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Effects of saikosaponins on hepatic damage induced by halothane and hypoxia in phenobarbital-pretreated rats

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Abstract: The effects of saikosaponins-a. -b_1, -b_2, -c, and -d on hepatic damage induced by halothane and hypoxia were investigated in the rat. Inhalation of halothane under a hypoxic condition significantly increased serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in rats pretreated with phenobarbital compared with rats pretreated without phenobarbital. Pretreatment with saikosaponin (especially -a and -d) and with phenobarbital suppressed the increase in serum GOT and GPT levels in comparison with the rats treated with phenobarbital, halothane, and hypoxia. Histological observation also confirmed that pretreatment with saikosaponin had a protective effect against liver cell damage caused by halothane and hypoxia. Saikosaponins-a and -d, the most effective saikosaponins against hepatic damage, inhibited the increases in cytochrome P450 and NADPH-cytochrome *c* reductase activity which are induced by phenobarbital treatment. Therefore, it is suggested that the cytoprotective effect of saikosaponin against halothane-induced hepatitis under hypoxia is caused by inhibition of phenobarbital stimulation of the enzyme system for hepatic drug metabolism.

Key words: Saikosaponin, Sho-saiko-to, Hepatitis

Introduction

Halothane anesthesia was introduced into clinical practice in 1956 and has been used widely since then. However, shortly after its introduction into clinical practice, Virtue et al. [1] reported the development of unexplained jaundice following halothane anesthesia. Subsequently, a similar phenomenon was reported by other researchers. In 1966, the National Halothane Study was conducted by the National Institutes of Health (NIH) in the United States. The findings of the study of hepatic disorders reported by Carney and Van Dyke [2] in 1972 were almost identical to those of the National Halothane Study. The mortality rate due to halothane-induced hepatitis is about 0.01%, but the treatment of halothane-induced hepatitis has rarely been reported. The saikosaponins used in the present study are active compounds of *Bupleuri radix*, an important crude drug contained in sho-saiko-to, which is widely used to treat viral hepatitis. The protective effects of the saiko-saponins against CCl_4-induced hepatitis [3] and D-galactosamine hepatitis [4] have been reported previously.

In the present study, saikosaponins were found to have a protective effect against hepatic damage induced by halothane and hypoxia in phenobarbital pretreated rats.

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Materials and methods

Preparation of saikosaponins

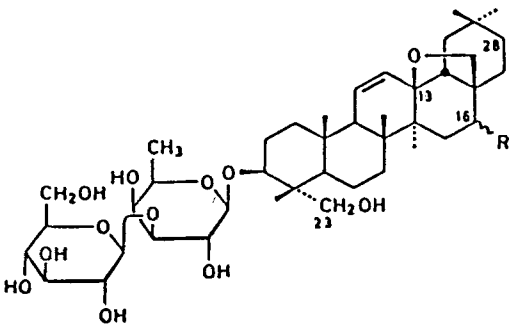
Saikosapoin-a, -b_1, -b_2, -c, and -d were extracted from the roots of *Bupleurum falcatum L.* by the method of Kubota and Tonami [5]. The structural formulae of these saikosaponins are shown in Fig. 1.

Animals

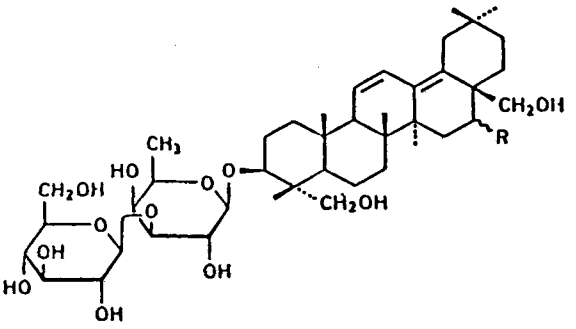
Male Wistar rats weighing 190–230 g 8 weeks of age were maintained on unrestricted quantities of a standard diet with free access to tap water. The animals were housed in metal cages with wire mesh floors in air-conditioned quarters with a regulated diurnal cycle (light from 7 a.m. to 7 p.m.).

