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Alleviation in protriptyline-photosensitized skin irritation by di-*O*-methyl-β-cyclodextrin complexation

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Protriptyline hydrochloride (PTL; N-methyl-5H-dibenzo[a,d]cycloheptene-5-propylamine), a tricyclic antidepressant, is known to cause cutaneous photosensitivity, resulting in serious skin irritation (Magnus, 1976), although its detailed mechanism has not been elucidated (Kochevar, 1980; Gasparro and Kochevar, 1982).

Recently, pharmaceutical applications of methylated cyclodextrins as drug carriers are intensively studied, since they are generally more able than parent cyclodextrins (CyDs) to improve various physicochemical properties such as solubility, stability and bioavailability of drug molecules (Szejtli, 1983; Uekama, 1985). In spite of their high pharmaceutical potential, little is known about the effects of methylated CyDs on the photosensitivity induced by drug molecules. In this preliminary study, therefore, effect of heptakis(2,6-di-O-methyl)-β-CyD (DM-β-CyD) on the cutaneous photosensitivity induced by PTL was investigated, in comparison with parent β-CyD, by evaluating skin irritation in guinea pigs in vivo.

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PTL hydrochloride solutions (0.5 mM, Merck, F.R.G.) in the absence and presence of β-CyD and DM-β-CyD (0.5 mM, Nippon Shokuhin Kako Ltd. and Toshin Chemical Ltd., Japan, respectively) in normal saline were irradiated under aerobic and anaerobic conditions for 60 min, through a pyrex filter by using a medical UV instrument (Dermaray M-DMR-1, Eisai Co., Japan). The irradiance of source was averaged at 7 mW/cm^2 at 305 nm. Deoxygenation of the test solutions was accomplished by purging with nitrogen for 20 min. The skin irritation studies were carried out by injecting the photoirradiated PTL solution into the dermal skin of clipped and shaved guinea pigs (Hartly females, weight about 400 g). The initial doses of PTL and CyDs to be injected were chosen to 0.05 μmol, where no appreciable skin-irritation was observed for both intact PTL and β-CyDs. The skin irritations such as erythema and edema were evaluated at regular time intervals by the modified Draize scoring method (Fukawa et al., 1982). Photolysis rate of PTL was monitored by TLC and HPLC under the following conditions; HPLC: Jasco BIP-I chromatograph (Japan), Whatmann Particil column (10 diameter × 250 mm, U.S.A.), methanol/chloroform (3 : 2) containing 0.5% n-propyl amine as a

mobile phase, 290 nm and 240 nm for detection of PTL and other degradation products, respectively, and imipramine as an internal standard. TLC: silica-gel plate (Kieselgel 60F 254, Merck, F.R.G.), *n*-butanol/H_2O/10% NH_4OH (3 : 2 : 1) as a developing solvent, and an UV lamp (365 nm) for detection.

Fig. 1 shows the time courses of the irritation score for 48 h after intradermal injection of the PTL solutions which were preirradiated in the presence and absence of β-CyDs. The inflammatory responses were most severe at about 12 h and persisted for at least 48 h after the injection, and from histological examinations (Irie et al., 1984) inflammatory cells such as polymorphs and macrophages were found to infiltrate into the dermis and subcutaneous fatty tissues. The PTL solution irradiated under anaerobic conditions elicited more severe erythema and edema in the depilated skin, compared with that irradiated under aerobic conditions. It is apparent from Fig. 1 that both β-CyDs reduced the irritation score and the alleviating effect was significantly greater in the DM-β-CyD system than in the β-CyD system, particularly in the anaerobic condition. On the other hand, little alleviation was observed when β-CyDs were added to the degradated PTL solution after the irradiation. These results suggest that the alleviating effects of β-CyDs may be due to the change in the photoreactivity of PTL itself rather than the inclusion of the toxic photoproducts of PTL (Uekama et al., 1983). Therefore, to gain insight into the alleviative mechanism, the effects of β-CyDs on the photochemical reactivity of PTL were preliminarily investigated in vitro.

