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Zhichan decoction induces differentiation of dopaminergic neurons in Parkinson's disease rats after neural stem cell transplantation

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Abstract
The goal of this study was to increase the dopamine content and reduce dopaminergic metabolites in the brain of Parkinson's disease rats. Using high-performance liquid chromatography, we found that dopamine and dopaminergic metabolite (dihydroxyphenylacetic acid and homovanillic acid) content in the midbrain of Parkinson's disease rats was increased after neural stem cell transplantation + *Zhichan* decoction, compared with neural stem cell transplantation alone. Our genetic algorithm results show that dihydroxyphenylacetic acid and homovanillic acid levels achieve global optimization. Neural stem cell transplantation + *Zhichan* decoction increased dihydroxyphenylacetic acid levels up to 10-fold, while transplantation alone resulted in a 3-fold increment. Homovanillic acid levels showed no apparent change. Our experimental findings show that after neural stem cell transplantation in Parkinson's disease rats, *Zhichan* decoction can promote differentiation of neural stem cells into dopaminergic neurons.

Key Words: *nerve regeneration; traditional Chinese medicine; neurodegeneration; Parkinson's disease; rat model; Zhichan decoction; stem cell transplantation; dopamine metabolite; dihydroxyphenylacetic acid; homovanillic acid; curve fitting equation; genetic algorithm; optimization model; NSFC grant; neural degeneration*

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Introduction
Parkinson's disease is pathologically characterized by progressive degeneration and necrosis of dopaminergic neurons in the substantia nigra (Beal, 2001). The ideal Parkinson's disease treatment not only improves dopamine content in the striatum, but also rescues, delays, and prevents degeneration of dopaminergic neurons. Current treatments include drug therapy, surgery, cell transplantation, and gene therapy. Parkinson's disease is one of the indications for stem cell transplantation (Qian et al., 2002; Rossi et al., 2002), and stem cell transplantation reconstructs dopaminergic neuronal function in Parkinson's disease patients.

As *in vitro* neural stem cell culture and purification techniques develop, neural stem cell transplantation is the most promising transplantation strategy for Parkinson's disease treatment. Neural stem cells bring new hope for the treatment of neurodegenerative diseases, as they are considered the most suitable cells for cell replacement and gene therapy in the central nervous system. Cell transplantation success largely depends on survival of the transplanted cells, acquisition of specific neuronal phenotypes, integration into the recipient tissue (Wang et al., 2010; Song et al., 2011; Li et al., 2011), and reconstruction of dopaminergic neuronal function in Parkinson's disease patients. However, understanding of how to promote differentiation of transplanted neural stem cells into dopaminergic neurons in the brain and change the brain microenvironment and transplanting area around signals, are urgently required to achieve this aim (Englund et al., 2002).

Zhichan decoction is an effective Chinese herbal drug for Parkinson's disease, and has obtained satisfactory clinical efficacy (Li et al., 2000; Liu et al., 2000; Cao et al., 2002; Li et al., 2002). In the Parkinson's disease rat brain, *Zhichan* decoction promotes differentiation of transplanted neural stem cells into dopaminergic neurons and inhibits dopamine excretion, thereby achieving a therapeutic effect. To determine the advantages of a traditional Chinese medicine compound recipe on improving the brain microenvironment for cell survival, it should first be determined if *Zhichan* decoction promotes differentiation of *in vitro* cultured and transplanted neural stem cells into dopaminergic neurons in the Parkinson's disease rat brain; secondly, if it increases proliferation and survival of transplanted neural stem cells and dopaminergic neurons *in vivo*; and ultimately, if it affects dopamine synthesis and metabolism (Li et al., 2009). At present, there is little evidence from modern medicine, addressing an effective method to promote differentiation and long-term survival of transplanted neural stem cells, therefore traditional Chinese medicine treatments are worthy of investigation. To further study the role of neural stem cell transplantation in Parkinson's disease, a contemporary intelligent optimization algorithm was applied to obtain global optimization of dopaminergic metabolites, specifically, dihydroxyphenylacetic acid, and homovanillic acid, in the midbrain of Parkinson's disease rats after neural stem cell transplantation.

Mathematical optimization requires selection of the best element, with regards certain criteria, from sets of available alternatives. In the simplest case, an optimization problem consists of maximizing or minimizing a real function by systematically choosing input values from within an allowed set and computing the function value. More generally, optimization includes finding "best available" values for objective functions given a defined domain, or set of constraints. Modern optimization algorithms include genetic algorithms, simulated annealing, and tabu search. These algorithms combine artificial intelligence, computer science, and operation research to analyze and solve complex optimization problems (Yan et al., 2013). Algorithms are characterized by (1) no requirement for resolving objective and constraint functions, or even explicit objective function expression, and function values alone are enough for the calculation; (2) obtaining discrete values for bound variables, such as integer or other specific values; and (3) allowing global optimization.