Experimental procedure

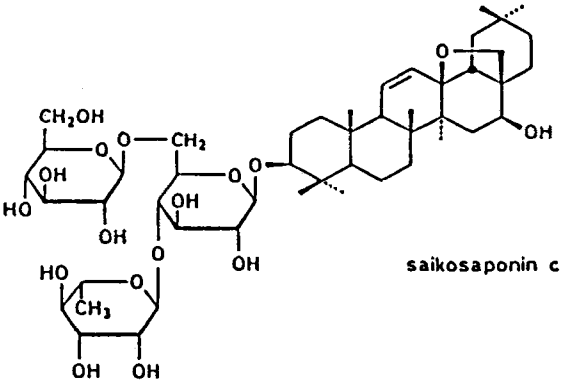
Experiment 1. Rats were randomly assigned to seven groups of 12 rats.



saikosaponin a : R= β-OH
saikosaponin d : R= α-OH



saikosaponin b_1: R= β-OH
saikosaponin b_2: R= α-OH



saikosaponin c

Fig. 1. Structure of saikosaponins

Group I: The animals were administered an equal volume of 0.9% NaCl (saline) by a single intramuscular injection daily for 3 days.

Group II: The rats were pretreated with 75 mg·kg^{-1} phenobarbital alone by a single intramuscular injection daily for 3 days.

Group III: The rats were pretreated with 75 mg·kg^{-1} phenobarbital and 1 mg·kg^{-1} saikosaponin-a by a single intramuscular injection daily for 3 days.

Group IV: The rats were pretreated with 75 mg·kg^{-1} phenobarbital and 1 mg·kg^{-1} saikosaponin-b_1 by a single intramuscular injection daily for 3 days.

Group V: The rats were pretreated with 75 mg·kg^{-1} phenobarbital and 1 mg·kg^{-1} saikosaponin-b_2 by a single intramuscular injection daily for 3 days.

Group VI: The rats were preteated with 75 mg·kg^{-1} phenobarbital and 1 mg·kg^{-1} saikosaponin-c by a single intramuscular injection daily for 3 days.

Group VII: The rats were pretreated with 75 mg·kg^{-1} phenobarbital and 1 mg·kg^{-1} saikosaponin-d by a single intramuscular injection daily for 3 days.

All rats were deprived of food for 24 h after the final injection on the 3rd day. One percent halothane in 10% oxygen and 90% nitrogen were admitted to the chamber at a flow rate of 3 l·minute^{-1} from a previously calibrated halothane vaporizer for 5 min. Halothane concentration in the chamber was comfirmed and monitored by a multiple gas monitor (Capnomax, Datex, Helsinki, Finland). Anesthesia was maintained for 1 h under this condition (1% halothane in 10% oxygen and 90% nitrogen). At 24 and 48 h after anesthesia, blood was taken from the cervical vein and separated into serum and blood cells.

Fourteen rats were killed to obtain a specimen of liver 24 h after anesthesia under inhalation of ether.

Experiment 2. Rats were randomly assigned to seven groups consisting of six rats each. The same pretreatment as in experiment 1 was performed without anesthesia. After being deprived of food for 24 h, the rats were killed under inhalation of ether and the livers were removed. Each liver was homogenized and microsomes were separated.

Serum assay

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were assayed us-

ing a commercial kit (Mako Pure Chemical Industries, Osaka, Japan).

Preparation of liver homogenate

The liver was perfused with ice-cold physiological saline to wash out the blood. It was then dissected and minced in ice-cold 0.25 M sucrose. The liver blocks were then homogenized with a Potter-Elvehjem homogenizer in 0.25 M sucrose.

The homogenate was used to determine NADPH-cytochrome *c* reductase activity in 15 mg protein·ml^{-1}. The protein content was determined by the method of Lowry et al. [6].

