

DP-155, a Lecithin Derivative of Indomethacin, is a Novel Nonsteroidal Antiinflammatory Drug for Analgesia and Alzheimer's Disease Therapy

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ABSTRACT

DP-155 is a lipid prodrug of indomethacin that comprises the latter conjugated to lecithin at position *sn*-2 via a 5-carbon length linker. It is cleaved by phospholipase A₂ (PLA)₂ to a greater extent than similar compounds with linkers of 2, 3, and 4 carbons. Indomethacin is the principal metabolite of DP-155 in rat serum and, after DP-155 oral administration, the half-life of the metabolite was 22 and 93 h in serum and brain, respectively, compared to 10 and 24 h following indomethacin administration. The brain to serum ratio was 3.5 times higher for DP-155 than for indomethacin. *In vitro* studies demonstrated that DP-155 is a selective cyclooxygenase (COX)-2 inhibitor. After it is cleaved, its indomethacin derivative nonselectively inhibits both COX-1 and -2. DP-155 showed a better toxicity profile probably due to the sustained, low serum levels and reduced maximal concentration of its indomethacin metabolite. DP-155 did not produce gastric toxicity at the highest acute dose tested (0.28 mmol/kg), while indomethacin caused gastric ulcers at a dose 33-fold lower. Furthermore, after repeated oral dosing, gastrointestinal and renal toxicity was lower (10- and 5-fold, respectively) and delayed with DP-155 compared to indomethacin. In addition to reduced toxicity, DP-155 had similar ameliorative effects to indomethacin in antipyretic and analgesia models. Moreover, DP-155 and indomethacin were equally efficacious in reducing levels of amyloid β (A β)₄₂ in transgenic Alzheimer's disease mouse (Tg2576) brains as well as reducing A β ₄₂ intracellular uptake, neurodegeneration, and inflammation in an *in vitro* AD model. The relatively high brain levels of indomethacin after DP-155 administration explain the equal efficacy of DP-155 despite its low systemic blood concentrations. Compared to indomethacin, the favored safety profile and equal efficacy of DP-155 establish the compound as a potential candidate for chronic use to treat AD-related pathology and for analgesia.

INTRODUCTION

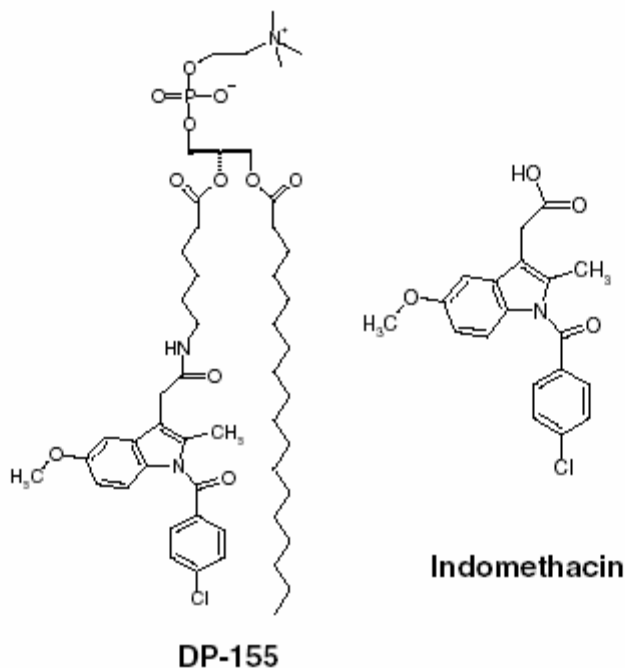
DP-155 is a lipid prodrug of the nonsteroidal antiinflammatory drug (NSAID), indomethacin. The chemical name for the active ingredient in DP-155 is a mixture of 1-steroyl and 1-palmitoyl-2-{6-[1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-3-indolyl acetamido]hexanoyl}-*sn*-glycero-3-phosphatidyl choline, and, hereafter, the whole compound is referred to as DP-155 (Fig. 1). The synthesis of DP-155 involves covalently linking indomethacin to lysolecithin via a 5-carbon length linker at the *sn*-2 position. Previously, phospholipid prodrugs were covalently attached to the phosphate group, but they demonstrated poor absorption and pharmacokinetic (PK) behavior (Lambert 2000). Recently, the first phospholipid with a fatty moiety replaced by a drug on the *sn*-2 position was reported (Kurz and Scriba 2000). Only conjugates with palmitic acid at *sn*-2 and the drug at *sn*-1 were recognized and degraded by phospholipase A (PLA)₂. Position *sn*-2 is relatively resistant to hydrolysis, which allows for the controlled and targeted delivery that is essential to the prodrug approach. Linker length has a major influence on the enzymatic degradation of the active drug as the *in vitro* studies presented in this review will demonstrate. Controlled cleavage of indomethacin from DP-155 improves its safety while exerting the same therapeutic effect as the parent compound, probably due to improved PK. The current review describes the chemistry of DP-155 and its unique PK behavior as well as its analgesic, antipyretic, and Alzheimer's disease (AD) therapeutic effects.

CHEMISTRY

Synthesis of DP-155 (Fig. 1) is a six-stage process. In the first stage, the aminogroup of the linker (6-aminohexanoic acid) is protected and then the second stage uses the protected amino acid to prepare the anhydride. Third, a lipid is produced from the protected linker and lysolecithin. The fourth and fifth stages involve removing the protected group from the aminogroup of the obtained lipid. Finally, indomethacin is connected to the linker in the sixth stage. The resulting product is a pale yellow wax and its chemical structure was identified using ¹H and ³¹P nuclear magnetic resonance, elemental analysis, and mass and ultraviolet spectrometry. The purity of DP-155 made by this process was 96.6%, as assessed by high-performance liquid chromatography and thin layer chromatography. The molecular weights of DP-155 and indomethacin are 976.7 and 359.8,

respectively. DP-155 is sparingly soluble in water (0.5 mg/mL) and highly soluble in organic solvents including ethanol, dimethylsulphoxide (DMSO), and octanol (>20 mg/mL). Compared to other sustained release prodrugs, DP-155 can be administered in liquid form, an advantage for use in elderly and pediatric patients. The formulation used for the drug in all the *in vivo* experiments was 4–10% oil (mixture of Labrafil, Tween 80, and Transcutol) and water phases.

