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Research Paper

In-vivo evaluation of prolonged release bilayer tablets of anti-Parkinson drugs in Göttingen minipigs

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Abstract

Objectives Patients with Parkinson's disease can benefit from controlled released levodopa dosage forms since there is a clear clinical advantage in obtaining sustained plasma concentrations. The purpose of this study was to obtain a tablet that prolonged the release of levodopa.

Methods A novel bilayer tablet, consisting of an immediate release layer containing nebicapone (100 mg) and an erosion-matrix type prolonged release layer containing levodopa (100 mg) and carbidopa (25 mg) was developed (LCN PR). A pharmacokinetic study in Göttingen minipigs was performed to evaluate this formulation.

Key findings LCN PR tablets prolonged the in-vitro release of levodopa in HCl 0.1 M for more than 3 h. In-vivo plasma levodopa levels peaked at a later time point with LCN PR tablets as compared with that obtained with Sinemet 100/25 (2.7 vs 0.5 h). Nebicapone increased the maximum plasma concentration and area under the plasma concentration–time curve values for levodopa.

Conclusions The results obtained suggested that LCN PR tablets may have decreased the number of tablets and daily intake in the treatment of patients with Parkinson's disease.

Keywords carbidopa; levodopa; nebicapone; Sinemet

Introduction

Parkinson's disease is a progressive neurodegenerative disorder attributed to selective loss of dopaminergic neurons in the substantia nigra, which causes the reduction of striatal dopamine.[1,2] Levodopa, introduced over 40 years ago, remains the most effective drug in the symptomatic treatment of Parkinson's disease.[3–5] Levodopa, administered in oral dosage forms, is mainly metabolized in the periphery by aromatic L-amino acid decarboxylase (AADC) to dopamine, which does not cross the blood–brain barrier, and can cause nausea, vomiting, orthostatic hypotension, and cardiac arrhythmia.[2,6] Therefore, at the present time, levodopa is invariably administered in association with an AADC inhibitor (either benserazide or carbidopa). Nevertheless, even when levodopa is co-administered with an AADC inhibitor approximately 90% is transformed by catechol-*O*-methyltransferase (COMT) to 3-O-methyldopa, an inactive metabolite.[7] The half-life of this metabolite in human beings is approximately 15 h.

COMT inhibitors as adjuvants to the levodopa/AADC inhibitor improve the clinical benefits of pharmacotherapy mainly by increasing levodopa disposition to the brain in a more consistent way.[2,6] Tolcapone and entacapone are COMT inhibitors that increase the area under the plasma levodopa concentration–time curve (AUC), leaving the maximum plasma concentration (C_{max}) without significant changes both in patients with Parkinson's disease and healthy volunteers.[2] The use of tolcapone is limited and requires specialized monitoring due to its hepatic toxicity and although entacapone is safer, it is sometimes referred to as less efficacious than tolcapone. So there is a real need for COMT inhibitors with safer and better therapeutic profiles.[8]

Nebicapone is a new reversible and mainly peripherally acting COMT inhibitor that is being developed as an adjunct to levodopa/AADC inhibitor therapy.[9,10] In rats this drug demonstrated a stronger and longer COMT inhibitory effect than entacapone.[11] In healthy

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Table 1 Composition of the prolonged release layer tablets containing levodopa and carbidopa

Layer type	LCN PR tablet	LCP PR tablet
Prolonged release layer	Levodopa	Levodopa
	Carbidopa monohydrate	Carbidopa monohydrate
	Hypromellose	Hypromellose
	Sodium stearyl fumarate	Sodium stearyl fumarate
Immediate release layer	Nebicapone	Microcrystalline cellulose
	Lactose monohydrate	Lactose monohydrate
	Ethylcellulose	Croscarmellose sodium
	Croscarmellose sodium	

Prolonged release layer contained 100 mg levodopa and 25 mg carbidopa, the immediate release layer contained either 100 mg nebicapone (LCN PR) or placebo (LCP PR).

male volunteers nebicapone, in single doses of 10–800 mg, was well tolerated.[6] Nebicapone C_{max} is reached within 0.5–2.5 h and the apparent elimination half-life ranged between 2–4 h.[8]

