Anesthetic Effects on the Glycerol Model of Rhabdomyolysis-Induced Acute Renal Failure in Rats

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Abstract. Isoflurane, the most widely used inhalational anesthetic, releases inorganic fluoride during its metabolism by the cytochrome P450 system. Recent experimental data indicate that when cultured proximal tubular cells are exposed to inorganic fluoride, they become relatively resistant to myoglobin- and ATP depletion-mediated attack. The present study was undertaken to assess whether isoflurane anesthesia might confer in vivo cytoprotection, possibly by causing renal tubular inorganic fluoride exposure, thereby mitigating a combined myoglobin/ATP depletion model of acute renal failure (glycerol-induced ARF). Rats were injected with hypertonic glycerol (50%; 9 ml/kg, intramuscularly) while undergoing 4 h of isoflurane anesthesia. Glycerol-injected rats anesthetized with a virtually nonfluorinated inhalational anesthetic (desflurane) or with a nonfluorinated anesthetic (pentobarbital) served as controls. The severity of ARF was assessed 24 h later (blood urea nitrogen, plasma creatinine [Cr], and renal histology). Anesthetic effects on extrarenal injury (plasma creatine phosphokinase, lactate dehydrogenase, and hematocrit levels), acute intrarenal heme loading (cast formation), and BP during the initiation phase of renal injury (0 to 4 h after glycerol injection) were also assessed. Glycerol induced severe ARF under pentobarbital anesthesia (Cr, 2.8 ± 0.3 mg/dl; severe tubular necrosis). Somewhat worse azotemia, but comparable tubular necrosis, resulted with desflurane use. Conversely, glycerol plus isoflurane anesthesia induced only mild renal damage (Cr, 0.9 ± 0.1, minimal tubular necrosis; P < 0.01). This reduction apparently was not due to differences in degrees of muscle necrosis, hemolysis, acute renal heme loading, or BP during the initiation phase of ARF, suggesting that a direct renal mechanism was operative. These results: (1) underscore that differing anesthetics can profoundly alter the expression of experimental renal injury; (2) raise the intriguing possibility that isoflurane could potentially protect surgical/trauma patients from rhabdomyolysis-induced ARF; and (3) further support the concept that renal fluoride exposure may confer proximal tubular cytoprotective effects. (J Am Soc Nephrol 9: 305–309, 1998)

The early postoperative period is one of the most common clinical settings for the onset of acute renal failure (ARF). Indeed, some degree of renal insufficiency, or severe ARF, has been reported in 0.1 to 30% of patients after various forms of surgery (1). It traditionally has been assumed that perioperative hypotension and/or nephrotoxin-induced acute tubular necrosis are the dominant causes of postoperative ARF. However, the high degree of variability in the incidence of postoperative renal failure suggests that multiple factors, in addition to hypotension and nephrotoxin exposure, may be involved.

An issue of considerable clinical relevance is whether the type of anesthesia used during surgery can modulate the expression of superimposed acute renal tubular damage. Observations from Finn almost 20 years ago provide experimental support for this possibility. They found that when rats were subjected to 60 min of renal artery occlusion under inactin (a thiobarbiturate) or pentobarbital (an oxybarbiturate) anesthesia, far less injury resulted in the former group (2). Although the explanation for this observation has remained elusive, it nevertheless graphically demonstrates that even structurally related anesthetics can have profoundly different effects on the expression of superimposed acute renal damage.

Volatile fluorinated anesthetics (e.g., isoflurane, desflurane, sevoflurane, and enfurane), rather than barbiturates, are the current mainstays of clinical anesthetic practice (3). Although structurally similar (all fluorinated methylethyl ethers), they have differing degrees and sites of metabolism (3–5). One of the major differences is the extent to which they are defluorinated via the cytochrome P450 system (4,5). Methoxyflurane, a previously used fluorinated anesthetic, undergoes marked hepatic and renal defluorination, resulting in the accumulation of purportedly nephrotoxic inorganic fluoride levels (>50 μM) (6–8). Indeed, this has been widely implicated as the explanation for an outbreak of ARF cases induced by methoxyflurane during its widespread use in the 1960s (culminating in its elimination from clinical practice). Isoflurane, now the most commonly used inhalational anesthetic, undergoes significantly less intrahepatic, and presumably intrarenal, defluorination than methoxyflurane (e.g., approximately 10 μM plasma fluoride levels in rats) (9,10). This may explain why it has little or no nephrotoxic effect. In contrast, desflurane, a relatively
new fluorinated anesthetic, is virtually completely resistant to cytochrome P450-mediated defluorination reactions (3,10).

