Introduction

Recent epidemiologic studies documented an increased hepatotoxicity and nephrotoxicity [1, 2], ulcerative colitis, and migraine in anaesthesiologists and an increased incidence of spontaneous abortion and congenital abnormalities in their offspring [3–5]. The mentioned health effects seem to be associated with an occupational exposure to anaesthesiologic drugs used in operation theatre. The inhalation drugs most commonly used in today's anesthesia practice are: halothane, enflurane, isoflurane, desflurane and sevoflurane. When inhaled into human cells they are metabolised by the group of enzymes from cytochrome P450 family. A role of cytochrome P450 enzymes in metabolism of inhalation anaesthetics and their involvement in generation of cytotoxic and organotoxic effects is presented in this review.

The metabolism of the inhalation anaesthetics takes place mainly in the liver, and to a lesser extent in the kidney and lung. All mentioned agents are metabolised in the liver by cytochrome P450, especially by the isoenzyme 2E1, and the dominating route is the process of oxidation [6]. The induction of liver microsomal enzymes, by e.g., phenytoin, isoniazid, phenobarbital, alcohol or halothane, increases the metabolism of anaesthetics. The metabolism is highest with halothane (15–40% of drug), followed by enflurane (2.4%), sevoflurane (2–5%), isoflurane (0.17–0.2%) and desflurane (0.02%) [7–10]. Some metabolites (compound A, compound B) of anaesthetic gases are associated with toxic effects in certain tissues (e.g., liver, kidney, brain) [1, 11, 12]. The metabolism of enflurane and sevoflurane produces an increased level of plasma inorganic fluoride, which is associated with nephrotoxicity. Volatile anaesthetics can also act as cytotoxic and genotoxic agents [13, 14 and references in the papers].

Cytochrome P450 proteins

The P450 proteins are expressed by the gene superfamily (CYPs) that currently contains more than 40 different members in humans (and 1000 in all the species). In humans the P450s play a critical role by, among other, catalyzing the metabolism of xenobiotics to reac-
tive metabolites (mostly free radicals) that interact with cellular macromolecules (DNA, RNA, proteins) or undergo detoxication by reaction with cellular constituents such as glutathione [15]. In most instances mammalian P450 enzymes catalyze reactions with activation of molecular oxygen for the oxidative metabolism of a vast variety of lipophilic organic chemicals [16].

CYP2E1 oxidizes small molecules and participates in the metabolism of general anaesthetics such as sevoflurane and halothane. Volatile anaesthetics by their chemical structure are halogenated aliphatic hydrocarbons and belong to rather relatively small organic molecules. Indeed CYP2E1 may convert halothane to a reactive metabolite that binds to hepatic proteins and has been implicated in halothane hepatotoxicity [17]. The metabolic activation of xenobiotics mediated by CYP2E1 may be modulated by such substances as trans-resveratrol and tannic acid present in diet [18]. In the serum of the patients autoantibodies directed against a xenobiotic metabolizing enzyme and among them frequently against cytochromes P450 can be detected. In some cases a xenobiotic, such as tannic acid, dihydrolazine, halothane, has been shown to be responsible for the disease but, in many instances, no toxic agent has been identified (polycyandlar syndrome or chronic active hepatitis) [15].

Numerous halogenated hydrocarbons are metabolized in liver by P450 enzymes to products that exert cytotoxic and/or carcinogenic effects. Such halogenated hydrocarbons include anaesthetics (e.g., halothane and enflurane) and industrial solvents (e.g., carbon tetrachloride, chloroform and vinylidine chloride). Formation of reaction intermediates from these compounds occurs via P450-promoted dehalogenation, reduction, or reductive oxygenation, with certain hydrocarbons undergoing all three reaction types. Of the multiple forms of P450 present in liver microsomes, P450 2E1 has been identified as the primary catalyst of hydrocarbon bioactivation in animals and, most likely, in humans as well. Exposure to 2E1-inducing compounds can play a role in halogenated hydrocarbon toxicity. Considering that metabolism governs the cytotoxicity and carcinogenicity of halogenated hydrocarbons repeated exposure to halothane in adult humans, particularly in obese, middle-aged women and over a short period of time may result in severe liver damage [19]. The cause of unexplained hepatitis following halothane anaesthesia is unclear. It seems unlikely to be a metabolic mechanism alone and most probably involves an immunological response in addition. Enflurane and isoflurane appear to induce no hepatotoxicity effect [20]. Sevoflurane appears to have a minimal effect on the guinea pig liver slices, which is consistent with in vivo studies in which minimal or no hepatotoxicity has been observed [21].

