 

# *Effects of Probenecid on Renal Function in Surgical Patients Anesthetized with Low-flow Sevoflurane*

*Hideyuki Higuchi, M.D.,* Hiroki Wada, M.D.,† Yumi Usui, M.D.,‡ Kohichiro Goto, M.D.,§ Masuyuki Kanno, M.D.,‖ Tetsuo Satoh, M.D.#*

***Background:*** **Dehydrofluorination of sevoflurane by carbon dioxide absorbents in anesthesia machines produces compound A, which is nephrotoxic in rats. Several clinical studies indicate that prolonged low-flow sevoflurane anesthesia is associated with an increased urinary excretion of biochemical markers, such as protein. Probenecid, a competitive inhibitor of organic anion transport, diminishes compound A nephrotoxicity in rats. The purpose of the present study was to examine the effects of low- and high-flow sevoflurane anesthesia on urinary excretion of biochemical markers in humans and to examine the effects of probenecid on urinary excretion of these markers.**

***Methods:*** **Elective surgical patients (n = 64) were assigned to four groups (n = 16 each): low-flow sevoflurane plus probenecid (LSP), low-flow sevoflurane (LS), high-flow sevoflurane plus probenecid (HSP), and high-flow sevoflurane (HS). Probenecid (2.0 g) was administered orally 2 h before the induction of anesthesia in both the LSP and HSP groups. Nothing was administered orally 2 h before the induction of anesthesia in either the LS or HS groups. All patients underwent prolonged low-flow (1 l/min) or high-flow (6 l/min) sevoflurane anesthesia. Urinary excretion of protein, albumin, $\beta_2$-microglobulin, glucose, and *N*-acetyl-$\beta$-D-glucosaminidase was measured for up to 7 days postoperatively.**

***Results:*** **Sevoflurane doses were similar in all four groups. There were no differences in blood urea nitrogen, creatinine, or creatinine clearance among the four groups after anesthesia. Average values for urinary excretion of protein, $\beta_2$-microglobulin, and *N*-acetyl-$\beta$-D-glucosaminidase in the LS group were significantly higher than those in the other groups (LSP, HSP, HS; $P < 0.05$). There was no significant difference between the LS and LSP groups in average values for urinary excretion of albumin and glucose, although there were significant differences between the LS and both high-flow sevoflurane groups (HSP, HS).**

***Conclusions:*** **Low-flow sevoflurane, which produces a sevenfold higher compound A exposure than high-flow sevoflurane, resulted in significant increases of several biochemical markers in half of the patients. Probenecid appears to provide protection against these renal effects.**

SEVOFLURANE is an inhalational anesthetic agent that has been used in Japan since 1991 and is now approved for use in 54 other nations. When sevoflurane is used in anesthetic circuits equipped with carbon dioxide absorbents, it undergoes dehydrofluorination, producing a fluoromethyl 2,2-difluoro-1-(trifluoromethyl) vinyl ether commonly referred to as compound A.[1] Compound A has dose-related nephrotoxic effects in rats,[2–6] but whether it is toxic in humans is a subject of much debate.[7–14] Some studies of humans report increased renal excretion of biochemical markers such as $\alpha$-glutathione-*S*-transferase, protein (albumin), and glucose after low-flow sevoflurane anesthesia, suggesting possible nephrotoxicity,[7–9] whereas others report no change.[10–14]

Probenecid, a uricosuric agent, is a selective inhibitor of organic anion transport.[15,16] Kharasch *et al.*[5,6] demonstrated that probenecid pretreatment completely prevented compound A–induced renal injury in rats, suggesting that probenecid-sensitive organic anion transport has an important role in compound A nephrotoxicity in rats. The purpose of the present study was to investigate further the potential nephrotoxicity of compound A by investigating renal function and several biochemical markers of nephrotoxicity in surgical patients anesthetized with low- and high-flow sevoflurane. We also studied whether probenecid diminishes urinary excretion of several biochemical markers of renal injury.

## Methods

The present study was conducted at the Self Defense Force Central Hospital in Tokyo, Japan, and was approved by its Hospital Ethics Committee. Written informed consent was obtained from each patient before participation in the study. The patients were 64 men undergoing anesthesia for orthopedic surgery with anticipated duration of more than 3 h. Patients whose medical history, physical examination, or laboratory test results yielded evidence of abnormal hepatic or renal function were excluded from the study. Patients were divided into one of four groups (n = 16 each): low-flow sevoflurane plus probenecid (LSP), low-flow sevoflurane (LS), high-flow sevoflurane plus probenecid (HSP), or high-flow sevoflurane (HS). The low-flow groups (LSP, LS) were enrolled first, then the high-flow groups (HSP, HS) were added. Patients in either the low- or high-flow

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*‡§ Staff Anesthesiologist, ‖ Chief Anesthesiologist, Department of Anesthesia, Self Defense Force Central Hospital. †Senior Resident, # Professor and Chair, Department of Anesthesiology, National Defense Medical College, Saitama, Japan.

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Address reprint requests to Dr. Higuchi: Department of Anesthesia, Self Defense Force Central Hospital, 1-2-24 Ikejiri, Setagaya, Tokyo 154-8532, Japan. Address electronic mail to: higu-chi@ka2.so-net.or.jp. 

**Table 1. Patient Demographics**

| Group | Age (yr) | Height (cm) | Weight (kg) | Duration of Anesthesia (min) | MAC-h | Mean MAC | Duration of Surgical Procedure (min) | Surgical Site (Knee/ Shoulder) | Duration of Tourniquet Inflation (min) |
|---|---|---|---|---|---|---|---|---|---|
| Low-flow sevoflurane plus probenecid | 25 ± 5 | 173 ± 6 | 70 ± 6 | 424 ± 82 | 11.1 ± 4.0 | 1.6 ± 0.4 | 312 ± 92 | 9/7 | 143 ± 25 |
| Low-flow sevoflurane | 24 ± 5 | 173 ± 5 | 69 ± 8 | 429 ± 69 | 11.6 ± 3.1 | 1.7 ± 0.4 | 310 ± 74 | 9/7 | 160 ± 37 |
| High-flow sevoflurane plus probenecid | 25 ± 5 | 169 ± 6 | 69 ± 13 | 426 ± 53 | 11.3 ± 2.0 | 1.6 ± 0.2 | 312 ± 50 | 9/7 | 156 ± 51 |
| High-flow sevoflurane | 25 ± 5 | 169 ± 7 | 69 ± 7 | 428 ± 74 | 11.5 ± 2.5 | 1.6 ± 0.2 | 327 ± 76 | 9/7 | 154 ± 65 |

* $P < 0.01$ compared with the low-flow sevoflurane group. † $P < 0.01$ compared with the low-flow plus probenecid sevoflurane group.

MAC = minimum alveolar concentration; pre = preanesthesia; lowest = the lowest mean arterial blood pressure during anesthesia; ave = average mean arterial blood pressure during anesthesia; AUC = area under the curve.

groups were assigned by surgery scheduled for either the knee or the shoulder (a tourniquet was inflated during the operation when the surgical site was the knee) using the stratified blocked randomization method. Probenecid (2.0 g) was administered orally 2 h before the induction of anesthesia in the probenecid groups (LSP and HSP). No probenecid was administered in the other two groups (LS and HS).