A recent *in vitro* study from this laboratory indicates that although high doses of inorganic fluoride can induce proximal tubular cell necrosis, in low concentrations it can exert a profound cytoprotective effect (11). For example, when cultured human proximal tubular (HK-2) cells were exposed to subtoxic inorganic fluoride challenges, a marked increase in resistance to concomitant nephrotoxic (myoglobin-) and ATP depletion- mediated tubular cell death resulted (an approximate 50% decrease in cell death). This observation raises the intriguing possibility that the use of fluorinated anesthetics that release subtoxic levels of inorganic fluoride might confer an *in vivo* cytoprotective effect.

The purpose of the present study was to gain experimental support for this hypothesis. To this end, rats were subjected to intramuscular glycerol injection, which induces a combined nephrotoxic (myohemoglobinuric)/ATP depletion form of tubular cell death (12). Because we previously found that inorganic fluoride exposure attenuates each of these forms of tubular cell injury *in vitro* (11), we postulated that isoflurane anesthesia, which produces subtoxic renal fluoride accumulation (9,10), might limit the severity of glycerol-induced ARF. As controls, rats were subjected to glycerol injection under either desflurane or pentobarbital anesthesia. The rationale for this was that: (1) desflurane would provide a virtual noninorganic fluoride-generating fluorinated anesthetic control (3,10); and (2) the use of pentobarbital anesthesia would allow for the assessment of whether fluorinated anesthetics in general (*i.e.*, independent of defluorination reactions) alter the expression of the glycerol model of ARF (by comparing the desflurane results with those obtained with pentobarbital, a nonfluorinated agent).

**Materials and Methods**

**Glycerol Model of Myohemoglobinuric ARF**

Male Sprague Dawley rats (175 to 200 g; B & K Universal, Kent, WA) maintained under standard laboratory conditions with free food and water access were used for all experiments. After being placed in an airtight 22-L chamber, they were anesthetized for 15 min with either isoflurane (*n* = 15) or desflurane (*n* = 8). These agents were delivered in 100% oxygen at a flow rate of 2 L/min using agent-specific vaporizers. The vaporizers were set to maintain chamber isoflurane and desflurane concentrations (monitored by infrared analysis) at 1.4 and 5.7%, respectively (equivalent anesthetic dosages) (3). A comparable level of anesthesia (defined as a complete inhibition of spontaneous, nonrespiratory body movements) was induced in additional rats (*n* = 12) by intramuscular pentobarbital injection (75 mg/kg). Fifteen minutes after inducing anesthesia, each rat was subjected to intramuscular glycerol injection (50% solution, 9 ml/kg), administered in equally divided doses into each upper hind limb. Anesthesia was then maintained for an additional 3 3/4 h (the pentobarbital-treated rats requiring a maintenance dose of 50 mg/kg at the 2-h time point). Body temperature, assessed with a rectal probe, was maintained at 36 to 37°C using an adjustable heating surface. After completing anesthesia, the rats were placed into standard cages permitting rapid recovery from anesthesia. Free food and water access were provided. Twenty-four h later, the rats were reanesthetized (75 mg/kg pentobarbital), the abdomens were opened via a midline incision, and exsanguination was performed by aortic puncture followed by kidney resection. Blood urea nitrogen and plasma creatinine concentrations were determined by autoanalyzer technology. Kidneys (from 5 to 8 rats per group, randomly selected) were bisected and fixed with 10% buffered formalin. Four-micrometer paraffin-embedded sections were then stained with hematoxylin and eosin for histologic assessments, as described below.