Preparation of microsomes

The liver perfused with ice-cold saline was homogenized with a Potter-Elvehjem homogenizer in 0.25 M sucrose. This homogenate was centrifuged at 10 000 g for 10 min. The supernatant was further centrifuged at 100 000 g for 60 min and the pellet was washed once 0.15 M KCl. The resuspension contained 2 mg protein·ml^{-1} of microsomes.

Enzyme assays

NADPH-cytochrome *c* reductase activity was measured according to the method of Williams and Kamin [7]. The cytochrome P450 content was determined from CO difference spectra of dithionite-reduced samples with an extinction coefficient of 91 cm^{-1}·mM^{-1} [8]. The difference spectra were recorded between 370 and 500 nm.

Light microscopy

A portion of liver tissue from each animal was fixed with 15% formalin, embedded in paraffin wax, and stained with hematoxylin and eosin.

Statistical analysis

Statistical analysis was performed using analysis of variance and Student's *t*-test, and differences of $P < 0.05$ were considered statistically significant. All values are expressed as mean ± SE.

Results

Experiment 1

Serum GOT and GPT levels. Inhalation of halothane under hypoxia produced maximal increases in serum GOT and GPT levels in rats pretreated with phenobarbital alone (group II) 24 h after the end of anesthesia. Serum GOT and GPT levels of these rats were significantly increased compared with the rats pretreated with saline alone (group I).

In contrast, saikosaponin-a (group III) or saikosaponin-d (group VII) pretreatment supressed increases in serum GOT and GPT compared with value of group II 24 h after inhalation of halothane under hypoxia as shown in Table 1. The serum GOT and GPT levels 48 h after inhalation of halothane showed similar values to those 24 h after inhalation of halothane under hypoxia. Saikosaponin-b_1 (group III), -b_2 (group V) and -c (group VI) pretreatment did not suppress increases in serum GOT and GPT levels.

Light microscopic observation. The rats pretreated with saline alone showed no centrilobular necrosis (Fig. 2a). The rats pretreated with phenobarbital alone and inhalation of halothane under hypoxia (group II) showed marked centrilobular necrosis with enlargement of hepatocytes in the centrilobular zone and loss of the normal architectural pattern. Numerous areas of hepatic necrosis were observed in the central regions of lobules with sparing of periportal cells. Foam cells were

Table 1. Effects of phenobarbital and saikosaponins on serum GOT and GPT 24 and 48 h after inhalation of helothane and hypoxia

Treatment	24 h after inhalation of halothane and hypoxia		48 h after inhalation of halothane and hypoxia	
	GOT (u/l)	GPT (u/l)	GOT (u/l)	GPT (u/l)
Group I (saline + halothane)	75 ± 4	44 ± 2	61 ± 5	56 ± 14
Group II (phenobarbital + halothane)	1152 ± 196^{++}	442 ± 63^{++}	996 ± 176^{+}	417 ± 61^{+}
Group III (phenobarbital + ssa + halothane)	553 ± 134$^{*,+}$	252 ± 53$^{*,+}$	397 ± 89$^{*,+}$	149 ± 18*
Group IV (phenobarbital + ssb$_1$ + halothane)	889 ± 167^{++}	338 ± 47^{++}	640 ± 109^{++}	379 ± 87
Group V (phenobarbital + ssb$_2$ + halothane)	722 ± 246	227 ± 62^{+}	618 ± 129^{+}	290 ± 65
Group VI (phenobarbital + ssc + halothane)	787 ± 146^{++}	286 ± 45^{++}	761 ± 130^{++}	278 ± 33^{+}
Group VII (phenobarbital + ssd + halothane)	268 ± 47$^{**,+}$	150 ± 24$^{*,+}$	191 ± 39**	94 ± 16**

$^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$, significant difference *vs* control (group I) treated with saline and halothane.
* $P < 0.05$, ** $P < 0.01$, significant difference *vs* control (group II) treated with phenobarbital and halothane.
ss, saikosaponin.

