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Pressor Response to Intravenous Tyramine in Healthy Subjects After Safinamide, a Novel Neuroprotectant With Selective, Reversible Monoamine Oxidase B Inhibition

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Summary: Safinamide is a novel neuroprotectant combining sodium and calcium channel blocking properties with selective, reversible monoamine oxidase type B (MAO B) inhibition. Phase 1 studies have demonstrated that in healthy volunteers, the ED50 (a dose that inhibits enzyme activity by 50% in 50% of treated subjects) for MAO B inhibition is 87.5 μg/kg/day orally, and that no MAO A inhibition occurs after 10-mg/kg oral dosing. To assess the risk of inducing the "cheese effect," the effect of safinamide and placebo on the pressor response to tyramine was investigated in a group of healthy male volunteers. The study was an open, single-dose placebo-controlled trial with the 2 treatments in sequence. An increase of 30 mm Hg systolic blood pressure was obtained by intravenous tyramine administered by 0.5-mg incremental boluses injected at 15-minute intervals. The amount of tyramine necessary to achieve such a blood pressure increase was the same after the safinamide 2-mg/kg oral load compared with placebo. These results suggest that dietary restrictions for food with high tyramine content should not be required under safinamide treatment. **Key Words:** safinamide, monoamine oxidase, tyramine

Safinamide is a novel chemical entity with several pharmacodynamic properties all converging toward a neuroprotecting effect. Safinamide use and frequency-dependent sodium channel block together with its calcium antagonism likely contribute to the glutamate release inhibition that has been observed in various experimental settings.[1] In addition, safinamide is a potent, selective, reversible inhibitor of monoamine oxidase type B (MAO B) and inhibits dopamine uptake. As such, safinamide has demonstrated antiparkinsonian and antiepileptic activity in several animal models, and it is in phase 2 clinical trials for both Parkinson and epilepsy indications.

MAO exists in two functional isoenzyme forms, A and B, which are different on the basis of their affinity for substrates and specificity toward inhibitors.[2] MAO A preferentially deaminates noradrenaline and serotonin; MAO B has a higher affinity for benzylamine and phenylethylamine. Monoamines, such as dopamine and tyramine (the serotonin precursor), are substrates of both types of MAO.[2]

Tyramine intestinal degradation mainly involves MAO A, whereas its neuronal degradation involves mainly MAO B.[3] Tyramine evokes its pharmacologic action by displacing noradrenaline from intraneuronal stores, subsequently causing an increase in blood pressure (BP).[4]

The use of nonselective MAO inhibitors or MAO A inhibitors can lead to life-threatening hypertensive crisis via the potentiation of peripheral adrenergic stimulation caused by tyramine ingested with food, such as seasoned cheese, chicken liver, soy sauce, red wine, and so forth.[5] This is the so-called "cheese effect," which can be assessed pharmacodynamically by the measurement of systolic BP.[6]

The tyramine challenge, by both the oral and intravenous routes, is largely used to simulate the clinical situation that may occur during treatment with MAO inhibitors when ingesting food with a high tyramine content.[4–11]

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As suggested by Pace et al,[12] the intravenous route should be preferred inasmuch as it provides less variable and more predictive results, with no first-pass metabolism problems.

Safinamide, a derivative of the chemical class of α-aminoamides, is an anticonvulsive and neuroprotectant drug that has also shown antiparkinsonian properties on the basis of its marked reversible inhibition of MAO B activity and dopamine uptake inhibition. Block of either endogenously or exogenously supplied dopamine degradation is an effective means to improve motor performance and to reduce oxidative stress in Parkinson disease.[13]

This open, single-dose, placebo-controlled study compared the effect of safinamide and placebo on the pressor response to tyramine in a group of healthy male volunteers.

METHODS

Subjects

The study was approved by the ethics committee of Canton Ticino, Switzerland, and detailed information about the protocol, including its benefits and risks, was provided before written informed consent was obtained. The study was conducted in accordance with the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice and in compliance with the Declaration of Helsinki and its amendments.

Eight male subjects, age 18 to 45 years, of white origin were enrolled in this trial. They were all healthy volunteers, according to their medical histories and results of physical examination, electrocardiograms, vital signs, chest radiographs, and routine laboratory tests (complete hematologic/biochemical profile; urinalysis; virologic tests for human immunodeficiency virus, hepatitis B and C; and drugs of abuse screen).