FIG. 1. Chemical structure of DP-155 and its parent compound, indomethacin.



IN VITRO STUDIES

Characterization of DP-155 Cleavage by PLA₂

PLA₂ belongs to a family of enzymes that catalyze the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids to liberate free fatty acid and lysophospholipid (Kudo and Murakami 2002). This enzyme has been shown to play a role in AD brain pathology. Cytosolic PLA₂ (cPLA₂) immunoreactivity associated with astrocytes is elevated in the AD brain (Stephenson et al. 1996), and cPLA₂ and COX-2 gene expression are increased in hippocampal cornu ammonis (CA1) region of AD patients (Colangelo et al. 2002).

Since PLA₂ is elevated in the AD brain, we aimed to utilize the enzyme to liberate indomethacin from a lipid vector in DP-155 in anticipation of using the compound as an AD treatment. To do this, indomethacin was linked by a carbonic linker to the lipid vector that can target the prodrug to the brain via its lipophilic nature. Since DP-155 bears the indomethacin moiety in the *sn*-2 position of lecithin, it is of importance to test whether pure secreted PLA₂ (sPLA₂), as well as PLA₂ obtained from biological samples, could indeed release indomethacin from the lipid vector. DP-155 cleavage was assessed using several enzymatic sources: pure type III sPLA₂ (from bee venom), pure type II sPLA₂ (from *Crotalus Atrox* venom), brain-derived enzymes (both cytosolic and membrane-derived), liver and pancreas homogenates, cerebrospinal fluid, and serum. To induce cleavage, a sonicated mixture of DP-155, L- α -PC, di-*o*-hexadecyl, and lecithin was incubated in buffer with a pH 7.4 at 37°C for the biological samples and, for pure type II and type III sPLA₂, in a buffer of pH 8.9 at 25°C. Afterward, cleavage products were analyzed using liquid chromatography mass spectroscopy and thin layer chromatography. As background controls, a reaction mixture without the enzymatic source and one that contained the enzymatic source, but not DP-155, were used. Under our experimental conditions, no spontaneous, nonenzymatic, cleavage was detected in the absence of an enzymatic source. Over 60% of DP-155 was cleaved by both type II and type III sPLA₂. The degree of the enzymatic cleavage by bee venom sPLA₂ depended on the length of the linker; for example, more cleavage product was produced from compounds with 4- compared to 2-carbon linkers. Thus, DP-155, which has a 5-carbon linker, was cleaved by bee venom sPLA₂ to a greater

extent than compounds with linkers containing fewer carbons. A compound in which indomethacin was directly linked to lecithin, but without a carbon linker, was not cleaved at all.

All the enzymes from various sources, mentioned previously, cleaved DP-155; thus it is of interest to determine which PLA2 group is involved in DP-155 cleavage in the brain. To this end, calcium-dependent and -independent cPLA2 was inhibited in brain homogenates and DP-155 cleavage was assessed. Methyl arachidonyl fluorophosphate, at 2 μM , which is a selective, active site-directed, irreversible inhibitor of both forms of cPLA2, almost completely blocked DP-155 cleavage by brain homogenates. Thus, one or both of the cytosolic types of PLA2 cleaved DP-155 in brain. The extent of cleavage by serum and brain was not correlated with the length of the linker, a result that differs from that seen with bee venom Type III sPLA2. However, compared to compounds with the shorter linkers, the 5-carbon linker appeared to be the optimal length for maximal cleavage by brain homogenate and pure PLA2 and minimal cleavage by serum. The main cleavage product produced by the biological samples and pure sPLA2 is indomethacin attached to its linker, consistent with cleavage by endogenous PLA2. In our *in vitro* system, cleavage by pure PLA2 was linear on the scale of minutes, while the scale was hours for linear cleavage by biological samples.

DP-155 Inhibits Cyclooxygenase (COX)-2, but Not COX-1, Activity

NSAIDs, such as indomethacin commonly exert their effects via COX inhibition (Roberts and Morrow 2001). Cyclooxygenases have three isoforms: COX-1, which is constitutive; COX-3, which is a splice variant of COX-1 (Schwab et al. 2003); and COX-2, which is induced at inflammatory sites and is responsible for the biosynthesis of prostaglandins under acute inflammatory conditions (Willoughby et al. 2000). The therapeutic, antiinflammatory effects of NSAIDs are attributed to their ability to inhibit COX-2, whereas their harmful side effects [including gastrointestinal (GI)-toxicity] are associated with inhibition of COX-1 (MacDonald 2000). Since indomethacin inhibits the activity of both COX-1 and -2, its antiinflammatory effects are accompanied by harmful GI effects (Arnold et al. 1974).

TABLE 1. Structure–function relationship between linker length and COX inhibition

TABLE 1. Structure–function relationship between linker length and COX inhibition

Compound	Lipid vector	Carbon linker length	Sn position	COX-2 inhibition ¹	COX-1 inhibition ²
DP-546	Lecithin	0	Sn 2	–	–
DP-157	Lecithin	2	Sn 2	–	–
DP-155	Lecithin	5	Sn 2	+	–
DP-499	None	5	Irrelevant	+	–

¹Maximum concentration tested was 20 μM .

²Maximum concentration tested was 1 μM .