The anesthetic protocol was designed to result in prolonged high compound A concentrations, as previously described.[8] Anesthesia was generally induced 120 min before the scheduled time of the surgical procedure to increase the period of anesthesia. Thirty minutes after an intramuscular injection of atropine (0.5 mg) and midazolam (0.08 mg/kg), an intravenous injection of thiopental (3–5 mg/kg) and succinylcholine (1 mg/kg) or vecuronium bromide (0.1 mg/kg) was administered to each patient to facilitate tracheal intubation. After tracheal intubation, anesthesia was maintained with sevoflurane, air, and oxygen (fraction of inspired oxygen = 0.4) at a total flow of 6 l/min. After 5 min, the fresh gas flow rate was reduced to 1 l/min in the low-flow sevoflurane groups (LSP and LS). In the high–flow sevoflurane groups (HSP and HS), the fresh gas flow rate was maintained at a total flow of 6 l/min during anesthesia. A semiclosed recirculating system with a soda lime absorbent (Drägersorb 800, Dräger, Luebeck, Germany) was used to absorb carbon dioxide. The carbon dioxide absorbent was changed before the administration of anesthetics to each patient. The anesthesia machine was a North American Dräger Narcomed IIB (Telford, PA). The anesthetic was administered *via* a Penlon PPV$\Sigma$ vaporizer (Penlon, Abingdon, United Kingdom). Two sevoflurane vaporizers linked in series were used, permitting the administration of high concentrations of sevoflurane to patients in the low-flow system. The flow meters in the anesthesia machine were calibrated with a Calibration Analyzer RT-200 (Allied Healthcare, St. Louis, MO) before each study. A radial arterial catheter was inserted to monitor arterial blood pressure and to obtain blood samples for analysis of arterial blood gases. The lungs were ventilated mechanically with a tidal volume of 8–10 ml/kg, with the ventilatory rate adjusted to maintain an end-tidal carbon dioxide partial pressure of 35–40 mmHg. End-tidal concentrations of sevoflurane were analyzed using a Capnomac Ultima gas analyzer (Capnomac, Datex, Finland), which was calibrated immediately before each study using a cylinder that contained a mixture of gases of known concentrations. Minimum alveolar concentration (MAC)-hours for sevoflurane exposures were calculated from the percent anesthetic concentration and the duration of anesthetic exposure. Using the Mapleson formula, MAC-hour values were calculated as MAC = 2.0% for the age group studied.[17] The anesthetic concentration was adjusted by the anesthesiologist to maintain a mean arterial blood pressure within ± 20% of baseline. No adjunct anesthetics or vasoactive drugs were used. A temperature probe (model DT-300, Intermedical Co., Tokyo, Japan) was inserted into the center of the upper absorbent canister, and the soda lime temperature was recorded at 5-min intervals. After postoperative radiographs of the surgical site were obtained, anesthetic administration was discontinued, and the fresh gas inflow rate was changed to 6 l/min of oxygen. After each patient opened his eyes and took a deep breath on verbal command, the endotracheal tube was removed. All patients received cefotiam intravenously twice a day (2.0 g/day) as an antibiotic perioperatively, from immediately after the induction of anesthesia to day 2 after anesthesia. Thereafter, 600 mg cefotiam was administered orally for 5 days.

Lactated Ringer's solution 5–6 ml · $kg^{-1}$ · $h^{-1}$ was administered during anesthesia and 2 ml · $kg^{-1}$ · $h^{-1}$ was administered for 16 h after cessation of anesthetic exposure. Clinical laboratory studies, including serum uric acid, blood urea nitrogen (BUN), and serum creatinine concentrations, were performed immediately before anesthesia and repeated at 1, 3, 5, and 7 days after initiation

**Table 1.** *Continued*

| Blood Loss (ml) | Mean Arterial Blood Pressure (mmHg) | | | Compound A Concentration (ppm) | | Compound A Inspired AUC (ppm-h) |
|---|---|---|---|---|---|---|
| | Pre | Lowest | Ave | Peak | Mean | |
| 72 ± 54 | 79 ± 9 | 66 ± 5 | 77 ± 6 | 45.8 ± 9.8 | 29.5 ± 6.9 | 211.5 ± 75.2 |
| 80 ± 73 | 78 ± 8 | 68 ± 7 | 78 ± 8 | 46.6 ± 16.3 | 29.9 ± 7.4 | 215.4 ± 65.4 |
| 67 ± 84 | 79 ± 7 | 67 ± 7 | 80 ± 8 | 7.2 ± 3.4*† | 3.9 ± 1.9*† | 27.3 ± 13.9*† |
| 106 ± 137 | 78 ± 9 | 68 ± 7 | 79 ± 8 | 7.2 ± 1.9*† | 4.0 ± 1.8*† | 27.9 ± 11.9*† |

of anesthesia. Urine samples (24 h) were collected before anesthesia and for at least 7 days after anesthesia. These samples were kept in room air for 24 h on the orthopedic surgery ward and were thereafter used for the measurement of urinary excretion of uric acid, protein, albumin, $\beta_2$-microglobulin, glucose, *N*-acetyl-$\beta$-D-glucosaminidase (NAG), and creatinine. Postanesthetic urine collection began at the end of anesthesia for each 24-h period from 0 to 168 h.

Gas samples were obtained from the inspiratory limbs of the anesthetic circuit distal to the one-way valves *via* a capped stopcock port, using gas-tight glass syringes for compound A analysis. Inspiratory limb gas samples were obtained from the inspiratory limb every 1 h after intubation and at the end of anesthesia using a gas-tight locking syringe. The gas was injected into the gas chromatograph (GC-14A, Shimazu, Tokyo, Japan). A glass column with a length of 5 m and an ID of 3 mm packed with 20% dioctyl phthalate on a Chromosorb WAW (GL Science Co., Tokyo, Japan) 80/100 mesh was maintained at 110°C in the gas chromatograph. The injection port was maintained at 130°C. A carrier stream of nitrogen flowing at 30 ml/min was delivered through the column to a hydrogen flame ionization detector. The gas chromatograph was calibrated by preparing standard calibration gases from stock solutions of compound A supplied by Maruishi Pharmaceutical (Osaka, Japan).

Routine laboratory tests were performed, and urinary protein, albumin, $\beta_2$-microglobulin, glucose, and NAG concentrations were measured in the clinical laboratories of the Self Defense Force Central Hospital. The methods of protein, albumin, and glucose measurement were changed from those in our previous work.[8] Urinary protein concentrations (24 h) were measured with an Eimax241 Spectrophotometer (Fuji, Tokyo, Japan). Urinary glucose concentrations were measured with a Hitachi 7170 Auto Analyzer (Hitachi, Tokyo, Japan). The lowest detectable level of protein or glucose was 1 mg/dl, far more sensitivity than in our previous work. Urinary albumin concentration was measured with a Nephelometer Analyzer II (Behring, Marburg, Germany). Urinary $\beta_2$-microglobulin was measured by radioimmunoassay ($\beta_2$-Micro-RIABEARS, Dainabot, Tokyo, Japan). Urinary NAG activity (24 h) was determined colorimetrically using a commercially available method (Shionogi, Osaka, Japan).

