Renal Function in Fischer 344 Rats with Chronic Renal Impairment after Administration of Enflurane and Gentamicin

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To assess the potential for producing nephrotoxicity in rats with abnormal renal function, the renal effects of enflurane or halothane anesthesia, 1 MAC for two hours, were examined in six groups of six Fischer 344 rats each with surgically induced chronic renal impairment. As an additional predisposing factor, gentamicin, 5 mg/kg/day, was administered for one week before and for one week after anesthesia to three of the groups, one anesthetized with enflurane, one anesthetized with halothane, and one unanesthetized. No significant change in renal function could be attributed to either anesthetic agent. Serum inorganic fluoride levels four hours and 24 hours after enflurane anesthesia were similar in the gentamicin-treated and the non-gentamicin-treated groups. Clinically small but statistically significant increases in serum creatinine concentration and urinary flow occurred in all three gentamicin-treated groups during the period of treatment. Anesthesia with either enflurane or halothane in rats with chronic renal impairment treated with gentamicin did not result in additional renal damage. (Key words: Anesthesia, volatile: enflurane; halothane. Antibiotics: gentamicin. Ions: Fluoride. Kidney: failure; toxicity. Toxicity: renal.)

In 1973, Mazze and Cousins described a patient with methoxyfluorane-induced F⁻ nephropathy in whom renal impairment was markedly aggravated by the postoperative administration of gentamicin. They subsequently demonstrated in Fischer 344 rats, an animal model for anesthetic and aminoglycoside antibiotic nephrotoxicity, that there was toxic interaction between methoxyfluorane and gentamicin. Enflurane is also metabolized to F⁻ but to a lesser extent than is methoxyfluorane. Its administration does not result in clinically significant renal dysfunction in surgical patients and volunteers without renal disease nor has toxic interaction between gentamicin and enflurane been reported. However, there have been three reports of patients with abnormal renal function whose conditions worsened following enflurane anesthesia. This study was carried out to determine whether enflurane anesthesia would aggravate surgically induced chronic renal impairment in Fischer 344 rats concurrently treated with gentamicin.

Methods

Sixty 11-month-old, male, Fischer 344 rats were bedded on ground corn cob, four to a cage, and quarantined for a week prior to experimentation. Room temperature was maintained at 21 ± 1°C and artificial light was provided from 6 A.M. to 7 P.M. each day. Rat chow containing 21 mg/kg of F⁻ and tap water containing 1 ppm of F⁻ (52.6 μM) were allowed ad libitum throughout the experiment, which lasted 154 days. Figure 1 depicts the experimental schedule. On day zero, rats were placed in metabolic cages. To measure renal function, three 24-hour urine samples were collected, one the day before, one the day of, and one the day after a 2-ml sample of tail blood was obtained. Serum and urinary samples were analyzed for sodium, potassium, F⁻, urea nitrogen and creatinine concentrations and osmolality. Hematocrit was measured on all blood samples using a micromethod; body weight was determined two to three times weekly. Sodium and potassium concentrations were determined with an Instrumentation Laboratories #143 Flame Photometer and F⁻ concentration was measured with an Orion Ion Specific F⁻ electrode and #801 Ionalyzer. Urea nitrogen and creatinine samples were analyzed with a Technicon AutoAnalyzer II and osmolality was measured with an Advanced Cryometric Osmometer, Model 3CII. Twenty-four-hour sodium, potassium, F⁻, urea nitrogen, creatinine and osmolal excretions were determined. Urea nitrogen and creatinine clearances were calculated and expressed in ml/min/100 g body weight.