To observe changes in dihydroxyphenylacetic acid and homovanillic acid levels, we fitted dopamine-dihydroxyphenylacetic acid and dopamine-homovanillic acid equations. Because the fitting equations were nonlinear, it was difficult to obtain global optimization using the conventional method, therefore we chose a genetic algorithm, reflecting the relationship between decisive and randomness (Bao, 2011). In addition, we also examined survival and proliferation of transplanted cells in Parkinson's disease rats, and the induction effect of *Zhichan* decoction on neural stem cell differentiation.

In this study, Parkinson's disease rat models were treated with *Zhichan* decoction and neural stem cell transplantation, and the content of dopamine and its metabolites in the Parkinson's disease rat brain detected using high-performance liquid chromatography. Global optimization of dihydroxyphenylacetic acid and homovanillic acid levels was obtained using a genetic algorithm.

Materials and Methods

Animals

Forty-eight healthy male Wistar rats, aged 10 weeks and weighing 260 ± 20 g, were provided by Shanghai SLAC Laboratory Animal Co., Ltd., Shanghai, China (license No. SCXK (Hu) 2007-0005). A healthy female Wistar rat, 14 days pregnant and weighing 250 ± 10 g, was used for embryo extraction. The experiment was approved by the Experimental Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine in China (Approval No. 09047).

Zhichan decoction

Zhichan decoction consists of 12 g *Radix astragali*, 15 g *Radix paeoniae alba*, 15 g *Radix salviae miltiorrhiae*, 15 g prepared *rhubarb*, 18 g *Uncaria tomentosa*, 12 g *Rhizoma anemarrhenae*, 9 g *Rhizoma cimicifugae*, and 10 g *Rhizoma gastrodiae*. These Chinese herbs are identified according to the quality standard of the Chinese Pharmacopoeia 2005 edition. The optimal dose in animal experiments was previously described as 1.2 mL/100 g (Li et al., 2000), although our decoction required a concentration dose up to 7 fold-higher. The herbs were decocted in 600 mL purified water, obtaining a 300 mL decoction, and then decocted again in 500 mL purified water, obtaining a 200 mL decoction. Both decoctions were mixed, filtered, and concentrated to 250 mL using a rotating distillator (Shanghai Aiming Equipment Co., Ltd., Shanghai, China).

Grouping, modeling, and intervention

The 48 experimental rats were randomly and equally divided into four groups, with 12 rats in each group and 4 rats each at 7, 14, and 28 days. Control group: Control rats were injected with equal amounts (5 μL) of DMEM/F12 medium (Sigma, St. Louis, MO, USA) into the substantia nigra, and simultaneously 4 mL saline was administered intragastrically twice per day (at 8:00 and 18:00). Model group: Parkinson's disease rat models were treated as for the control group. Neural stem cell transplantation group: Parkinson's disease rat models were injected with neural stem cells into the substantia nigra corpus striatum at the injured side, and simultaneously 4 mL saline was administered intragastrically twice per day (at 8:00 and 18:00). *Zhichan* decoction group: Parkinson's disease rat models were injected with neural stem cells into the substantia nigra corpus striatum at the injured side, and simultaneously 4 mL saline containing 12 μL/g *Zhichan* decoction was administered intragastrically twice per day (at 8:00 and 18:00). Behavioral testing of all rats at 7, 14, and 28 days after continuous administration was performed. Dopamine, dihydroxyphenylacetic acid, and homovanillic acid contents were determined using high-performance liquid chromatography (Murase et al., 2006). All standards were from Sigma.

Parkinson's disease rat model established by stereotaxic 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) injection

Apart from rats in the control group, the rest of the rats were anesthetized and their heads fixed in a stereotaxic apparatus (Huaibei Zhenghua Biological Qquipment Co., Ltd, Suixi, Anhui Province, China). Using the Rat Brain Atlas of Paxinos & Watson, the position of the left substantia nigra pars compacta was defined at the bregma (anterioposterior 5.0 mm, lateral 2.1 mm). Subsequently, the skull surface was drilled and 2 μL (10 μg) MPTP injected through a microsyringe 7.7 mm below bregma, at an injection speed of 0.4 μL/min. The needle was maintained for 5 minutes and then withdrawn (Rubio-Osornio et al., 2009). In the control group, rats were injected with 2 μL saline instead of MPTP. At 7, 14, and 28 days after administration, behavioral

Table 1 Behavioral changes in Parkinson's disease (PD) rats (number of rats rotating to the left side: rotations/30 minutes) after *Zhichan* decoction and neural stem cell (NSC) transplantation

Time after administration (day)	7	14	28
NSC transplantation group	288.8±7.5	278.5±6.5	265.0±4.1
Model group	294.0±5.3	287.8±7.7[a]	295.0±7.2[b]
Zhichan decoction group	282.6±3.9	265.0±4.4	248.8±7.3
Control group	0	0	0

*P < 0.05, ** P < 0.01, *vs. Zhichan* decoction group. Data are expressed as mean ± SD of four rats at each time point in each group. Differences between groups were compared using analysis of variance and least significant difference tests. The PD model was defined as successful if rats rotated to the injured (left) side > 210 times within 30 minutes (> 7 rotations/minute). The number of rotations reflects disease severity and a reduction in rotations indicates improved symptoms. Our results show that the PD model was successfully established in the NSC transplantation, model, and *Zhichan* decoction groups. Compared with the model group, PD rat behavior was significantly improved 14 and 28 days after *Zhichan* decoction + NSC transplantation.

testing was performed. In brief, rats were subcutaneously injected with apomorphine (0.5 mg/kg; Sigma), and the total number of rats rotating to the injured (left) side with in 30 minutes was measured. Parkinson's disease model establishment was defined as successful if the rats rotated > 210 times within 30 minutes (> 7 rotations/min) (Liu et al., 2006; Zou et al., 2006). The number of rotations reflects the disease severity, and a reduction in rotations indicates improved symptoms.

