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# Negligible Effect of *Ginkgo Biloba* Extract on the Pharmacokinetics of Cilostazol

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**Abstract** – *Ginkgo biloba* (*G. biloba*) extract is a widely used phytomedicine for the oral treatment of peripheral vascular disease. Cilostazol is a synthetic antiplatelet and vasodilating agent for the treatment of intermittent claudication resulting from peripheral arterial disease. It is likely to use concomitantly *G. biloba* extract and cilostazol for the treatment of peripheral arterial disease, which raises a concern of increasing their adverse effects of herbal-drug interactions. To clarify any possible herbal-drug interaction between *G. biloba* extract and cilostazol, the effect of the *G. biloba* extract on the pharmacokinetics of cilostazol was investigated. As cilostazol is known to be eliminated mainly by cytochrome P450 (CYP)-mediated metabolism, we investigated the effects of *G. biloba* extract on the human CYP enzyme activities and the effect of *G. biloba* extract on the pharmacokinetics of cilostazol after co-administration of the two agents to male beagle dogs. The *G. biloba* extract inhibited more or less CYP2C8, CYP2C9, and CYP2C19 enzyme activities in the *in vitro* microsomal study with $IC_{50}$ values of 30.8, 60.5, and 25.2 μg/ml, respectively. In the pharmacokinetic study, co-administration with the *G. biloba* extract had no significant effect on the pharmacokinetics of cilostazol in dogs, although CYP2C has been reported to be responsible for the metabolism of cilostazol. In conclusion, these results suggest that there may not be a pharmacokinetic interaction between *G. biloba* extract and cilostazol.

**Keywords:** *Ginkgo biloba* extract, Cilostazol, Cytochrome P450, Pharmacokinetic interaction, Dogs

## INTRODUCTION

A standardized *Ginkgo biloba* (*G. biloba*) extract, which is a phytomedicine for the oral treatment of senile dementia and peripheral vascular disease, is one of the most frequently prescribed phytomedicines in Europe and Asia, and one of the most widely sold herbal dietary supplements in the United States (Blumenthal *et al*., 1998; Blumenthal, 2001). *G. biloba* extract is known to contain ginkgo flavone glycosides, terpene lactones, and proanthocyanidins (Chan *et al*., 2007). Although the mechanism of the action is not clear, it is generally agreed that it protects against oxidative cell damage from free radicals (Sastre *et al*., 1998), improves blood flow, including microcirculation in small capillaries, to most organs (Jung *et al*., 1990), and blocks many of the effects of the platelet-activating factor that have been related to the development of a number of cardiovascular, renal, respiratory, and CNS disorders (Oberpichler *et al*., 1990).

Cilostazol is a synthetic antiplatelet and vasodilating agent for the treatment of intermittent cloudication resulting from peripheral arterial disease. Cilostazol is a selective cAMP phosphodiesterase inhibitor. It inhibits platelet aggregation and also acts as a direct arterial vasodilator (Kimura *et al*., 1985). Its main effects are dilation of the arteries supplying blood to the legs and decreasing platelet coagulation (Fontana and Reny, 2007).

Both antiplatelet agents of *G. biloba* extract and cilostazol are used to treat peripheral arterial disease and improve blood circulation. Many studies have demonstrated that the combination treatment of aniplatelet agents could be beneficial in cerebral and peripheral arterial disease (Kim *et al*., 1998; Aldandashi *et al*., 2007; Fontana and

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Reny, 2007). Moreover, *G. biloba* extract enhanced the antiplatelet and antithrombotic effects of cilostazol without the prolongation of bleeding time (our unpublished data). Thus, it is likely that *G. biloba* extract is prescribed in combination with cilostazol for their possible synergic effects. However, such a combination treatment may lead to herb-drug interactions. Therefore, it is necessary to evaluate the drug interaction potential between these two agents.