Renal insufficiency was produced surgically by the method of Kaufman et al. Rats were operated upon on two occasions, employing pentobarbital anesthesia, 6 mg/kg, ip. At the first procedure, day 21, the upper and lower poles of the left kidney were excised. Seven
days later, a right nephrectomy was performed. Four
weeks later, renal function of the 39 surviving rats
was again determined. On the basis of these studies,
three rats were discarded from the experimental
group: two had severe renal failure and were unlikely
to survive the experiment; the third had only minimal
renal impairment. The remaining 36 rats were
randomly allocated into the six groups of six animals
each (table 1). On day 69 of the experiment a 15-day
course of gentamicin, 2.5 mg/kg, ip b.i.d., was com-
menced in rats of Groups 2, 4 and 6. In a pilot study,
it had been established that this dose resulted in
blood levels in the clinical therapeutic range (5–10
μg/ml).10 Control animals received equivalent volumes
of physiologic saline solution by ip injection. On day 8
of gentamicin treatment, rats of Groups 1 and 2 were
placed in a plexiglass chamber and were exposed to
compressed air, 4–6 l/min, for two hours. Rats of
Groups 3 and 4 were placed in a similar chamber and
received 1 per cent halothane in compressed air.
Rats of Groups 5 and 6 were placed in a third chamber
and exposed to 2 per cent enflurane in compressed
air for two hours. Inspired anesthetic concentrations
were monitored with an Hewlett-Packard model
5830A gas chromatograph and inspired oxygen
concentration with an Instrumentation Laboratories
#401 oxygen analyzer. Rectal temperatures of repre-
sentative rats were monitored with a multichannel
Yellow Springs Tele-Thermometer; body temperature
was maintained in the normothermic range with the
aid of a heated water mattress placed under the
chamber floor. After recovering from anesthesia, rats
were returned to their metabolic cages.

Daily 24-hour urine samples were collected for two
weeks beginning with the first day of gentamicin
treatment. Blood samples were collected 5, 9, 11, and
15 days after the start of gentamicin treatment (days
73, 77, 79, and 83) for assessment of renal function.
An additional 0.5-ml sample was obtained from every
animal four hours after anesthesia (day 76) for
measurement of F–; peak F– levels after enflurane
anesthesia usually occur at this time in rats.11 Serum
gentamicin levels were determined one hour after
injection on days 73, 79, and 83 and one hour before
injection on day 72, using a radioimmunoassay
method.‡‡

After gentamicin treatment was completed, rats
were housed in communal cages, four animals to a
cage. They were returned to metabolic cages for six-
day periods beginning two weeks, six weeks, and ten
weeks later, to assess any late changes in renal func-
tion. On day 154, rats were killed by decapitation
and a blood sample was obtained from each for
biochemical studies.

Group means ±SE for each variable were deter-
mined and differences among groups established
by analysis of variance and Newman-Keuls post-hoc
test. Intragroup differences were determined by t
tests for paired data. P < 0.05 was considered
significant.

Results

After operation, urinary flow increased by approxi-
mately twofold and osmolality decreased a reciprocal
amount in every group (fig. 2, table 2); serum urea
nitrogen increased by 85 per cent, serum creatinine
increased by 65 per cent, and creatinine clearance
decreased by 20 per cent (fig. 3, table 2). Hematocrit
was slightly reduced, body weight remained stable,
and urinary sodium and potassium concentrations
decreased (data not shown). These changes are
consistent with moderate impairment of renal func-
tion. Renal function was relatively stable throughout
the period in which gentamicin and anesthetic treat-
ments were administered, but had deteriorated
perceptibly by day 126. Two rats treated with genta-
icin and halothane (Group 4) died from renal
failure, one each on days 152 and 153, prompting
sacrifice of the remaining 34 animals on day 154. At
that time it was found that several animals from
every group, including those that were not anesthes-
tized, had marked elevations of blood urea nitrogen

‡‡ Monitor Science Corporation, Newport Beach, California.
and serum creatinine and were in terminal renal failure.

No significant change in renal function could be attributed to treatment with either enflurane or halothane. Exposure to enflurane resulted in an elevation of serum fluoride (F⁻) four hours after anesthesia (fig. 4), and an increase in urinary F⁻ excretion (fig. 5). No significant difference in mean serum F⁻ concentrations could be demonstrated between the saline–enflurane-treated group (25.4 ± 2.8 μM) and the gentamicin–enflurane-tested group (21.7 ± 2.1 μM); by 24 hours serum F⁻ had almost returned to pre-anesthetic values. Urinary F⁻ excretion in the two groups were similar.