Total compound A exposure was calculated from the area under the curve (AUC) of compound A concentration *versus* time using the trapezoid rule. Values are expressed as mean ± SD. Patients' demographic data

**Table 2. Preoperative and Postoperative Serum BUN, Creatinine Concentrations, and Creatinine Clearance**

| | Group | Preanesthesia | Day 1 | Day 3 | Day 5 | Day 7 |
|---|---|---|---|---|---|---|
| Serum BUN (mg/dl) | Low-flow sevoflurane plus probenecid | 13 ± 3 | 11 ± 3* | 11 ± 2* | 13 ± 3 | 14 ± 4 |
| | Low-flow sevoflurane | 14 ± 3 | 10 ± 3* | 10 ± 3* | 14 ± 4 | 15 ± 4 |
| | High-flow sevoflurane plus probenecid | 14 ± 3 | 10 ± 4* | 11 ± 3* | 15 ± 4† | 16 ± 4† |
| | High-flow sevoflurane | 13 ± 4 | 10 ± 4* | 10 ± 2* | 13 ± 3 | 14 ± 3 |
| Serum creatinine (mg/dl) | Low-flow sevoflurane plus probenecid | 0.9 ± 0.2 | 0.9 ± 0.1 | 0.8 ± 0.1* | 0.9 ± 0.1† | 0.9 ± 0.1 |
| | Low-flow sevoflurane | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.2† | 0.9 ± 0.2 | 0.9 ± 0.1 |
| | High-flow sevoflurane plus probenecid | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.1* | 0.8 ± 0.1† | 0.8 ± 0.1 |
| | High-flow sevoflurane | 0.9 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1† | 0.8 ± 0.1 | 0.9 ± 0.1 |
| Creatinine clearance (ml/min) | Low-flow sevoflurane plus probenecid | 110 ± 24 | 127 ± 32† | 113 ± 24 | 104 ± 18 | 104 ± 23 |
| | Low-flow sevoflurane | 106 ± 18 | 127 ± 27† | 98 ± 19 | 104 ± 24 | 110 ± 30 |
| | High-flow sevoflurane plus probenecid | 109 ± 35 | 129 ± 49 | 127 ± 47 | 112 ± 49 | 106 ± 37 |
| | High-flow sevoflurane | 113 ± 28 | 144 ± 26† | 128 ± 41 | 113 ± 30 | 104 ± 19 |

* $P < 0.01$ compared with preanesthesia value. † $P < 0.05$ compared with preanesthesia value.
BUN = blood urea nitrogen.

**Table 3. Time Course of Urinary Excretion of Protein, Albumin, $\beta_2$-Microglobilin, Glucose, and NAG**

| | | | Days after Anesthesia | |
|---|---|---|---|---|
| | Group | Pre | 1 | 2 |
| 24-h urinary protein excretion (mg) | Low-flow sevoflurane plus probenecid | 60 ± 33 | 94 ± 32* | 106 ± 44*† |
| | Low-flow sevoflurane | 54 ± 18 | 279 ± 508† | 293 ± 296† |
| | High-flow sevoflurane plus probenecid | 65 ± 16 | 73 ± 31†‡ | 62 ± 32‡ |
| | High-flow sevoflurane | 47 ± 17 | 144 ± 222*† | 84 ± 47‡ |
| 24-h urinary albumin excretion (mg) | Low-flow sevoflurane plus probenecid | 8 ± 4 | 12 ± 7* | 14 ± 7† |
| | Low-flow sevoflurane | 7 ± 5 | 55 ± 87† | 67 ± 84† |
| | High-flow sevoflurane plus probenecid | 8 ± 7 | 15 ± 12*§ | 9 ± 6‡ |
| | High-flow sevoflurane | 8 ± 5 | 20 ± 24§ | 10 ± 6‡ |
| 24-h urinary $\beta_2$-microglobulin excretion ($\mu$g/g · creatinine) | Low-flow sevoflurane plus probenecid | 45 ± 14 | 146 ± 234*† | 218 ± 299† |
| | Low-flow sevoflurane | 44 ± 18 | 3,073 ± 10,294† | 2,691 ± 8,743† |
| | High-flow sevoflurane plus probenecid | 54 ± 36 | 118 ± 115§ | 156 ± 128 |
| | High-flow sevoflurane | 46 ± 23 | 443 ± 1,317† | 285 ± 408† |
| 24-h urinary glucose excretion (mg) | Low-flow sevoflurane plus probenecid | 55 ± 42 | 225 ± 270† | 446 ± 931† |
| | Low-flow sevoflurane | 65 ± 19 | 535 ± 1,000† | 750 ± 1,471† |
| | High-flow sevoflurane plus probenecid | 66 ± 77 | 145 ± 110§ | 117 ± 96* |
| | High-flow sevoflurane | 62 ± 30 | 205 ± 194† | 111 ± 51* |
| 24-h urinary NAG excretion (U/g · creatinine) | Low-flow sevoflurane plus probenecid | 1.6 ± 0.7 | 1.6 ± 1.1‖ | 1.9 ± 1.2‡ |
| | Low-flow sevoflurane | 1.5 ± 0.8 | 2.9 ± 4.9 | 4.6 ± 4.1† |
| | High-flow sevoflurane plus probenecid | 1.9 ± 0.8 | 2.2 ± 0.9 | 2.1 ± 1.3* |
| | High-flow sevoflurane | 2.0 ± 0.6 | 2.6 ± 1.3 | 3.8 ± 5.0 |

* $P < 0.05$ compared with the low-flow sevoflurane group. † $P < 0.01$ compared with preanesthesia value. ‡ $P < 0.01$ compared with the low-flow sevoflurane group. § $P < 0.05$ compared with preanesthesia value. ‖ $P < 0.05$ compared with the high-flow sevoflurane group.

NAG = *N*-acetyl-$\beta$-glucosaminidase; pre = preanesthesia; max = maximum value after anesthesia; ave = average value after anesthesia.

were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls *post hoc* test. Intergroup and intragroup comparisons of laboratory data were analyzed using a two-way repeated-measures analysis of variance followed by the Student-Newman-Keuls *post hoc* test for multiple comparison. Comparison of the excretion of urinary-sensitive markers among the four groups was performed using the Friedman test or the Kruskal-Wallis test followed by the Dunn *post hoc* test. Regression analysis was used to evaluate the correlation between inspired compound A AUC and maximum or average values of several markers after anesthesia, using the Spearman rank correlation. Differences were considered statistically significant if the *P* value was less than 0.05.

## Results

The data for the individual patients in tables 1–3 can be found on the Journal's Web site. Patient demographic data are presented in table 1. The four groups were identical with respect to general clinical characteristics, including age, height, weight, duration of anesthesia, and anesthetic dosage. Measurements of blood pressure and heart rate did not differ among the four groups. The individual peak concentrations of compound A were 45.8 ± 9.8 ppm (LSP), 46.6 ± 16.3 ppm (LS), 7.2 ± 3.4 ppm (HSP), and 7.2 ± 1.9 ppm (HS). The individual mean concentrations were 29.5 ± 6.9 ppm (LSP), 29.9 ± 7.4 ppm (LS), 3.9 ± 1.9 ppm (HSP), and 4.0 ± 1.8 ppm (HS). There was no difference in inspired compound A AUC between the LSP and LS groups or between the HSP and HS groups (table 1).

Mean serum uric acid concentrations in the groups receiving probenecid (LSP, 1.8 ± 0.6 mg/dl; HSP, 1.9 ± 0.8 mg/dl) and the other two groups (LS, 4.2 ± 1.0 mg/dl; HS, 4.8 ± 1.2 mg/dl) 1 day after anesthesia were significantly different from each other ($P < 0.0001$). Urinary excretion of uric acid during anesthesia was significantly greater in the probenecid-treated groups (LSP, HSP) than in the other groups (LS, HS; fig. 1).

The four groups did not differ in clinical laboratory baseline values, and no abnormal changes in the results of the renal function studies were noted during the study period in any of the four groups. BUN and serum creatinine concentration did not increase, nor did creatinine clearance decrease in any patient (table 2).

