive BZK via PO, JV or FA. Rats were under anesthesia throughout the experimental procedures. A mix of droperidol 1.25 mg/ml and fentanyl 0.025 mg/ml was used as anesthetic. A dose of 2 ml/kg (droperidol 2.5 mg/kg and fentanyl 0.05 mg/kg) was initially administered intramuscularly and was given at 0.3–0.6 ml/kg thereafter as needed.

2.4.1. Infusion via jugular vein (JV)

A total of 15 rats were used in this study, in which six and nine of them were to determine the elimination kinetics and distribution of BZK, respectively. The rats for the kinetic study were implanted with two catheters for a dose (right JV) and blood sampling (left femoral vein), and the rats for the distribution study were implanted with a single catheter for a dose (right JV). A dose of 15 mg/kg-BZK [= 1.5 ml/kg of 10-fold diluted Osvan® (10 mg/ml of BZK solution)] was infused over 1 min via JV, and the catheter was removed after the dose. This dose (15 mg/kg) was based on the LD_{50} (14 mg/kg i.v. in rats) reported by Kamiya [24]. In the kinetic study, the blood samples of 0.1, 0.3, 0.5, 0.5, 0.8, 1.0, 1.5 ml were collected at 2, 5, 10, 30, 60, 120 and 180 min, respectively, and the same volume of saline was substituted each time for the blood loss. Approximately 15–17% of the total volume of blood was taken [25]. The rats were decapitated at 4 h to collect trunk blood and tissue samples. In the distribution study, six and three rats were decapitated

at 2 min and 24 h to determine the early and late distribution of BZK, respectively. Trunk blood and tissue samples were collected and the samples were stored at −20 °C until the BZK assay. The kinetic parameters were estimated by model-independent analysis using an online available program (Moment.xls, ver. 1.0, Day Three Institute, Tsukuba, Japan) with the blood BZK concentrations until 4 h in the kinetic study.

2.4.2. Infusion via femoral artery (FA)

A total of 15 rats were used in this study. The design of study was identical to that of JV except the sites for a dose (left FA) and blood sampling (right femoral vein).

2.4.3. Oral administration (PO)

A total of 34 rats were used in this study. The rats fasted overnight on the night before the experiment. A dose of 250 mg/kg BZK [=2.5 ml/kg of Osvan® (100 mg/ml of BZK solution)] was given by stomach tube. This dose was based on the smallest LD_{50} (234 mg/kg PO in rats) reported by Alfredson et al. [26]. Blood samples were collected by cardiac puncture at 1, 2, 4, 8 and 24 h (six rats of each sampling point and 30 rats in total) and the rats were then sacrificed by injecting the anesthetic (0.2 ml) into the heart. Lung, liver, kidney were harvested after death. Samples were stored at −20 °C until the BZK assay. Since four rats died before their due times, additional rats were used to compensate for the loss.

3. Results

3.1. Chromatography

Fig. 1A shows the chromatogram of standard BZK. C_{12}, IS and C_{14} detected at 24, 28 and 36 min, respectively. Standard C_{16} homologue was detected at 57 min, but a peak of C_{16} was not detected either in standard BZK or in Osvan®.

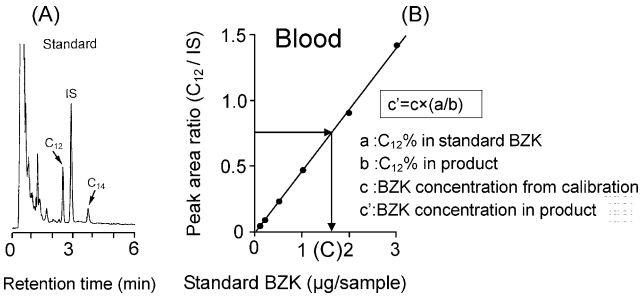

Fig. 1. Typical chromatogram obtained from standard BZK (A) and a calibration curve of BZK in blood (B). Domiphen was used as an IS. The peak (C_{12}) area ratio to IS (*y*-axis) was plotted against the amount of total BZK in the sample (*x*-axis). The concentration of BZK derived from a commercial product was calculated by equation: $c' = c \times (a/b)$, in which, c' is the BZK concentration in the biological sample derived from the product, c the BZK concentration obtained from the calibration curve, a the proportion of C_{12} in standard BZK used for the calibration curve (%), and b the proportion of C_{12} in the product (%).