**Assessment of Anesthetic Effects on the Extent of Extrarenal Tissue Injury**

The following experiment was undertaken to test for the possibility that differing anesthetic effects on the severity of glycerol-induced ARF were due to differences in the extent of glycerol-induced: (1) muscle necrosis; (2) hemolysis; (3) intrarenal heme protein loading; (4) hemoconcentration (due to fluid “third” spacing into injured muscle); or (5) differences in BP during the induction phase of this ARF model. A total of 12 rats was injected with glycerol under isoflurane, desflurane, or pentobarbital anesthesia, as described above (*n* = 4 per group). The animals that received isoflurane or desflurane had their systemic BP monitored during the 4-h anesthesia period via tail BP cuff technology (13). [Note: This method is not suitable for gauging systemic BP under pentobarbital anesthesia (14,15). Hence, in pilot experiments, we demonstrated that BP, assessed by direct intracarotid artery monitoring (16), is stable under pentobarbital anesthesia ± glycerol injection.] After completing the 4-h anesthesia protocols, the rats were killed by aortic puncture, and plasma was obtained for quantification of: (1) creatine phosphokinase (CPK; as an index of muscle necrosis; Sigma, 520C, St. Louis, MO); (2) lactate dehydrogenase (LDH; as a combined index of muscle necrosis/hemolysis); and (3) hemoglobin (as an index of hemoconcentration, recognizing that a degree of hemolysis complicates interpretation of this assessment). The kidneys were resected, and tissue sections were prepared as noted above and then used to microscopically assess the extent of intraluminal heme pigment loading/cast formation during the induction phase of ARF.

**Statistical Analyses**

Group values are given as mean ± 1 SEM. Statistical comparisons were made by ANOVA with after testing by unpaired *t* test with Bonferroni correction. The extent of morphologic injury at 24 h postglycerol injection was gauged semiquantitatively and in a blinded manner (by Dr. Zager) by assessing the approximate percentage of cortical proximal tubular segments that manifested necrosis, as follows: 0, none; 1+, <10%; 2+, 10 to 25%; 3+, 25 to 50%; 4+, ≥50%. Histologic comparisons were made by Wilcoxon rank sum test with Bonferroni correction.

**Results and Discussion**

Rats subjected to glycerol injection under pentobarbital developed severe ARF, as denoted by marked blood urea nitrogen and serum creatinine increments (Figure 1). This result was not simply due to a uniquely adverse effect of pentobarbital on the glycerol model, because even worse azotemia resulted when the rats were challenged with glycerol under desflurane anesthesia (Figure 1). In contrast, rats subjected to glycerol injection under isoflurane anesthesia manifested a marked attenuation of glycerol-induced ARF (approximately two-thirds to three-fourths reduction in the degree of azotemia, compared with the other anesthetic groups). Renal histologic assessments at 24 h indicated that isoflurane use was associated
with morphologic, and not simply renal functional, protection. Whereas glycerol injection induced severe and equivalent renal morphologic injury (12) under conditions of pentobarbital and desflurane anesthesia (heme cast formation, extensive proximal tubule necrosis; 24-h assessments), the isoflurane/glycerol group sustained only mild morphologic damage (Figure 2 and Figure 3 A through C). The isoflurane-associated decrease in renal injury could not simply be attributed to a reduction in muscle necrosis, hemolysis, or hemoconcentration during the induction phase of injury, because CPK, LDH, and hematocrit values at this time did not statistically differ among the three experimental groups (Figure 4). Given the similar CPK and LDH increments, it was not surprising that no discernible differences in the degree of intraluminal heme accumulation (cast formation) were apparent among the three experimental groups at 4 h postglycerol injection (Figure 3D). Finally, systolic BP after glycerol injection were highly comparable for the desflurane (95 ± 2 mmHg) and isoflurane (91 ± 4 mmHg; NS) groups, consistent with comparable systemic hemodynamic profiles. (Nevertheless, this does not rule out the possibility that differences in intrarenal hemodynamics could have existed among the experimental groups, potentially affecting the induction of renal damage.)

Although it is virtually impossible to assign direct "cause-and-effect" relationships in whole animal experiments, the present results are at least consistent with the hypothesis that isoflurane anesthesia, via its release of inorganic fluoride, can elicit a direct proximal tubular cytoprotective effect. In this regard, it is noteworthy that fluoride affects at least three pathways that we have previously documented to be important mediators of myoglobin cytotoxicity. First, it exerts a ouabain-like effect on proximal tubule Na,K-ATPase activity (17), an action that can directly attenuate the cytotoxic effects of myoglobin (18). Second, it can alter mitochondrial electron transport (17). In this regard, it is noteworthy that both rotenone and antimycin A, mitochondrial respiratory chain inhibitors, can mitigate myoglobin cytotoxicity in a cell culture system, presumably by decreasing mitochondrial-driven oxidant stress (18). Third, inorganic fluoride can acutely deplete proximal tubule cytosolic phospholipase A2 activity (11), an action that can induce or contribute to a cytoprotected state (11). Thus, although it remains to be proven that the observed differences in glycerol-induced renal injury were mediated by inorganic