Cilostazol is extensively metabolized in rats, dogs, and humans (Akiyama *et al.,* 1985b). It was reported that CYP isoforms 3A4, 1B1, 3A5, 2C19, and 2C8 are involved in the metabolism of cilostazol (Hiratsuka *et al*., 2007). Cilostazol is known to be eliminated mainly by CYP-mediated metabolism and consequently to interact with some drugs *in vitro* and *in vivo* (Bramer and Suri 1999; Suri and Bramer 1999; Abbas *et al*., 2000), indicating that cilostazol carries a considerable potential for drug interactions. Co-administration of cilostazol with a CYP inhibitor (such as omeprazol, erythromycin, and quinidine) may result in an increase in the systemic exposure of cilostazol in humans and consequently cause adverse effects (Bramer and Suri, 1999; Suri and Bramer, 1999; Suri *et al*., 1999).

*G. biloba* extract is often taken in combination with prescription and conventional medications, causing herb-drug interactions (Gurley *et al*., 2005; Hu *et al*., 2005). There are controversial reports on the CYP inhibition by *G. biloba* extract. In some reports, ginkgolic acids, one of the components of *G. biloba* extract, were shown to be potent inhibitors of CYP1A2, CYP2C9, and CYP2C19 using cDNA expressed CYP enzymes (Zou *et al*., 2002). Although flavonol aglycones did show significant inhibitory activity against CYP1A2, 2C9, and 3A, other findings show that the most abundant components of ginkgo preparations in clinical use (terpene trilactones and flavonol glycosides) do not significantly inhibit major CYP enzymes in human liver microsomes (von Moltke *et al*., 2004).

As mentioned above, cilostazol is metabolized via CYP3A4, 1B1, 3A5, 2C19, and 2C8. Therefore, the pharmacokinetics of cilostazol could be affected by co-administration with *G. biloba* extract if the *G. biloba* extract possesses inhibitory activity on these CYP subtypes. Therefore, it is necessary to characterize the inhibitory activity of *G. biloba* extract on the CYP enzymes and investigate pharmacokinetic herb-drug interactions between the *G. biloba* extract and the cilostazol. This paper presents the effect of *G. biloba* extract on human CYP enzyme activity and the pharmacokinetics of cilostazol after the co-administration of these two agents to male beagle dogs.

## MATERIALS AND METHODS

### Chemicals

The standardized *G. biloba* extract, cilostazol, and 4-isopropylantipyrine were obtained from SK Chemical Co., Ltd. (Seoul, Korea). The Standardized *G. biloba* extract contains 22.0-27.0% ginkgo-flavonol glycosides and 5.4-12.0% terpene lactones (2.6-5.8% bilobalide and 2.8-6.2% ginkgolides). Pooled human liver microsome, phenacetin, coumarin, paclitaxel, diclofenac, mephenytoin, dextromethorphan, midazolam, *O*-deethylphenacetin, 7-hydroxycoumarin, 6-hydroxypaclitaxel, 4-hydroxydiclofenac, 4-hydroxymephenytoin, *O*-demethyldextromethorphan, and 1-hydroxymidazolam were purchased from BD Biosciences (Woburn, MA). Glucose 6-phosphate, β-$NADP^+$, and glucose 6-phosphate dehydrogenase were supplied by Sigma Corporation (St. Louis, MO). All other chemicals used were of analytical grade.

### Animals

Male beagle dogs (weighing 9-11 kg) were purchased from Central Lab. Animal Inc. (Seoul, Republic of Korea). Dogs were kept at an environmental temperature of 23 ± 2°C and relative humidity of 55 ± 5% with a 12 h light-dark cycle. Each dog was unrestrained and individually housed in a stainless steel cage, and allowed access to food and water *ad libitum*. Washout periods of one week were used between studies. Animals were deprived of food for 12 h before dosing. The protocol of animal study was in accordance with the regulations of the Korea Institute of Science and Technology.

### Microsomal incubation

Incubation studies were designed to determine the effects of *G. biloba* extract on various CYP isozyme activities. The incubation mixture contained 1 mg/ml microsomal protein and various CYP isoform-specific substrates in 0.2 ml of 0.1 M potassium phosphate buffer (pH 7.4). Reactions were initiated by the addition of the NADPH generating system (10 mM glucose 6-phosphate, 0.67 mM β-$NADP^+$, and 1 U/ml glucose-6-phosphate dehydrogenase), at various concentrations (10, 20, 40, 60, 80, and 100 μg/ml) of *G. biloba* extract and continued for 30 min in a water bath at 37°C. After the incubation, the reaction was stopped by adding 0.4 ml of 0.1% acetic acid. The following substrate concentrations were used (Tucker *et al*., 2001): 40 μM for phenacetin, 2.5 μM for coumarin and midazolam, 10 μM for paclitaxel and diclofenac, 160 μM for mephenytoin, and 5 μM for dextromethorphan.