Table 1
Recovery, intra-day precision and accuracy when 1 μg/g of BZK was spiked in the whole blood or tissue samples (n = 6)

	Recovery		Intra-day		
	BZK (%)	IS (%)	Observed (μg/g)	Precision CV (%)	Accuracy (%)
Whole blood	91.4	91.1	1.00 ± 0.05	5.36	100.4
Lung	71.6	82.9	1.03 ± 0.02	1.84	103.2
Liver	71.4	67.2	0.99 ± 0.05	4.95	99.0
Kidney	75.4	74.2	1.00 ± 0.02	1.95	100.1

The percentage of C_{12} in BZK was calculated by the equation described in USP NF: 100A/B, in which A is the area obtained from C_{12} homologue multiplied by its molecular weight and B is the sum of those of all homologues [3]. The calibration curves were obtained by plotting the C_{12} area ratio to IS against the amount of standard BZK spiked in blood and tissue samples (n = 6) (Fig. 1B shows the calibration curve in blood). Good linearity was obtained in the range of 100 ng–3 μg. The limit of detection was 20 ng on column, when the signal-to-noise ratio was set at 3. The recovery, intra-day precision and accuracy for the whole blood and the tissue samples measured in this study are summarized in Table 1. Good inter-day precision (4.2% of C.V.) and accuracy (98.8%) was also obtained when 1 μg/g of BZK was spiked in whole blood. The validation data for serum and the rest of tissue samples have been reported in our previous article [23].

Since C_{12} was the most dominant component in pharmaceutical products of BZK, this homologue was selected to quantify BZK. The existence of a secondary main peak of C_{14} was checked on the chromatogram to qualify presence of BZK. To calculate the concentration of BZK derived from a commercial product, the concentration obtained from the calibration curve was adjusted based on the percentage of C_{12} in standard BZK and the product using the equation in Fig. 1B. The standard BZK and Osvan® used in this study contained 68.7% (a) and 89.8% (b) of C_{12} homologue, respectively.

3.2. Infusion via JV and FA

After JV administration, most of the rats stopped breathing immediately after the dose, but recovered in 30–40 s. However, three rats died within 11 min without recovery and other rats survived until their euthanasia. After FA administration, all rats appeared to be normal in the initial period, but did not urinate after the dose and gradually became cyanotic. No FA-rat died within 4 h, but all three rats assigned for distribution study over 24 h died at 7, 10 and 13 h (no sample at 24 h). Though the toxic manifestations were different between JV- and FA-rats, the time-course changes in the blood BZK concentrations were similar (p = 0.16 by repeated measure ANOVA) (Fig. 2), and kinetic parameters such as elimination half-life ($t_{1/2}$) [1.30 h (JV) and 1.56 h (FA)], AUC and

Table 2
BZK concentrations in blood and tissues following JV, FA and PO administration

Route	Sampling		Concentrations of BZK (μg/g)			
	Time	*N*	Whole blood	Liver	Lung	Kidney
JV	2 min	6(1)	26.1 ± 8.4	16.5 ± 4.6	126.7 ± 46.2^{*ABc}	39.9 ± 11.7
	4 h	6(1)	0.31 ± 0.07	0.23 ± 0.03	7.9 ± 1.1^{**AB}	7.0 ± 1.5^{*AB}
	24 h	3(1)	0.02	N.D.	2.32	2.86
FA	2 min	6	13.5 ± 1.0	12.5 ± 1.9	25.7 ± 3.6	29.1 ± 2.8
	4 h	6	0.26 ± 0.02	0.15 ± 0.03	3.2 ± 0.4^{AB}	3.8 ± 0.4^{AB}
	24 h	3	–	–	–	–
PO	1 h	6	0.06 ± 0.02$^{\#\#}$	0.22 ± 0.08	0.84 ± 0.32	1.09 ± 0.74
	2 h	6	0.08 ± 0.02$^{\#\#}$	0.78 ± 0.46	1.94 ± 0.85	2.46 ± 1.54
	4 h	6	0.06 ± 0.01$^{\#\#}$	0.72 ± 0.26	0.50 ± 0.12$^{\#}$	0.83 ± 0.30
	8 h	6	0.09 ± 0.05	0.70 ± 0.31	0.39 ± 0.14$^{\#}$	1.08 ± 0.64
	24 h	6	0.34 ± 0.13	0.72 ± 0.39	2.75 ± 1.17	5.25 ± 2.26^{ab}