Subjects had a body weight within 10% of the ideal height/frame size as reported in the height and weight tables for Metropolitan Life Insurance and were nonsmokers, nonvegetarians, with no history of allergic response to anticonvulsant drugs. In addition, they should not have used any prescription or over-the-counter medication, not consumed food or liquid containing tyramine 3 days before study initiation and until the end of the study, and not ingested caffeine-containing beverages, fresh grapefruit, grapefruit juice, or alcohol 48 hours before and until the end of the study. In addition, they should not have been receiving treatment with any known enzyme inhibiting or inducing agent, and should not have made a blood donation of 250 mL or more, or taken part in a clinical trial in the previous 3 months.

Subjects were in fasting conditions starting 12 hours before and lasting until the end of the pressor test. They were hospitalized the evening before the day of administration until 24 hours after dosing, and their diet was controlled during hospitalization. No medications were allowed 7 days before enrollment and for the entire duration of the study period. All subjects gave their written informed consent before the study started.

Study Design

The study was an open, single-dose, placebo-controlled trial with the 2 treatments in sequence. Each subject was treated first with placebo and then with safinamide at the 2.0-mg/kg body weight dose. All subjects had a 2-week run-in period during which study eligibility was determined, followed by 2 treatment sessions separated by a 3-day washout period. Tyramine challenges were conducted 3 hours after treatment administration, at a time when, according to previous studies, time to peak concentration (Tmax) of safinamide is expected. For safety purposes, subjects were followed after treatment ended.

Drugs and Test Substance

Safinamide and a matched placebo were administered as 10- and 50-mg capsules at the dose of 2.0 mg/kg body weight (approximated by defect). Study drugs were dispensed by the clinical investigator or under his direct supervision 3 hours before the tyramine pressor test. Subjects were assigned consecutively to a progressive number and were administered placebo during the first period and safinamide during the second. One tyramine hydrochloride vial (167.7 mg) was diluted in 100 mL saline and was administered as an intravenous bolus to fasting subjects, in ascending doses from 0.5 to 6.0 mg (volume range, 0.3–3.6 mL), increasing gradually according to the pressor response at approximately 15-minute intervals, until a systolic BP increase of 30 mm Hg was recorded.

Rationale for the Choice of Safinamide Dose

In a previous study, single ascending doses from 2.5 to 10 mg/kg were administered orally to healthy male volunteers.[14] Blood and urine samples were collected for as long as 48 hours after dosing. Safinamide blood levels and MAO B activity in the platelet-enriched plasma fraction were measured at fixed times after dosing, as was the urinary excretion of phenylethylamine, an MAO B substrate.

MAO A was evaluated at the highest tested dose (10 mg/kg) by measuring plasma levels of 3-methoxy-4-hydroxy-phenylglycol levels.

At all doses, platelet-enriched plasma fraction MAO B activity was inhibited reversibly (>90%), and urinary phenylethylamine was augmented remarkably as long as 48 hours after drug intake, whereas MAO A was unaffected after 10 mg/kg.

To provide a more accurate analysis of the dose–response curve for MAO B inhibition, in separate trials[14] single oral doses of safinamide ranging from 25 to 600 μg/kg were administered to healthy male volunteers.

A significant, reversible, progressive inhibition of platelet-enriched plasma fraction MAO B activity was noted with an ED50 (a dose that inhibits enzyme activity by 50% in 50% of treated subjects) of 87.54 μg·g/kg (Fig. 1). Considering these findings, the dose chosen in the current study (2.0 mg/kg), which is more than 20 times higher than ED50, is very predictive for the therapeutic doses to be used in phase 2 to 3 trials.

Tyramine Challenge

The tyramine pressor test was performed with the same procedure both after placebo and safinamide administration. Subjects rested in a supine position for at least 30 minutes (equilibration period) before and until the end of the pressor test.

Tyramine hydrochloride was administered by intravenous bolus to fasting subjects, starting 3 hours after placebo or safinamide administration, in the following dose sequence: 0.5, 1.0, 2.0, 3.0, 3.5, 4.0, 5.0, 5.5, and 6.0 mg, with each intravenous injection separated from the next by 15 minutes. Baseline conditions for BP were considered to be attained when the difference between 3 consecutive readings, at 5-minute intervals, were not greater than 5 mm Hg.