**CYP substrate metabolism assays**

The metabolites of the CYP isozyme specific substrate were analyzed by a liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. Four microliter of 16 μM terfenadine in acetonitrile was added as an internal standard (IS) to the reaction mixture. The reaction mixture was loaded to a solid-phase extraction cartridge (a preconditioned $C_{18}$ Sep-pak® with methanol and deionized water). The cartridge was washed with 1 ml of 0.1% acetic acid three times. The analytes were then eluted with 1 ml of methanol. The methanol extract was evaporated under a gentle stream of nitrogen gas. The residue was reconstituted in a 40-μl aliquot of 30% acetonitrile and a 10-μl aliquot was injected directly onto a reversed-phase ($C_{18}$) HPLC column (Xterra®, 3.5 μm, 2.1×150 mm). The LC-MS/MS system consisted of a LC-10ADvp binary pump system (Shimadzu, Japan) with an API2000 triple quadrupole mass spectrometer (Applied Biosystems-SCIEX, Concord, Canada) equipped with an electrospray ionization (ESI) source. HPLC mobile phases consisted of (A) 0.1% aqueous formic acid and (B) 90% acetonitrile in 0.1% formic acid. The initial composition was 5% B, programmed linearly to 70% B after 3 min, and held for 1 min. The gradient was then changed back to the initial condition in 0.5 min and kept at the initial condition for 2 min. The total run time was 6 min. A gradient program was used for the HPLC separation with a flow rate of 0.2 ml/min. ESI was performed in the positive mode with nitrogen as the nebulizing, turbo spray, and curtain gas, with the optimum values set at 40, 75, and 40 (arbitrary units). The multiple reaction monitoring (MRM) detection method was employed for the detection of metabolites. Transitions monitored for metabolites were *m/z* 152 →110 for *O*-deethylphenacetin, *m/z* 163 →107 for 7-hydroxycoumarin, *m/z* 870 →286 for 6-hydroxypaclitaxel, *m/z* 312 →230 for 4-hydroxydiclofenac, *m/z* 235→150 for 4-hydroxymephenytoin, *m/z* 258 → 157 for *O*-demethyldextromethorphan, *m/z* 342 →324 for 1-hydroxymidazolam, and *m/z* 472 →436 for terfenadine (IS).

**Data analysis**

The $IC_{50}$ values for the activity-concentration curves from individual experiments were calculated with Sigma-plot software (Systat Software Inc., Chicago, IL), using a non-linear regression method.

**Oral administration of cilostazol and *G. biloba* extract to dogs**

Six male beagle dogs were randomly divided into three groups and each group received three treatments for 3-period, in a 3×3 Latin Square Design: 10 mg/kg oral cilostazol without *G. biloba* extract, 10 mg/kg oral cilostazol with 8 mg/kg *G. biloba* extract, and 10 mg/kg oral cilostazol with 16 mg/kg *G. biloba* extract. Each experimental period consisted of a one-day single dose treatment and a one week wash-out period. The foot cephalic vein of each dog was cannulated with sterile intravascular catheters for blood sampling.

Cilostazol at a dose of 10 mg/kg with or without *G. biloba* extract was orally administered to the dogs using a capsule. Co-administration groups received *G. biloba* extract and cilostazol simultaneously. A blood sample (approximately 1 ml) was collected via the cephalic vein at 0, 0.5, 1, 2, 3, 4, 6, and 8 h after the oral administration of the drug(s). Blood samples were centrifuged immediately and a 500 μl aliquot of plasma sample was stored in a −20°C freezer until the LC-MS/MS analysis of the cilostazol.