Data express mean ± S.E. except the samples at 24 h in JV-rats. (1) One of the rats died prior to the due time and the data were analyzed with the rest of five or two rats. (–) All rats died prior to the due time and no sample was collected at 24 h. N.D., under the detection limit. (*) $p < 0.05$ and (**) $p < 0.01$ when compared between JV and FA by factorial ANOVA. (^{a}) $p < 0.05$ and (^{A}) $p < 0.01$ when compared with the blood concentrations at the same sampling time. (^{b}) $p < 0.05$ and (^{B}) $p < 0.01$ when compared with the liver concentrations at the same sampling time. (^{c}) $p < 0.05$ when compared with the kidney concentrations at the same sampling time. ($^{\#}$) $p < 0.05$ and ($^{\#\#}$) $p < 0.01$ when compared with the concentration at 24 h in the same kind of samples in PO-rats. No significant of difference was observed between any other groups. A dose of 15 mg/kg of BZK was administered via JV or FA and 250 mg/kg was administered orally (PO).

clearance (Cl_{tot}) were also similar except the volume of distribution (Fig. 2). Fig. 3 shows the chromatograms obtained from a JV-rat sacrificed at 24 h. There was no peak derived from BZK detected in liver and very small peaks in blood, while large peaks of C_{12} and C_{14} were detected in lung and kidney. In both JV- and FA-rats, lung and kidney had significantly higher BZK concentrations than blood and/or liver at 2 min and/or 4 h (Table 2). The BZK concentrations in lung and kidney in JV-rats were significantly higher than those in FA-rats at 2 min and/or 4 h (Table 2). The BZK concentrations in the rats that died before their euthanasia did not appear to be high compared to those in the survived rats (data not shown), though they were hard to compare because of different sampling times.

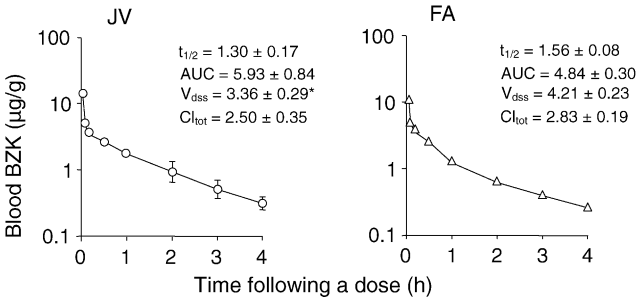

Fig. 2. The time-course changes in blood BZK concentrations following JV ($n = 5$) and FA ($n = 6$) administration (15 mg/kg). There was no significant difference in the BZK concentrations over 4 h between JV and FA ($p = 0.16$ when compared by repeated ANOVA). The kinetic parameters were calculated by model-independent analysis. The AUCs were calculated by the trapezoidal rule. Data express mean ± S.E., but most of the error bars are within symbols. (*) $p < 0.05$, compared individually between JV and FA; $t_{1/2}$, half-life of elimination (h); AUC, area under the blood concentration–time curve until 4 h (μg/(ml h)); Cl_{tot}, total body clearance (l/(h kg)); V_{dss}, volume of distribution at the steady state (l/kg).

