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Relationship Between Pharmacological Potency and Surface Activity of Cholic Acid Analogs

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Abstract: The aim of this study was to elucidate whether changes of surface activity of cholic acid analogs may lead to alteration of its pharmacological potency. The potencies of various cholic acid analogs were explored by two mice models, Perfluoroisobutylene induced pulmonary edema and Xylene induced ear edema. Determination of the Critical Micelle Concentration (CMC), an index for the comparison of the surface activity, of cholic acid analogs was also conducted. The results showed that administration of cholic acid analogs decreased the water content in the organ with a potency of chendehydroxycholic acid>dehydroxycholic acid>ursodehydroxycholic acid>hyodehydroxycholic acid>cholic acid. This is in accordance with the sequence of CMC. We suggest, that the effects of cholic acid analogs might be closely related with their surface activity.

Key words: Cholic acid, critical micelle concentration, surface activity, edema, perfluoroisobutylene, xylene

INTRODUCTION

Cholic acid, an effective component of Calculus Bovis (dry gallstone of *Bos taurus domesticus* Gmelin.), is proved to possess versatile pharmacological effects, such as sedation, anti-hyperspasmia, relieving fever and anti-inflammation (Yan *et al*., 2007). Therefore, it was commonly used clinically in China, as a major ingredient in many composite preparations of Traditional Chinese Medicine (TCM). However, it still unknown why cholic acid has such abundant pharmacological effects and how it acts pharmacologically.

Cholic acid, in fact, is well known as an amphiphilic molecule with surface active nature. There are two faces in cholic acid stereo-structure, a hydrophilic face and a hydrophobic face (Fig. 1). Cholic acid analogs differ by varying position, number and configuration of the hydroxy groups, which determine the surface activity of each cholic acid analogs. Hitherto, no published literature has been available on the relationship between the pharmacological effects and the surface activity of cholic acid. It was postulated that changes of surface activity of cholic acid analogs may lead to alteration of its pharmacological potency.

To proof this hypothesis, Critical Micelle Concentration (CMC) was selected as an indicator to represent the surfactant activity of a certain amphiphile. According to the theories in surface chemistry, surface active materials will tend to arrange as monolayer at the surface of the solution at low concentrations. At a distinct concentration, the surface becomes completely loaded with surfactants and any further addition will self-aggregate as micelles in the interior of the solution. This concentration is called CMC, which varies in different experimental medium conditions. The physical-chemistry properties (i.e., surface tension, electrical Conductivity and light scattering) of the surfactant solution at CMC changes apparently. The lower the CMC is, the higher the strength of surface activity is.

Two mice models, Perfluoroisobutylene (PFIB) induced pulmonary edema and Xylene induced ear edema, were established to explore the pharmacological potency of cholic acid analogs.

PFIB, a colorless gas at normal temperature, is a typical lung damaging agent whose route of action is similar to Phosgene. Animal studies showed, that a sub-lethal dose inhalation is followed by an asymptomatic latent period of about 8-12 h and the severe pulmonary edema could be observed at a peak time of approximate 24 h (Zhang *et al*., 2005).

Xylene induced ear edema is a commonly used animal models to examine anti-inflammatory effect of drugs. According the reported method, the increase in weight caused by the irritant was measured by

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subtracting the weight of the untreated left ear section from that of the treated right ear sections (Hosseinzadeh *et al*., 2003). However, present pilot study showed that the process of sample collection influenced the data accuracy very much. To avoid this, the percentage of water content was used in present test.

The present study was conducted to compare the pharmacological potency with the surface activity, to provide a new insight to the quantitative action of cholic acid analogs.

MATERIALS AND METHODS

PFIB was obtained from Shanghai Institute of Organic Fluorine Materials (Shanghai, China) at a purity of 98%. Cholic Acid (CA), Hyodehydroxycholic Acid (HDCA), Ursodehydroxycholic Acid (UDCA), Dehydroxycholic Acid (DCA), Chendehydroxycholic Acid (CDCA) and pinacyanolchlorid were all purchased from SIGMA.