**Analytical method of cilostazol in dog plasma**

Concentrations of cilostazol in the dog plasma samples were determined by a slight modification of the reported LC-MS/MS method (Nirogi *et al*., 2006). An aliquot of 20-μl internal standard (0.1 μg/ml 4-isopropylantipyrine in 50% methanol) was added to a 100-μl aliquot of plasma sample. The mixture was then extracted with 1 ml of extraction solvent (ethyl acetate:methylene chloride=7:3). The organic layer was transferred into a clean microcentrifuge tube and evaporated under a gentle stream of nitrogen gas. The residue was reconstituted in a 50-μl aliquot of the mobile phase and a 5-μl aliquot was injected directly onto a LC-MS/MS system. The mobile phase, 5 mM ammonium formate buffer (pH 4.0):acetonitrile (90:10, v/v), was run at a flow-rate of 0.2 ml/min. The LC-MS/MS system was the same as that used in the CYP substrate assay. The MRM detection was employed; the transitions monitored were *m/z* 370 →125 for cilostazol, *m/z* 231 →56 for 4-isopropylantipyrine (IS). Retention times of the cilostazol and IS were approximately 2.2 min and 2.4 min, respectively. Calibration curves of cilostazol were linear over the ranges studied with $r^2 > 0.999$. The lower limit of quantification of the cilostazol in the dog plasma was 1 ng/ml. The coefficients of variations of the cilostazol (intra-day and inter-day) were below 10.7% and the accuracy ranged from 91.7 to 113%.

**Pharmacokinetic analysis**

The total area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated using the trapezoidal rule-extrapolation method (Chiou, 1978). The area from the last datum point to time infinity

was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the pharmacokinetic parameters using a noncompartmental analysis (WinNonlin®; Pharsight Corporation, Mountain View, CA). The peak plasma concentration ($C_{max}$) and time to reach $C_{max}$ ($T_{max}$) were directly read from the experimental data. The apparent oral clearance of cilostazol (CL/F) was calculated as dose/AUC.

**Table I.** $IC_{50}$ of the *G. biloba* extract on CYP isozyme specific marker activities in pooled human liver microsomes

| P450 isozyme | $IC_{50}$ (μg/ml) mean ± S.D. |
|---|---|
| CYP1A2 | > 100 |
| CYP2A6 | > 100 |
| CYP2C8 | 30.8 ± 1.1 |
| CYP2C9 | 60.5 ± 8.3 |
| CYP2C19 | 25.2 ± 3.0 |
| CYP2D6 | > 100 |
| CYP3A4 | > 100 |

### Statistical analysis

A *p*-value of less than 0.05 was considered statistically significant using a Duncan's multiple range test of the Statistical Package for the Social Sciences *posteriori* analysis of the repeated measure analysis of variance test. All results are expressed as mean ± standard deviation (S.D.) except median (ranges) for $T_{max}$.

## RESULTS

### Microsomal incubation

The inhibitory effects of *G. biloba* extract on the CYP isoform-specific activities were evaluated in human liver microsomes at various concentrations of *G. biloba* extract. The $IC_{50}$ values of the *G. biloba* extract for various CYP enzyme marker activities are presented in Table I. The *G. biloba* extract showed inhibitory activities on specific isozymes. The *G. biloba* extract did not inhibit the phenacetin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), dextromethorphan *O*-demethylation (CYP2D6), or the midazolam 1-hydroxylation (CYP3A4) activities ($IC_{50}$ > 100 μg/ml). The *G. biloba* extract inhibited the paclitaxel 6-hydroxylation (CYP2C8), diclofenac 4-hydroxylation (CYP2C9),

**Table II.** Pharmacokinetic parameters (mean ± S.D.) of cilostazol after oral administration (10 mg/kg) of cilostazol alone or co-administration of *G. biloba* extract (8 mg/kg or 16 mg/kg) to dogs (n=6)

| Parameters | Without *G. biloba* extract | With *G. biloba* extract (8 mg/kg) | With *G. biloba* extract (16 mg/kg) | *F* value | *p* value[a,b] |
|---|---|---|---|---|---|
| $C_{max}$ (ng /ml) | 417 ± 290 | 418 ± 168 | 499 ± 280 | 0.177 | 0.841 |
| $T_{max}$ (h)[c] | 1.5 (1-4) | 1.5 (1-3) | 2 (1-2) | 0.020 | 0.980 |
| $t_{1/2}$ (h) | 2.55 ± 1.10 | 2.22 ± 0.873 | 2.29 ± 1.25 | 0.182 | 0.836 |
| CL/F (ml/min/kg) | 131 ± 62.7 | 145 ± 94.3 | 119 ± 45.1 | 0.200 | 0.822 |
| AUC (ng h/ml) | 1,610 ± 972 | 1,410 ± 507 | 1,670 ± 837 | 0.155 | 0.858 |

[a]There was no significant difference among the three groups, [b]95% confidence intervals, [c]$T_{max}$ is expressed as median (ranges).