Halothane

Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) is a commonly used anaesthetic, which may result in an unpredictable hepatotoxicity, possibly due to a reactive free radical metabolite. The prevalence of hepatitis is 1:3000–1:30 000 with halothane and 1:80 000 with enflurane, a few cases have been reported with isoflurane but only a single case of liver damage in relation with desflurane anaesthesia [9, 10].

Halothane is extensively metabolized in humans and undergoes both oxidative and reductive cytochrome P450-catalyzed hepatic biotransformation. Oxidation is catalyzed by P450s 2E1 and 2A6, with no apparent role for P450 3A4. Anaerobic reduction is catalyzed by P450s 2A6 and 3A4, with no apparent role for P450 2E1 [22, 23].

Through an oxidative pathway, halothane may generate a reactive metabolite, trifluoroacetyl chloride TFA (CF3COCl), with concomitant loss of bromine (Fig. 1). This unstable intermediate can undergo hydrolysis to yield the nontoxic metabolite TFA, to bind to phospholipids and to acetylate tissue proteins to form the trifluoroacetylated protein (TFA-protein) neoantigens. Most of TFA is excreted by the kidney but a small percentage binds covalently to lipoproteins and proteins, including P450 enzymes, to form a TFA-hapten, which, in susceptible individuals, stimulates formation of anti-TFA-protein antibodies, and hepatic necrosis in halothane re-exposure [17, 24]. To date, a number of the TFA-modified proteins have been identified. These include protein disulfide isomerase, microsomal carboxylesterase, calreticulin, stress protein Erp72 and Erp99/endoplasm/GRP 94 in microsomes [25], and glutathione-S-transferase in cytosol. Both P450s 2A6 and 2E1 are the isoforms that catalyze oxidative halothane metabolism in human liver microsomes. These results suggest further that at low halothane concentrations, P450 2E1 is the predominant catalyst and at high concentrations, P450 2A6 is the predominant catalyst of halothane oxidation. At clinically relevant concentrations, both P450 2E1 and 2A6 participate in halothane oxidation. P450 2A6 and P450 3A4 catalyze reductive halothane metabolism. Halothane is a unique substrate in that it readily undergoes both oxidative and reductive metabolism [17].

Fig. 1. Metabolism of halothane.

Halothane anaesthesia uncommonly induces cytolytic hepatitis with a high mortality rate. In halothane hep-
totoxicity the predisposing factors are: obesity, age above 40 years, female sex and previous anaesthesia with this compound. A hereditary susceptibility is suggested by the observation of halothane-induced hepatitis in several females from the same family. Halothane hepatotoxicity is mediated by a reactive metabolite (producing trifluoroacetylation of hepatocyte proteins), which triggers an immune reaction against hepatocytes [26].

Trifluoroacetylchloride binds to several proteins generating a neoantigen. CYP2E1 is the main enzyme responsible for the formation of the reactive metabolite and, therefore, of the neoantigens. The sera of patients are able to recognize some halothane-altered proteins and contain autoantibodies against CYP2E1. These autoantibodies were also found in some subjects chronically exposed to this kind of anaesthetic. Halothane is metabolized by CYP2E1 into a reactive metabolite which binds to CYP2E1, generating a neoantigen that triggers an immune response characterized by the presence of autoantibodies directed against CYP2E1. This disease is multifactorial combining metabolic and immunological factors, the metabolic factors being much better known. However, it is not yet known whether the autoantibodies are directly involved in liver cell injury. A direct impact of the autoantibodies on hepatic injury would require the autoantibodies to bind directly to the hepatocytes and activate cytotoxic T cells and the complement system [15].

Environmental exposure of anaesthesiology personnel to certain inhaled anaesthetics can induce the formation of autoantibodies ERp58 and P450 2E1 that have been associated with anaesthetic hepatitis. Female anaesthesiologists have high levels of these autoantibodies; however, a majority of these individuals do not develop hepatitis, suggesting that autoantibodies may not have a pathological role in volatile anaesthetic-induced hepatitis [27].