3.3. PO administration

The physiological responses following oral administration varied greatly among the individual rats. Approximately half of them appeared to be normal throughout the experimental period, while the other half coughed or vomited some of the dose, followed by sneezing or difficulty in breathing. Four rats died at 1, 6, 7 and 24 h by respiratory irritation. In Fig. 4, the BZK concentrations in blood and tissues of individual rats are plotted against time. The rats with some symptoms (shaded symbols) had significantly higher concentrations of BZK than the rats that appeared normal (open symbols) ($p < 0.01$ in blood, liver and lung; $p < 0.001$ in kidney). The concentrations of BZK in the rats that died before their due times (dark symbols) were higher than others, except for the one rat that died at 24 h. The average BZK concentrations were even higher at 24 h in blood, lung and kidney

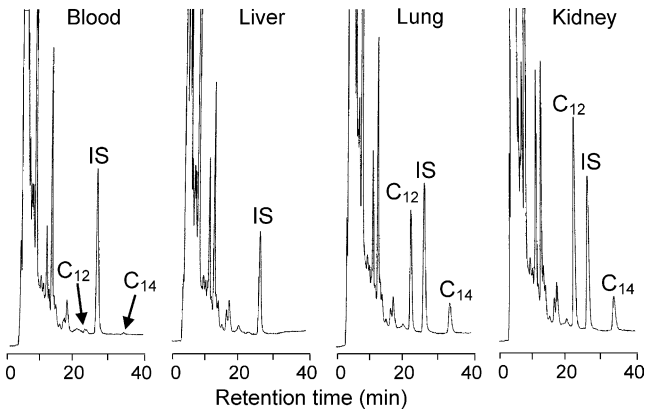

Fig. 3. Chromatograms obtained from blood, liver, lung and kidney in a JV-rat sacrificed at 24 h. Large peaks of C_{12} and C_{14} were detected in lung and kidney, while no peak was detected in liver.

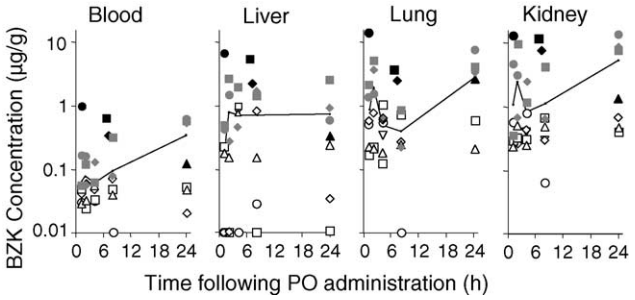

Fig. 4. Individual BZK concentrations in blood and tissues are plotted against the time following PO administration (250 mg/kg). The open, shaded and dark symbols correspond to normal, sick and dead rats, respectively. The lines express the average concentration of BZK at each time point, but without the rats that died. The sick rats coughed or vomited some of the dose, followed by having symptoms such as sneezing or difficulty in breathing. There was a positive correlation between the BZK concentrations and the degree of toxicity.

compared to those in the same kind of samples at earlier times (Table 2).

4. Discussion

Dozens of pharmaceutical BZK products are adopted as prescribed drugs in Japan [5], and many other BZK products are commercially available as over-the-counter drugs [4]. Many poisoning cases have occurred by accidental or intentional ingestion of high concentrations of BZK (10% or more) over the years due to its easy availability [7–13]. Accidental intravascular administrations are estimated to occur more frequently than the cases reported [27]. Intravenous injection of BZK instead of a drug or intra-arterial injection of BZK instead of an angiographic agent occurs very occasionally even though these are not officially reported due to malpractice concerns [28]. In spite of numbers of poisoning cases that occurred, there have been few reports concerning the BZK concentrations in biological samples [11,22–23] and no report regarding systematic study on kinetics and distribution of BZK in vivo.

The LD_{50} of BZK in rats was reported as 234–525 and 14 mg/kg when administered orally and intravenously, respectively [6,26,29,30]. In humans, an oral dose of 100–400 mg/kg [13] or a parenteral dose of 5–15 mg/kg [9,31] is thought to be fatal. In this study, four out of 34 rats (12%) died by 250 mg/kg-PO and six out of 30 rats (20%) died by 15 mg/kg of intra-vascular injection.

