SOUTHERN RESEARCH

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In-vitro and In-vivo Drug-Drug Interaction Studies with SPINALON, a Combination of Buspirone and Levodopa/Carbidopa for Spinal Cord Injury

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Objective

SPINALON is a combination of three generic drugs (buspirone, levodopa, and carbidopa) and has been designed to activate the spinal locomotor network (also called Central Pattern Generator, CPG) for treatment of patients that are paralyzed due to chronic spinal cord injury. SPINALON has been found to activate the CPG and sustain involuntary stepping movement in chronic and completely paraplegic mice and turtles. The objective of these investigations was to determine whether any drug-drug interactions occur in vitro or in vivo between buspirone and levodopa/carbidopa (4/1, w/w) when they are used in combination.

Experimental

In Vitro Studies: The components of SPINALON (levodopa, carbidopa, and buspirone) were added alone or in combination (levodopa/carbidopa/buspirone or levodopa/carbidopa) to rat plasma at concentrations of: 5000 ng/mL levodopa, 1250 ng/mL carbidopa, and 500 ng/mL buspirone. Triplicate samples were collected from each mixture immediately upon preparation of the individual mixtures and after a 1-hour incubation of the mixtures at 37°C, or after a 2-hour incubation at room temperature in the presence and absence of stabilizers [25 µL/mL of plasma of a solution containing 10% (w/v) sodium metabisulfite and 10% (w/v) hydrazine dihydrochloride]. Samples were analyzed by HPLC/MS/MS.

In Vivo Studies: Male Sprague Dawley (CD® IGS) rats, with indwelling jugular vein cannulas, were purchased from Charles River Laboratories (Raleigh, NC). Animals were maintained on Teklad Certified Rodent Diet #2016 (Harlan; Madison, WI) and tap water (Birmingham, AL city) *ad libitum*. On the day of dosing, the mean body weight of the animals in each drug-treated group was between 272 and 282 g. Animals were assigned to dose groups as follows:

Group No.	Dose Group	Dose Volume (mL/kg)	Dose Route	Dose (mg/kg)	Dose Formulation Conc. (mg/mL)	No. of Rats
1	Buspirone	10	Oral gavage	5	0.5	10
2	Levodopa	10	Oral gavage	50	5	10
3	Carbidopa	10	Oral gavage	12.5	1.25	10
4	SPINALON	10	Oral gavage	5/50/12.5[a]	0.5/5/1.25[b]	10
8	Levodopa/ carbidopa	10	Oral gavage	50/12.5[c]	5/1.25[b]	10
5	None	--	--	--	--	3

[a]Dose of buspirone, levodopa, and carbidopa, respectively
[b]Concentration of buspirone, levodopa, and carbidopa, respectively
[c]Dose of levodopa and carbidopa, respectively

Dose formulations of individual or combination drugs were prepared as suspensions in 0.5% carboxymethyl cellulose.

Blood (plasma) samples were collected from each rat at 0, 0.25, 0.5, 1, 2, 4, and 8 hours into tubes containing EDTA and drug stabilizers [approximately 25 µL/mL of 10% (w/v) sodium metabisulfite and 10% (w/v) hydrazine dihydrochloride. Plasma samples were analyzed for levodopa, carbidopa, and/or buspirone and its metabolite, 1-(2-pyrimidinyl)-piperazine (1-PP), using HPLC/MS/MS.

Mean plasma drug concentration versus time data were subjected to non-compartmental analysis using WinNonlin® (Pharsight Corporation; Mountain View, CA).

Analytical Conditions

HPLC conditions:

Analytical Column:	Thermo EC Aquasil C18 5µm, 150 mm × 2.1 mm ID
Guard Column:	C18 Security Guard cartridge
Elution Flow rate:	400 µL/min.
Injection volume:	10 µL
Mobile phase:	A: 5 mM ammonium acetate with 0.5% formic acid
	B: Acetonitrile with 0.5% formic acid
Gradient Profile:	0.0 – 1.5 min. 95%A : 5%B
	1.5 - 5.0 min 30%A : 70%B Linear
	5.0 – 5.5 min 30%A : 70%B
	5.5 – 5.6 min 95%A : 5%B
	5.6 – 8.5 min 95%A : 5%B
Column Temperature:	Ambient
Sample Temperature:	Ambient

Mass Spectrometer Conditions:

Instrument:	Sciex 3000		
Polarity:	Positive		
Ion Source Temp.:	450°C		
Ion Spray Voltage:	5 kV		
Collision Gas:	Nitrogen		
	Levodopa	Carbidopa	Buspirone
Mass Transitions:	197.9 to 152.3	227.2 to 181.0	386.1 to 122.3
Dwell Time (ms)	150	150	150
	1-PP	Lidocaine (IS)	
Mass Transitions:	165.2 to 122.3	235.0 to 86.2	
Dwell Time (ms):	150	150	