Fig. 2 shows the time course of the photolysis of PTL in the presence and absence of β-CyDs under aerobic and anaerobic conditions. It is apparent that both β-CyDs inhibited the photolysis of PTL, where the deceleration effect of DM-β-CyD was significantly larger than that of β-CyD. This order was well correlated with the magnitude of the stability constants of PTL-β-CyD complexes (20600 M^{-1} and 46700 M^{-1} for β- and DM-β-CyD complexes, respectively). The photolysis of PTL in the presence of oxygen is reported to yield PTL-10,11-epoxide, 10-hydroxy-PTL and 10,11-dihydro-PTL-diol as main products, where the epoxide has higher phototoxicity (Jones and Sharples, 1984). Without oxygen, however, a cyclobutyl dimer of PTL is formed as one of the main products, which has considerably higher hemolytic activity than the epoxide (Gasparro and Kochevar, 1982). Thus, the higher irritation score obtained under anaerobic conditions (Fig. 1) may be due to the difference in the photodegradated species of PTL. From HPLC and TLC analyses, the photodimerization of PTL was found to be significantly suppressed by DM-

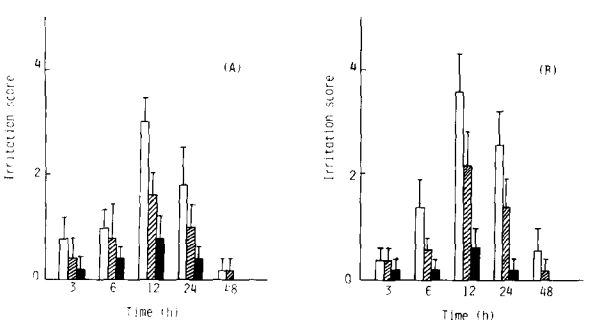

Fig. 1. Effects of β-CyDs (0.5 mM) on the irritating reactions produced by intradermal injection of photoirradiated PTL in dorsal skin of guinea pigs. □, PTL alone; ▨, PTL + β-CyD; ■, PTL + DM-β-CyD. A: 0.5 mM PTL irradiated for 60 min under anaerobic conditions. B: 0.5 mM PTL irradiated for 60 min under anaerobic conditions. The irritation score indicates the degree of erythema and edema around the injection site. Each result represents the mean ± S.E. of 5 guinea pigs.

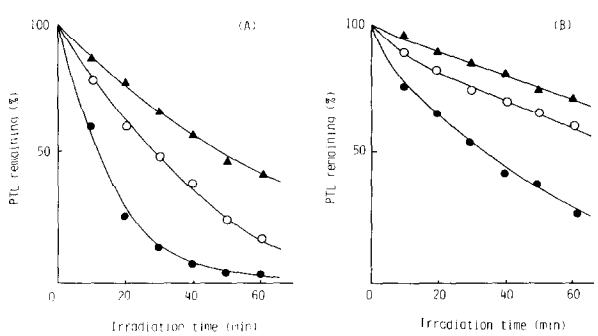

Fig. 2. Effects of β-CyDs (0.5 mM) on the photolysis of PTL under aerobic and anaerobic conditions. ●, PTL alone; ○, PTL + β-CyD; ▲, PTL + DM-β-CyD. A: 0.5 mM PTL irradiated under aerobic conditions. B: 0.5 mM PTL irradiated under anaerobic conditions.

β-CyD, leading to strong alleviation in the photosensitized skin irritation. The alleviating effects of β-CyDs, therefore, may be attributable to the deceleration in the photolysis of PTL, together with the alternation in the photoreaction pathway to suppress the dimerization by means of the inclusion complex formation.

The present results indicate that DM-β-CyD is particularly useful in alleviating the photosensitized skin-damage of PTL from the safety point of view. Further studies are now in progress to elucidate the detailed alleviating mechanism of β-CyDs.

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