Determining whether the ability of indomethacin to inhibit COX-1 and -2 was altered by linking it to the lipid moiety to produce DP-155 was important to explain the effects of DP-155. Thus, the effect of DP-155 and free indomethacin on COX activities was tested using a commercial COX (ovine) inhibitor screening assay, a colorimetric assay that utilizes the peroxidase component of COX. Although DP-derivatives of indomethacin were tested for the inhibition of ovine COX, they are most likely also relevant to rat and human COX, since the interspecies homology for these enzymes is high. Free indomethacin (1 μM) completely (100%) inhibits COX-1 activity with an IC₅₀ of $0.016 \pm 0.002 \mu\text{M}$, while DP-155 concentrations of up to 20 μM do not. Both DP-155 (10 and 20 μM) and free indomethacin (20 μM) inhibit COX-2 activity. DP-155 inhibits $\approx 63\%$ of COX-2 activity (with an IC₅₀ of $1.68 \pm 0.24 \mu\text{M}$), while free indomethacin completely inhibits this activity with an IC₅₀ of $1.19 \pm 0.28 \mu\text{M}$. Thus, DP-155 is a specific COX-2 inhibitor that may prevent side effects correlated with *in vivo* COX-1 inhibition. Linking indomethacin directly (without a linker) to the lipid vector or through a 2-carbon linker abolished its ability to inhibit COX-2 activity (Table 1).

Effect of DP-155 on Alzheimer’s Disease–Related Pathologies *in Vitro*

Inflammatory reactions contribute importantly to the pathogenesis of many neurodegenerative diseases including AD (Zipp and Aktas 2006), and COX-2 expression is elevated in the hippocampal CA1 region of AD patients (Colangelo et al. 2002). It was of interest, therefore, to test whether inhibiting COX-2 via DP-155 would produce therapeutic effects in models of AD.

AD is characterized by intraneuronal amyloid β ($A\beta$) accumulation, $A\beta$ plaque formation, neuronal and synaptic loss, and inflammatory processes (microglial and astrocytic reactions) (McGeer and McGeer 1999). The latter contribute significantly to AD pathology (Rogers et al. 1996), and in epidemiological and clinical studies, administering certain NSAIDs, including indomethacin, inversely correlates with the prevalence, and severity of AD (t' Veld et al. 2001). Indomethacin's ameliorative action in AD may be due to its inhibitory effects on $A\beta$ plaque formation via blocking α -secretase, which is a COX-dependent process (Weggen et al. 2001) that inhibits $A\beta$ aggregation into fibrils (Thomas et al. 2001; Agdeppa et al. 2003). Furthermore, NSAIDs have COX-dependent antiinflammatory and neuroprotective effects (Halliday et al. 2000a, 2000b; Weggen et al. 2001).

In our studies, we examined the effects of DP-155 and indomethacin on neuronal uptake of $A\beta$, microglial reactions and neurodegeneration in an *in vitro* model that recreates these AD-related pathologies in just 6 days (Bi et al. 2002). Cultured rat hippocampal slices were exposed to $A\beta_{42}$ and RGD (H-Gly-Arg-Gly-Asp-Ser-Pro-OH), an integrin antagonist that enhances neuronal $A\beta_{42}$ uptake (Bi et al. 2002) and treated with either: vehicle; $A\beta_{42}$; $A\beta_{42}$ + RGD; $A\beta_{42}$ + RGD + DP-155; $A\beta_{42}$ + DP-155, or DP-155 alone. DP-155 was tested at 10, 40, and 80 μM ($n = 4$ rats/concentration). The same study design was repeated with commercial indomethacin at 5, 10 and 80 μM . Slices were stained with FluoroJade-B to detect degenerating neurons or antibodies against $A\beta_{42}$ or ED1, a microglia marker. Data were expressed as a percentage of the $A\beta_{42}$ or $A\beta_{42}$ + RGD group mean. Treatment groups were compared using one-way ANOVA and the Bonferroni/Dunn post hoc test. As predicted from a previous report (Bi et al. 2002), intraneuronal $A\beta$ uptake doubled when slices were treated with the integrin ligand RGD ($P < 0.001$). Treating slices with DP-155 reduced this enhanced (with RGD) $A\beta$ uptake by about 40% at both 40 and 80 μM

FIG. 2. (A) Effects of DP-155 and indomethacin (IND) on the area of enhanced $A\beta$ uptake (top panel) and its accompanying microglial response, as assessed with ED1 immunostaining (bottom panel). Data expressed as a percentage of the $A\beta_{42}$ + RGD group mean (denoted by the dashed line) \pm standard error of the mean. $P < 0.05$; $..P < 0.005$. (B,C) Photomicrographs showing the effect of DP-155 on neurodegeneration, as assessed with Fluoro-Jade B staining in CA1 region of cultured hippocampal slices. (B) Treating with $A\beta$ and RGD caused marked increases in degenerating neurons. (C) This increase was reduced by about half with DP-155 at 80 μM . Scale bar = 50 μm .