In Vitro Stability

In Vitro Drug Stability in Rat Plasma at 37°C

Incubation Conditions	% Drug Remaining after 1 hour at 37°C		
	Buspirone	Levodopa	Carbidopa
Individual Drugs	91.3	32.7	42.5
SPINALON	116	48.1	52.5
Individual Drugs	--	52.6	54.1
Levodopa/carbidopa	--	40.3	55.4

In Vitro Drug Stability in Rat Plasma at Room Temperature In the Presence and Absence of Stabilizers[a]

Incubation Conditions	% Drug Remaining after 2 hours at Room Temperature					
	Without Stabilizers			With Stabilizers		
	Buspirone	Levodopa	Carbidopa	Buspirone	Levodopa	Carbidopa
Individual	84.6	74.7	60.4	95.7	91.4	97.7
SPINALON	94.2	58.7	76.6	108	105	113

[a]25 µL/mL of plasma of a solution containing 10% (w/v) sodium metabisulfite and 10% (w/v) hydrazine dihydrochloride

In Vivo Pharmacokinetics

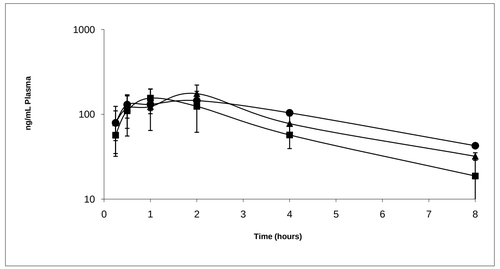

Figure 1. Plasma concentrations of **carbidopa** following po administration of carbidopa (■), levodopa/carbidopa (▲) or SPINALON (●) to rats

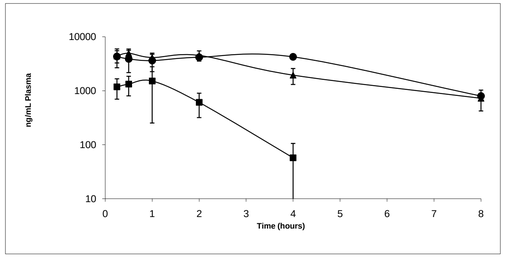

Figure 2. Plasma concentrations of **levodopa** following po administration of levodopa (■), levodopa/carbidopa (▲) or SPINALON (●) to rats

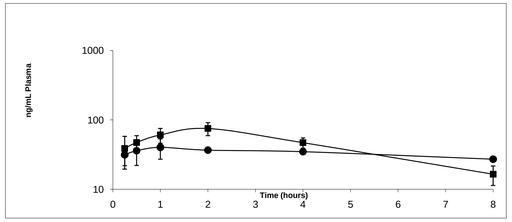

Figure 3. Plasma concentrations of the buspirone metabolite, **1-PP**, following po administration of buspirone (■) or SPINALON (●) to rats.

No unchanged buspirone was quantifiable in plasma at any time point after administration of buspirone alone or SPINALON.

Pharmacokinetic Parameters Calculated from Plasma Concentrations of Levodopa, Carbidopa, and 1-PP following Single or Combined Administration of Levodopa, Carbidopa, or Buspirone to Rats

Group	Compound	Drug Combination	Half-life[a] (hours)	Tmax[b] (hours)	Cmax[c] (ng/mL)	AUCall[d] (hr·ng/mL)
1	1-PP[e]	None	NC	2	75.2	347
4	1-PP	SPINALON	13.4	1	40.2	264
2	Levodopa	None	NC	1	1517	2749
4	Levodopa	SPINALON	NC	0.25	4310	23998
3	Carbidopa	None	NC	1	155	520
4	Carbidopa	SPINALON	NC	2	145	761
8	Levodopa	Levodopa/ carbidopa	2.3	0.5	4943	19337
8	Carbidopa	Levodopa/ carbidopa	NC	2	175	689

NC: a reliable half-life could not be estimated from the plasma drug concentration data
[a]Half-life of the terminal elimination phase
[b]Time the mean peak plasma concentration of drug was observed
[c]Mean peak plasma concentration
[d]Area under the plasma drug concentration time curve calculated from 0 to the last time point
[e]Rats were administered buspirone; plasma concentrations of the metabolite, 1-PP, were measured

Conclusions

No drug/drug interactions occur in vitro when carbidopa, levodopa, and buspirone are incubated together in rat plasma.

Buspirone exhibits poor oral bioavailability in rats.

No drug/drug interactions occur between buspirone, levodopa, and carbidopa when given orally in combination to rats.

The increased plasma concentrations of levodopa observed for rats given po doses of SPINALON, as compared with rats given levodopa alone, are likely related to the inhibition of the metabolism of levodopa by carbidopa.

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