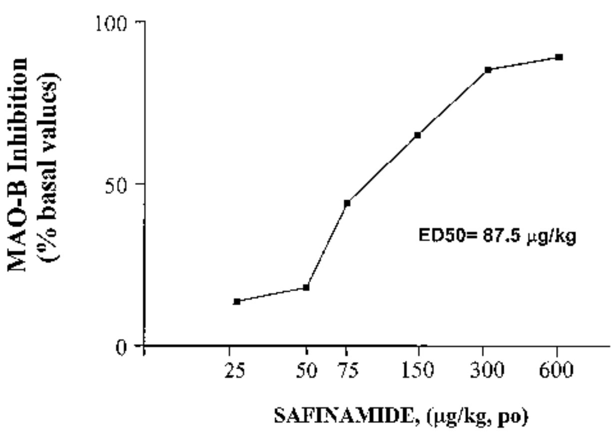

FIGURE 1. Dose–response curve of monoamine oxidase (MAO) B inhibition in platelet-enriched plasma fraction (PRP) after a single oral dose of safinamide in healthy volunteers (dose on the abscissa). ED50, a dose that inhibits enzyme activity by 50% in 50% of treated subjects.

An antecubital vein was cannulated with a Teflon cannula, ending in a 3-way cock. The cock was connected to a bottle of sterile isotonic saline, and another part was used for the injection of tyramine hydrochloride. Each tyramine injection was followed immediately by 3 mL saline to wash out the catheter.

Starting from 0.5 mg, the dose of tyramine hydrochloride was increased gradually according to the pressor response at approximately 15-minute intervals, after systolic BP returned within 5 mm Hg of baseline. The test was stopped when an increase in systolic BP of 30 mm Hg over the baseline value was attained. The dose of tyramine that increased systolic BP by 30 mm Hg was called PD30. During the test, heart rate and electrocardiograms were monitored by telemetry, and BP was recorded using a mercury sphygmomanometer at 1-minute intervals for 10 minutes or longer after each tyramine injection (ie, up to the end of the pressor effect and restoration of the baseline values).

Appropriate measures to counteract possible sustained increase of BP or other untoward effects (ie, nifedipine or intravenous phentolamine) were ready at hand. Volunteers were asked to report any adverse events.

Safety

Urine was collected before drug treatment (placebo or safinamide) and 0 to 6 hours, 6 to 12 hours, and 12 to 24 hours after dosing. Vital signs (blood pressure, heart rate, respiratory rate, and body temperature), 12-lead electrocardiograms, and laboratory tests were measured during the run-in period and at the end of the study. Adverse events were recorded during the course of the trial.

Statistical Analysis

Descriptive statistics (mean, standard deviation, coefficient of variation) were performed on PD30 values. A comparison between PD30 values observed with tyramine challenge after safinamide administration and those observed after placebo treatment were performed using the Student *t*-test and 1-way analysis of variance.

All statistical tests and comparisons were evaluated at the 5% significance level ($P < 0.05$) using STAT 100 version 1.2 (BioSoft USA).

RESULTS

Demographic Data

Demographic data of the volunteers are reported in Table 1.

TABLE 1. *Volunteers' demographic data and treatment sequence*

Subject no.	Gender	Age, y	Height, cm	Weight, kg	Body frame	Treatment sequence
1	M	22	177	81	Large	P/SAF
2	M	24	178	70	Small	P/SAF
3	M	23	170	62	Small	P/SAF
4	M	33	165	60	Small	P/SAF
5	M	27	180	82	Large	P/SAF
6	M	33	174	80	Large	P/SAF
7	M	25	175	72	Medium	P/SAF
8	M	27	170	70	Large	P/SAF

M, male; P, placebo; SAF, safinamide.

Effect of Treatment Drugs on Systolic BP After Intravenous Tyramine Administration

A dose-dependent increase of systolic BP was obtained with successive injections of increasing doses of tyramine. The planned 5.5-mg and 6.0-mg tyramine doses were not administered because the results were obtained with a maximum 5.0 mg in all subjects.

The PD30 obtained in the study were 4.1 ± 0.82 mg (mean ± standard deviation, $n = 8$) and 4.3 ± 0.77 mg ($n = 8$) after administration of placebo and safinamide respectively.

Table 2 summarizes the single values obtained and reports the means and standard deviations. Statistical analysis (Student *t*-test for paired data and 1-way analysis of variance) showed that the slight difference between the PD30 value after placebo administration and after safinamide administration is not significant.

Vital Signs

Vital signs were measured at screening and at the end of the study. No abnormalities occurred as far as BP, heart rate, body temperature, and respiratory rate were concerned. In addition, electrocardiographic tracking did not show any abnormality.