Blood gentamicin levels were in the therapeutic range (table 3). Antibiotic treatment resulted in clinically trivial but statistically significant changes in renal function in all three antibiotic-treated groups. These first became evident nine days after gentamicin treatment was initiated and persisted until the end of the antibiotic treatment period. Urinary flow (fig. 2) was maintained at a higher volume and serum creatinine (fig. 6) was elevated when compared with the non-gentamicin-treated groups. There was no renal toxic interaction between gentamicin treatment and either of the anesthetic treatments.

Discussion

The results of the present study indicate that either enflurane or halothane administered to Fischer 344 rats with moderate renal impairment is well tolerated and does not lead to further deterioration of renal function. The results agree with those of an earlier investigation by Sievenpiper et al., who administered enflurane initially for two hours, and then three weeks later for six hours, to Fischer 344 rats that were sham-operated, had unilateral nephrectomy, or had the same mass of renal tissue removed as in this study. In both studies the specific toxic effects of the fluoride load resulting from enflurane biotransformation and the nonspecific stress of anesthesia were well tolerated. In the present study, the administration of gentamicin caused additional minor deterioration of some measures of renal function. This was not unexpected, because in a previous study with Fischer 344 rats with normal renal function, similar gentamicin dosage resulted in toxic renal morphologic changes. There was, however, no evidence of toxic interaction between gentamicin and either halothane or enflurane.

What then is the potential for fluoride-induced renal impairment after anesthesia? Dose-related, polyuric, vasopressin-resistant nephropathy following methoxyflurane anesthesia was first reported in 1966, by Crandell et al. Subsequent studies showed that fluoride, released as a result of methoxyflurane biotransformation, was the cause of the renal lesion, and that the severity of nephrotoxicity was proportional to the concentration of serum fluoride. In almost all reports, peak serum fluoride levels occurred within three days after exposure to methoxyflurane. Patients with the highest levels had the greatest changes in renal function; most patients appeared to recover completely. However, in a case reported by Mazze and
Cousins, the clinical course became atypical after the initiation of gentamicin therapy on the third postoperative day. There was a further increase in serum F with the peak value, 250 µM, reached on day 6; blood urea nitrogen and creatinine concentrations increased; creatinine clearance decreased; polyuria occurred, with urinary volumes of as much as 9 l/day. Three months later, creatinine clearance was still reduced by more than 60 per cent compared with preoperative values. Toxic interaction between methoxyflurane and gentamicin in Fischer 344 rats was confirmed by Barr et al. Therapy with gentamicin at the time of exposure to methoxyflurane produced greater renal damage than did treatment with either gentamicin or methoxyflurane alone. They concluded that methoxyflurane should not be administered to patients likely to need other potentially nephrotoxic drugs.

Studies of renal function following enflurane administration generally have failed to demonstrate abnormalities. Cousins et al. administered enflurane (mean exposure, 2.7 ± 0.3 MAC hours; mean peak F level, 22 µM) to surgical patients and compared postoperative renal concentrating ability in response to vasopressin administration with that of a control group of patients anesthetized with halothane; there was no difference between the two groups. Mazzie et al. administered prolonged enflurane anesthesia (mean exposure 9.6 ± 0.1 MAC hours; 0.14 hours).