Specific pathogen-free male mice (18-22g b.w.) were obtained from the Center of Medical Experimental Animals, the Academy of Military Medical Sciences (Beijing, P.R. China). The animals were maintained under standard environmental conditions and fed with laboratory pellet food and water *ad libitum*. All the animal experiments were performed in accordance with the Guidelines for Animal Experiments at the Chinese Academy of Medical Sciences (Beijing, P.R. China).

Mice were allotted to 7 groups of 12 animals each in both experimental models.

In model A, a flow-past whole-body exposure apparatus was used for small animals to expose to PFIB at an inhalation dose of 130 mg m^{-3}×5 min, which were chosen for the convenient observation of the pathophysiological changes without fatal consequences in 24 h (Wang *et al*., 2001). All drugs (CA, HDCA, UDCA, DCA and CDCA), were administrated intraperitoneally at a dosage of 80 mg kg^{-1}, after 1 h of PFIB exposure. Twenty four hours later, mice were anesthetized by an intraperitoneal injection of pentobarbital (50 μg g^{-1}) and exsanguinated via abdominal aorta transection. The tracheae and lungs were then excised en bloc and cleared from all extrapulmonary tissue, rinsed within saline and blotted dry, total lung wet weight was determined. After drying at 80°C for over 24 h, the dry lung weight was finally scaled, the water content within the lung was calculated as (wet lung-dry lung) / wet lung×100%.

In model B, edema was induced in each mouse by applying 20 μL xylene to the inner surface of both ears, 30 min after oral treatment of mice with CA, HDCA, UDCA, DCA and CDCA (600 mg kg^{-1}). After 15 min, the animals were killed under anesthesia and both ears cut off and weighed. After drying, the water content within the ear was calculated similarly to the lung.

The CMC value of each anionic surfactant was determined by the dye titration method (Schott, 1966). High concentration of surfactant solutions were prepared with the dye solution (Pinacyanolchlorid 0.1 g was added to 100 mL distilled water and filtered) and then titrated with the same dye solution. The CMC value is the concentration of the surfactant when the color of the solution turned from blue to red.

RESULTS AND DISCUSSION

Table 1 showed that all the cholic acid analogs, administrated intraperitoneally at the dose of 80 mg kg^{-1}, could significantly decrease the pulmonary edema induced by PFIB inhalation. The potency of DCA and CDCA were higher than those of the others, as shown by the lung water content at 24 h after PFIB exposure. However, 6 and 4 mice died after DCA and CDCA administration, respectively and the data of the dead subjects were excluded because they did not survive till 24 h after PFIB exposure. The reason of death could be related to the toxicity of DCA and CDCA rather than that of PFIB in that the mice expressed severe sedation before death, one of the typical symptoms of the pharmacological effects of cholic acid and that the autopsy of these animals revealed no pulmonary edema. Briefly, the potency of pharmacology might be concomitant with that of toxicology in cholic acid analogs.

Cholic acid analogs when administrated orally, as was shown in Table 1, could also significantly decrease the ear edema induced by xylene. The pharmacological potency sequence was CDCA>DCA>UDCA>HDCA>CA, same as that in the PFIB inhalation model, suggesting an innovative idea that the pharmacological effect of cholic acid analogs in the two models maybe in a common mechanism.