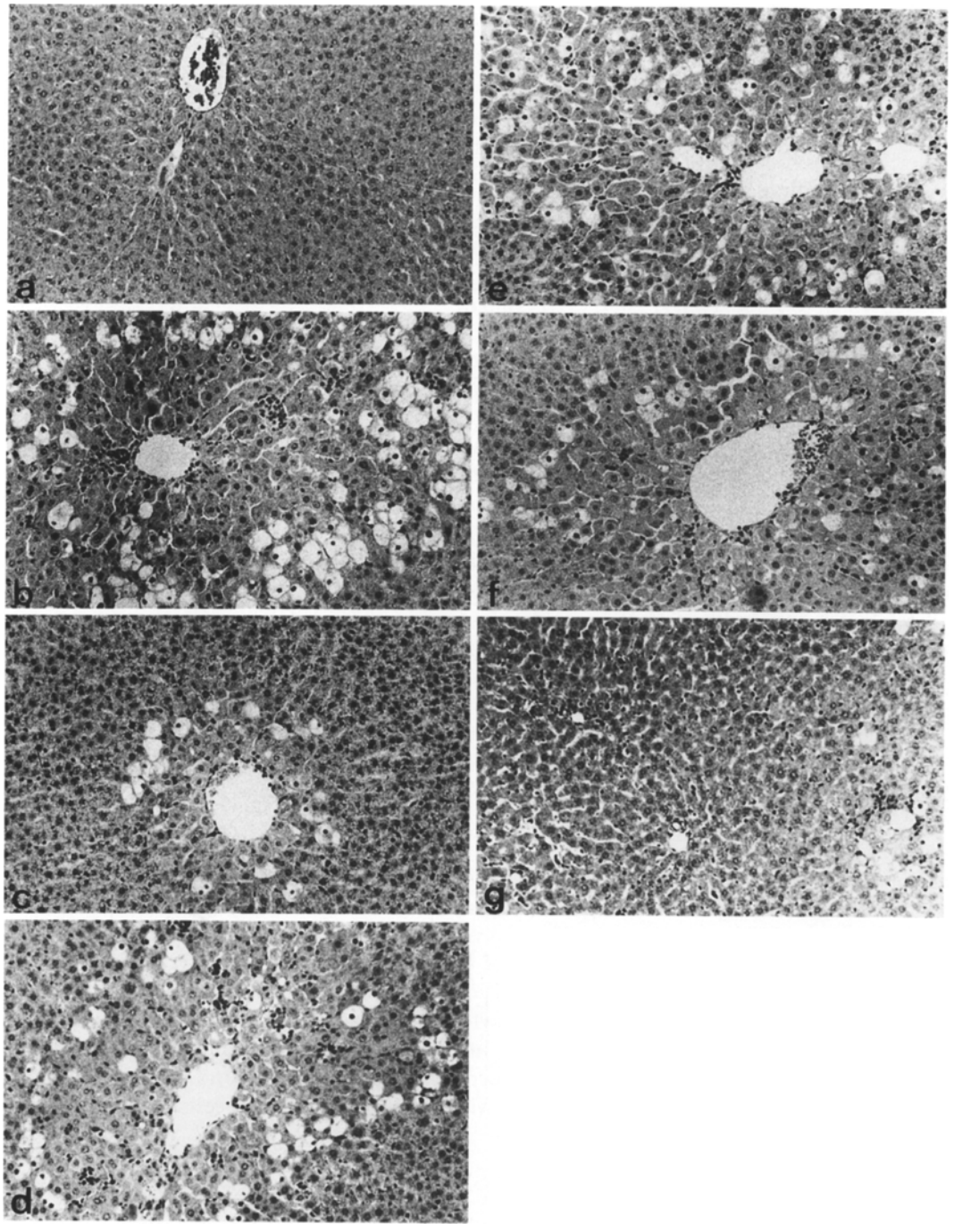


Fig. 2a–g. Histological findings by light microscopy. **a** Liver of a saline-pretreated rat (group I) 24 h after inhalation of halothane under hypoxia (×25). Centrilobular necrosis and inflammatory infiltration are not noted. **b** Liver of a phenobarbital-pretreated rat (group II) 24 h after inhalation of halothane under hypoxia (×25). Extensive centrilobular necrosis and inflammatory infiltration are noted. **c** Liver of a rat pretreated with phenobarbital and saikosaponin-a (group III) 24 h after inhalation of halothane under hypoxia (×25). Less extensive necrosis is present. **d** Liver of a rat pretreated with phenobarbital and saikosaponin-b_1 (group IV) 24 h after inhalation of halothane under hypoxia (×25). Centrilobular necrosis is present. **e** Liver of a rat pretreated with phenobarbital and saikosaponin-b_2 (group V) 24 h after inhalation of halothane under hypoxia (×25). Centrilobular necrosis is present. **f** Liver of a rat pretreated with phenobarbital and saikosaponin-c (group VI) 24 h after inhalation of halothane under hypoxia (×25). Centrilobular necrosis is present. **g** Liver of a rat pretreated with phenobarbital and saikosaponin-d (group VII) 24 h after inhalation of halothane under hypoxia (×25). Centrilobular necrosis is less extensive and periportal zone is present

observed at the margins of the necrotic areas (Fig. 2b).

Pretreatment with saikosaponin-a or -d resulted in marked protection against the liver cell damage caused by inhalation of halothane under hypoxia (Figs 2c and 2g). In the rats pretreated with saikosaponin-b_1, -b_2 or -c, the small necrotic area was almost the same as that in rats pretreated with phenobarbital alone. These necrotic areas were observed in the central regions of lobules.

Experiment 2