Adverse Events

No adverse events occurred during the study. Nor were there any study discontinuations. No concurrent medications were administered to the subjects.

DISCUSSION

Tyramine is an indirectly acting sympathomimetic amine present in high concentrations in certain foods,

TABLE 2. *Systolic BP results*

	V1	V2	V3	V4	V5	V6	V7	V8	*Mean (SD)
Placebo	4.0	4.5	5.5	3.2	4.5	4.3	3.0	3.5	4.1 (0.8)
NW-1015	4.0	4.7	5.0	4.0	5.0	5.0	3.0	3.5	4.3 (0.8)

* mg of i.v. tyramine needed to raise BP by 30 mm Hg

and it evokes its pharmacologic action by displacing noradrenaline from intraneuronal stores, resulting in an increase in BP.

The risk of hypertensive crises during therapy with nonspecific MAO inhibitors or pure MAO A inhibitors is a major drawback that restricts their therapeutic use. Specific MAO B inhibitors have been reported to be without this effect. However, selegiline, for which extensive clinical experience exists, has been reported to have cardiovascular toxicity that includes hypertensive paroxysms.[15–17] A role in this effect can be played by those amphetaminelike metabolites found in notable amounts during selegiline treatment.[18,19] Moreover, in contrast to the findings of the DATATOP study,[20] others have reported a greater mortality in parkinsonian patients under selegiline treatment.[21] One of the hypotheses advanced to explain this suspected increased mortality relates to the higher incidence of cardiovascular adverse events, including hypertensive reactions, in selegiline treated versus L-dopa-treated patients.[22] Despite the failure to replicate the data of increased mortality in the British study,[23–25] many European neurologists refrain from widespread use of selegiline in Parkinson disease.

Thus, it is important that any new MAO B inhibitor be devoid of such a negative property. In this regard it should be stressed that the selectivity of safinamide compared with either selegiline or rasagiline is much higher. In fact, the respective MAO A-to-MAO B ratios are approximately 5000 for safinamide versus 127 for selegiline and 103 for rasagiline.[23,24] In addition, selegiline and rasagiline are irreversible inhibitors. On the other hand, safinamide is fully reversible, as demonstrated by the absence of a time-dependent inhibition in human platelets and the complete recovery of MAO B activity after washing of the membrane. Because of this, in case of an adverse event there is no need for prolonged maintenance of the therapeutic remedies.

The results of this study showed that there are no significant differences in the PD30 values after administration of placebo and safinamide, confirming the specificity of safinamide's MAO B inhibition, as seen in previous trials. In human plasma, safinamide ED50 for MAO B inhibition has been calculated to be 87.5 μg/kg orally. Supramaximal inhibition of MAO B activity in healthy volunteers was seen from single doses equal to 0.6 mg/kg, which is definitely lower than the dose tested in the current experiment. The reversible MAO B inhibition of safinamide without effect on MAO A activity should provide a significant increase of both endogenously and exogenously supplied dopamine. Ongoing studies in parkinsonian patients have been designed to provide an answer to this hypothesis.

Safinamide at the dose of 2.0 mg/kg was well tolerated throughout the study by all volunteers. No subjective complaints were reported and no abnormalities were detected in the laboratory tests. A detailed analysis of each volunteer's response evidenced that to reach the same BP elevation observed after placebo, 4 volunteers required a greater amount of tyramine (0.5 mg) after safinamide, 3 required the same amount, and only 1 necessitated a lower amount (0.5 mg).

It can be concluded that no dietary restrictions concerning tyramine-containing food have to be recommended in future clinical trials or during routine therapy with safinamide. The risk of association with other drugs interfering with catecholamine levels and their metabolism remains to be assessed in future clinical studies.