Table 1 also showed the CMC values of various cholic acid analogs. Although CDCA and UDCA are stereo isomers with different configuration at 7-hydroxyl

Table 1: CMC values of different test drugs and effects on the water content in the organ in two animal models

		Water content in the organ (%)	
Group	CMC (mM)	PFIB induced lung edema	Xylene induced ear edema
Negative control		83.69±2.62	74.58±3.52
CA	12.06	82.47±2.02*	73.20±2.03*
HDCA	10.92	82.00±1.88*	71.56±3.47*
DCA	5.00	79.54±1.74*@	69.89±3.14*
UDCA	10.28	81.24±1.90*	71.54±2.34*
CDCA	4.80	79.37±0.82*#	69.80±4.94*
Normal control		77.91±0.97	65.42±3.37

Results are shown as the Mean±SEM.*represents $p<0.05$ compared with negative control group using one-way analysis of variance followed by Dunnett's test. n = 12. @: Six mice dead after administration. #: Four mice dead after administration. The data of the table did not contain the dead

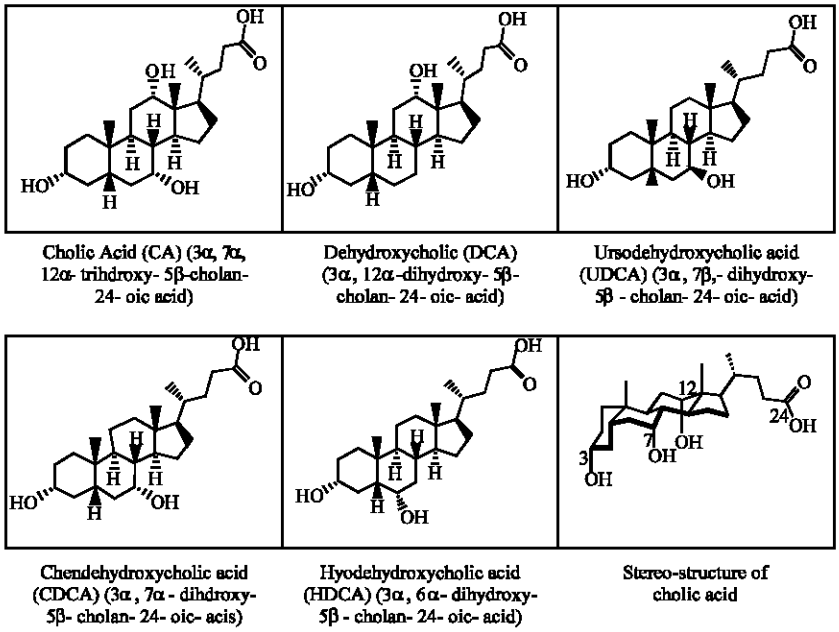

Fig. 1: Chemical structures of cholic acid analogs

substituent (Fig. 1), the CMC value of CDCA was lower than that of UDCA because hydrophilic substituents (7-OH and 3-OH) of CDCA are at the same face in stereo conformation. DCA and CDCA had lower CMC values, correspondingly the potency of decreasing water content in the organ were higher. Both parameters showed a significantly positive correlation (Correlation coefficient $r^2 = 0.98441$, $p<0.01$.), i.e., the lower is the CMC, the more potent is the drug. It was concluded that the pharmacological effects of cholic acid analogs against organic edema might be related with their surface activities.

A theoretical hypothesis can be made concerning the anti-edematous effect of cholic acid analogs at the present time: Cholic acid, due to its surface activity, might prefer to distribute to the interface between cell membrane and matrix or incorporate into cell membrane, exerted the effects by virtue of shielding the membran-proteins or other bio-molecules. This resulted in the inhibition of the recognition and interaction between molecules in such processes as proteolysis, phosphorylation and the like, and, finally, contributions to maintain the integrity of the blood-air barrier. However, direct experimental proof to this theory still needs to be provided.

Since the surfactant effect is considered as non-specific, the abundant pharmacological effects of any TCM preparation containing cholic acid or Calculus Bovis could not be totally dissociated, in this aspect, from their adverse reactions or toxicological effects of sedation, even death. It seemed to be obvious, that the general pattern for the surfactant containing preparations, at least in the current mice pulmonary edema model of PFIB inhalation, might be that the stronger the pharmacological effect is, the more severe is the toxicological reaction. This suggests that priorities must be put to find a very good balance between the pharmacological effects versus the toxicological ones in the research and development of surfactant containing preparations.

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