Nebicapone increases the bioavailability of levodopa and reduces the formation of 3-O-methyldopa (3-OMD) when administered concomitantly with levodopa/carbidopa 100/25 mg (Sinemet 100/25) or with controlled-release levodopa/carbidopa 200/50 mg (Sinemet CR 200/50).[6,12]

The optimization of the bioavailability of drugs used in treatment of Parkinson's disease may have important implications for their clinical utility.[13] In fact, there is a clear therapeutic advantage in obtaining sustained plasma concentrations of levodopa which supports the view that the development of a formulation that provides stable and sustained plasma levels should be achieved.[13,14]

A patient with Parkinson's disease needs to take medication several times a day and so compliance can be significantly improved by using a fixed dose combination (this is especially important in patients with tremor).

Currently the immediate release oral solid dosage forms that contain levodopa are either an association of levodopa and an AADC inhibitor or a triple combination of levodopa/carbidopa/entacapone (Stalevo). There are also modified release oral solid dosage forms that combine levodopa and an AADC inhibitor. However, it is not yet available as a dosage form that combines the benefits of having a sustained levodopa concentration with the advantage of a triple combination.

To simplify the treatment of Parkinson's disease, a novel bilayer tablet consisting of an immediate release layer containing nebicapone (100 mg) and an erosion-matrix type prolonged release layer containing levodopa (100 mg) and carbidopa (25 mg) was developed (LCN PR). This study reports a pharmacokinetic evaluation of plasma levodopa and 3-OMD in minipigs treated with the LCN PR formulation and immediate release formulations of nebicapone and Sinemet 100/25. This novel bilayer tablet is intended to be an option for improving therapeutic outcomes for patients with Parkinson's disease.

Materials and Methods

Tablet preparation

Bilayer tablets consisting of an immediate release layer and an erosion-matrix type prolonged release layer were produced. The prolonged release layer contained 100 mg levodopa and 25 mg carbidopa, the immediate release layer contained either 100 mg nebicapone or placebo (LCN PR and LCP PR, respectively). Placebo and nebicapone immediate release tablets (nebicapone IR) were also prepared. Placebo tablets were obtained by direct compression of microcrystalline cellulose, lactose and sodium croscarmellose. Nebicapone IR tablets were identical to the immediate release layer of LCN PR tablets. Additional details can be found in Table 1. All tablets were prepared in a hydraulic press equipped with 13 mm punches.

In-vitro drug release study

Levodopa release from the LCN PR tablets was evaluated using USP apparatus 2 (Sotax model AT 7) at 50 rev/min. The dissolution medium was HCl 0.1 M (900 ml) at 37.0 ± 0.5°C. The amount of levodopa released was determined by HPLC with UV detection at 280 nm. Filtrate samples were removed at 30, 60, 150, 240 min and injected (20 µl) onto a HPLC system that consisted of a pump (Varian model 912), an injection valve (Rheodyne model 7725(i)), a Symmetry C8 5 µm, 4.6 × 250 mm column (Waters) and a detector (Varian model 9050). Separation of the three drugs was achieved by gradient elution at a flow rate of 1 ml/min (T_o [minutes] = 90% A, 10% B; T_5 = 30% A, 70% B; $T_{5,5}$ = 30% A, 70% B; T_{11} = 30% A, 70% B; $T_{11,5}$ = 90% A, 10% B; T_{15} = 90% A, 10% B). The mobile phase A was phosphate buffer at pH 2.2 and the mobile phase B was acetonitrile.

Study design and bioanalysis

In a nonrandomized single dose four phase study, Göttingen minipigs (Ellegard Göttingen minipig A/S, Denmark) received orally the tablets as described in Table 2.

Sinemet 100/25, containing 100 mg levodopa and 25 mg carbidopa, were immediate release tablets commercially available in Portugal.

The minipigs were all male ($n = 4$, 27–32 kg). Between each phase there was a seven-day washout period. Minipigs were deprived of food for at least 8 h before each administration. A standard diet was given to the minipigs approximately 2 h post dosing.

