Study on Pharmacological Effect of Bile Salts, Sodium Scymnol Sulfate, from *Rhizoprionodon acutus*. I. Effect of Scymnol, Chimaerol and Sodium Scymnol Sulfate on Cerebral Anoxia

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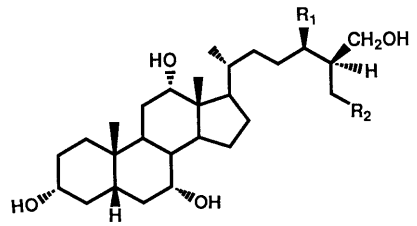
The effects of scymnol, chimaerol and sodium scymnol sulfate, prepared from the bile of *Rhizoprionodon acutus*, on cerebral anoxia were investigated in experimental models of hypoxia, ischemia and histotoxic anoxia in mice. Scymnol, at a dose of 100 mg/kg, showed a significant protective action against cerebral anoxia in all of the models studied and significantly increased the partial oxygen pressure of the arterial blood. The anti-anoxic activity of scymnol was found to be slightly greater than that of idevenone. A similar protective effect of sodium scymnol sulfate was seen at doses higher than 100 mg/kg. The survival time on hypoxia was significantly prolonged in the animals pretreated with chimaerol.

Keywords scymnol; chimaerol; sodium scymnol sulfate; *Rhizoprionodon acutus*; hypoxia; anoxia

In traditional Chinese medicine, the gall bladders of some mammals, especially bear (*Ursus torquatus* SCHL., *U. tibetanus* CUW.) and cow (*Bos taurus* L.), and gallstone of cow (*Bos taurus* L.) are important crude drugs, which have been used as a sedative for biliary calculus, as an antiphlogistic for liver, and as a remedy for jaundice, *etc.*[1,2] Bile from various fishes, such as shark (*Isuropsis glauca*, *Heterodontus japonicus*), carp (*Cyprinus carpio* L.), mackerel (*Scomber japonicus*) and crucian (*Carassius carassius* L.), has also been used as a crude drug to treat dyspnea due to disorders of the throat, and pharynx, eye diseases, *etc.*, and as an analgetic.[1]

There have been many studies on the structures and pharmacological effects of bile and gallstone components, especially on bile of bear and cow, in which C-24 bile acids such as ursodeoxycholic acid,[3] chenodeoxycholic acid[4] and cholic acid[5] are major components. In contrast, less work has been done on chemical elucidation of sodium bile alcohol sulfates and bile alcohols, such as scymnol,[6] chimaerol,[7] cyprinol[8] and petromyzonol,[9] which are major components of fish biles. To our knowledge, no pharmacological study on these compounds has yet been reported.



R_1	R_2	
H	OH	cyprinol (5β-cholestane-3α,7α,12α,26,27-pentol)
OH	OH	scymnol ((24*R*)-(+)-5β-cholestane-3α,7α,12α,24,26,27-hexol)
OH	H	chimaerol (24*R*,25*R*)-(+)-5β-cholestane-3α,7α,12α,24,27-pentol
OH	OSO_3Na	sodium scymnol sulfate ((24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate)

Fig. 1

During the course of our study on bile salts of sharks, *Rhizoprionodon acutus* and *Lamna ditropis*, we have so far reported the isolation and stereochemistry of the bile salts, sodium scymnol sulfates and sodium chimaerol sulfate, and preparation of bile alcohols, scymnol and chimaerol, from these salts.[10] As there is a great interest in the pharmacological use of shark bile to treat throat diseases,[1] the effects of scymnol, chimaerol and their sodium sulfates on various functional disorders due to hypoxia were investigated. In this report we describe the protective actions of scymnol, chimaerol and sodium scymnol sulfate against various experimental models of cerebral anoxia in mice.

Experimental

Animals Male dd mice (20—25 g, 5 weeks old and 30—40 g, 30 weeks old) were starved for 18 h, then used for the experiments. Animals were housed in a room with a constant temperature of 24 °C and a 12 h light/dark cycle.