Liver weight, protein content and microsomal content. Liver weight, protein content and microsomal content showed no significant difference between groups treated with phenobarbital and saikosaponins and with phenobarbital alone without inhalation of halothane (Table 2).

Enzyme activity in liver. The activities of NADPH-cytochrome *c* reductase of liver homogenate and microsomal NADPH-cytochrome *c* reductase and the P450 content were compared (Table 2).

NADPH-cytochrome *c* reductase activity of liver homogenate in the rats treated with phenobarbital and saikosaponin-a (group III) or saikosaponin-d (group VII) was lower than that in the rats treated with phenobarbital alone (group II). In the rats pretreated with phenobarbital and saikosaponin-b_1 (group IV), -b_2 (Group V), or -c (group VI), no significant difference was observed compared with the rats pretreated with phenobarbital alone. Microsomal NADPH-cytochrome *c* reductase activity in the rats pretreated with phenobarbital and saikosaponins, except for saikosaponin-c, was lower than that in the rats pretreated with phenobarbital alone. The content of P450 in the rats pretreated with phenobarbital alone was significantly increased compared with rats treated with saline alone ($P < 0.001$). The increase in the P450 content was inhibited significantly in the rats pretreated with phenobarbital and saikosaponin-a ($P < 0.05$) or -d ($P < 0.01$). The increase in the P450 content was not inhibited in the rats pretreated with phenobarbital and saikosaponin-b_1, -b_2, or -c.

Discussion

The mechanism of hepatic damage by halothane is unknown, but it is thought to be closely related to various factors such as heredity, sex, hypoxia, immunologic response, and mobilization of intracellular Ca^{2+}. To explain the mechanism of halothane-induced hepatic damage, many investigators [9,10] have used animal models, and identified the following four factors necessary for halothane-induced hepatic damage: male rats, induction by phenobarbital, deprivation of food before anesthesia, and anesthesia under hypoxia.