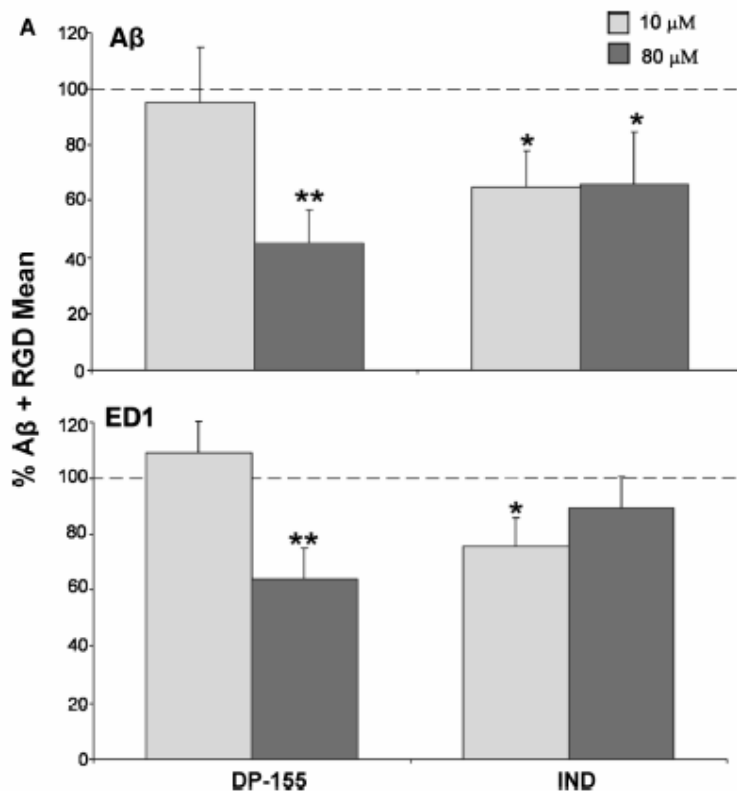
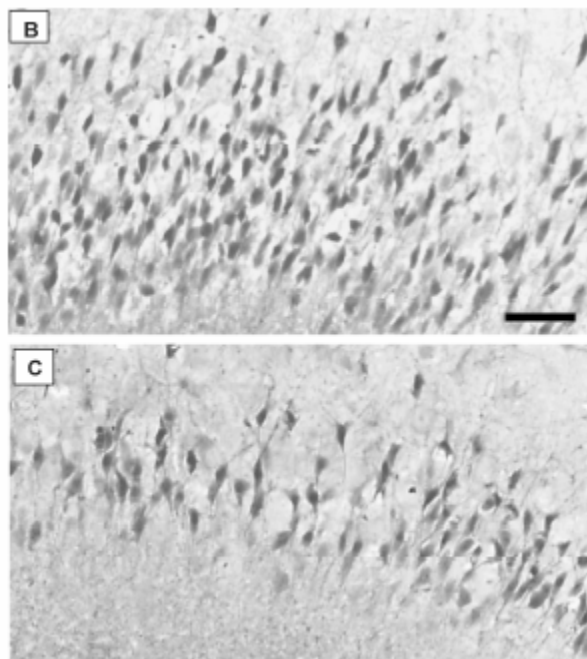


FIG. 2. Continued.



($P < 0.005$ and $P < 0.05$, respectively), but not at $10 \mu\text{M}$ (Fig. 2). Indomethacin showed a similar effect at $80 \mu\text{M}$ ($P = 0.05$), but also decreased the area of A β staining at 5 and $10 \mu\text{M}$ (both $P < 0.05$). Basal (without RGD) A β uptake was reduced by treating slices with $80 \mu\text{M}$ of DP-155 (52% decrease, not significant) or indomethacin (72% decrease; $P < 0.001$).

Enhanced A β uptake was accompanied by marked increases in activated microglia; the area they occupied was increased by 30% relative to slices treated with A β alone (both $P < 0.001$), consistent with a previous study (Bi et al. 2002). At the highest dose tested ($80 \mu\text{M}$), DP-155 eliminated the increase in area and number of reactive microglia induced by A β uptake in both the basal (45% decrease, $P < 0.05$) and enhanced (35% decrease, $P < 0.005$) conditions (Fig. 2). Indomethacin at $80 \mu\text{M}$ reduced the number of microglia induced by enhanced A β uptake by 20% ($P < 0.05$; Fig. 2) and the area occupied by the microglia with basal uptake by 35% ($P < 0.05$). It also had similar effects at $10 \mu\text{M}$.

Degenerating neurons increased two-fold in slices with enhanced compared to basal A β uptake ($P < 0.05$). DP-155 and indomethacin reduced neurodegeneration accompanying enhanced A β uptake to a similar extent. Both compounds decreased the area of degenerating neurons by about 35% at $10 \mu\text{M}$ (not significant) and by 50% at $80 \mu\text{M}$ (both $P = 0.01$, Fig. 2). DP-155 also decreased neurodegeneration by more than half at $40 \mu\text{M}$ ($P = 0.01$), but did not affect this measure in the basal A β uptake condition. Indomethacin markedly decreased degenerating neurons observed with basal A β uptake at $5 \mu\text{M}$ (60% decrease, $P = 0.05$). At concentrations of 10 and $80 \mu\text{M}$, the slices were nearly devoid (95% decrease) of degenerating neurons (both $P < 0.001$).

DP-155 may preferentially block integrin-driven increases in currents gated by *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (Lin et al. 2003). This mechanism underlies the enhanced A β uptake, and DP-155 effects were larger in the enhanced uptake model. Indomethacin also reduced A β uptake, but it was not influenced by the integrin antagonist (i.e., it affected A β uptake in both the enhanced and basal conditions). Thus, DP-155 may act directly on integrins or their effector cascades. This suggestion is supported by the reported link between NSAIDs and integrins in regard to cancer research, since integrins are involved in cancer biology and NSAIDs reduce cancer risk in humans and tumor growth in animal models (Yazawa et al. 2005). Investigations into these links found that NSAIDs block integrin activation in neutrophils (Garcia-Vicuna et al. 1997) and integrin signaling in endothelial cells (Weyant et al. 2000; Dormond et al. 2001).

Reduction of A β uptake is a new mechanism for indomethacin in the treatment of AD, in addition to the well-described inhibitory effect on α -secretase breakdown of amyloid precursor protein to A β 42 (Weggen et al. 2003). This novel action may be particularly important, since intraneuronal A β accumulation precedes and may contribute to plaque formation given that it occurs in human AD brains (Gouras et al. 2000) and AD transgenic mice (Wirhth et al. 2001; Takahashi et al. 2002) that do not show plaques. Moreover, brain function declines as A β levels rise before plaques appear (Holcomb et al. 1998; Chapman et al.