Results of 24-h urinary excretion of protein, albumin, $\beta_2$-microglobulin, glucose, and NAG for the four groups before and 1–7 days after anesthesia are shown in figs. 2–6. Proteinuria, glucosuria, and enzymuria were observed after anesthesia in all groups (table 3; figs. 2–6). Urinary excretion of protein, albumin, $\beta_2$-microglobulin, glucose, and NAG were significantly greater in the LS group than in the other three groups (LSP, HSP, HS) after anesthesia (table 3; figs. 2–6). Maximum and average values for urinary excretion of the biochemical markers are shown in table 3 and figure 7. There were significant differences in the average values of urinary excretion of protein, $\beta_2$-microglobulin, and NAG after anesthesia between the LSP and the LS groups (table 3 and figs. 7F, 7H, and 7J). Although there were significant differences between LS and high-flow sevoflurane groups (HSP, HS) in the average for urinary excretion of albumin and glucose, there was no significant difference between the

**Table 3.** *Continued*

| Days after Anesthesia | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 4 | 5 | 6 | 7 | Max | Ave |
| 106 ± 50 | 89 ± 52 | 64 ± 39 | 60 ± 37 | 65 ± 35 | 129 ± 55* | 83 ± 30‡ |
| 339 ± 403† | 240 ± 267§ | 140 ± 117 | 97 ± 63 | 88 ± 44 | 466 ± 562 | 211 ± 214 |
| 65 ± 24‡§ | 53 ± 36‡ | 42 ± 24‡ | 56 ± 57‡ | 49 ± 37‡ | 100 ± 51‡ | 57 ± 22‡ |
| 77 ± 39‡ | 48 ± 31‡ | 50 ± 28‡ | 44 ± 27‡ | 48 ± 31‡ | 150 ± 219‡ | 70 ± 47‡ |
| 12 ± 8 | 12 ± 6 | 9 ± 6 | 9 ± 4 | 8 ± 3 | 17 ± 7* | 11 ± 5 |
| 115 ± 165† | 129 ± 186† | 48 ± 75 | 21 ± 29 | 9 ± 6 | 145 ± 191 | 63 ± 86 |
| 12 ± 7 | 11 ± 10‡ | 8 ± 5 | 8 ± 4 | 7 ± 4 | 20 ± 14* | 10 ± 5* |
| 9 ± 5* | 9 ± 7‡ | 7 ± 3* | 8 ± 4 | 7 ± 4 | 21 ± 24* | 10 ± 5* |
| 172 ± 247† | 67 ± 39 | 54 ± 26* | 52 ± 23 | 41 ± 20 | 285 ± 370 | 107 ± 99* |
| 1,111 ± 2,514† | 339 ± 942 | 189 ± 364 | 97 ± 115 | 61 ± 37 | 3,411 ± 10,863 | 1,075 ± 3,346 |
| 248 ± 341 | 115 ± 184 | 138 ± 236 | 100 ± 135 | 116 ± 200 | 341 ± 374 | 141 ± 139 |
| 159 ± 151† | 88 ± 103 | 55 ± 48‡ | 79 ± 102 | 53 ± 36 | 609 ± 1,324 | 166 ± 244 |
| 144 ± 108† | 102 ± 59 | 145 ± 305 | 58 ± 30* | 58 ± 24 | 555 ± 934 | 168 ± 194 |
| 732 ± 1,395† | 588 ± 1,067† | 196 ± 282 | 117 ± 83 | 94 ± 94 | 833 ± 1,498 | 430 ± 749 |
| 190 ± 231 | 85 ± 94‡ | 46 ± 30‡ | 67 ± 60* | 52 ± 21 | 241 ± 216 | 100 ± 66* |
| 101 ± 74 | 62 ± 27‡ | 79 ± 82 | 64 ± 43* | 58 ± 22 | 239 ± 194 | 97 ± 40* |
| 2.7 ± 1.1 | 3.0 ± 1.8§ | 2.5 ± 1.4 | 2.6 ± 1.4 | 2.3 ± 1.6 | 3.8 ± 1.5 | 2.4 ± 1.1* |
| 6.1 ± 5.2† | 4.2 ± 2.8† | 4.2 ± 3.0† | 3.1 ± 1.5 | 3.1 ± 1.8 | 7.5 ± 5.2 | 4.0 ± 2.4 |
| 2.5 ± 1.5* | 2.8 ± 1.0† | 3.0 ± 1.7§ | 3.2 ± 2.4† | 3.0 ± 2.4 | 3.8 ± 2.3* | 2.7 ± 1.3 |
| 4.0 ± 4.0 | 3.8 ± 2.4† | 3.8 ± 1.9 | 3.3 ± 2.1 | 2.8 ± 1.0 | 5.7 ± 5.1 | 3.4 ± 2.1 |

LSP and LS groups in average values for urinary excretion of albumin and glucose (table 3 and figs. 7G and 7I).

Figure 8 shows the relation between maximum and average values for urinary excretion of the biochemical markers and inspired compound A AUC in patients in the LS and HS groups (n = 32). There were statistically significant correlations between the maximum or average values for urinary excretion of several biochemical markers and inspired compound A AUC (maximum: protein, albumin, $\beta_2$-microglobulin, and glucose; average: protein, albumin, $\beta_2$-microglobulin, and NAG; figs. 8A–H). However, the correlations were strengthened by patients anesthetized with high-flow sevoflurane and were not statistically significant without the data from these patients.

## Discussion

The present study demonstrated that prolonged low-flow sevoflurane anesthesia is associated with increases in urinary excretion of several biochemical markers of nephrotoxicity in surgical patients, consistent with the findings of Eger *et al.*[7] and Goldberg *et al.*,[9] as well as our previous studies.[8] However, conflicting data from human studies have been reported. Ebert *et al.*[10] observed no significant change in urinary biochemical markers in volunteers exposed to 3% sevoflurane for 8 h. Bito *et al.*[11] and Kharasch *et al.*[12] reported that there were no significant differences in urinary biochemical markers between low-flow sevoflurane and low-flow isoflurane anesthesia in surgical patients who underwent

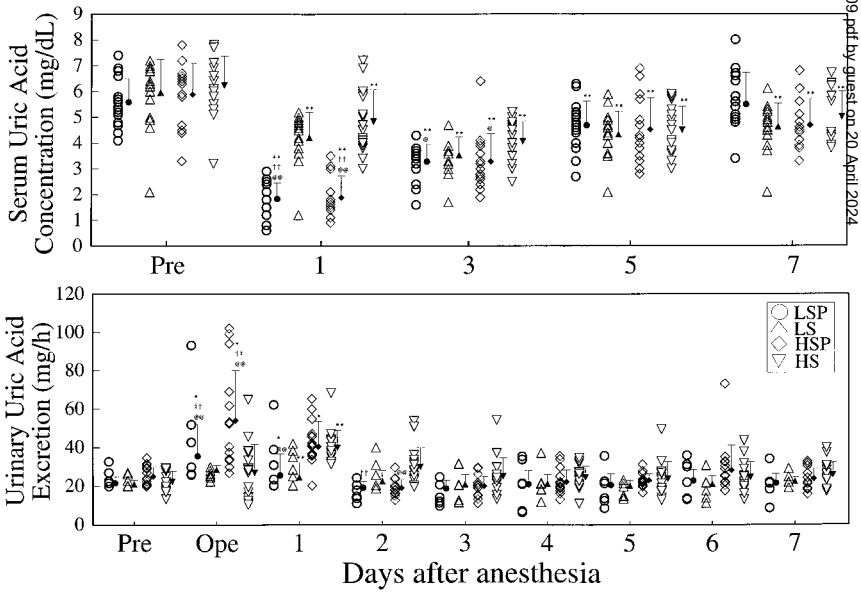


**Fig. 1. Changes over time in serum uric acid concentration (*top*) and urinary excretion of uric acid (*bottom*) in the four groups. Individual (open symbols) and mean ± SD values (closed symbols) are shown. Serum uric acid concentrations in the groups that received probenecid (low-flow sevoflurane plus probenecid [LSP], high-flow sevoflurane plus probenecid [HSP]) were significantly lower than in the control groups (low-flow sevoflurane [LS], high-flow sevoflurane [HS]) on day 1 after anesthesia. Urinary excretions of uric acid in the probenecid-treated groups were significantly higher than in the control groups during anesthesia. *$P$ < 0.05 compared with each preoperative value. **$P$ < 0.01 compared with each preoperative value. †$P$ < 0.05, ††$P$ < 0.01 compared with the LS group. @$P$ < 0.05, @@$P$ < 0.01 compared with the HS group.**

