Expressions of genes encoding drug-metabolizing enzymes are altered after sevoflurane, isoflurane, propofol or dexmedetomidine anesthesia

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ABSTRACT
We previously showed that sevoflurane anesthesia affected the expression ratios of 177 of 10,000 genes in multiple organs of rats by microarray analyses. The maximum number of altered genes was detected in the liver, and included several genes characterized as encoding drug-metabolizing enzymes (DMEs). Here, we investigated whether alterations of pharmacokinetic gene expressions after anesthesia differed between inhalation and intravenous anesthesia, and how long the alterations persisted after awakening from anesthesia. Livers were obtained from rats (n = 6 per group) anesthetized with sevoflurane, isoflurane, propofol or dexmedetomidine for 0 or 6 h, and rats awakened for 24 h after anesthesia for 6 h. The mRNA expression ratios of eight genes encoding DMEs that showed the greatest alterations in the previous study, namely Cyp7a1, Cyp2b15, Por, Nr1i2, Ces2, Ugt1a7, Abcb1a and Abcc2, were measured by quantitative real-time reverse transcriptase-polymerase chain reaction. The expression ratios were mostly increased after 6 h of anesthesia and returned to their control levels at 24 h after awakening from anesthesia. However, the expression ratios of some genes remained elevated for 24 h after awakening from anesthesia. There were differences between inhalation and intravenous anesthesia, and interestingly, between sevoflurane and isoflurane and between propofol and dexmedetomidine.

General anesthesia has been routinely used for surgical operations, and its safety has been assessed and established by clinical outcomes for sevoflurane (18), isoflurane (16), propofol (17) and dexmedetomidine (7). However, only a few studies have reported on the effects of anesthesia at the molecular level (2, 15, 19, 25).

Halothane (CF₃CHBrCl), sevoflurane (CHF₂-O-CHF-CF₃) and isoflurane (CHF₂-O-CHCl-CF₃) are halogenated inhalation anesthetics that are metabolized by hepatic cytochrome P450, family 2, subfamily E, polypeptide 1 (CYP2E1) (13, 14). Approximately 25% of administered halothane, 5% of sevoflurane and 0.2% of isoflurane is metabolized, and the incidence of liver injury is correlated with the extent of their oxidative metabolism (21). Several hundred cases of hepatotoxicity have been attributed to halothane (5, 34), and the risks of hepatitis are increased after repeated exposure to halothane, whereas hepatotoxicity appears to be rare for sevoflurane (31, 37) and isoflurane (3, 33). Sevoflurane and isoflurane both have low solubilities in blood and tissues (rapid recovery from anesthesia) and are frequently used. In patients with severe liver dysfunction, however, isoflurane is preferred because it undergoes less metabolism in the liver.

Propofol (C₁₂H₁₆O) is the most commonly used intravenous anesthetic, and is administered as an alkyphenol formulated in a lipid emulsion (32). Propofol provides rapid and smooth induction of anesthesia and exhibits rapid clearance from the body. It is rapidly metabolized in the liver by conjugation to glucuronide and sulfate to produce water-soluble compounds, and 68.3% of these compounds

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are excreted in the urine within 24 h (9). In patients with cirrhosis, propofol anesthesia is associated with a prolonged recovery time after discontinuation of the infusion, but does not affect the postoperative liver function (27, 28).

Dexmedetomidine (C₁₀H₁₄N₂HCl) is the most ideal intravenous anesthetic. It is a highly selective alpha₂-adrenergic agonist that produces sedation, hypnosis and analgesia, and has a minimal effect on respiration. Dexmedetomidine is rapidly distributed and extensively metabolized in the liver and excreted in both the urine and feces. It undergoes conjugation (41%), N-methylation (21%) or hydroxylation followed by conjugation, and 85% of the resulting compounds are excreted in the urine within 24 h (8, 11). In patients with liver dysfunction, dexmedetomidine anesthesia is associated with a significantly prolonged distribution half-time in parallel with the severity, although hematological variables show no differences after dexmedetomidine or propofol anesthesia (8).

In a previous study, we showed that sevoflurane anesthesia affected the expression ratios of 177 of 10,000 (1.8%) genes in multiple organs of rats by microarray analyses (25). Above all, the maximum number of altered genes (99 genes; approximately 56% of the total affected genes) was detected in the liver. These liver-specific alterations included genes encoding drug-metabolizing enzymes (DMEs). In the present study, we measured the expression ratios of eight genes encoding DMEs that showed the greatest alterations among the affected genes in the previous study.