REFERENCES

1. Salvati P, Maj R, Caccia C, et al. Biochemical and electrophysiological studies on the mechanisms of action of PNU-151774E, a novel antiepileptic compound. *J Pharmacol Exp Ther*. 1999;288: 1151–1159.
2. Johnston JP. Some observation upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol*. 1968;17:1285–1297.
3. Fowler CJ, Ross SB. Selective inhibitors of monoamine oxidase A and B: biochemical, pharmacological and clinical properties. *Med Res Rev*. 1984;4:323–358.
4. Hinze C, Kaschube M, Hardenberg J. Pharmacodynamics of MDL 72974A: absence of effect on the pressor response to oral tyramine. *J Neurol Transm*. 1994;41:371–375.
5. Walker SE, Shulman KI, Tailor SAN, et al. Tyramine content of previously restricted foods in monoamine oxidase inhibitor diets. *J Clin Psychopharmacol*. 1996;16:383–388.
6. Bieck PD, Antonin KH. Tyramine potentiation during treatment with MAO inhibitors; brofaromine and moclobemide vs irreversible inhibitors. *J Neurol Transm*. 1989;28:21–31.
7. Simpson GM, Gratz SS. Comparison of the pressor effect of tyramine after treatment with phenelzine and moclobemide in healthy male volunteers. *Clin Pharmacol Ther*. 1992;52:286–291.
8. Colombo F, Sega R, Mailland F, et al. Beta-blockade antagonism of tyramine-induced rise in blood pressure. *Eur J Clin Pharmacol*. 1988;34:263–266.
9. Wilkins MR, Wynne RD, Kendall MJ. A placebo controlled comparison of the effects of pirenzepine and amitriptyline on the tyramine pressor test in healthy volunteers. *Br J Clin Pharmacol*. 1985; 19:829–831.
10. Reimann IW, Firkusny L, Antonin KH, et al. Intravenous amine pressor tests in healthy volunteers. Within- and between subject variances and sex differences. *Eur J Clin Pharmacol*. 1992;42:137–141.
11. Cusson JR, Goldenberg E, Larochelle P. Effect of a novel monoamine-oxidase inhibitor, moclobemide, on the sensitivity to intravenous tyramine and norepinephrine in humans. *J Clin Pharmacol*. 1991;31:462–467.
12. Pace DG, Reele SB, Rozik LM, et al. Evaluation of methods of administering tyramine to raise systolic blood pressure. *Clin Pharmacol Ther*. 1988;44:137–144.
13. Youdim MH, Finberg JPM. New directions in monoamine oxidase A and B selective inhibitors and substrates. *Biochem Pharmacol*. 1991;41:155–162.
14. Caccia C, Tocchetti P, Dal Bo L, et al. *Safinamide (NW-1015): Effects on Platelets MAO-B Activity in Healthy Volunteers*. Presented at the 5th International Conference on Progress in Alzheimer's and Parkinson's Disease. Kyoto, Japan. March 31–April 5 2001.
15. Ito D, Amano T, Sato H, et al. Paroxysmal hypertensive crises induced by selegiline in a patient with Parkinson's disease. *J Neurol*. 2001;248:33–34.
16. Hinds NP, Hiller CE, Wiles CM. Possible serotonin syndrome arising from an interaction between nortriptyline and selegiline in a lady with parkinsonism. *J Neurol*. 2000;247:811.
17. Montastruc JL, Chaumerliac C, Desboeuf K, et al. Adverse drug reactions to selegiline: a review of the French pharmacovigilance database. *Clin Neuropharmacol*. 2000;23:271–275.
18. Rose LM, Ohlinger MJ, Mauro VF. A hypertensive reaction induced by concurrent use of selegiline and dopamine. *Ann Pharmacother*. 2000;34:1020–1024.
19. Reynolds GP, Elsworth JD, Blau K, et al. Deprenyl is metabolized to methamphetamine and amphetamine in man. *Br J Clin Pharmacol*. 1978;6:542–544.
20. The Parkinson Study Group. Effect of deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med*. 1989;321: 1364–1371.
21. Lees AJ, on behalf of the Parkinson's Disease Research Group of the United Kingdom. Comparison of therapeutic effects and mortality data of levodopa and levodopa combined with selegiline in patients with early, mild Parkinson's disease. *BMJ*. 1995;311:1602–1607.
22. Reynolds GP, Riederer P, Sandler M, et al. Amphetamine and 2-phenylethylamine in post mortem parkinsonian brain after deprenyl administration. *J Neural Transm*. 1978;43:271–277.
23. Larsen JP, Boas J, Erdal JE. Does selegiline modify the progression of early Parkinson's disease? Results from a five-year study. The Norwegian–Danish study group. *Eur J Neurol*. 1999;6:539–547.
24. Donnan PT, Steinke DT, Stubbings C, et al. Selegiline and mortality in subjects with Parkinson's disease: a longitudinal community study. *Neurology*. 2000;55:1785–1789.
25. Shoulson I, Oakes D, Fahn S, et al. Impact of sustained deprenyl (selegiline) in levodopa-treated Parkinson's disease: a randomised placebo-controlled extension of the deprenyl and tocopherol antioxidative therapy of parkinsonism trial. *Ann Neurol*. 2002;51:604–612.