The liver is susceptible to hypoxia. According to the thesis of Shingu et al. [11], hypoxia per se may be more important than halothane metabolism in causing liver damage. In our previous experiment, we comfirmed that hypoxia (under 15% oxygen) was necessary for halothane-induced hepatitis. Halothane is metabolized as follows [12]. Volatile metabolites appear as a result of reductive metabolism requiring microsomal cytochrome P450 in the presence of NADPH-cytochrome *c* reductase and the absence of oxygen. Two reductive metabolites (difluorochloroethylene [CDE] and trifluorochloroethane [CTE]) are used. The mechanism by which the liver is injured by CDF and CTF is unknown. Brown [13] found that lipid peroxidation occurs in the hepatic microsome during the process of halothane metabolism. During this process, free radicals [14,15] were observed and the binding of microsomal lipids [16] and protein was reported. This covalent binding may contribute to liver cell damage. On the other hand, a unique immune response is observed in patients following halothane anesthesia. In patients

Table 2. Effects of phenobarbital and saikosaponins on liver weight and microsomal protein content

Treatment	Liver weight (g)	Liver weight (% Body weight)	Microsomal protein content (mg/ml liver homogenate)
Group I (saline)	7.37 ± 0.20	3.95 ± 0.05	2.35 ± 0.23
Group II (phenobarbital)	9.32 ± 0.47	4.95 ± 0.17	2.82 ± 0.58
Group III (phenobarbital + ssa)	8.77 ± 0.38	4.93 ± 0.23	2.57 ± 0.47
Group IV (phenobarbital + ssb$_1$)	9.86 ± 0.65	4.73 ± 0.18	3.07 ± 0.53
Group V (phenobarbital + ssb$_2$)	9.43 ± 0.30	4.87 ± 0.06	2.92 ± 0.30
Group VI (phenobarbital + ssc)	9.21 ± 0.44	5.12 ± 0.18	2.74 ± 0.39
Group VII (phenobarbital + ssd)	7.49 ± 0.16	4.65 ± 0.12	2.20 ± 0.30

ss, saikosaponin.

with halothane-induced hepatitits, anti-mitochondrial and anti-smooth muscle antibodies [17], anti-thyroid antibodies [18], and anti-nucleic acid antibodies [19] were found. Halothane or its metabolites bind covalently to liver tissue, and could potentially act as haptens and evoke an immune response. According to the immune response theory, liver protein thus becomes immunogenic, changing from self to non-self protein. Antibodies are induced against this non-self protein, creating an immune response.

Phenobarbital induces microsomal enzymes in the rat [20]. Halothane-induced hepatitis is produced only in rats pretreated with phenobarbital under hypoxia [21]. The administration of phenobarbital increases the activity of various enzymes in the liver microsomes of various animals [22]. Our results indicate that saikosaponins decrease liver microsomal enzyme activity. In particular, saikosaponin-a and -d caused marked decreases of microsomal enzymes. Serum GOT and GPT in rats pretreated with saikosaponin-a or -d were significantly lower than those in rats pretreated with phenobarbital alone. George et al. [23] reported that induction of the microsomal drug-metabolizing enzymes is necessary to make a rat model of halothane-induced hepatitis under hypoxia. Accordingly, it is suggested that the inhibitory action of saikosaponin in microsomal emzyme activity induced by phenobarbital plays the most important role in the inhibition of halothane-induced hepatitis under hypoxia. However, since saikosaponins have been known to have various pharmacological effects [24–26], including stabilization of cell membranes [27], the protective action of saikosaponin against halothane-induced hepatitis may be partly due to stabilization of the cell membrane of hepatocytes.