In reductive biotransformation halothane is reduced anaerobically by cytochrome P450 (P450) to the volatile metabolites 2-chloro-1,1,1-trifluoroethane (CTE), 2-chloro-1,1-difluoroethene (CDE) and inorganic fluoride. Human liver microsomal reductive halothane metabolism is catalyzed predominantly by P450 2A6 and 3A4 [28]. In the standard Ames test volatile metabolites of halothane CTE (CF3CH2Cl) and CF2CBrCl are not mutagenic, but CDE (CF2CHCl) and CF2CBrCl are weakly mutagenic [29].

It is generally accepted that halothane radicals initiate lipid peroxidation, at least in animals. Halothane causes also lipid peroxidation in human liver microsomes, which is catalyzed by CYP2A6, and inhibition of halothane reduction prevents halothane-dependent lipid peroxidation in vitro [30].

**Sevoflurane**

Sevoflurane (fluoromethyl 2,2,2-trifluoro-1-[trifluoromethyl]ethyl ether) is an inhalational anaesthetic agent that is used worldwide. This anaesthetic is a subject of oxidative degradation by cytochrome P450 2E1 with separation of the methoxy and isopropyl moieties (Fig. 2). The isopropyl moiety is biodegraded to a harmless glucuronide conjugate. The hexafluoroisopropanol is an anaesthetic and could delay a recovery from anesthesia. In adults more than 99% of the hexafluoroisopropanol is degraded to a glucuronide conjugate and excreted in the urine. The methoxy moiety is metabolised in the liver to carbon dioxide and inorganic fluoride [21, 31].

**Fig. 2.** Metabolism of sevoflurane.

When used in anaesthesia machines equipped with carbon dioxide absorbents containing strong base, sevoflurane undergoes base-catalyzed elimination of hydrogen fluoride to form degradation product fluoromethyl-2,2-difluoro-1-(trifluoromethyl) vinyl ether (FDVE = compound A). In the closed anaesthetic circuit there was found also the second product fluoromethyl 2-methoxy-2,2-difluoro-1-(trifluoromethyl)ethyle ether (compound B) [32, 33]. Patients may be exposed to 20 to 50 ppm of FDVE under these conditions. Like several other haloalkenes, FDVE is nephrotoxic in rats [32, 34]. Nephrotoxicity of inhaled compound A in rats is associated with a multistep pathway that includes hepatic glutathione S-conjugate formation, enzymatic hydrolysis of the glutathione S-conjugates to cysteine S-conjugates, renal uptake of cysteine S-conjugates, and bioactivation by renal cysteine S-conjugate β-lyase to reactive species, in which the reaction with cellular proteins is associated with cell damage and death [34–37].

FDVE metabolism via glutathione conjugation and the β-lyase pathway has been established in rats and humans. In rats, in vivo FDVE undergoes a reaction with GSH to form two alkane diastereomers and two alkene glutathione conjugates, which undergo cleavage to the cysteine S-conjugates. The cysteine S-conjugates are also metabolized by rat renal β-lyase in vitro and in vivo to reactive intermediates, which may bind to cellular macromolecules or undergo hydrolysis to 3,3,3-trifluoro-2-(fluoromethoxy) propanoic acid. Cysteine S-conjugates are metabolized by human kidney cytosol and mitochondria in vitro. The 3,3,3-
trifluoro-2-(fluoromethoxy)propanoic acid is unstable and decomposed to trifluorolactic acid [36–39].

The nephrotoxicity in rats of several haloalkenes is most likely mediated by the biosynthesis and renal processing of glutathione S-conjugates. Although FDVE undergoes metabolism in humans by the GSH- and β-lyase-catalyzed pathway, interindividual variability has not been reported. It has been shown that FDVE undergoes GSH- and β-lyase-dependent biotransformation in rats and humans in vitro and in vivo. The cysteine S-conjugates were biotransformed to pyruvate, a known product of the β-lyase-catalyzed β-elimination reaction of cysteine S-conjugates [35–38, 40].