**Figure 1.** Blood urea nitrogen (BUN; left panel) and plasma creatinine (right panel) values 24 h after anesthesia under isoflurane (Iso; n = 15), desflurane (Des; n = 8), or pentobarbital (Pent; n = 12) anesthesia. Whereas desflurane and pentobarbital anesthesia were associated with severe acute renal failure (ARF), glycerol injection under isoflurane anesthesia induced only mild ARF. (For comparison, normal BUN and creatinine values for rats, as obtained previously in this laboratory, are 10 to 20 mg/dl and 0.3 to 0.5 mg/dl, respectively). The attenuation of glycerol-induced injury under isoflurane or pentobarbital anesthesia was not simply due to the use of an inhalational or a noninhalational agent, because desflurane (an inhalational agent) was associated with slightly worse ARF than pentobarbital anesthesia (P < 0.05).

**Figure 2.** Acute tubular necrosis (ATN) scores 24 h after glycerol injection. Whereas glycerol induced extensive ATN under pentobarbital (Pent) or desflurane (Des) anesthesia (3 to 4+ scores, or >25 to 50% necrotic tubule segments; see text), only modest ATN (0 to 2+ scores, or 0 to 25% necrotic tubules) resulted with isoflurane anesthesia (P < 0.01). (Eight, five, and six randomly selected kidneys from the isoflurane, desflurane, and pentobarbital groups, respectively, were prepared for histologic examination and scoring.)
fluoride, the available data are at least consistent with this hypothesis.

It is noteworthy that isoflurane anesthesia in rats generates approximately 5- to 10-μM plasma fluoride levels (9,10) (our unpublished observations). Because low millimolar inorganic fluoride levels were needed to produce cytoresistance in our previous cell culture experiments (11), it might appear that the inorganic fluoride levels generated from isoflurane would be far too low to induce an analogous cytoresistant state. However, two points must be noted in this regard. (1) An approx-

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**Figure 3.** (A through C) Representative renal histology 24 h after glycerol injection. Minimal tubular necrosis is apparent in renal cortex from an isoflurane/glycerol-treated rat (A) compared with a pentobarbital (B)- or desflurane (C)-treated rat. (D) Intraluminal heme accumulation 4 h after glycerol injection. Marked heme accumulation is apparent, the extent of which did not noticeably differ among the three experimental groups (isoflurane depicted).

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**Figure 4.** Plasma creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and hematocrit levels 4 h after glycerol injection for the three experimental groups. No significant differences were observed. Dotted lines represent normal mean values (CPK, 70 ± 22; LDH, 21 ± 5; hematocrit, 40 ± 1%). Abbreviations as in Figure 1.
imate 1000:1 extracellular:intracellular inorganic fluoride gradient exists; thus, low millimolar inorganic fluoride additions to cell culture likely produce only low micromolar intracellular fluoride concentrations (11,17). (2) Isoflurane is highly membrane-permeable, allowing for high micromolar/low millimolar intracellular concentrations to be achieved (17). This presumably allows for micromolar (i.e., cytoprotective) intracellular fluoride levels to be achieved as a result of intracellular P450 metabolism. Given these considerations, it is apparent that intracellular fluoride generation, rather than tubular fluoride uptake from plasma or urine, is presumably the critical determinant of the cytoprotective effects of fluoride.

In conclusion, the results of the present studies indicate that isoflurane, an agent that releases subtoxic doses of inorganic fluoride, can markedly attenuate the expression of myohemoglobinuric ARF, compared with a minimally defluorinated (desflurane) or a nonfluorinated (pentobarbital) anesthetic. This finding has a number of potential implications. (1) It underscores that the type of anesthetic used in studies of experimental ARF can have a profound impact on the results obtained. This makes it mandatory to scrutinize anesthetic regimens when interpreting and comparing results in the experimental literature. (2) It raises the intriguing possibility that isoflurane could potentially decrease the risk of ARF in selected surgical patients (e.g., those with rhabdomyolysis), at least relative to other anesthetics. (3) It suggests that our previous in vitro observations of inorganic fluoride-induced proximal tubule cytoprotection might, in fact, have a clinically relevant in vivo correlate. We believe that each of these issues provides a compelling rationale for further exploration of fluoride effects on proximal tubular homeostasis.

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