1999; Moechars et al. 1999; Kawarabayashi et al. 2001; Pratico et al. 2001) and A β accumulation in neurons has been associated with lysis events that result in plaque formation (D'Andrea et al. 2001). Thus, blocking the uptake and/or accumulation of A β inside neurons could substantially reduce amyloid toxicity.

In addition to their effects on A β uptake, DP-155 and indomethacin also reduced inflammation, as assessed by microglial activation. Microglia are persistently active in AD patients and occur in proximity to amyloid plaques (Rogers et al. 1996; Halliday et al. 2000a). They can cause cellular damage and death by generating proteolytic enzymes and releasing reactive oxygen and nitrogen species and cytokines (Rogers et al. 1996; Halliday et al. 2000a). Although DP-155 reduced inflammation, its neuroprotective effects may be due mostly to its effects on A β uptake rather than to its antiinflammatory actions, since DP-155 reduced neurodegeneration by about 50% at the same concentrations at which it decreased A β uptake (40 and 80 μ M), but only decreased inflammation at 80 μ M. Indomethacin also significantly reduced neurodegeneration at doses as low as 5 μ M. These neuroprotective effects may be independent of its effects on inflammation and A β uptake; however, the correlation with reduced A β uptake was better.

As indomethacin and DP-155 effects have many similarities and few differences in their effect in this *in vitro* model, it is unclear whether the DP-155 effect is due to its indomethacin metabolite or to specific features of DP-155. In any case, it is evident that DP-155 can act in brain tissue to reduce neurodegeneration associated with intraneuronal A β and/or inflammation.

IN VIVO (ANIMAL) STUDIES

Pharmacokinetics

Following oral administration of DP-155 to rats, free indomethacin, but not DP-155, was detected in plasma (Dvir et al. 2006) probably after indomethacin was liberated in the gut lumen by PLA₂ (Dahan et al. 2007). Following degradation, the DP-155 derived indomethacin metabolite is absorbed throughout the intestine including the colon (Dahan et al. 2007). This mechanism of enzyme degradation of the prodrug is unique and optimized by the 5-carbon linker. The PK profile of indomethacin derived from DP-155 is very different from the one obtained with commercial indomethacin. The former has a prolonged absorption and elimination half-life (Table 2) and broader C_{max}. At later stages of the degradation process, flip-flop kinetics occurred in that absorption exceeded elimination, probably due to continuous degradation of the drug in the colon (Dahan et al. 2007). Although the total amount of indomethacin absorbed decreased when administered as DP-155, serum indomethacin concentrations were relatively constant and maintained for 24 h. This finding suggests that indomethacin continues to be absorbed after 24 h. Unlike oral administration of the nonmodified form, indomethacin derived from DP-155 did not produce high initial serum concentrations (C_{max}), which can cause many adverse effects.

Surprisingly, the indomethacin metabolite of DP-155 had a higher brain to plasma concentration ratio than nonmodified indomethacin when administered orally. This ratio was 2.5-fold higher following administration of DP-155 than that of indomethacin. Similarly, the C_{max} brain to plasma ratio was also greater, with values that were 3.5-fold higher after DP-155 administration (0.07 and 0.02 for DP-155 and indomethacin, respectively). These ratios indicate that although orally administering nonmodified indomethacin results in higher plasma concentrations of the drug, DP-155 produces relatively higher concentrations in the brain. Brain uptake of indomethacin is limited by cerebral vasoconstriction and a consequent reduction in blood flow to the brain (McCulloch et al. 1982; Markus et al. 1994). The low plasma levels of indomethacin derived from DP-155 may produce less vasoconstriction enabling relatively higher therapeutic brain levels of indomethacin to be achieved.

Distribution studies after oral administration were performed in mice using radioactively labeled DP-155, [³H]-DP-155, in which one hydrogen atom of indomethacin was substituted with tritium. After acute administration, most of the tritiated compound accumulated in the liver, kidney, and lungs [30%, 32%, and 20% of the area under the plasma concentration curve over time (AUC)]. Six repeat doses every 12 h revealed minor accumulation in serum, kidney, and liver in a pattern similar to that seen with acute administration. Levels of [³H]-DP-155 reached a steady state at 30 h in the above-mentioned regions; however, in brain, accumulation persisted throughout administration, and plateaued thereafter for up to 30 h after the last dosing. Indomethacin is particularly suitable for selective central nervous system activity because it has a relatively slow brain elimination rate compared to other NSAIDs (Gasparini et al. 2004). Furthermore, brain targeting of the drug should be facilitated by metabolism of DP-155 by brain PLA₂, as observed in *in vitro* studies. Future investigations will determine whether different routes of administration will diminish degradation of DP-155 in the GI tract.

TABLE 2. Brain and plasma indomethacin PK parameters after administration of DP-155 or indomethacin

PK parameter	Brain		Plasma	
	DP-155	Indomethacin	DP-155	Indomethacin
Dose [mmol/kg (mg/kg)]	0.01 (10)	0.01 (3.54)	0.01 (10)	0.01 (3.54)
AUC 0–24 h (h·μg/g)	6.4	9.0	86.2 ± 4.0	283.6 ± 45.5
t _{1/2} (h)	93	24	21.7 ± 3.7	9.9 ± 0.4
T _{max} (h)	4	2	4	2
C _{max} (μg/g)	0.31 ± 0.03	0.48 ± 0.09	4.4 ± 0.2	23.7 ± 3.6

Data are expressed as mean ± STD.