Recent studies in vitro revealed cytochrome P4503A-catalyzed formation of novel sulphoxide metabolites of FDVE cysteine-S and mercapturic acid conjugates in rat liver and kidney microsomes. FDVE-mercapturic acid sulphoxides were more toxic than other FDVE conjugates to renal proximal tubular cells in culture. FDVE S-conjugates undergo P450-catalyzed and nonenzymatic sulphoxidation and that enzymatic sulphoxidation is catalyzed predominantly by P450 3A. This sulphoxidation pathway contributes to nephrotoxicity. FDVE S-conjugates sulphoxidation constitutes a newly discovered mechanism of FDVE bioactivation and toxicification in rats, in addition to beta-lyase-catalyzed metabolism of FDVE-cysteine S-conjugates [41, 42]. FDVE undergoes both P450-catalyzed and nonenzymatic deflourination by human liver microsomes. P4502E1 is implicated in the enzymatic deflourination. Nonenzymatic deflourination may result from FDVE addition to protein thios. Enzymatic and/or nonenzymatic deflourination may be etiologic factors in FDVE nephrotoxicity in rats. In contrast, P450-dependent FDVE deflourination may be of less clinical consequence in humans, because it is inhibited by the parent anaesthetic, sevoflurane [43].

Humans, compared with rats, have shown no evidence of nephrotoxicity from FDVE [44]. The toxicification pathway is many times more intense in rats than in humans. Species differences in dose and metabolism may influence compound A renal effects [37, 45, 46]. FDVE undergoes GSH-dependent conjugation in human liver and kidney microsomes and cytosol as well as blood, which may account for the detection of corresponding mercapturic acids in the urine of patients exposed to FDVE [47]. Rat FDVE nephrotoxicity is attributed to FDVE glutathione conjugation and bioactivation of subsequent FDVE-cysteine S-conjugates, in part by renal β-lyase. Human proximal tubular cells are relatively resistant to FDVE and FDVE S-conjugate cytotoxicity. This may partially explain the lack of FDVE nephrotoxicity in humans [36, 37, 48]. Compound A causes renal proximal tubular injury in rats but has had no effect on blood urea nitrogen, creatinine concentrations, and creatinine clearance in patients [49, 50]. Sevoflurane undergoes also extrahepatic metabolism in the kidneys, lungs or gut [51].

Amount of inorganic fluoride released after anaesthesia with sevoflurane depends on the dose of adminis-

[Diagram of Enflurane Metabolism]

Enflurane, isoflurane and sevoflurane prolonged anaesthesia in humans resulted in peak of concentrations in serum inorganic fluoride exceeding 50 microM, but there were no significant changes in renal function. Enflurane anaesthesia can also result in significantly diminished urine concentrating ability at plasma fluoride concentrations less than 50 microM [20].

Isoflurane

The biodegradation pathways for isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) and desflurane are thought to be parallel. Cytochrome P450 2E1 is the predominant P450 isoform responsible for human clinical isoflurane metabolism in vivo [57]. P450 2E1 may insert an active oxygen atom between the alpha ethyl carbon of isoflurane and its hydrogen, producing HCl and an unstable product. The resulting unstable product degrades ultimately to two free fluoride ions, trifluoroacetic acid TFA, carbon dioxide, and water (Fig. 4). The biodegradation of TFA may be of importance because of the connection between its hepatic production and hepatotoxicity via an immune pathway [31].
Fig. 4. Metabolism of isoflurane.

Isoflurane sedation is associated with an increase in plasma fluoride concentration without any clinical deterioration of renal function [58], but the caution is recommended using isoflurane when prolonged anaesthesia and surgery are planned [59].

Desflurane

In desflurane (fluoromethyl-2,2,2-trifluoro-1-(trifluoromethyl)-ethyl ether) cytochrome P450 2E1 may insert an active oxygen atom between the alpha ethyl carbon and its hydrogen, producing HF and unstable product, which degrades ultimately to two free fluoride ions, trifluoroacetic acid TFA, carbon dioxide, and water (Fig. 5). The biodegradation of TFA may be of importance because of the connection between its hepatic production and hepatotoxicity via an immune pathway [31].

Fig. 5. Metabolism of desflurane.

Conclusions

Metabolism of inhalation anaesthetics is today considerably well known and it does not differ from metabolism of many other xenobiotics. The critical role plays cytochrome P450, which is responsible for two pathways: oxidative and reductive. Finally, potentially nontoxic anaesthetics could be metabolised to simple organic substances and to inorganic fluorides, which could have toxic properties. However, toxicity depends on metabolic pathway and quantity of substance. It should be admitted that nephrotoxicity was studied almost exclusively on rats and the results cannot be directly transmitted to human beings.

References

Role of cytochrome P450 in metabolism of inhalation anesthetics


37. Iyer R.A., Anders M.W.: Cysteine conjugate beta-lyase-dependent biotransformation of the cysteine S-conjugates of the sevoflurane degradation product 2-(fluoromethoxy)-1,3,3,3-