Materials Scymnol, 24*R*-(+)-5β-cholestane-3α,7α,12α,24,26,27-hexol, anhydroscymnol, (24*R*,25*S*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol, 5β-chimaerol, (24*R*,25*R*)-(+)-5β-cholestane-3α,7α,12α,24,27-pentol, and sodium scymnol sulfate, (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate, were prepared from the bile of *Rhizoprionodon acutus* according to the procedures reported previously.[10] 5β-Cyprinol, 5β-cholestane-3α,7α,12α,26,27-pentol, was prepared from the bile of *Mola mola* L.[11] Commercial bile acids used were cholic acid (3α,7α,12α-trihydroxy-5β-cholanic acid) (Wako Chemicals), deoxycholic acid (3α,12α-dihydroxy-5β-cholanic acid) (Kanto Chemical Co., Inc.), ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholanic acid), chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanic acid) (Tokyo Tanabe Pharmaceutical Industries, Ltd.), glycocholic acid (*N*-[3α,7α,12α-trihydroxy-24-oxocholan-24-yl]glycine), taurodeoxycholic acid (2-[[3α,12α-dihydroxy-24-oxo-5β-cholan-24-yl]amino]ethanesulfonic acid) and glycodeoxycholic (*N*-[3α,12α-dihydroxy-24-oxocholan-24-yl]glycine) (Sigma). As reference materials, idevenone (Takeda Pharmaceutical Industries, Ltd.) and indeloxazine (Yamanouchi Pharmaceutical Industries, Ltd.) were used.[12,13] Each compound was administered orally or intraperitoneally after being suspended in 0.5% methylcellulose–physiological saline. The dosage was adjusted to 0.1 ml/20 g of body weight of mouse.

Statistics The results are expressed as arithmetic mean values ±S.E.

The statistical analysis was conducted by using Student's t test.

Methods 1. Normobaric Hypoxia The method was based on that described by Arnfred and Secher.[14] Test compounds were administered intraperitoneally or orally to 5-week-old mice and then 30, 60 or 90 min later each animal was individually placed in a transparent box (1 l) aerated with a gas mixture of N_2 and O_2 (96 : 4) at a flow rate of 5 l/min. The time to respiratory failure was recorded.

2. Histotoxic Anoxia Induced by KCN Injection Five-week-old mice were administered test material intraperitoneally or orally and then 90 min later a lethal dose (3 mg/kg) of KCN saline solution was given intravenously. The inhibitory effect of test material on the lethality of KCN was determined.

3. Complete Ischemia by Decapitation Cerebral ischemia was produced by decapitation, according to the method of Holowach-Thurston *et al.*[15] Five-week-old mice were given test compounds intraperitoneally or orally 90 min before decapitation. The duration and number of gasping movements of the isolated heads were determined.

4. Effect on Partial Pressures of Oxygen and Carbon Dioxide and Blood pH in Mice Test compounds (100 mg/kg) were administered orally to 30-week-old mice. Ninety min later the mice were laparotomized under anesthesia with sodium pentobarbital (50 mg/kg, i.p.) and the blood of each animal was immediately collected from the aorta in the hypogastric region. The partial pressures of oxygen and carbon dioxide and the pH were measured with a blood gas analyzer (Type 170, Ciba-Corning).

Results

Normobaric Hypoxia In the hypoxic condition, the average survival time of the control animals ($n=33$) was 96 s. All animals tested died within 300 s. The effect of scymnol and sodium scymnol sulfate on survival time of mice subjected to hypoxia is shown in Table I. A significant and dose-dependent prolongation of the survival time was produced by scymnol, whether administered orally or intraperitoneally. A similar protective action against hypoxia was observed in mice treated with idevenone (Table I), as has been reported.[12]

As shown in Table II, the prolongation effect of scymnol on survival time under hypoxia lasted for at least 90 min at the dose of 100 mg/kg (*p.o.*).

Table III shows the activities of various C-27 bile alcohols and C-24 bile acids. Protective effects against hypoxia were observed in the animals pretreated with chimaerol, cholic acid, chenodoxycholic acid, taurodeoxycholic acid and glycodeoxycholic acid, besides scymnol. Scymnol, chimaerol, chenodeoxycholic acid and taurodeoxycholic acid were the most potent protectors.

Histotoxic Anoxia Induced by KCN Injection Tables IV and V show the effects of scymnol, sodium scymnol sulfate and taurodeoxycholic acid against histotoxic anoxia induced by KCN injection. The control animals died within 30 to 60 s after intravenous injection of KCN (3 mg/kg) and mortality was 100%. The pretreatment with scymnol resulted in both a decrease in mortality and a significant, dose-dependent prolongation of the survival time.

