Pharmaceutical and Biomedical Sciences, Raabe College of Pharmacy, Ohio Northern University, Ada, Ohio, and Department of Pharmaceutics, Krupanidhi College of Pharmacy, Koramangala, Bangalore, India

# n-Octyl-β-thioglucoside enhances the transdermal permeation of ketotifen

S. Narasimha Murthy, B. A. Vishwanath

*Received April 11, 2005, accepted July 18, 2005*

*Prof. S. Narasimha Murthy, Pharmaceutical and Biomedical Sciences, Raabe College of Pharmacy, Ohio Northern University, Ada, Ohio 45810, USA*

*Pharmazie 61: 75–76 (2006)*

The ability of octyl-β-thioglucoside (OTG), to enhance the skin permeation of ketotifen fumarate was assessed across the rat skin model. OTG at 0.2% w/v enhanced the transdermal flux of ketotifen by 80 fold from 1.28 ± 0.12 $\mu$mol/cm$^2$ to 102.40 ± 10.36 $\mu$mol/cm$^2$. The penetration enhancing effect of OTG was concentration dependent up to 2% w/v in PBS buffer. The flux at 2% w/v concentration of OTG and flux across the tape stripped (no stratum corneum) skin were not significantly different (~184 $\mu$mol/cm$^2$, $P = 0.0.64$). The nonionic nature as well as its ability to interact with both lipid and protein domains of the stratum corneum is most likely the reason for its potent transdermal penetration enhancer characteristics.

From the pharmacokinetic perspective, ketotifen is an ideal candidate for formulation of transdermal drug delivery systems for prolonged therapeutic activity in chronic asthmatic patients. The transdermal drug delivery of ketotifen is also believed to help in overcoming the nocturnal attacks in the patients by maintaining constant therapeutic levels (Bhattacharya and Ghosal 2000). Ketotifen is hydrophilic and its skin penetrability is poor. Hence it is a drug of interest to study the effect of transdermal penetration enhancers.

Several surfactants such as sodium lauryl sulfate, n-decyltrimethylammonium bromide have been reported to be good penetration enhancers (1–2% w/v), and the dermal sensitivity to anionic and cationic surfactants at those concentrations is somewhat concerning. The dermal safety of most of the nonionic surfactants is well established and hence they are preferred over the ionic surfactants. Unfortunately not many nonionic surfactants are good penetration enhancers. n-Octyl-β-thioglucoside (OTG) is a nonionic detergent which was demonstrated to increase the permeation of macromolecules (Ogiso et al. 1991). In this study we assessed the ability of OTG to enhance the skin permeation of ketotifen fumarate (Both procured from Sigma Aldrich, USA).

The *in vitro* diffusion studies were carried out using a Franz diffusion cell with an active diffusion area of 0.64 cm$^2$. Freshly excised rat skin was used as the diffusion barrier for in vitro permeation studies. Sprague dawley rats were sacrificed by carbon dioxide asphyxiation and the dorsal and flank area was shaved using fine clippers, taking care to avoid damaging the skin. The area was carefully excised and any underlying fat or muscle tissue was removed. The skin was cut into the required size and used immediately. Rat skin was sandwiched between donor and receiver compartments with the stratum corneum part facing the donor compartment. The barrier integrity of rat skin was confirmed prior to transport studies, by measuring the electrical conductance at 24 °C using two 4-mm Ag/AgCl disk electrodes introduced into the diffusion cell, one in the receiver compartment and the other in the donor compartment of the diffusion apparatus (Inone et al. 2000). The skin samples having a resistivity $>30$ k$\Omega$ cm$^2$ were only used for the transport studies. Both the compartments were filled with PBS for resistance measurements. During the transport studies, the receiver compartment was filled with PBS, whereas the donor compartment was filled with drug solution (25 mM) prepared in PBS (control). To study the influence of OTG on the permeation of ketotifen, OTG was dissolved in the drug solution in different concentrations. The receiver compartment buffer was withdrawn at different time intervals to measure ketotifen. Ketotifen was measured by HPLC with a UV detector at 301 nm (Nakamura et al. 1996). The column used was C18 reverse phase and the mobile phase was methanol, water and phosphoric acid (750 : 1250 : 1 v/v) at a flow rate of 0.5 ml/min. The detection limit of ketotifen was 0.1 $\mu$g/ml and the coefficient of variation of the method was 4.1%.