The toxic effects of BZK differed according to the routes of administration. Fatal toxicity appeared soon after the dose in JV-rats, and was delayed in FA- or PO-rats. Since the drug injected via JV goes into lungs directly, the sudden cessation of breathing soon after the dose in JV-rats was considered to be a sudden blockage of the oxygen supply in lungs by the large bolus concentrated dose of BZK, this may be reflected by the high lung BZK concentrations observed in JV-rats (Table 2). This extremely high lung concentrations achieved in the initial phase in JV rats could result in higher concentrations even at later time since the elimination half-life was not different between JV and FA in blood (Fig. 2). The effect on respiration, however, appeared to be transient and 80% of the rats could recover relatively quickly once breathing restarted and survived for at least 2 weeks (data not shown). In FA administration, toxic effects appeared a few hours later and no rat survived 1 day, which suggests that FA administration of BZK is extremely toxic. Despite the difference in the toxic effects of BZK between JV- and FA-rats, the blood concentration profiles and the kinetic parameters such as $t_{1/2}$, AUC and clearance (Cl_{tot}) were similar. The BZK concentrations in lung and kidney in JV-rats were even higher than those in FA-rats. These results are in contradiction to the generally accepted idea that higher drug concentrations usually mean greater toxicity. BZK has a strong effect on biological membranes due to its detergent property, which results in hemolysis and mucosal necrosis [6]. These effects of BZK may induce systemic problems and irreversible fatal failures, i.e. dysfunction of kidney (no urination) or imbalance of physiological ions after a FA-dose. However, the exact mechanism of action of BZK was not addressed in this study and further investigation is clearly needed.

As an additional small experiment, five rats of each were given BZK via femoral vein (FV) or jugular artery (JA) to compare the difference in kinetics and toxicity of BZK between venous and arterial administration as well as between jugular and femoral administration. The toxic effects of BZK infused via FV and JA revealed similar symptoms to those in JV-rats, and unlike in FA-rats. Three out of 10 rats died within 10 min. The blood and tissue BZK concentrations were detected similar to those in JV- and FA-rats, though it was hard to compare due to limited number of rats used (data not shown). These indicated that only the injection of BZK via FA resulted in completely different manifestations among four routes of intra-vascular administration.

After PO administration, aspiration of even a small amount of BZK into the bronchia resulted in systemic problems. If no aspiration occurred, the rats appeared to be normal and the BZK concentrations in blood and tissues were relatively low, suggesting that only a small amount of BZK was systemically absorbed (Fig. 4). If aspiration occurred, fatal damage to lung cells resulted from aspirated BZK being absorbed from the pulmonary blood vessels, and resulted in higher concentrations of BZK than those observed in non-aspirated rats. The systemic cellular changes and an imbalance of blood gases simultaneously developed with the cytolytic effects of BZK, and resulted in fatal failures. These results indicated that there was a positive correlation between the degree of toxicity and BZK concentrations in blood and tissues following oral administration of BZK. However, the detailed toxicokinetic analysis was hard to perform in this study since the rats were sacrificed at each time point and the serial blood concentrations in the same rat were not obtained. Though highly variable concentrations were observed between rats, the higher concentrations of BZK at 24 h compared with those

at earlier times may result from slow absorption through gastrointestinal mucosa or redistribution of absorbed BZK. The toxic peak after PO administration may occur 1 day later, but not later than a few days. This is based on the result that non-aspirated rats survived for 2 weeks at least, though the rats appeared to be slow for a few days (data not shown).

In conclusion, the kinetic characteristics and toxic effects of BZK were experimentally investigated using rats utilizing HPLC technique with solid phase extraction. The fatal effects appeared soon after the dose in JV-rats, but were delayed in FA- and PO-rats. In PO-rats, the toxic effects of BZK were correlated with the blood or tissue BZK concentrations. Though no FA-rat survived 1 day and 80% of JV-rats survived, the blood BZK concentrations were similar between JV- and FA-rats and the BZK concentrations in lung and kidney were even higher in JV-rats. The degree of toxicity was hard to determine from the BZK concentrations in JV- or FA-rats. Lung and kidney had significantly higher concentrations of BZK than blood and liver in intravascular administration. These tissues may be the target organs of BZK and could be useful specimens in forensic analysis.

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