Anesthesia is often repeated within a short period of time with the same anesthetic agent or a different agent. If the alterations of gene expression do not return to their baseline levels after awakening from anesthesia, as well as the influences of other anesthetic agents, such as isoflurane, propofol and dexmedetomidine. Using quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR), we measured the mRNA expression ratios of pharmacokinetic genes during anesthesia with sevoflurane, isoflurane, propofol or dexmedetomidine and after awakening from anesthesia with these agents.

MATERIALS AND METHODS

Animal preparation. Approval for this study was granted by the Animal Experimental Ethical Review Committee of Nippon Medical School. Six-week-old male Wister rats (Saitama Experimental Animals Supply, Saitama, Japan) weighing 210 ± 10 g were maintained under temperature-controlled environmental conditions and had free access to food and water. A venous catheter was inserted into the caudal vein of all rats, and each rat was housed in an individual plastic box. Rats undergoing inhalation anesthesia were placed in plastic boxes supplied with sevoflurane (Maruishi Pharmaceutical, Osaka, Japan; 4% gas-air mixture) (22) or isoflurane (Abbott Japan, Tokyo, Japan; 2.5% gas-air mixture) (30) at a rate of 6 L min⁻¹, and normal saline was administered via the venous catheter at a rate of 1 mL h⁻¹. Rats undergoing intravenous anesthesia were housed in plastic boxes supplied with air alone at a rate of 6 L min⁻¹, and administered propofol (AstraZeneca, Osaka Japan; 600 μg kg⁻¹ min⁻¹) (23) or dexmedetomidine (Hospira Japan, Osaka, Japan; 1 μg kg⁻¹ min⁻¹) (1) via the venous catheter at a rate of 1 mL h⁻¹. Rats anesthetized by inhalation or intravenous anesthesia were allowed to breathe spontaneously.

The rats were randomly assigned to nine groups (n = 6 per group). In the control group, rats were not administered any anesthetics and were immediately sacrificed. In the 6-h anesthesia groups with sevoflurane (S6), isoflurane (I6), propofol (P6) or dexmedetomidine (D6), the rats were taken from their plastic boxes and sacrificed after 6 h of anesthesia. In the groups awakened for 24 h after 6 h of anesthesia with sevoflurane (S24), isoflurane (I24), propofol (P24) or dexmedetomidine (D24), the rats were awakened within 12 min after 6 h of anesthesia and sacrificed at 24 h after awakening. The left lateral lobe of the liver was obtained from each rat within 3 min after death, immediately frozen in liquid nitrogen and lysed with ISOGEN reagent (Nippon Gene, Tokyo, Japan). All liver samples were stored at −80°C until analysis. The mRNA expression ratios of eight genes encoding DMEs, namely cytochrome P450, family 7, subfamily a, polypeptide 1 (Cyp7a1); cytochrome P450, family 2, subfamily b, polypeptide 15 (Cyp2b15); P450 (cytochrome) oxidoreductase (Por); nuclear receptor subfamily 1, group I, member 2 (NrlI2); carboxylesterase 2 (Ces2); UDP glycosyltransferase 1 family, polypeptide A7 (Ugt1a7); ATP-binding cassette, sub-family B (MDR/TAP), member 1A (Abcb1a); and ATP-binding cassette, sub-family C (CFTR/ MRP), member 2 (Abcc2), were measured by real-time RT-PCR. DMEs are categorized into phase I, II and III enzymes. Phase I enzymes modify substrates
directly via oxidation, hydroxylation and dealkylation, while phase II enzymes facilitate clearance by conjugating drugs to sulfate, glutathione or carbohydrates, and phase III enzymes directly transport the conjugates from the liver into the bile or urine. Cyp7a1, Cyp2b15, Por, Nr1i2 and Ces2 are categorized as phase I, Ugt1a7 is categorized as phase II, and Abcb1a and Abcc2 are categorized as phase III.

**RESULTS**

All rats survived until sacrifice, and the data for all animals were used. The expression ratios of the genes encoding DMEs are presented in Fig. 1A–H.