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References

1. Virtue RW, Kathleen WP, Caramna LJ (1958) Observation during experimental and clinical use of halothane. Anesthesiology 19:478–487
2. Carney MT, Van Dyke RA (1972) Halothane hepatitis: a critical review. Anesth Analg 51:135–160
3. Abe H, Orita M, Odashima S, et al. (1982) Protective effect of saikosaponin-d isolated from Bupleurum L on CCl_4-induced liver injury in the rat. Naunyn-Schmiedebergs Arch Pharmacol 320:266–271
4. Arichi S, Konishi H, Abe H (1978) Studies on the mechanism of action saikosaponin-induced by D-galactosamine. Acta Hepatol Jpn 19:430–435
5. Kubota T, Tonami F (1967) Triterpenoids from Bupleurm falcatum L.II: Isolation of saikosaponin Band longspinogen. Tetrahedron 23:3333
6. Lowry OH, Rosebrough NJ, Fan AL, et al. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
7. Williams GH Jr, Kamin H (1962) Microsomal triphosphopyridine nuclectite-cytochrome C reductase of liver. J Biol Chem 237:587–595
8. Omura T, Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. J Biol Chem 239:2379–2385
9. Sipes IG, Brown BR Jr (1976) An animal model hepatotoxicity associated with halothane anesthesia. Anesthesiology 45:622–628
10. Ross WT Jr, Daggy BP, Cardell RR Jr (1979) Hepatic necrosis caused by halothane and hypoxia in phenobarbital treated rats. Anesthesiology 51:327–333
11. Shingu K, Eger II EI, Johnson BH (1982) Hypoxia may be more important than reductive metabolism in halothane-induced hepatic injury. Anesth Analg 61:824–827
12. Van Dyke (1983) Halogenated anaesthetic hepatotoxicity—Is the answer close at hand? Clinics in Anesthesiololgy, Vol 2. Saunders, London, 485–506
13. Brown BR (1972) Hepatic microsomal lipoperoxidation and inhalation anesthetics: a biochemical and morphologic study in the rat. Anesthesiology 36:458–465
14. Poyer JI, McCay PB, Weddle CC, et al. (1981) In vivo spin-trapping of radicals formed during halothane metabolism. Biochem Pharmacol 30:1517–1519
15. Fujii K, Miki N, Kanashiro M, et al. (1982) A spin trap study on anaerobic dehalogenation of halothane by a reconstituted liver microsomal cytochrome P450 enzyme system. J Biochem 91:415–418
16. Van Dyke RA, Wood CL (1975) In vitro studies on irrversible binding of halothane metabolites to microsomes. Drug Metab Dispos 3:57–57
17. Rodriguez M, Paronetto F, Schaffner F, et al. (1980) Anti-mitochondrial antibodies in jaundice following drug administration. JAMA 303:104–104
18. Walton B, Simpson BR, Strunin L, et al. (1976) Unexplained hepatitis following halothane. Br Med J 1:1171–1176
19. Moult PJA, Sherlock S (1975) Halothane related hepatitis. A clinical study of 26 cases. QJ Med 44:99–114
20. Willam TR Jr, Bruce PD, Robert RC Jr (1979) Hepatic necrosis caused by halothane and hypoxia in phenobarbital-treated rats. Anesthesiology 51:327–333
21. Adachi Y, Yamamoto T (1976) Influence of drugs and chemicals upon hepatic enzymes and protein-I. Biochem Pharmacol 25:663–668
22. Yoshimura H, Ozawa N, Saeki S (1978) Inductive effect of polychlorinated biphenyls mixture and individual isomers on the hepatic microsomal enzymes. Chem Pharm Bull 26:1215–1221
23. George EM, Glenn S, Burnell RB Jr (1979) An animal model of halothane hepatotoxicity Anesthesiology 51:321–326
24. Abe H, Sakaguchi M, Arichi S (1982) Pharmacological studies on a prescription containing Bupleuri Radix (IV). Effect of saikosaponin on the anti-inflammatory action of glucocorticoid. Fol Pharmacol Jpn 80:155–161
25. Abe H, Sakaguchi M, Arichi S (1983) Pharmacological studies on prescription containing Bupleuri Radix (V). Effect of adrenolectomy on pharmacological actions of saikosaponin-d. Med J Kinki Univ 8:379–383
26. Abe H, Orita M, Konishi H, et al. (1985) Effect of saikosaponin-d on aminonucleoside nephrosis in the rats. Eur J Pharmacol 120:171–178
27. Abe H, Sakaguchi M, Anno M, et al. (1981) Erythrocyte membrane stabilization by plant saponin and sapogenins. Naunyn Schmiedebergs Arch Pharmacol 316:262–265