The data in the graphs represent the mean readings of 6 trials with the error bars representing the standard deviation. The t-test was selected as the test for significance and a P value less than 0.05 was considered statistically significant.

The study showed that OTG at (0.2% w/v) enhanced the transdermal flux of ketotifen across rat skin by ~80 fold from 1.3 ± 0.1 $\mu$mol/cm$^2$ to 102.4 ± 10.4 $\mu$mol/cm$^2$ (Fig. 1). Further it was found that the penetration enhancing effect of OTG was concentration dependent up to 2% w/v in PBS buffer (Fig. 2). The enhancement factor increased with the increase in concentration of the surfactant up to 2% and then attained a plateau indicating the saturation of the skin layer with the drug. The lag time to achieve steady state diffusion was significantly reduced from 6 h to 1 h (Fig. 1). Transdermal diffusion studies of

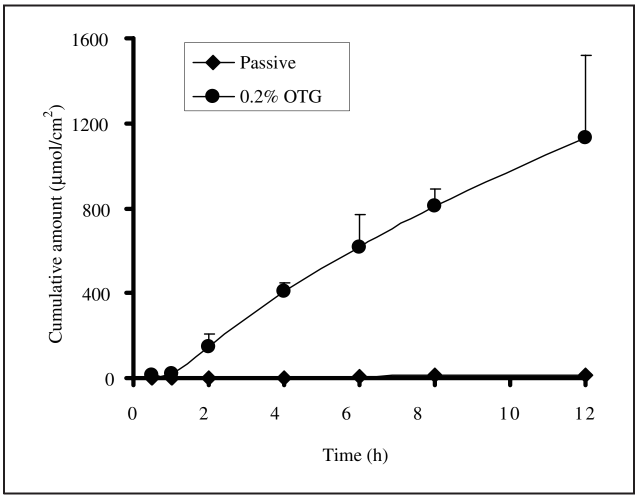

Fig. 1: Influence of 0.2% w/v OTG on the passive permeation of ketotifen across rat skin from 25 mM Ketotifen solution prepared in PBS. (◆) Passive, (●) With 0.2% OTG

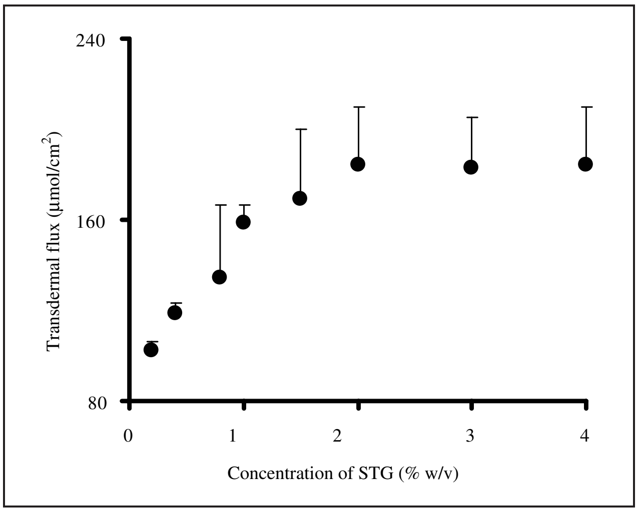

Fig. 2: Concentration dependent transdermal penetration enhancement activity of OTG