The study was conducted according to Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. The study was

Table 2 Treatment allocation to Göttingen minipigs

Phase	Administration	Dosages
I	1 LCP PR tablet	100 mg levodopa, 25 mg carbidopa, placebo
	Washout (7 days)	
II	1 LCN PR tablet	100 mg levodopa, 25 mg carbidopa, 100 mg nebicapone
	Washout (7days)	
III	1 Sinemet 100/25 + 1 placebo	100 mg levodopa, 25 mg carbidopa
	Washout (7days)	
IV	1 Sinemet 100/25 + 1 nebicapone IR tablet	100 mg levodopa, 25 mg carbidopa, 100 mg nebicapone

The immediate release layer contained either 100 mg nebicapone (LCN PR) or placebo (LCP PR).

checked and approved by the Animal Experimentation Ethics Committee at RCC CIDA S.A. Barcelona.

During each phase of the study, blood samples were collected at predose and 0.5, 1, 2, 4, 6 and 8 h post-dose for determining plasma levels of levodopa and 3-OMD. Blood samples were taken into polypropylene vials, transferred to heparinized test tubes and kept in cryoracks until centrifugation at 1620*g* for 10 min at 4°C. Sodium metabisulphite (50 µl 20% (w/v)) was added to each sample of the plasma obtained from centrifugation, which was then transferred to two fresh polypropylene test tubes and frozen in dry ice. The samples were stored at −20 ± 5°C until assayed. Plasma levodopa and 3-OMD were quantified by HPLC with electrochemical detection. Briefly, plasma samples were deproteinized by adding 200 µl perchloric acid 1 M and 500 µl perchloric acid 0.2 M to 300 µl plasma. After 10-min incubation on ice, samples were centrifuged at 9000*g* for 5 min at 4°C and supernatants were filtered through 0.22-µm filters (Costar Spin-X). Filtrate samples (50 µl) were injected onto a HPLC system that consisted of a pump (Gilson model 302; Gilson Medical Electronics, Villiers le Bel, France) connected to a manometric module (Gilson model 802 C) and a 50 Spheri-5 RP18 5 µm, 4.6 × 250 mm, column (Perkin Elmer). Samples were injected by means of an automatic sample injector (Gilson model 231) connected to a Gilson dilutor (model 401). The mobile phase was a degassed solution of citric acid (0.1 mM), sodium acetate (0.1 M), EDTA (0.17 mM), sodium octylsulphate (0.5 mM), dibutylamine (2 mM) and methanol (10% v/v), adjusted to pH 3.5 with perchloric acid 2 M and pumped at a rate of 1.0 ml/min. The detection was carried out electrochemically with a glassy carbon electrode, an Ag/AgCl reference electrode and an amperometric detector (Gilson model 142); the detector cell was operated at 0.75 V.

A calibration curve with the range 100–1000 ng/µl in minipig blank plasma was used and the limit of quantification for each analyte was 100 ng/ml.

Pharmacokinetic analysis

The pharmacokinetic parameters were determined from individual levodopa plasma concentrations by the noncompartmental approach. These parameters were: maximum plasma concentration (C_{max}), time to attain C_{max} (t_{max}), area under plasma concentration–time curve from zero to the last sampling time ($AUC_{0\text{-}t}$), area under plasma concentration–time curve from zero to infinity ($AUC_{0\text{-}\infty}$) and mean residence time (*MRT*). $AUC_{0\text{-}t}$ was calculated by the linear trapezoidal rule and $AUC_{0\text{-}\infty}$ from:

$$AUC_{0-t} + C_{last} / \lambda_z$$

where C_{last} is the last quantifiable concentration and λ_z is the apparent terminal rate constant. This constant (λ_z) was calculated by log-linear regression of the terminal segment of the plasmatic concentration versus time curve. The apparent terminal half-life ($t^1/_2$) was calculated from:

$$\ln 2 / \lambda_z$$

The *MRT* was calculated according to the following equation:

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$$

where $AUMC_{0\text{-}\infty}$ is the area under first moment calculated by the following equation:

$$AUMC = \sum_{i=1}^{n} \left[\frac{t_i C_i + t_{i+1} C_{i+1}}{2} (t_{i+1} - t_i) \right] + \frac{C_n}{\lambda_z^2} + \frac{t_n C_n}{\lambda_z}$$

Comparisons of t_{max}, *MRT* for levodopa and *AUC* for 3-OMD assuming a non-normal distribution were done using a nonparametric approach (Friedman and Dunn's tests). A *P*-value ≤ 0.05 was considered statistically significant.