<table>
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<tr>
<th>Table 2. Laboratory Results</th>
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<tr>
<td><strong>Experimental Day</strong></td>
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<tr>
<td>14 Preoper-</td>
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<tr>
<td>Gentamicin</td>
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<tr>
<td>Group 1, control-saline</td>
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<tr>
<td>± 60 ± 109 ± 95 ± 107 ± 81 ± 134 ± 80 ± 84 ± 87</td>
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<tr>
<td>± 124 ± 78 ± 109 ± 97 ± 73 ± 100 ± 57 ± 29 ± 23</td>
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<td>± 155 ± 82 ± 89 ± 92 ± 87 ± 99 ± 64 ± 57 ± 56</td>
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<td>± 103 ± 62 ± 79 ± 73 ± 89 ± 66 ± 48 ± 45 ± 34</td>
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<tr>
<td>Group 4, halothane-gentamicin</td>
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<tr>
<td>± 96 ± 52 ± 77 ± 83 ± 58 ± 70 ± 84 ± 83 ± 65</td>
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<tr>
<td>± 150 ± 42 ± 61 ± 28 ± 39 ± 55 ± 39 ± 43 ± 30</td>
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<td>± 157 ± 85 ± 87</td>
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<tr>
<td>± 42 ± 82 ± 53 ± 52 ± 52 ± 37 ± 31 ± 34 ± 27</td>
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<tr>
<td>Serum urea nitrogen (mg/100 ml)</td>
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<tr>
<td>Group 1, control-saline</td>
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<tr>
<td>± 1.3 ± 1.5 ± 3.1 ± 3.5 ± 3.5 ± 5.4 ± 5.9 ± 9.8 ± 16.6</td>
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<td>Group 2, control-gentamicin</td>
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<td>± 1.2 ± 4.1 ± 3.7 ± 4.5 ± 3.9 ± 2.9 ± 5.0 ± 8.4 ± 32.9</td>
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<td>Group 3, halothane-saline</td>
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<td>± 1.6 ± 1.5 ± 2.7 ± 2.7 ± 1.9 ± 1.8 ± 4.8 ± 7.3 ± 52.7</td>
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<tr>
<td>± 1.4 ± 1.0 ± 3.9 ± 2.5 ± 2.4 ± 5.0 ± 4.6 ± 7.7 ± 16.9</td>
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<tr>
<td>Group 4, halothane-gentamicin</td>
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<tr>
<td>± 1.1 ± 1.4 ± 0.7 ± 0.8 ± 2.4 ± 0.9 ± 2.3 ± 4.1 ± 6.7</td>
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<tr>
<td>± 1.3 ± 1.4 ± 2.2 ± 2.2 ± 2.5 ± 3.2 ± 3.8 ± 8.9 ± 19.9</td>
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<tr>
<td>Creatinine clearance (ml/min/100 g)</td>
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<td>Group 1, control-saline</td>
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<td>± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01</td>
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<td>± 0.02 ± 0.01 ± 0.01 ± 0.02 ± 0.02 ± 0.02 ± 0.02 ± 0.04 ± 0.05</td>
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<td>± 0.16 ± 0.12 ± 0.22 ± 0.23 ± 0.23 ± 0.23 ± 0.23 ± 0.23 ± 0.23</td>
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<td>± 0.04 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01 ± 0.01</td>
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<td>± 0.17 ± 0.13 ± 0.24 ± 0.24 ± 0.24 ± 0.24 ± 0.24 ± 0.24 ± 0.24</td>
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<td>± 0.13 ± 0.10 ± 0.21 ± 0.21 ± 0.21 ± 0.21 ± 0.21 ± 0.21 ± 0.21</td>
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Fig. 3. Serum creatinine concentrations during the experiment. After operation values increased in all groups but were relatively stable during the period of gentamicin and anesthetic treatments. There was a marked increase in creatinine values in all groups on days 126 and 154. Two gentamicin–halothane-treated animals died of renal failure prior to day 154. The day 154 data point for that group, 1.6 ± 0.4 mg/100 ml, is not included in the figure. The area within the box is enlarged and shown as figure 6, below.

Fig. 4. Serum fluoride values during the experiment. There was no difference between the gentamicin–enflurane (ENF)-treated group and the saline–enflurane-treated group. Rats anesthetized with halothane had no increase in F⁻.
mean peak F⁻ level, 33.6 μM) to normal volunteers and studied its effect upon urinary concentrating ability. A transient but consistent reduction in urinary osmolality, to an average of 264 mOsm/kg from a mean preanesthetic value of 1034 mOsm/kg, was observed one day after anesthesia. This is a clinically insignificant impairment of urinary concentrating ability. Volunteers exposed to halothane had a slight increase in concentrating ability. It may be concluded from these data that surgical patients with normal renal function will not have clinically significant urinary concentrating defects following enflurane anesthesia.