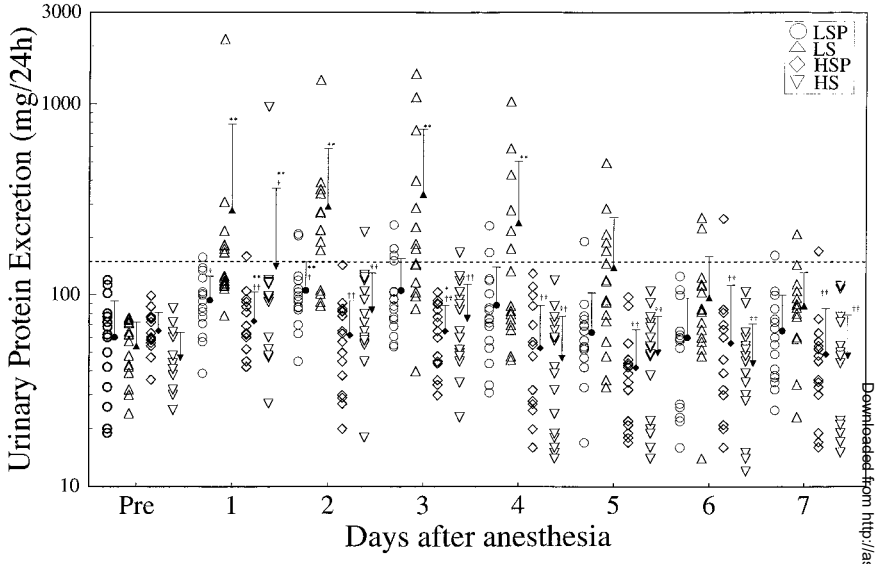


**Fig. 2. Changes over time in urinary excretion of total protein in the four groups. Individual (open symbols) and mean ± SD values (closed symbols) are shown. Note the logarithmic scale used in this graph. The dotted line represents the upper limit of the reference range (150 mg/24 h). Urinary excretion of protein in the low-flow sevoflurane (LS) group was significantly higher than in the other three groups (low-flow sevoflurane plus probenecid [LSP], high-flow sevoflurane plus probenecid [HSP], high-flow sevoflurane [HS]) on days 1–2 or in the high-flow groups (HSP, HS) on days 3–7 after anesthesia. *$P$ < 0.05 compared with each preoperative value. **$P$ < 0.01 compared with each preoperative value. †$P$ < 0.05, ††$P$ < 0.01 compared with the LS group.**

mostly major abdominal surgery. Furthermore, other studies reported that there was no difference in renal effects between low-flow sevoflurane and the other anesthetics.[13,14] There are no obvious explanations to completely explain the discrepancy regarding urinalysis changes in these studies. However, the difference in inspired compound A AUC might partially account for the discrepancy. Compound A nephrotoxicity in rats is dose-dependent,[2–6] and the dose-dependent effect might be applicable to humans.[9,18] Goldberg *et al.*[9] determined that the inspired compound A AUC in the study by Ebert *et al.*[10] was 220 ppm-h. If so, the compound A inspired AUCs in the studies by Bito *et al.*,[11] Kharasch *et al.*,[12] and Ebert *et al.*[10] were 122 ppm-h, 79 ppm-h, and 220 ppm-h, respectively, whereas the corresponding values in our previous study, the present study, and the studies by Eger *et al.*[7] and Goldberg *et al.*[9] were 192 ppm-h, 219 ppm-h, 328 ppm-h, and 253 ppm-h, respectively. The differences in biochemical changes in the study by Ebert *et al.*[10] and our studies might be explained by the antibiotics administered or concurrent imposition of surgery. In the present study, all patients received cefotiam. Cefotiam-treated patients displayed increases in protein and urinary excretion of lysosomal enzymes (leucine aminopeptidase).[19] The extent of the increase in urinary excretion of NAG is proportional to the stress induced by surgery.[20]

Other differences among several clinical studies were the average maximum compound A concentration, postural change during the study, and study conditions that varied from routine clinical anesthesia (high sevoflurane concentration, hypotension, and volume restriction); however, it is unknown whether these differences can account for the discrepancy for the results among the studies. The volunteers and patients in the previous and present studies were not necessarily in the recumbent position throughout the study: hypotension of volunteers was treated with a head-down tilt, and the positions of our patients changed in accord with the sur

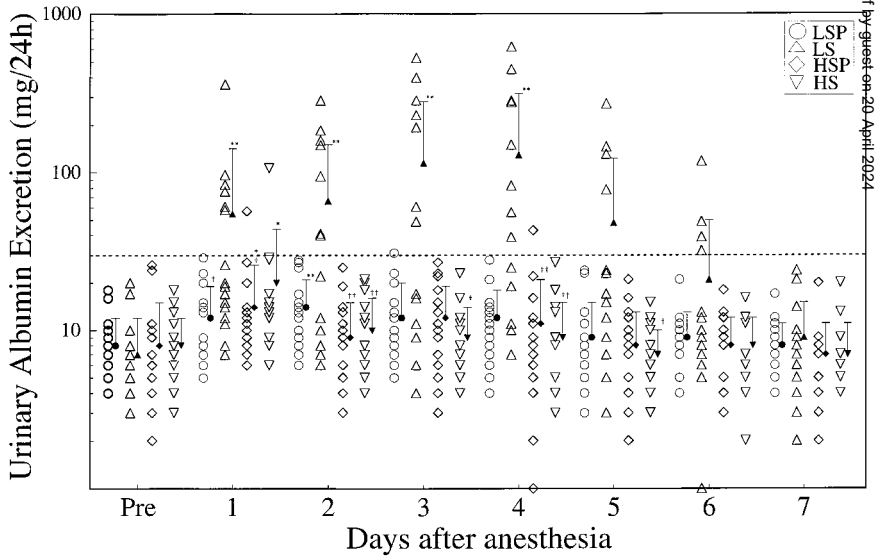


**Fig. 3. Changes over time in urinary excretion of albumin in the four groups. Individual (open symbols) and mean ± SD values (closed symbols) are shown. Note the logarithmic scale used in this graph. The dotted line represents the upper limit of the reference range (30 mg/24 h). Urinary excretion of albumin in the low-flow sevoflurane (LS) group was significantly higher than in the other groups on days 1–5 after anesthesia. *$P$ < 0.05 compared with each preoperative value. **$P$ < 0.01 compared with each preoperative value. †$P$ < 0.05, ††$P$ < 0.01 compared with the LS group. LSP = low-flow sevoflurane plus probenecid; HSP = high-flow sevoflurane plus probenecid; HS = high-flow sevoflurane.**