Results and Discussion

The focus of this study was to evaluate the LCN PR formulation kinetic profile used in phase II. Levodopa is absorbed 80–90% in the proximal part of the small intestine by an active saturable carrier system for large neutral amino acids.[15,16] Prolonged release formulations of levodopa allow more sustained plasma levels of the drug than those obtained with immediate release dosage forms, which may delay the occurrence of motor fluctuations in levodopa therapy.[17] In addition, Dempski *et al*.[18] suggested that there was no considerable advantage in prolonging the release of levodopa for more than 2–3 h, because modified released dosage forms of the drug with slower in-vitro release produced lower plasma levels. Bearing these premises in mind, LCN PR tablets were developed to sustain the release of levodopa and carbidopa for 3–4 h, which could lead to a prolongation in levodopa absorption. As can be observed in Figure 1, LCN PR formulation allowed a prolongation of the in-vitro release of levodopa for more than 3 h.

As shown in Figure 1, Sinemet 100/25 released 90% of levodopa in 0.5 h whereas LCN PR released 90% of levodopa in approximately 3 h.

Lag time (t_{lag}), an empirical parameter that reflects the delay between the time of dosing and the time the drug appears in systemic circulation, is used to characterise the delay in absorption of orally given drugs due to slow dissolution. Accordingly, it was expected that immediate

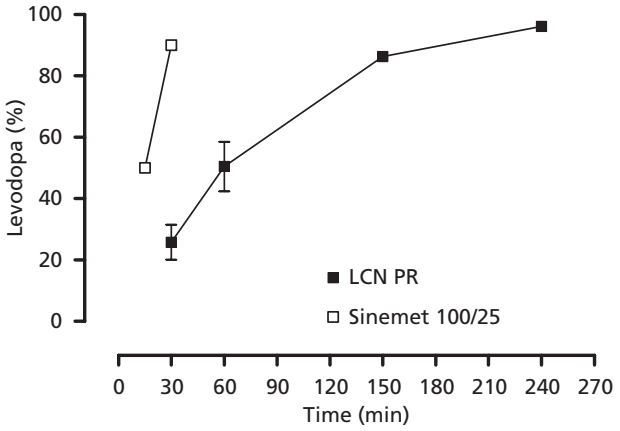

Figure 1 In-vitro release of levodopa from the prolonged release layer in bilayer tablets and Sinemet 100/25 tablets. Sinemet profile was obtained using data from Dempski *et al*.[18]; similar dissolution test conditions were used. LCN PR, prolonged release bilayer tablets, containing nebicapone in the immediate release layer and levodopa/carbidopa in the prolonged release layer.

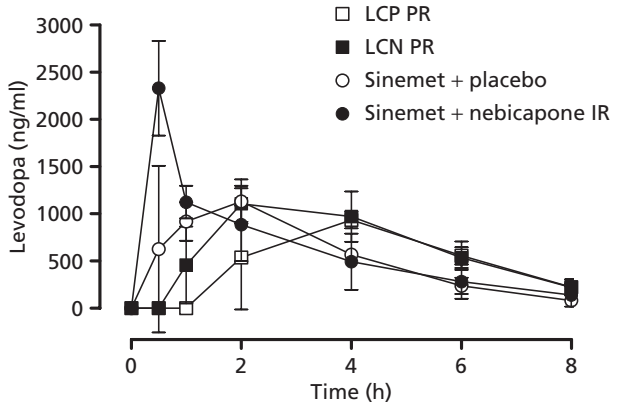

Figure 2 Mean plasma levodopa concentration–time profiles after single oral administration of various immediate and prolonged release layer tablets. Tablets used were : LCP PR, prolonged release (PR) bilayer tablets, containing placebo in the immediate release layer and levodopa/carbidopa in the prolonged release layer; LCN PR, prolonged release bilayer tablets, containing nebicapone in the immediate release layer and levodopa/carbidopa in the prolonged release layer; Sinemet (immediate release tablet containing 100 mg levodopa and 25 mg carbidopa) + placebo; or Sinemet + nebicapone immediate release (IR). Symbols represent average of $n = 3$.

release formulations (used in phases III and IV) would have a low t_{lag} (0.50 and 0.13 h, respectively) and the modified release formulations (used in phases I and II) would have increased t_{lag} (1.33 and 1.00 h, respectively), but not so high assuming the presence of an acceptable correlation between in-vitro versus in-vivo dissolution test profiles.