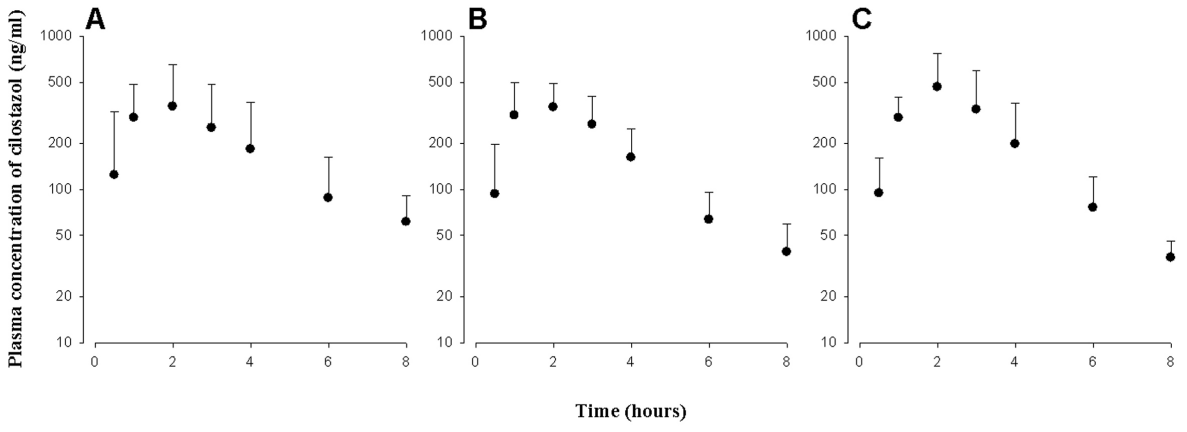


**Fig. 1.** Mean plasma concentration-time profiles of cilostazol after oral administration of cilostazol without *G. biloba* extract (A) and with *G. biloba* extract (B; 8 mg/kg and C; 16 mg/kg) to dogs (n=6). Bars represent standard deviation.

and mephenytoin 4-hydroxylation (CYP2C19) activities with $IC_{50}$ of 30.8, 60.5, and 25.2 μg/ml, respectively.

**Pharmacokinetics of cilostazol after oral administration of cilostazol with or without administration of *G. biloba* extract to dogs**

The mean plasma cilostazol concentration-time profiles from the three treatments are shown in Fig. 1 and relevant pharmacokinetic parameters are summarized in Table II. After oral administration, the plasma concentrations of the cilostazol reached peak concentration ($C_{max}$) at 1-4 h and declined (Fig. 1) with mean terminal half-lives of 2.55, 2.22, and 2.29 hours for the cilostazol only, with 8 mg/kg *G. biloba* extract, and with 16 mg/kg *G. biloba* extract, respectively (Table II). Pharmacokinetic parameters were comparable in terms of $C_{max}$, $T_{max}$, $t_{1/2}$, AUC, and CL/F (Table II). Co-administration with the *G. biloba* extract had no significant effect on the pharmacokinetics of the cilostazol.