Sodium scymnol sulfate and taurodeoxycholic acid prolonged the survival time at 100 mg/kg (i.p.), like scymnol. Idevenone showed a similar effect,[12] but was

Table I. Effect of Scymmol, Sodium Scymnol Sulfate and Idevenone on Survival Time of Mice Subjected to Normobaric Hypoxia

Treatment (mg/kg)		Survival time (s)
Control-1	— (*p.o.*)	94.8± 2.5
Scymnol	50 (*p.o.*)	103.5± 2.4
	100 (*p.o.*)	134.7± 3.9**
	200 (*p.o.*)	108.2± 2.4*
Sodium scymnol sulfate	50 (*p.o.*)	94.8± 2.5
	100 (*p.o.*)	105.2± 3.2
	200 (*p.o.*)	103.6± 1.4
Idevenone	100 (*p.o.*)	108.8± 3.1*
Control-2	— (i.p.)	99.8± 3.2 (13)
Scymnol	50 (i.p.)	122.4± 4.0 (10)***
	100 (i.p.)	144.8±10.3 (9)*
	200 (i.p.)	237.7±37.3 (10)*
	400 (i.p.)	155.4±13.2 (10)*

Test compounds were given 90 min before each animal was placed in a transport box aerated with low oxygen gas. Values represent mean±S.E. for 10 animals. *, ** and *** show significant differences ($p<0.05$, 0.01 and 0.001) from the control.

Table II. Changes in Protective Effect as a Function of Time after Treatment with Scymnol

Treatment / Time after treatment (min)	Survival time (s)			
	0	30	60	90
Scymnol (100 mg/kg, *p.o.*)	95.8±2.5	105.6±2.2	108.4±2.4**	108.3±3.4**

Values represent mean±S.E. for 10 animals. ** shows significant difference ($p<0.01$) from the control.

Table III. Effect of Bile Alcohols, C-24 Bile Acids and Idevenone on Survival Time of Mice Subjected to Normobaric Hypoxia

Treatment (mg/kg, i.p.)		Survival time (s)
Control	—	99.8± 3.2 (13)
Scymnol	100	144.8±10.3 (9)*
Anhydroscymnol	100	102.9± 5.0 (9)
5β-Cyprinol	100	117.2± 8.1 (10)
5β-Chimaerol	100	154.1±13.7 (10)*
Cholic acid	100	159.5±16.2 (8)
Deoxycholic acid	100	97.4± 5.0 (9)
Ursodeoxycholic acid	100	112.0±10.9 (7)
Chenodeoxycholic acid	100	187.0±17.5 (7)*
Glycocholic acid	100	116.8± 9.3 (9)
Glycodeoxycholic acid	100	117.1± 3.9 (9)*
Taurodeoxycholic acid	100	143.5± 8.4 (10)*

Test compounds were given 90 min before each animal was placed in a transport box aerated with low oxygen gas. Values represent mean±S.E. for 7—13 animals. ( ) indicates number of animals. * shows significant difference ($p<0.05$) from the control.

Table IV. Effect of Scymnol, Sodium Scymnol Sulfate and Idevenone on KCN-Induced Anoxia in Mice

Treatment (mg/kg)		Survival time (s)	No. of survivors/used
Control-1	—	35.8± 1.3	0/10
Scymnol	50 (*p.o.*)	63.6± 2.1***	0/10
	100 (*p.o.*)	105.8± 3.2***	0/10
	200 (*p.o.*)	81.8± 2.2**	1/10
Sodium scymnol sulfate	50 (*p.o.*)	38.5± 0.5	0/10
	100 (*p.o.*)	39.6± 0.8	0/10
	200 (*p.o.*)	64.7± 4.1	0/10
Idevenone	100 (*p.o.*)	42.3± 1.9*	0/10
Control-2	— (i.p.)	53.8± 4.3	0/10
Scymnol	50 (i.p.)	95.6± 7.1***	0/9
	100 (i.p.)	159.0±10.8*	2/8
	200 (i.p.)	123.0± 7.2**	2/8
Idevenone	100 (i.p.)	69.3± 3.6**	0/9

Values represent mean±S.E. for 8—10 animals. *, ** and *** show significant differences ($p<0.05$, 0.01 and 0.001) from the control.

TABLE V. Effect of Scymnol, Sodium Scymnol Sulfate, and Taurodeoxycholic Acid on KCN-Induced Anoxia in Mice

Treatment (mg/kg, i.p.)		Survival time (s)	No. of survivors/used
Control	—	31.4±1.0	0/7
Scymnol	100	44.5±1.5***	1/8
Sodium scymnol sulfate	100	40.0±1.1**	0/8
Taurodeoxycholic acid	100	52.8±4.6*	0/6
Idevenone	100	46.4±2.3**	0/8

Values represent mean±S.E. for 8—10 animals. *, ** and *** show significant differences ($p<0.05$, 0.01 and 0.001) from the control.