Figure 2 shows mean plasma levodopa concentration–time profiles after oral administration of tablets of phase I, II, III and IV. The corresponding pharmacokinetic parameters are shown in Table 3.

After oral administration the *MRT* depends on the mean absorption time (*MAT*), which incorporates the dissolution

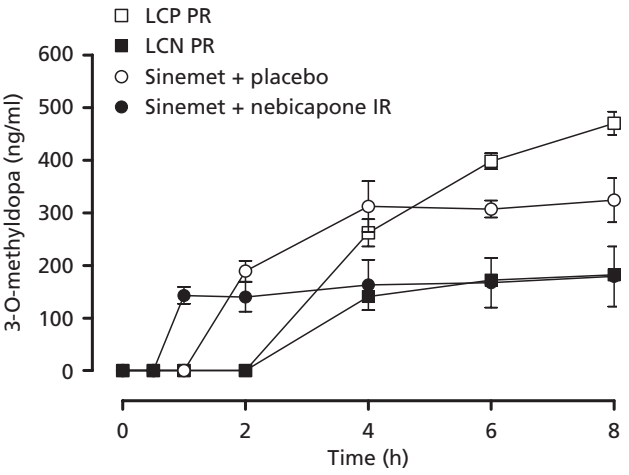

Figure 3 Plasma concentration–time profiles of 3-O-methyldopa after single oral dose administration of various immediate and prolonged release layer tablets. Tablets used were: LCP PR, prolonged release (PR) bilayer tablets, containing placebo in the immediate release layer and levodopa/carbidopa in the prolonged release layer; LCN PR, prolonged release bilayer tablets, containing nebicapone in the immediate release layer and levodopa/carbidopa in the prolonged release layer; Sinemet (immediate release tablet containing 100 mg levodopa and 25 mg carbidopa) + placebo; or Sinemet + nebicapone immediate release (IR). Symbols represent average of $n = 3$.

time. Therefore the *MRT* depends on the release characteristics of the dosage forms. The *MRT* while using immediate release formulations was 3.88 h (Sinemet 100/25 mg) and 3.15 h (Sinemet 100/25 mg + nebicapone IR); this value increased to 5.33 h (LCP) and 4.56 h (LCN) with the prolonged release formulations.

The t_{max} of formulations used in phases II (2.7 h) and III (1.5 h) were in agreement with their in-vitro release profiles, as well as the observed significant differences in t_{max} ($P = 0.0174$) and *MRT* ($P = 0.0174$) of levodopa, however the post hoc test (Dunn's test) could not distinguish the phases.

Minipigs have a digestive tract that is anatomically and physiologically similar to the human adult tract.[19] Hence, it was felt worthwhile to analyse the trend of pharmacokinetic results reported here with those reported in the literature. In humans the bioavailability of levodopa from levodopa/carbidopa PR is approximately 70–75% that of levodopa/carbidopa IR oral solid dosage forms.[20] There were no significant differences in $AUC_{0-\infty}$ or C_{max} of the four formulations ($P \geq 0.05$). This may be considered an advantage of the modified release tablets developed (LCN PR and LCP PR).

It appeared that nebicapone may have decreased the time to onset of levodopa absorption (with both immediate release and prolonged release formulations), without affecting the duration of its release. This was not an unexpected observation, since the decrease in the *O*-methylation of levodopa may have increased the amount of levodopa in the digestive tract available for absorption. In addition, a decrease in 3-OMD in the surrounding milieu was expected to facilitate the uptake of levodopa at the level of the absorptive epithelium.[21,22]

Mean plasma 3-OMD concentration–time profiles after oral administration of tablets of phase I, II, III and IV are

Table 3 Pharmacokinetic parameters of levodopa after oral administration of tablets of phases I, II, III and IV