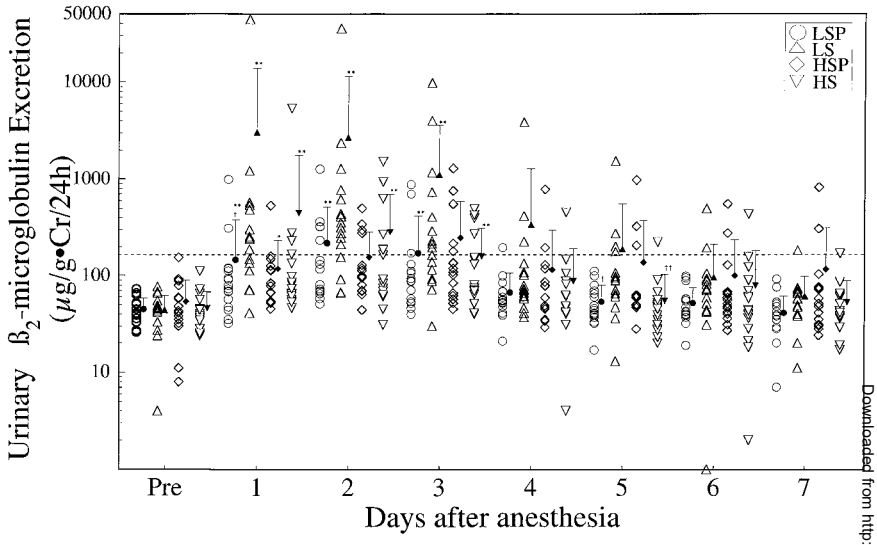


**Fig. 4. Changes over time in urinary excretion of ß$_2$-microglobulin in the four groups. Individual (open symbols) and mean ± SD values (closed symbols) are shown. Note the logarithmic scale used in this graph. The dotted line represents the upper limit of the reference range (0.25 mg/24 h). Urinary excretion of ß$_2$-microglobulin in the low-flow sevoflurane (LS) group was significantly higher than in the low-flow sevoflurane plus probenecid (LSP) group on days 1 and 5 or in the high-flow sevoflurane (HS) group on day 5 after anesthesia. *$P$ < 0.05 compared with each preoperative value. **$P$ < 0.01 compared with each preoperative value. †$P$ < 0.05, ††$P$ < 0.01 compared with the LS group. HSP = high-flow sevoflurane plus probenecid.**

geon's orders (often head-down position). It has been reported that postural stress or physical exertion causes proteinuria.[21] Finally, although Eger *et al.*[7] and Goldberg *et al.*[9] theorized that blood pressure was not an important contributor to the transient renal injury observed in their studies, Ebert *et al.*[10] insisted that low blood pressure should not be ruled out as a contributor or cofactor.

The increased albumin concentration observed in the present study might have been caused by a reduction in reabsorption by the renal tubules, because albuminuria was almost always less than 500 mg/day.[22] Including albumin, the increased excretion of sensitive markers in the present study may suggest that the effects observed are caused by compound A, because the only major difference between the LS and HS groups was exposure to compound A. However, toxicity is closely related to exposure to inhaled toxins.[12] In the present study, there was no significant relation between compound A exposure in patients anesthetized with low-flow sevoflurane and the excretion of sensitive markers. Consequently, we could not definitively demonstrate that compound A was responsible for the observed effects.

Probenecid is completely absorbed after oral administration. Peak concentrations in plasma are reached 2–4 h after oral administration. The plasma half-life ranges from 4 to 17 h in volunteers given 2.0 g probenecid orally.[15,16] In the present study, a single oral dose of probenecid (2.0 g) administered 2 h before induction of anesthesia significantly increased urinary excretion of uric acid during anesthesia and diminished the serum uric acid concentrations on day 1 in the probenecid-treated groups (LSP, HSP) compared with the control groups (LS, HS; fig. 1). These findings suggest that the inhibition of organic anion transport by a single-dose probenecid was sufficient during anesthesia. The four groups did not differ in urinary excretion of creatinine (data not shown). To our knowledge, probenecid does not alter the levels of the biochemical markers measured in the present study.[23] Consequently, it is unlikely that urinary excretion levels of biochemical markers, including those that were ex

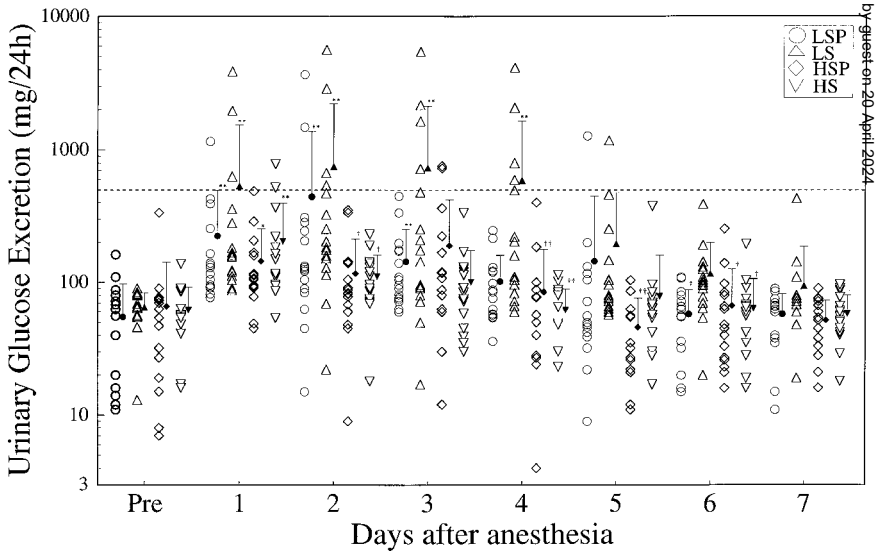


**Fig. 5. Changes over time in urinary excretion of glucose in the four groups. Individual (open symbols) and mean ± SD values (closed symbols) are shown. Note the logarithmic scale used in this graph. The dotted line represents the upper limit of the reference range (500 mg/24 h). Urinary excretion of glucose in the low-flow sevoflurane (LS) group was significantly higher than in the low-flow sevoflurane plus probenecid (LSP) group on day 6, in the high-flow sevoflurane plus probenecid (HSP) group on days 2 and 4–6, or in the high-flow sevoflurane (HS) group on days 2, 4, and 6 after anesthesia. *$P$ < 0.05 compared with each preoperative value. **$P$ < 0.01 compared with each preoperative value. †$P$ < 0.05, ††$P$ < 0.01 compared with the LS group.**

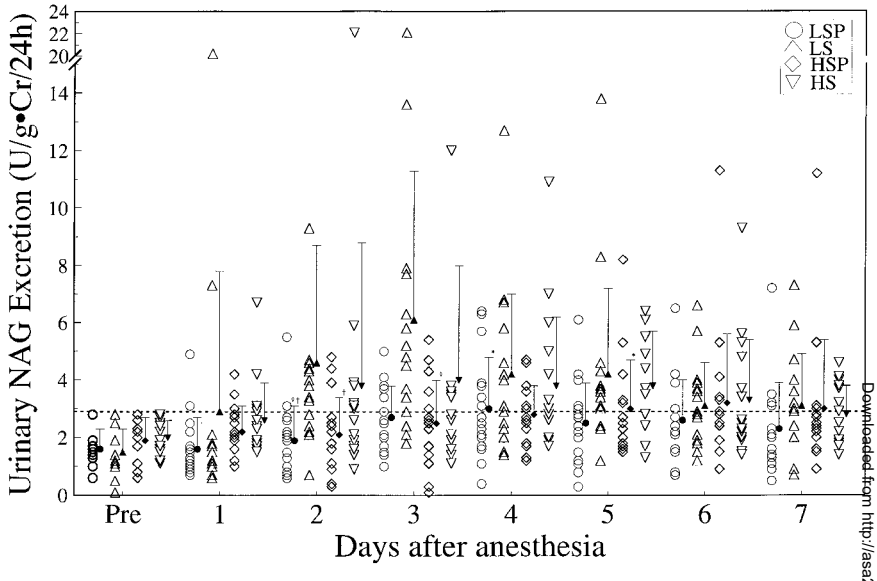


**Fig. 6. Changes over time in urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG) in the four groups. Individual (open symbols) and mean ± SD values (closed symbols) are shown. The dotted line represents the upper limit of the reference range (2.9 U/g · creatinine/24 h). Urinary excretion of NAG in the low-flow sevoflurane (LS) group was significantly higher than in the low-flow sevoflurane plus probenecid (LSP) group on day 2 or in the high-flow sevoflurane plus probenecid (HSP) group on days 2–3 after anesthesia. *$P < 0.05$ compared with each preoperative value. **$P < 0.01$ compared with each preoperative value. †$P < 0.05$, ††$P < 0.01$ compared with the LS group. @$P < 0.05$ compared with the high-flow sevoflurane (HS) group.**

pressed per urinary gram of creatinine, were altered by probenecid.