TABLE VI. Effect of Scymnol and Indeloxazine on Decapitation-Induced Gasping in Mice

Treatment (mg/kg)		Gasping	
		No.	Duration (s)
Control-1	— (i.p.)	9.7±1.1	18.4±0.7
Scymnol	100 (i.p.)	14.6±0.8**	20.9±0.7*
Indeloxazine	20 (i.p.)	16.4±0.8***	24.2±0.9***
Control-2	— (*p.o.*)	11.3±0.7	16.5±0.6
Scymnol	100 (*p.o.*)	14.1±0.4*	17.1±0.5
Indeloxazine	20 (*p.o.*)	14.7±0.7**	19.3±0.4**

Each value represents mean±S.E. of 10 animals. *, ** and *** show significant differences ($p<0.05$, 0.01 and 0.001) from the control.

TABLE VII. Effect of Scymnol, Sodium Scymnol Sulfate and Idevenone on the Partial Pressures of Oxygen and Carbon Dioxide in Blood of Mice and Blood pH

Treatment (mg/kg, *p.o.*)		PO_2	PCO_2	pH
Control	—	73.9±5.8	41.0±2.8	7.26±0.02
Scymnol	100	98.6±4.1**	41.3±1.8	7.23±0.01
Sodium scymnol sulfate	100	88.5±6.6	41.1±1.9	7.23±0.02
Idevenone	100	98.7±5.5**	40.0±1.6	7.22±0.01

Values represent mean±S.E. for 10 animals. ** shows significant difference ($p<0.01$) from the control.

less potent than scymnol at 100 mg/kg (*p.o.* or i.p.).

Complete Ischemia by Decapitation In the control animals the number of gasps and the duration from decapitation to the last gasp of the isolated heads were 9.7 and 18.4 s (*p.o.*) and 11.3 and 16.5 s (i.p.) on average (Table VI). The treatment with scymnol (100 mg/kg) produced an increase of gasp number and a prolongation of gasp duration. A similar effect on anoxic survival was observed in indeloxazine-treated mice (20 mg/kg, *p.o.*). The effect of indeloxazine was more potent than that of scymnol.[13]

Effect on Partial Pressures of Oxygen and Carbon Dioxide and Blood pH in Mice Table VII shows the effect of scymnol, sodium scymnol sulfate and idevenone on the partial pressures of oxygen and carbon dioxide and pH of arterial blood. Both compounds, scymnol and idevenone significantly elevated the partial pressure of oxygen of the blood in comparison with the control, but they had no effect on the partial pressure of carbon dioxide or the blood pH. A similar, but less potent, effect on partial oxgen pressure was observed in the animals treated with sodium scymnol sulfate.

Discussion

Scymnol showed a significant protective effect in the various models of cerebral anoxia, *i.e.*, normobaric hypoxia under 4% oxygen gas and anoxias caused by KCN (3 mg/kg, i.v.) and by decapitation, all of which are established pharmacological screening methods for circulatory drugs,[16] and it was more potent than idevenone. Scymnol also elevated the partial oxygen pressure of the arterial blood; this effect may account for the above protective effect against anoxia. The protective effect against normobaric hypoxia and KCN-induced anoxia was decreased at high doses of 200 (*p.o.*) and 400 (i.p.) mg/kg of scymnol, respectively.

Sodium scymnol sulfate and chimaerol showed similar effects to those of scymnol on anoxic or hypoxic models, but cyprinol and anhydroscymnol did not, suggesting that the hydroxyl group at C-24 in the effective compounds plays an important role in the protective actions.

The above results are consistent with the use of shark bile in traditional Chinese medicine, and indicate that scymnol and chimaerol are the main effective ingredients in the bile.[1] Some C-24 bile acids, such as chenodeoxycholic acid, taurodeoxycholic acid and glycodeoxycholic acid are also present in cow's gallstone, which has been used for treatment of amnesic symptoms.[1] To our knowledge, the present report is the first to describe the protective effect of C-24 bile acids against hypoxia.

It is well known that the brain is highly sensitive to oxygen deficiency and the oxygen dissociation of hemoglobin is proportional to the partial pressure of oxygen.[17] The effects of scymnol on both the anoxic and ischemic models and the partial oxygen pressure of arterial blood, suggest that it may be effective for improving blood rheology and for treatment of anoxic or ischemic diseases. Further investigation to clarify the mechanisms of the protective effect of scymnol in anoxic or ischemic models and a study on the effect of bile alcohol on blood rheology are in progress.[18]

In summary, it has been demonstrated that C-27 bile alcohols with a hydroxyl group at C-24, scymnol and chimaerol, show a protective effect against cerebral anoxia.

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