Toxicity

Long-term use of indomethacin is limited by its significant GI and renal toxicities (Tabet and Feldman 2002). GI complications are the most common side effect of NSAIDs, and chronic NSAID consumption by patients with rheumatoid arthritis results in hospitalization and death in 1.5% and 0.2% of them, respectively (Fries et al. 1998). Typical GI toxicity manifests as mucosal erosion and ulceration, especially in the stomach. Mechanistically, these lesions have mainly been attributed to reduced prostaglandin E₂ (PGE₂) levels that occur secondarily to indomethacin's inhibition of COX-1 activity. Some NSAIDs, including indomethacin, also have local toxic effects on the GI mucosa (Whittle 1998). In addition to the GI tract, the kidney is a frequent target of NSAIDs toxicity, occurring in 18% of NSAID-treated patients, with severe symptoms in 1% (Stichtenoth and Fr̄olich 1998). NSAIDs alter the autoregulation of glomerular blood flow in hypovolemia, a response mediated by PGE₂, which keeps the afferent arterioles dilated. In the renal tubules, PGE₂ also mediates optimal diuresis; reduced PGE₂ can cause antidiuresis, sodium retention, and hyperkalemia (Ayano et al. 1984).

The considerable side effects that occur with chronic indomethacin treatment prompted the question of whether it could be modified to diminish its toxic effects. As mentioned previously, observations that DP-155 inhibits COX-2 but not COX-1 were suggestive of this idea. Tests for toxicity comparing DP-155 and indomethacin in rats corroborated this suggestion and were recently described in detail (Dvir et al. 2006). DP-155 did not produce GI toxicity 4 h after a single administration of 0.28 mmol/kg (the highest dose soluble in 10 mL/kg vehicle). In contrast, indomethacin caused gastric ulceration at doses from 0.0084 mmol/kg and higher (Table 3). This 33-fold difference is in part probably related to differences in PK profiles: DP-155-derived indomethacin had a significantly lower C_{max} and a delayed time to maximal concentration compared to unmodified indomethacin. These results, however, clearly indicate that DP-155 lacks local toxicity, in marked contrast to indomethacin,

TABLE 3. Summary table of the highest nontoxic and lowest GI toxic doses after single and repeated administrations of DP-155 and indomethacin

Type of study	Highest nontoxic dose (mmol/kg)		Lowest toxic dose (mmol/kg)	
	Indomethacin	DP-155	Indomethacin	DP-155
Single administration 4 h observation	0.0028	>0.28	0.0084	>0.28
Single administration 24 h observation	0.007	0.14	0.014	0.28
Repeated administrations	<0.007	0.035	0.007 ¹	0.07
			0.014 ²	

¹Microscopic examination.

²Macroscopic examination.

for which local toxicity is considerably higher than for other NSAIDs, as described previously (Giraud et al. 1999; Ivanov and Tzaneva 2002). The relative safety of DP-155 may be related to its lack of effect on COX-1 activity. This property remains for hours until indomethacin is degraded by PLA₂, primarily in the intestine, as shown in our *in vivo* PK studies. The phosphatidyl choline within the DP-155 molecule may also contribute to the absence of local toxicity, as it was described previously that phosphatidyl choline when it is combined with indomethacin reduced gastric toxicity (Leyck et al. 1985). DP-155 is less toxic than indomethacin 24 h after a single dosing and repeated administrations. After a single administration of escalating doses, DP-155 revealed a 20-fold lower toxicity compared to indomethacin, and after three repeated daily doses

5–10-fold lower toxicity (Table 3). Toxic signs were stomach ulcers, melena (black feces due to digested blood pigments), enteritis, and low serum albumin. Indomethacin as a DP-155 metabolite had a relatively constant serum concentration over 24 h that would allow less frequent dosing; this might further improve its safety in patients. The toxicity of DP-155 following administration for more than 3 days, however, awaits further investigation.

Renal toxicity was also assessed after three daily repeated administrations of escalating doses of indomethacin and DP-155 by measuring urine output, creatinine clearance, and urine N-acetyl glycosaminidase (NAG), a parameter for renal tubular damage, over 24 h. Indomethacin (0.028 mmol/kg) and DP-155 (0.14 mmol/kg) yielded reduced urine output and an increased NAG to creatinine ratio, indicating that DP-155 exhibited a 5-fold greater renal safety (Dvir et al. 2006). The tubular damage expressed by an increased NAG to creatinine ratio is most likely due to ischemia and this may indicate reduction in renal blood flow (Arnold et al. 1974). Nephrotoxicity was further studied by a model developed by Ayano (Ayano et al. 1984). Briefly, rats were treated with an equimolar dose of indomethacin or DP-155 (0.028 mmol/kg), followed by vasopressin (for vasoconstriction and water reabsorption, mimicking hypovolemic stress) and furosemide (enhancing renal PGE₂ synthesis and excretion). After furosemide treatment, urine was collected for 1 h and the following were measured: urine volume, PGE₂, protein and NAG, and creatinine clearance. Indomethacin decreased urine PGE₂ and diuresis, similar to previous reports (Ayano et al. 1984; Stichtenoth and Fr̄olich 1998), while DP-155 had no such effect (Dvir et al. 2006). Volume retention and antiuretic effects are serious complications in the treatment of renal and heart failure, and DP-155 has the potential to decrease this risk, especially in the treatment of elderly patients. Cardiovascular toxicity is a major problem with COX-2 selective inhibitors (Psaty and Furberg 2005), but it has not been described with indomethacin. In the 3-day repeated dose study, various organs including the heart were investigated macro- and microscopically. No pathology was observed at any dose; however, to rule out cardiovascular toxicity, more specific cardiovascular assessment is warranted.