Pharmacokinetic parameters	LCP PR	LCN PR	Sinemet + placebo	Sinemet + nebicapone IR
t_{max} (h)	3.3 ± 1.2	2.7 ± 1.2	1.5 ± 0.87	0.5 ± 0.0
C_{max} (ng/ml)	1081 ± 201	1165 ± 139	1388 ± 511	2331 ± 614
$AUC_{0\text{-}t}$ (ng.h/ml)	4010 ± 689	5239 ± 850	4393 ± 524	4987 ± 1817
$AUC_{0\text{-}\infty}$ (ng.h/ml)	4673 ± 776	5913 ± 799	4714 ± 451	5600 ± 2238
$t^1/_2$ (h)	1.93 ± 0.44	1.99 ± 0.52	1.65 ± 0.33	2.33 ± 0.48
MRT (h)	5.33 ± 0.86	4.56 ± 1.02	3.88 ± 1.86	3.15 ± 1.07

Values are means ± standard deviation ($n = 3$). $AUC_{0\text{-}t}$, area under plasma concentration–time curve from zero to the last sampling time; $AUC_{0\text{-}\infty}$, area under plasma concentration–time curve from zero to infinity; C_{max}, maximum plasma concentration; IR, immediate release; LCN PR, prolonged release (PR) bilayer tablets, containing nebicapone in the immediate release layer and levodopa/carbidopa in the prolonged release layer; LCP PR, prolonged release bilayer tablets, containing placebo in the immediate release layer and levodopa/carbidopa in the prolonged release layer; *MRT*, mean residence time; $t^1/_2$, half life; t_{max}, time to attain C_{max}.

Table 4 Pharmacokinetic parameters of 3-O-methyldopa after oral administration of tablets of phases I, II, III and IV

Pharmacokinetic parameters	LCP PR	LCN PR	Sinemet + placebo	Sinemet + nebicapone IR
t_{max} (h)	8.0 ± 0.0	7.3 ± 1.2	6.7 ± 2.3	7.3 ± 1.2
C_{max} (ng/ml)	470 ± 27.5	183 ± 11.0	343 ± 34.9	179 ± 64.7
$AUC_{0\text{-}t}$ (ng.h/ml)	1861 ± 167	947 ± 81.8	1906 ± 235	1195 ± 391
$AUC_{0\text{-}\infty}$ (ng.h/ml)	NC	NC	NC	NC
$t^1/_2$ (h)	NC	NC	NC	NC
MRT (h)	NC	NC	NC	NC

Values are means ± standard deviation ($n = 3$). $AUC_{0\text{-}t}$, area under plasma concentration–time curve from zero to the last sampling time; $AUC_{0\text{-}\infty}$, area under plasma concentration–time curve from zero to infinity; C_{max}, maximum plasma concentration; IR, immediate release; LCN PR, prolonged release (PR) bilayer tablets, containing nebicapone in the immediate release layer and levodopa/carbidopa in the prolonged release layer; LCP PR, prolonged release bilayer tablets, containing placebo in the immediate release layer and levodopa/carbidopa in the prolonged release layer; *MRT*, mean residence time; NC, not calculated; $t^1/_2$, half life; t_{max}, time to attain C_{max}.

shown in Figure 3**.** The formation of 3-OMD was markedly attenuated due to the inhibition of COMT by nebicapone. This was also evidenced by statistical significant decreases in both C_{max} ($P = 0.0174$) and *AUC* 3-OMD ($P = 0.0330$) values (Table 4). These results were similar to those reported in humans using nebicapone and Sinemet 100/25 or Sinemet CR 200/50.[6,12] Another aspect that deserves to be underlined is that nebicapone appeared to anticipate the formation of 3-OMD, as shown by the early detection of 3-OMD in plasma (phase II and IV). This fits well the result of the increase in levodopa systemic exposure early after administration, which was observed while employing both immediate release and prolonged release levodopa formulations. However, it appeared that nebicapone was more effective in reducing the 3-OMD systemic exposure when levodopa was administered as LCP PR tablets versus Sinemet (49% versus 37% decrease 3-OMD *AUC*).

Conclusions

LCN PR tablets prolonged the absorption of levodopa, increasing the value of t_{max} and inhibiting 3-OMD production. These results sustain the hypothesis that the use of LCN PR tablets may decrease the number of tablets and daily intakes in the treatment of patients with Parkinson's disease.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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