## DISCUSSION

Recently, several reports have been published that combination therapies of antiplatelet agents were more effective than a single drug administration (Kim *et al*., 1998; Aldandashi *et al*., 2007; Fontana and Reny, 2007). However, for such a combination therapy, drug interactions should be considered to prevent likely side-effects caused by concomitant use of these agents. It is well known that inhibition or induction of drug metabolizing enzyme is responsible for drug interaction when one agent changes pharmacokinetic properties of a concurrently administered drug. For example, hydrocortisone significantly enhanced the bioavailability of orally administered loratadine in rats (Choi *et al*., 2009). Actually, it was one of the most important reasons for the early termination of the clinical trial (furafylline), the refusal of approval (mibefradil in Sweden), the withdrawal from the market (sorivudine, terfenadine, astemizole, cisapride), and the restriction of use (ketoconazole) (Tucker *et al*., 2001). There are also many cases of herb-drug interactions (Fugh-Berman, 2000): bleeding when warfarin is combined with ginkgo (*G. biloba*), garlic (*Allium sativum*), dong qual (*Angelica sinensis*), or danshen (*Salvia miltiorrhiza*); mild serotonin syndrome in patients who mix St John's wort (*Hypericum perforatum*) with serotonin-reuptake inhibitors; and, decreased bioavailability of digoxin, theophylline, cyclosporin, and phenprocoumon when these drugs are combined with St John's wort.

This study was designed to determine the effect of *G. biloba* extract on the pharmacokinetics of cilostazol. Cilostazol is cleared mainly via the metabolic route in rats, dogs, and humans (Akiyama *et al*., 1985b). Several metabolites of cilostazol were identified (Akiyama *et al*., 1985b) and CYP3A4, 1B1, 3A5, 2C19, and 2C8 have been known to involve the formation of the major metabolites, OPC-13015 and OPC-13213 (Abbas *et al*., 2000). Pharmacokinetic changes of cilostazol may be possible with the co-administration of the CYP inhibitor because it is metabolized mainly via CYP. There are some reports that *G. biloba* extract inhibited *in vitro* CYP 3A mediated metabolism (Zou *et al*., 2002; Hellum and Nilsen, 2008). The results from the present study (Table I) indicated that *G. biloba* extract mildly inhibits CYP2C8, CYP2C9, and CYP2C19 activities with $IC_{50}$ of 30.8, 60.5, and 25.2 μg/ml, respectively, in human liver microsomes. These results suggest CYP2C8, CYP2C9, or CYP2C19-mediated drug metabolism, especially the biotransformation of cilostazol by CYP2C8 or CYP2C19 might be affected by *G. biloba* extract. Therefore, pharmacokinetic interaction between these two reagents could not be excluded and pharmacokinetic studies in dogs were followed to determine whether the pharmacokinetics of cilostazol is affected by the co-administration of *G. biloba* extract *in vivo*. The *in vitro* assay was carried out using human liver microsomes for the best prediction of pharmacokinetic interactions in human but the *in vivo* study was carried out using animals because of safety concerns. The major two canine CYP2Cs, CYP2C21 and 2C41, were known to have 74-83% nucleotide and 67-76% amino acid identity with the human CYP2C8, 2C9, and 2C19 (Blaisdell *et al*., 1998) thus, canine CYP2C21 and 2C41 may play a similar role to human CYP2Cs. In our study, there was no difference in the pharmacokinetic parameters of cilostazol when cilostrazol was administered alone or with *G. biloba* extract and the pharmacokinetic parameters of cilostazol obtained from the present study were comparable to those previously reported (Akiyama *et al*., 1985a; Jinno *et al*., 2006). The $IC_{50}$ of *G. biloba* extract for CYP2C8 or CYP2C19 may be much higher than its level in plasma after oral administration of 8 or 16 mg/kg *G. biloba* extract. Therefore, *G. biloba* extract could not actually affect the metabolism of cilostazol. Taken together, it is considered that there is no pharmacokinetic interaction between *G. biloba* extract and cilostazol, which is partly consistent with the previous report regarding the pharmacodynamic interaction of *G. biloba* with cilostazol (Aruna and Naidu, 2007). They reported that the possibility of pharmacodynamic interaction between cilostazol and *G. biloba* was not shown regarding an inhibitory effect of platelet aggregation although the bleeding time prolongation effect of

cilostazol was potentiated by a co-administration with *G. biloba*.

## CONCLUSIONS

The *G. biloba* extract mildly inhibited the CYP2C8 and CYP2C19 activities *in vitro*. However, co-administration of *G. biloba* extract with cilostazol did not alter the pharmacokinetics of cilostazol in dogs. Further studies should be followed to characterize herb-drug interactions of *G. biloba* extract with cilostazol in human.

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