Analgesic Effect

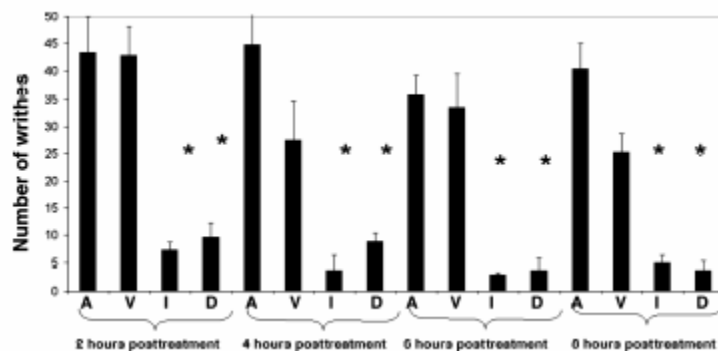
Analgesic effects of DP-155 were studied using the writhing test, a model of acute pain in which visceral afferent nerve fibers are activated in the abdominal viscera, in CD-1 mice. Mice react to pain that is chemically induced with acetic acid (AA) by writhing, which entails stretching the abdominal muscles, pulling one or both hind legs backward and laying the abdomen on the floor (Vogel and Vogel 1997). The abdominal constriction test has been largely employed as an assay of visceral pain, a major clinical problem (McMahon 1997).

Tests for analgesia were performed 1, 2, 4, 6, and 8 h after orally administering DP-155 or indomethacin at doses of 0.00008, 0.00025, and 0.01 mmol/kg, (n = 6 mice per dose). Each animal was injected with 0.06% AA in saline (i.p.; 0.1 mL/10 g body weight). Control groups included: sham mice treated with AA only and vehicle + AA treated. After injections, mice were released into a test chamber, allowed to rest for 5 min, and then all writhing incidents were recorded for 10 min. Noxious effects of AA were validated daily by assessing the first three mice in the AA control group; if they performed less than a mean of 30 writhes/10 min then the AA solution was discarded and the three mice were excluded. Student's t-test was used to compare control and experimental groups.

The analgesic effects of DP-155 and indomethacin were similar. The minimum effective dose, evaluated 2 h after drug administration, was found to be 0.25 μ mol/kg, while the next lowest dose tested, 0.08 μ mol/kg, did not have an effect (data not shown). At 2, 4, 6, and 8 h after treatment, both drugs had similar analgesic effects at a dose of 0.01 mmol/kg (Fig. 3); however, only indomethacin had an effect 1 h posttreatment (data not shown). Thus, the analgesic effects of DP-155 are slightly delayed compared to indomethacin.

The analgesic effect of indomethacin in this visceral pain model is well described and is attributed to its antiinflammatory actions via COX-2 inhibition in the periphery (Roberts and Morrow 2001). DP-155 and indomethacin showed similar analgesic effect from the second hour after oral administration, despite the lower plasma levels of indomethacin following DP-155 administration. This may be partially explained by the constant serum concentration of DP-155-derived indomethacin and possible saturation of the analgesic effect. According to the PK studies, the relative concentration of the indomethacin metabolite of DP-155 in the brain is high and consequently the brain area under the curve (AUC) of the metabolite is similar to commercial indomethacin. Several investigations found that indomethacin has a central analgesic effect even in visceral pain (DeLeo et al. 1989; Jurna and Brune 1990). This effect is mediated by α -adrenoreceptors or prostaglandins of the central nervous system (Hu et al. 1994). The central analgesic effect of indomethacin may contribute to the similar effect seen after DP-155 and indomethacin administration.

FIG. 3. Analgesic effect of 0.01 mmol/kg DP-155 or indomethacin, 2, 4, 6, and 8 h after oral administration, on the number of writhing episodes in a model of acute visceral pain induced by intraperitoneal injection of 0.06% acetic acid (0.1 mL/10g). (A) represents acetic acid; (V) represents vehicle; (I) represents indomethacin, and (D) represents DP-155. Data presented as mean \pm standard error. Asterisk denotes significance at $P < 0.05$.

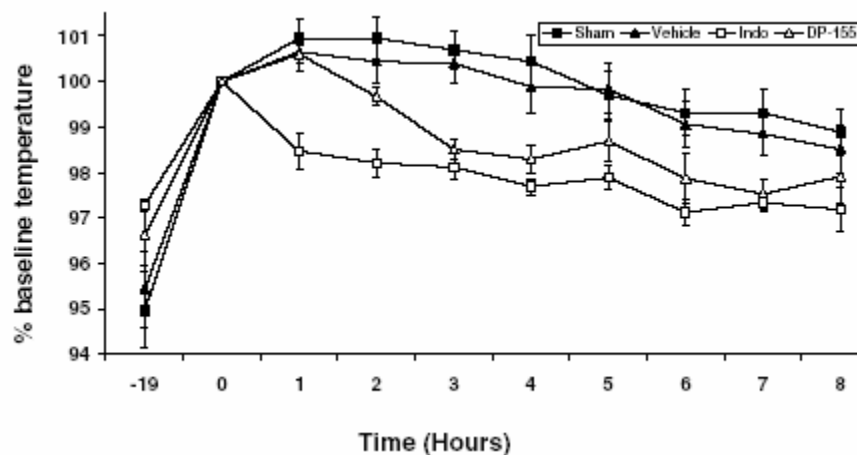


Antipyretic Effect

Indomethacin has very potent antipyretic effects compared to other fever-reducing agents, such as paracetamol and salicylic acid (Clark and Cumby 1975). To study the antipyretic activity of DP-155, fever was induced in Wistar rats by subcutaneously injecting a Brewer's yeast suspension (15%, prepared in 0.9% saline, 10 mL/kg) below the nape area (Vogel and Vogel 1997). Rectal temperature was measured 18–20 h later and only rats in which body temperature increased by more than 1°C were used in the study. At the time of the initial temperature reading, DP-155 or indomethacin was orally administered at a dose of 0.03 mmol/kg; $n = 6$ rats/group). Two control groups were included ($n = 6$ rats/group): vehicle treated (10 mL per/kg) and sham (no treatment). The animals' body temperatures were recorded at 1 h intervals for 8 h. Mean temperature was standardized to the temperature before treatment (%). The differences between groups were calculated using the repeated ANOVA with Bonferroni's posthoc test.