The expression ratios of Cyp7a1 at S6, I6 and D6 were higher than their control levels, and those at S24, I24 and D24 had decreased to their control levels, although that at I24 remained slightly elevated. The expression ratios of Cyp2b15 at S6, I6 and P6 were markedly increased, while that at D6 was not changed. Those at S24, I24 and P24 came close to their control levels, although that at P24 remained slightly increased, and that at D24 was somewhat decreased compared with its control level. The expression ratios of Por were increased at S6, I6, P6 and D6, and had returned to their control levels at S24, P24 and D24, while that at I24 remained elevated. The expression ratios of Nr1i2 at S6, I6, P6 and D6 were greater than their control levels, and had decreased at S24, I24, P24 and D24, although some differences remained compared with their control levels. The expression ratios of Ces2 were increased at S6, I6, P6 and D6, and those at S24, I24 and D24 persisted at the S6, I6 and D6 levels, respectively, while that at P24 was further increased compared with the P6 level. The expression ratios of Ugt1a7 at S6, I6 or P6 were not changed, and that at D6 was slightly decreased. That at S24 was somewhat decreased, and that at P24 was increased, and that at D24 had returned to its control level. The expression ratios of Abcb1a at S6, I6 and P6

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were higher than their control levels, while that at D6 was suppressed to below the control level. These changes returned to the control level at S24 or were slightly elevated at I24, P24 and D24. The expression ratios of Abcc2 were increased at I6, and that at I24 persisted at the I6 level.

DISCUSSION

In the present study, we have demonstrated that the expression ratios of genes encoding DMEs in the rat liver were mostly increased after anesthesia for 6 h, and returned to their control levels at 24 h after awakening from anesthesia. However, the expression ratios of some genes remained elevated for 24 h after awakening from anesthesia or were not altered by anesthesia. Furthermore, the expression ratios of the genes differed among individual anesthetics. Our results suggest that anesthesia may affect drug metabolism at the gene level.

The observed increases in the expression ratios of Cyp7a1, Cyp2b15, Por, Nr1i2, Ces2 and Abcb1a at S6 were consistent with those in our previous microarray study (25). Several reports have suggested that the activation levels of cytochrome P450s are increased after anesthesia with sevoflurane (19), isoflurane, enflurane (2) and phenobarbital (19). On the other hand, the levels of cytochrome P450s in the mouse liver were diminished after chronic isoflurane administration (15).

Mammalian cytochrome P450s play major roles in the metabolism and deposition of drugs and envi-
Gene expressions after anesthesia

Cyp7a1 catalyzes the first reaction in the cholesterol catabolic pathway in the liver, which converts cholesterol to bile acid. This reaction is the rate-limiting step and the major site of regulation of bile acid synthesis, which represents the primary mechanism for removal of cholesterol from the body. The expression ratios of Cyp7a1 were increased after anesthesia for 6 h, except for propofol anesthesia, and this effect continued at 24 h. Pandak et al. (24) demonstrated that overexpression of CYP7A1 resulted in marked activation of the classic pathway of bile acid biosynthesis in human hepatocytes. As a consequence, HMG-CoA reductase and acyl CoA:cholesterol acyltransferase activities were decreased, and cholesteryl ester hydrolase activity and low-density lipoprotein receptor mRNA expression were increased. After anesthesia with sevoflurane, isoflurane or propofol, Cyp7a1 overexpression may result in activation of the classic pathway of bile acid synthesis and this activation may last for 24 h after awakening from isoflurane anesthesia.

Cyp2b15 catalyzes reactions involved in drug metabolism and cholesterol biosynthesis, and is a metabolic oxidative stress-related gene (20). The expression ratios of Cyp2b15 were markedly increased after anesthesia with sevoflurane, isoflurane or propofol. Overexpression of Cyp2b15 may have obvious effects on drug oxidative metabolism.

Por plays a role in electron transport. Por-null mice develop normally and are fertile, but exhibit a number of phenotypic changes associated with the loss of P450 function, including hepatic lipid accumulation, reduced bile acid production, increased constitutive P450 expression and decreased levels of plasma cholesterol and triglycerides (6). The expression ratios of Por were increased after anesthesia for 6 h and returned to the control level at 24 h after awakening, except for isoflurane anesthesia. After anesthesia with sevoflurane, isoflurane, propofol or dexmedetomidine for 6 h, hepatic lipid accumulation, bile acid production and P450 expression ratios may change.

In rats, mice and humans, xenobiotic-mediated induction of the Cyp3a family of genes occurs through activation of Nr1i2, which is localized in the liver and intestinal tract. The liver and intestine express similar DMEs and drug transport proteins.
that are regulated by Nr1i2, including rodent and human Cyp3a, Ugt1a, Mrp2 (Abcc2) and Mdr1 (Abcb1). Nr1i2-mediated induction of these drug-management genes facilitates the excretion of potentially harmful chemicals from the body (10). Activation of Nr1i2 also results in decreased expression of CYP7A1 (12). The expression ratios of Nr1i2 were increased after 6 h of anesthesia and decreased at 24 h after awakening from anesthesia, but did not return to the control levels. These changes may have effects on the expression ratios of various genes encoding DMEs.