ketotifen were carried out across tape stripped (no stratum corneum) skin. This is apparently the maximum flux that can be achieved across the skin if the penetration enhancer was to absolutely compromise the barrier property of the skin. The flux at 2% concentration of OTG and flux across the tape stripped (no stratum corneum) skin were not different significantly ($\sim 184\ \mu mol/cm^2$, $P = 0.0.64$). This indicates that the surfactant compromises the barrier property of the skin almost completely. This also indicates that the surfactant could disrupt the lipid layers as well as permeabilizing the coenocytes layers. Our interpretations are somewhat in agreement with Inoue et al. who studied the mechanism of transdermal transport of ketotifen at different pH conditions (2000). They conclude that both lipid as well as proteinacoues phases of stratum corneum contribute to the poor permeability of ketotifen. This study demonstrates that OTG could be a choice as penetration enhancer for hydrophilic molecules like ketotifen. The nonionic nature as well as its ability to interact with both lipid and protein domains of the stratum corneum is most likely the reason for its potent transdermal penetration enhancer properties. Incorporation of OTG in a transdermal therapeutic system of $\sim 10\ cm^2$ area is anticipated to deliver the therapeutically necessary quantities of ketotifen.

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[1] Guangdong Key Laboratory of Marine Material Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, and [2] Hainan Key Laboratory of Tropical Marine Biotechnology, Chinese Academy of Science, Sanya, P.R. China

## New prenylated flavones from *Pongamia pinnata*

HAO YIN[1], JUN WU[1], HAIHAN NAN[1], SI ZHANG[1,2]

*Received May 24, 2005, accepted June 26, 2005*

*Si Zhang, Guangdong Key Laboratory of Marine Material Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, P.R. China*
*zhsimd@hotmail.com*

*Pharmazie 61: 76–78 (2006)*

From the stem bark of *Pongamia pinnata*, two new prenylated flavones (**1**, **2**) were isolated, along with seven known compounds (**3**–**9**). Compounds **3** and **4** are isolated for the first time from this plant. The structures of the new compounds were elucidated on the basis of spectroscopic data.

*Pongamia pinnata* (Linn) Pierre (Leguminosae, Papilionaceac; synonym, *Pongamia glabra* Vent) is a medium sized glabrous tree, growing in the littoral regions of South Eastern Asia and Australia. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds, ulcers (Tanaka et al. 1992). Extracts of the plant possess significant anti-diarrhoeal, antifungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory, and analgesic activities (Dahanukar et al. 2000; Shoba et al. 2001; Simonsen et al. 2001; Srinivasan et al. 2001; Misra et al. 1977). Previous phytochemical investigation of this plant indicated the presence of abounding prenylated flavonoids such as furanoflavones, franoflavonols, chromenoflavones, furanochalcones, and pyranochalcones (Carache-Blanco et al. 2003; Yadav et al. 2004). In this paper, we reported on isolation and identification of some compounds in the stem bark of this plant.

The EtOH extract of *Pongmia pinnata* stem bark was submitted to successive chromatography, affording two new prenylated flavones, 3-methoxy-(3″,4″-dihydro-3″,4″-diacetoxy)-2″,2″-dimethylpyrano-(5″,6″:8,7)-flavone (**1**) and 3-methoxy-5″-(2-hydroxypropan-2-yl)-furano-(2″,3″:7,8)-flavone (**2**). The C-5 side attachment of compound **2** is a new prenylation pattern encountered in flavones. In addition, seven known compounds, caryophyllene oxide (**3**) (de Oliveira Chaves et al. 2002), 8-hydroxy-6-methoxy-3-pentyl-1*H*-isochromen-1-one (**4**) (Kijjoa et al. 1991), stigmasterol (**5**) (Kjima et al. 1990), pongapin (**6**) (Aneja et al. 1958), demethoxykanugin (**7**), kanugin (**8**) (Sibrahmanyam et al. 1977), and 3,3′,4′,7-tetramethoxyflavone (**9**) (Ferreira et al. 1974), were obtained and identified by means of spectroscopic analysis and comparison with published data. Compounds **3** and **4** are isolated for the first time from this plant.

Compound **1,** a yellow plate, showed a molecular ion $[M]^+$ at m/z 452.14719 in the HREIMS, corresponding to the molecular formula $C_{25}H_{24}O_8$ (calcd. 452.14712). Together with HMQC spectra, the 1D NMR ($^{13}C$, $^1H$ and DEPT, Table) spectra of compound **1** displayed reso-