Indomethacin and DP-155 demonstrated significant antipyretic effects compared to sham rats; however, indomethacin's effect appeared earlier (at 1 h) than DP-155 (at 2 h; Fig. 4). These results are in agreement with the brain PK profile of DP-155. Previous studies claimed that the main antipyretic effect of indomethacin is central, inhibiting the effects of pyrogens on the brain and not their release in the periphery or their entry to the brain (Clark and Cumby 1975). Indomethacin directly inhibits the pyrogenic effect of intracerebroventricular injected cytokines, such as interleukin-1 α and tumor necrosis factor α (De Souza et al. 2002). The antipyretic activity of indomethacin has generally been ascribed to its COX-1 or COX-2 inhibition and consequently inhibition of PGE2 synthesis in the preoptic area of the anterior hypothalamus, but it is also mediated through increasing the antipyretic response to arginine vasopressin via its receptor (De Souza et al. 2002). Since the antipyretic

FIG. 4. Effect of DP-155 and indomethacin (0.03 mmol/kg) on body temperature after induction of pyrexia. Data presented as mean \pm standard error of the mean.



effects of DP-155 and indomethacin are similar and coincide with the brain PK of the DP-155 derived indomethacin, it is likely that the antipyretic mechanism is similar.

Effects of DP-155 on Pathology in an AD Transgenic Mouse Model

In addition to the effects of DP-155 on AD pathology *in vitro* (described earlier), its effects were also studied *in vivo* in a transgenic (Tg2576) mouse model of AD (Dvir et al. 2006). These mice have a mutation of the amyloid precursor protein (APP) gene (APPK670N,M671L), known as the “Swedish mutation,” which causes increases in secreted A β 42 and A β 40 in brain (Citron et al. 1994; Scheuner et al. 1996). They show an increase in soluble A β 42 at 3 months and develop amyloid plaques and progressive cognitive deficits at the age of 10–12 months (Hsiao et al. 1996; Kawarabayashi et al. 2001). DP-155 or indomethacin was administered to 3–4 month old female Tg2576 hAPP-transgenic mice at two equimolar doses, and brain levels of SDS-soluble A β 40 and A β 42 were measured (Dvir et al. 2006). Each of the two doses was given multiple times a day for 3 days, in equally divided doses and volumes. The high dose was 0.14 mmol/kg/day divided into six daily doses; the low dose was 0.046 mmol/kg/day divided into two daily doses. This dosing paradigm was chosen because a previous study (Weggen et al. 2001) showed that indomethacin and other NSAIDs delivered in this manner to Tg2576 mice reduced A β 42 levels.

Both DP-155 and indomethacin reduced soluble A β 42 levels to a similar extent in the brains of 3-month-old Tg2576 mice. Levels of SDS-soluble A β 42 were significantly decreased with DP-155 treatment by 23.6% with the high dose and 11.2% with the low dose. Likewise, indomethacin reduced A β 42 levels by 29.7% and 22.5% with the high and low doses, respectively. Neither treatment affected the level of A β 40 (Dvir et al. 2006).

DP-155 could reduce soluble A β 42 levels by inhibiting β -secretase cleavage of APP to A β 42, since this is a well-described effect of indomethacin (Eriksen et al. 2003; Weggen et al. 2003) and is most likely responsible for reducing soluble A β 42 after short-term treatment in young mice. Furthermore, Weggen et al. (2001) demonstrated that selective NSAIDs alter the cleavage pattern of β -secretase, leading to reduced A β 42 production without affecting A β 40 levels, consistent with our results. Another potential mechanism by which DP-155 could act to reduce A β 42 is by inhibiting NF-kappa B. NF-kappa B activation was blocked in Tg2576 mice by indomethacin and insoluble A β 42 and amyloid plaque burden were concurrently reduced (Sung et al. 2004).

The only NSAID to show some effect in the treatment of AD is indomethacin (Rogers et al. 1993). Although somewhat controversial (Tabet and Feldman 2002), this study does indicate the potential of indomethacin for treating AD. Indomethacin is a nonselective COX inhibitor, and such NSAIDs have shown better results compared to selective COX-2 inhibitors in various AD mouse models and retrospective epidemiological studies in humans (McGeer and McGeer 2007). DP-155 is unique in the sense that it initially shows COX-2 selective inhibition and, therefore, has fewer side effects, but the active metabolite is indomethacin with its proven therapeutic benefits. Long-term cognition studies in mouse models will indicate the potential for DP-155 therapy in AD patients.

SUMMARY

Toxicity remains a limiting factor for the chronic use of NSAIDs including indomethacin. An opportunity to overcome this limitation is presented in the form of an indomethacin prodrug, DP-155, which is indomethacin connected to lecithin via a 5-carbon length linker at the *sn*-2 position. DP-155 can be cleaved to indomethacin by PLA₂ in the brain and the GI tract. After oral administration, the cleavage in the GI tract results in constant low serum levels and low maximal concentration, facilitating 5–10-fold better GI and renal safety.

Importantly, delivery of DP-155 in this manner does not cause local gastric toxicity, probably because it selectively inhibits COX-2 and not COX-1. Although the serum concentration of DP-155-derived indomethacin remained low after oral administration, the indomethacin concentration reached in the brain was similar to that of the parent compound. Thus, due to its unique PK, DP-155 demonstrates more than a 5-fold reduction in toxicity, but maintains effects in the central nervous system similar to that of indomethacin. These effects included reduced AD-related pathology in *in vitro* and *in vivo* models, as well as analgesia and antipyrexia.

The studies summarized in this review establish DP-155 as a feasible candidate for future clinical studies in elderly patients for AD and for chronic analgesia. Before proceeding in this direction, DP-155 awaits further preclinical investigation for its effects after prolonged periods of administration, including additional safety and efficacy studies. Future preclinical

investigations will also include behavior and cognitive studies in AD models. In all, DP-155 is a safer alternative to indomethacin for the treatment of AD and for use as an antipyretic and/or analgesic.

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