These studies, however, did not address the question of whether individuals with preexisting renal disease might be harmed by exposing their already damaged kidneys to a F⁻ load. There have been three case reports to suggest that this might occur.⁵–⁷ Hartnett et al.⁵ described a 42-year-old woman with a low preoperative creatinine clearance (55 ml/min) in whom nonoliguric renal insufficiency developed after a three-hour exposure to enflurane; F⁻ levels were not documented. She had a period of mild intraoperative hypotension and was treated during and after operation with several potentially nephrotoxic antibiotics. Loehning and Mazze⁸ reported the case of a patient in whom enflurane anesthesia may have been a factor in the deterioration of function of a transplanted kidney. In this case, insufficient perioperative fluid replacement probably contributed to the problem. Peak F⁻ concentration was only 16 μM. The authors suggested that the threshold of F⁻ nephrotoxicity might be lower in diseased or transplanted kidneys, although they presented no evidence to support this view. The third case was reported by Eichhorn et al.,⁷ who described a patient who had renal failure following a six-hour exposure to enflurane for creation of an ileal loop urinary diversion. The patient's postoperative course was unusual in two respects: first, anuria developed on the second day, suggesting that he may have had complete urinary tract obstruction; second, serum F⁻ was 96 μM on day 2 after operation, and required two weeks to return to normal values. All of these reports, therefore, are equivocal in their indictment of enflurane as the cause of postoperative renal failure.

In the only clinical study of enflurane anesthesia in surgical patients with abnormal renal function, Carter et al.⁹ examined fluoride kinetics after enflurane administration in healthy and anephric patients, and in patients with poor renal function. They

![Fig. 5. Urinary fluoride excretion during the experiment. There was no difference between the gentamicin–enflurane-treated group and the saline–enflurane-treated group.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931465/)

### Table 3. Serum Gentamicin

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<tr>
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<th>Day 72</th>
<th>Day 73</th>
<th>Day 79</th>
<th>Day 83</th>
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<td>Trough</td>
<td>Peak</td>
<td>Peak</td>
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<tr>
<td>Group 2, control</td>
<td>1.4</td>
<td>± 0.1</td>
<td>± 1.0</td>
<td>± 1.2</td>
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<tr>
<td>Group 4, halothane</td>
<td>1.2</td>
<td>± 0.1</td>
<td>± 0.5</td>
<td>± 2.1</td>
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<tr>
<td>Group 6, enflurane</td>
<td>1.4</td>
<td>± 0.1</td>
<td>± 1.2</td>
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Fig. 6. Serum creatinine values from days 62–98 of the experiment. Beginning on day 77, serum creatinine in the three gentamicin-treated groups was significantly higher than that in the three saline-treated groups, but the difference was not maintained after day 83.

were unable to demonstrate any clinically or statistically significant difference among the groups with respect to maximum serum F– concentration or the rate of reduction of serum F– after anesthesia. They concluded that enflurane was not contraindicated for surgical patients with preexisting renal disease.

Should the results of the present study be extrapolated to the clinical situation? Fischer 344 rats have been used by us and others for several years as animal models for investigation of the nephrotoxicity of both the fluorinated inhalational anesthetic agents and gentamicin.2,11–13,16,17,20,21 The renal lesions associated with methoxyflurane administration are similar in rats22 and man.23 They are characterized by proximal tubular dilatation and focal necrosis, with interstitial fibrosis and oxalate crystal deposition present in cases that have progressed beyond the acute stage. The gentamicin-induced lesion is characterized by formation of proximal tubular lysosomal cytosesomes, many of which contain myeloid bodies.14 Functionally, methoxyflurane,17 sodium fluoride,16,20,21 and gentamicin16,17 produce polyuric, vasopressin-resistant renal insufficiency. The renal lesion associated with the animal model in this study is characterized initially by loss of renal mass and then by a glomerular lesion resembling glomerulonephritis; functionally, it is a high-output renal insufficiency.12 The morphologic components of these lesions are not in all cases similar, but animal models in renal failure research have only occasionally resembled their human counterpart. What is significant is that the functional changes have correlated well with the responses of humans to these agents. For this reason and because of the absence of well-documented cases of adverse renal effects following enflurane anesthesia in surgical patients, we believe this study has clinical relevance. We postulate that further deterioration of already impaired renal function is not likely to occur following exposure to enflurane, and, when indicated, enflurane may be used with safety for surgical patients who have minimal or moderate renal disease. Furthermore, concurrent therapy with gentamicin does not appear to contraindicate the use of enflurane.

References