The mechanism of compound A nephrotoxicity in rats is still debated.[4–7,10,24–29] It is thought by some investigators that the β-lyase pathway also underlies this nephrotoxicity.[4–6,10,24–26,29] The β-lyase pathway consists of glutathione conjugate formation, cleavage to cysteine conjugates, renal uptake of cysteine and glutathione conjugates, and intrarenal metabolism by cysteine conjugate β-lyase to toxic reactive intermediates. This mechanism of renal injury, which involves the β-lyase pathway, has been suggested for several haloalkenes, structurally related compounds (*e.g.*, tetrafluoroethylene).[30–32] Probenecid prevents haloalkene-induced nephrotoxicity, which is mediated by the β-lyase pathway, indicating a role for the renal organic anion transport system in nephrotoxicity.[30–32] Diminished urinary excretion of the biochemical markers of nephrotoxicity after probenecid administration in the present study may suggest that organic anion transport might have a central role in compound A–related increases in biochemical markers in humans. However, evidence that probenecid-treated patients have lower urinary excretion of compound A–derived conjugates, corresponding mercapturates, or the organic acid metabolite of β-lyase–catalyzed metabolism of compound A–derived cysteine conjugates, is required before concluding that probenecid prevents the renal uptake of compound A–derived conjugates and corresponding mercapturates and, hence, compound A nephrotoxicity.

Cephalosporins are potentially nephrotoxic, although they do not commonly have nephrotoxic effects at therapeutic doses.[33] Riegel and Hörl[19] reported that patients treated with cefotiam (5 g/day), which was administered in our previous[8] and present studies (2 g/day), displayed proteinuria and enzymuria. Consequently, cefotiam might also be associated with proteinuria and enzymuria in patients anesthetized with low-flow sevoflurane in our present and previous studies.[8] Cephalosporins are transported by renal organic anion transport, and cephalosporin nephrotoxicity in animals can be prevented by probenecid.[34,35] Probenecid might prevent transportation of cefotiam and diminish cefotiam-related increases in urinary protein and enzyme excretion. However, there was no significant difference in urinary excretion of sensitive markers between the HSP and HS groups (figs. 2–8). In the present study, patients in the probenecid groups received probenecid only once, whereas all patients received cefotiam for 7 days. Therefore, if probenecid was administered for a longer duration, different results might be achieved.

There are several potential limitations to the present investigation. Our patients were administered cefotiam and subjected to surgery. Although the dose of cefotiam remained in the therapeutic range and the sites of surgery in our healthy patients were always in the extremities, we cannot exclude the possibility of effects of cefotiam or surgical trauma on the excretion of the markers. Our findings concerning changes in the biochemical markers of renal function must be interpreted carefully.[8,10,12,33,37] As discussed in our previous report,[8] there are some reports that urinary NAG is variable and is therefore not a reliable marker,[12,36,37] because urinary excretion of NAG is not specific and is affected by many factors, such as surgical stress and hypertensive episodes.[37,38] It is also possible that urinary enzymes or low-molecular-weight proteins are too sensitive, in that elevations are sometimes present in the absence of other measurable abnormalities.[38] Although increased urinary excretion of protein is a reliable marker of renal impairment, compared with urinary enzymes, proteinuria can occur in completely benign conditions and is not necessarily predictive of subsequent renal disease.[39] Furthermore, we must define the "normal limits" for specific