Ces2 is an enzyme responsible for the hydrolysis of drugs containing ester and amide bonds. It also hydrolyzes long-chain fatty acid esters and thioesters in rats and humans, and plays roles in lipid metabolism, detoxification of xenobiotics and the blood-brain barrier. Flurbiprofen and propranolol derivatives are hydrolyzed in liver microsomes. Ces2 is expressed in the liver, kidney and intestine (36). The expression ratios of Ces2 were increased after anesthesia at S6, I6, P6 and D6, and remained elevated at S24, I24, P24 and D24. The specific functions of this gene have not yet been determined, but alterations in its expression may have influences on lipid metabolism and xenobiotic detoxification.

Ugt7a1 is a phase II biotransformation enzyme of the glucuronidation pathway that transforms small lipophilic molecules, such as steroids, bilirubin, hormones and drugs, into water-soluble excretable metabolites (29). The expression ratios at D6 and S24 were suppressed to below the control levels. Although the effects of decreases in the expression of this gene have not yet been determined, these alterations may affect the glucuronidation of various drugs.

The ATP-binding cassette (ABC) superfamily of glycoprotein membrane transporters maintain the efflux from cells of a variety of endobiotics and xenobiotics, ranging from peptides, lipids and toxins to dyes. ABC transporters pumping out drugs confer multidrug resistance on cells. Abcb1a transporters can extrude signaling molecules and toxins from normal and tumor cells, including the side-population of stem cells. Abcb1a is activated during liver regeneration and hepatocarcinogenesis. In addition, Abcb1a is overexpressed in side-populations of hematopoietic and epidermal cells, and considered to be a stem cell molecular marker (26). The expression ratios of Abcb1a were increased at S6, I6 and P6, while that at D6 was decreased, and those at I24, P24 and D24 were slightly elevated compared with their control levels. These alterations in Abcb1a expression may affect the homeostasis of various signaling molecules, expulsion of toxins and blood-organ barriers.

Abcc2 transports various molecules across extracellular and intracellular membranes and is involved in multidrug resistance. Abcc2 is expressed in the apical part of hepatocytes and functions in biliary transport. The substrates of Abcc2 include the glutathione, glucuronide and sulfate conjugates of many drugs, some non-conjugated organic anions and various neutral or positively charged drugs. Known high-affinity physiological substrates of Abcc2 are glucuronides of the hemoglobin breakdown product bilirubin. In humans, mutations in the MRP2 gene can result in the autosomal recessive Dubin-Johnson syndrome (4). The expression and functions of Abcc2 are modulated by pharmacotherapeutics, pregnancy, lactation, bile duct ligation, disease states and other factors (38). The expression ratios of Abcc2 were only increased after isoflurane anesthesia, and this effect persisted at I24. These alterations may affect biliary transport and drug conjugation. The expression ratios of Nr1i2 and Ces2 remained elevated for 24 h after awakening from anesthesia with sevoflurane, isoflurane, propofol and dexmedetomidine. In particular, many genes encoding DMEs and drug transport proteins are regulated by Nr1i2, and continued alterations in the expression of this gene may affect the metabolism of other drugs used within 24 h after awakening from anesthesia.

Our findings provide the first assessment of the influences of inhalation and intravenous anesthesia on the expression ratios of genes encoding DMEs. We confirmed that the expression ratios of some genes encoding DMEs remained elevated for 24 h after awakening from anesthesia, and found that the gene expression ratios differed among individual anesthetics. The present results suggest that we should adjust the anesthetic dose when patients, especially those with liver dysfunction, are anesthetized within 24 h after awakening from anesthesia, and that we should consider the influence of anesthesia when various drugs, particularly those affecting liver function, are administered in the postoperative period. The alterations to genes encoding DMEs due to anesthesia may affect the metabolisms of other drugs. It is therefore necessary to investigate whether the alterations in gene expression after anesthesia affect drug metabolites and protein expression, and why these alterations in gene expression occur after anesthesia. Further studies may also clarify whether the gene expression changes after anesthesia influence the body as a whole.
In conclusion, the expression ratios of some genes encoding DMEs remained elevated for 24 h after awakening from anesthesia, and the gene expression ratios differed among individual anesthetics.

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