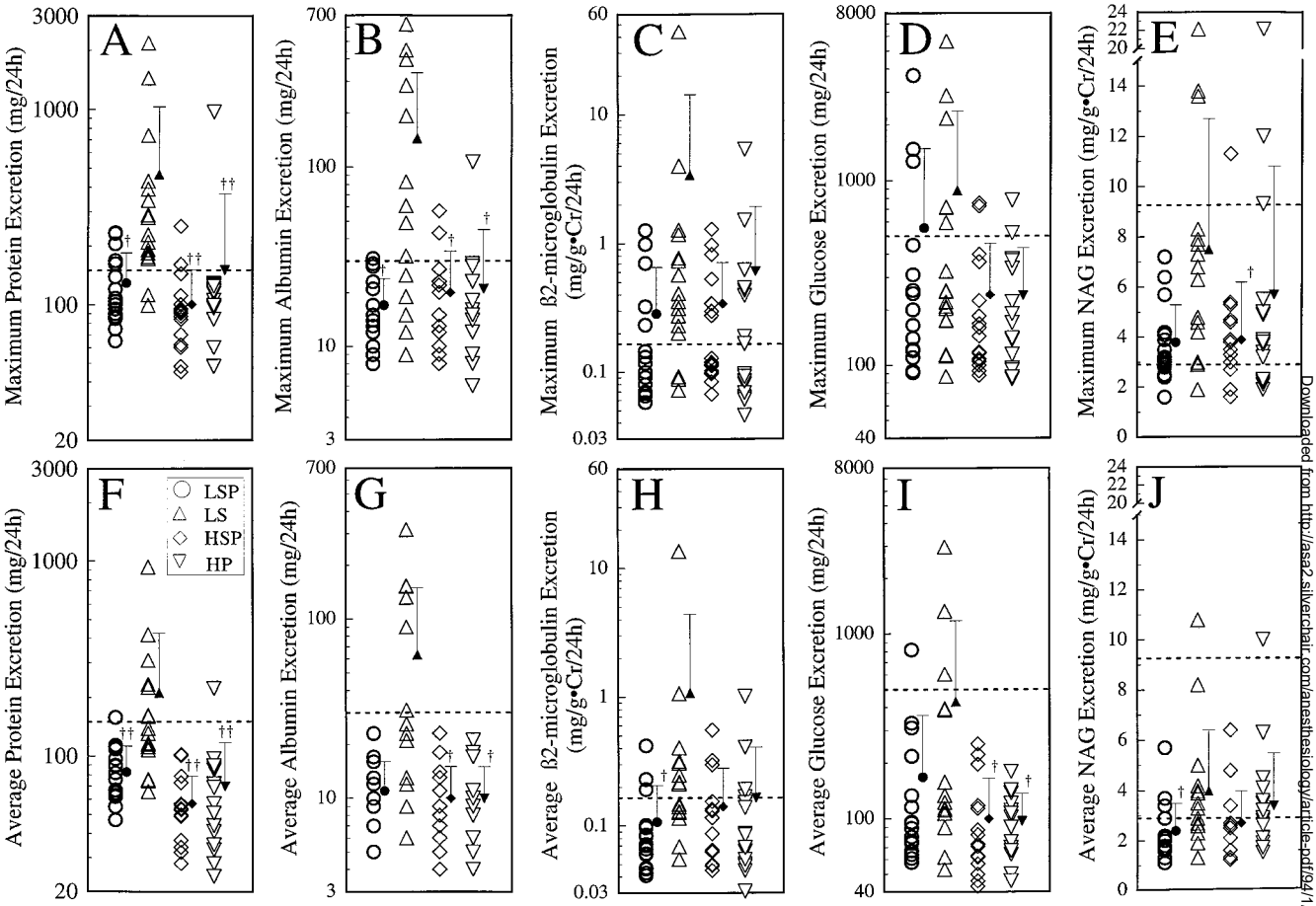


**Fig. 7. Maximum values of biochemical markers after anesthesia (*top*; *A–E*) and average values during 7 days after anesthesia (*bottom*; *F–J*). Individual (open symbols) and mean ± SD values (closed symbols) are shown. Note the logarithmic scale used in *A–D* and *F–I*. The dotted line represents the upper limit of the reference range. There were significant differences in the maximum urinary excretion of protein (*A*) and albumin (*B*) after anesthesia between the low-flow sevoflurane (LS) and the other three groups (low-flow sevoflurane plus probenecid [LSP], high-flow sevoflurane plus probenecid [HSP], high-flow sevoflurane [HS]) and *N*-acetyl-*β*-D-glucosaminidase (NAG; *E*) after anesthesia between the LS and the HSP groups. There were significant differences in the average urinary excretion of protein (*F*) between the LS and the other three groups (LSP, HSP, HS); both albumin (*G*) and glucose (*I*) between the LS and the HSP, HS groups; and both $\beta_2$-microglobulin (*H*) and NAG (*J*) between the LSP and LS groups. †$P < 0.05$, ††$P < 0.01$ compared with the LS group.**

populations (*i.e.*, surgical patients). The normal limits of the sensitive markers used in the present study were derived from healthy persons who were not undergoing anesthesia or surgery in the recumbent position. Elevations of the parameters above these normal limits do not reflect renal dysfunction because it is reported that the average excretion of protein, glucose, and NAG in patients receiving isoflurane for surgical procedures exceeded the laboratory normal limits, although the cause is unknown.[12] There were some patients in which excretion of $\beta_2$-microglobulin increased 20–1,000-fold after anesthesia, whereas 24-h creatinine clearance (the gold standard of renal function) actually increased. These findings cast doubt on the validity of protein, $\beta_2$-microglobulin, glucose, and NAG excretion using normal limits derived from healthy young subjects as valid measures of postoperative renal function in surgical patients. Consequently, we might simply measure the changes of these sensitive markers preoperatively in the previous and present studies, and probenecid might exert a nonspecific effect that is independent of anesthetic or flow rate used.[8] Finally, we must also consider how postoperative renal function should be assessed in surgical patients, which was the question raised by Mazze and Jamision[36] in an editorial accompanying the articles by Bito *et al.*[11] and Kharasch *et al.*[12] Certainly, serum creatinine concentration is not a good marker of increased glomerular permeability nor tubular integrity, as Bedford and Ives warned.[40] However, measurements of BUN and serum creatinine are easily performed, inexpensive, and prognostically significant in clinical medicine.[36] In contrast, obtaining consecutive 24-h urine collections to measure urinary excretion of sensitive markers requires great effort from patients and medical workers and is expensive (particularly for $\beta_2$-microglobulin assays). Despite the cost and effort, sensitive markers do not provide conclusive information, because the interpretations of sensitive markers are not straight-

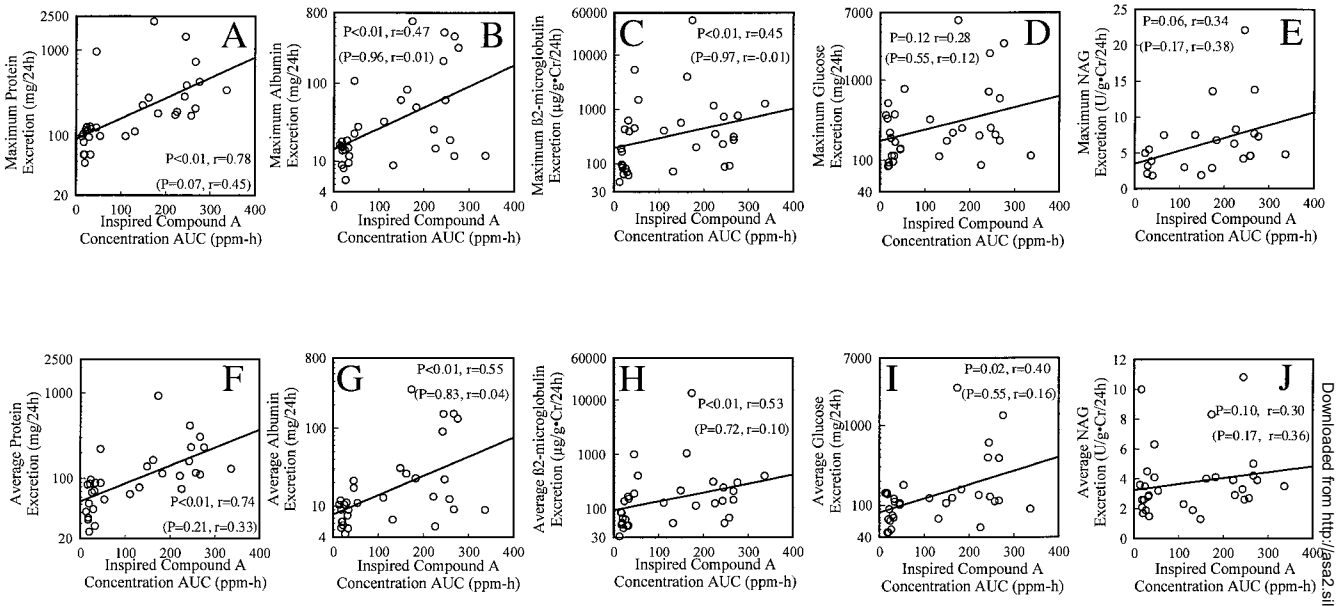


**Fig. 8. Relation between maximum (*top*; *A–E*) or average values (*bottom*; *F–J*) for urinary excretion of biochemical markers and inspired compound A area under the curve (AUC) in both the low-flow sevoflurane (n = 16; closed circles) and high-flow sevoflurane (n = 16; open circles) groups. *P* values and *r* coefficients for all patients (n = 32) are presented. Corresponding values for only the low-flow sevoflurane group (n = 16) are presented in parentheses. Although *P* values and correlation coefficients were calculated using the Spearman rank correlation with raw data (not log-transformed), logarithmic scale and the log linear regression lines are used in *A–D* and *F–J*. In *E* and *J*, linear axes are used, and the regression lines are linear. NAG = *N*-acetyl-β-D-glucosaminidase.**

forward, and the validity of sensitive markers as a reliable indicator of clinically significant renal injury has not been established. Thus, further studies investigating renal function in surgical patients using the sensitive markers are not warranted, *i.e.*, measurement of BUN and creatinine concentration might be sufficient.[41]

In summary, low-flow sevoflurane anesthesia and exposure to 30 ppm compound A for 7 h was associated with increased urinary excretion of biochemical markers of nephrotoxicity in young, healthy surgical patients without any changes in BUN, creatinine concentration, and creatinine clearance. A single dose of probenecid (2.0 g) administered 2 h before the induction of anesthesia diminished urinary excretion of the biochemical markers of nephrotoxicity in surgical patients anesthetized with low-flow sevoflurane, although the underlying mechanism is unclear.

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