Neural stem cell culture

Neural stem cells were isolated, cultured, and identified as previously described (Li et al., 2002). In brief, embryos were extracted from Wistar rats at pregnant 14 days, the midbrain was isolated, seeded onto a plastic culture flask, and mechanically triturated into a single cell suspension. The prepared suspension was cultured in plastic culture flasks at 5% CO_2, 37°C. After primary colonies appeared at 7–10 days, neurospheres were collected by centrifugation (at 1,000 r/min for 5 minutes), and trypsinized into single cell suspensions by pipetting. Prepared suspensions were cultured at a density of 1×10^8/L, and the culture medium replenished every 7 days for passaging. The neurospheres already formed were collected by centrifugation, and seeded onto poly-L-lysine-coated glass slides for immunocytochemical staining. Expression of nestin, a neural stem cell-specific protein, was determined. The number and growth of cultured neural stem cells were measured. A cell density of 1×10^6/mL and passage 3 cells were used for all following experiments (Encinas et al., 2008).

Detection of dopamine, dihydroxyphenylacetic acid, and homovanillic acid content in Parkinson's disease rat brain

The day after behavioral testing, Parkinson's disease rats were anesthetized and decapitated. Midbrain tissue was harvested, weighed, homogenized, and centrifuged (at 1,000 r/min for 5 minutes). Supernatants were collected and stored at –80°C until further use. For high performance liquid chromatography detection, 10 μL of each sample was thawed at room temperature. Peak areas were measured using the external standard method and then averaged. Linear regression lines were analyzed using the peak area (Y) and concentration (X). Recovery rates and precision degrees were also measured and then divided by the corresponding grams of brain tissue (Liu, 2002).

Establishing an optimization model

Genetic algorithm analysis was performed using Matlab 2011a software (MathWorks, Natick, MA, USA) to establish optimization models. The number of individuals selected from the population was 10, and we used a binary code length of 20, crossover probability of 0.95, and mutation probability of 0.08.

Statistical analysis

Measurement data are expressed as mean ± SD, and were statistically analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA). Differences between groups were compared using one-way analysis of variance and least significant difference tests. A P < 0.05 value was considered statistically significant.

Results

Quantitative analysis of experimental animals

There were no deaths or exclusions, and all 48 rats were included in the final analysis.

Behavioral changes in Parkinson's disease rats after *Zhichan* decoction and neural stem cell transplantation

The Parkinson's disease model was successfully established in neural stem cell transplantation, model, and *Zhichan* decoction groups. The number of rotations significantly increased and Parkinson's disease rat behavior significantly improved at 14 and 28 days after *Zhichan* decoction + neural stem cell transplantation, compared with the model group (P < 0.05, P < 0.01; **Table 1**).

Dopamine, dihydroxyphenylacetic acid, and homovanillic acid content in the midbrain of Parkinson's disease rats after *Zhichan* decoction and neural stem cell transplantation

Dopamine and dihydroxyphenylacetic acid content in the *Zhichan* decoction group were significantly higher than in the other three groups (P < 0.01). Dopamine content peaked at 7 days, gradually decreasing from 14 days, and was significantly decreased at 28 days, but still higher than in the neural stem cell transplantation group (P < 0.01). At 28 days after *Zhichan* decoction + neural stem cell transplantation, homovanillic acid levels were significantly increased compared with neural stem cell transplantation alone (P < 0.01). There were no significant differences in homovanillic acid levels at the other time points (P > 0.05). Lowest homovanillic acid levels were found in the neural stem cell transplantation group at 28 days (**Table 2**).

Global optimization of dopamine, dihydroxyphenylacetic acid, and homovanillic acid content in Parkinson's disease rats after *Zhichan* decoction and neural stem cell transplantation

In the *Zhichan* decoction group, the fitting equation between dopamine and dihydroxyphenylacetic acid is:

Table 2 Dopamine, DOPAC, and HVA content (nmol/g) in Parkinson's disease rats at 7, 14, and 28 days after *Zhichan* decoction and neural stem cell (NSC) transplantation

	Time after administration (day)	*Zhichan* decoction group	NSC transplantation group	Model group	Control group
Dopamine	7	86.8±4.7	50.2±2.8[a]	33.5±5.1[a]	59.8±3.3[a]
	14	81.8±27.1	49.9±4.8[a]	31.6±7.6[a]	60.0±4.4[a]
	28	46.5±1.1	39.5±2.6[a]	31.9±3.7[a]	60.1±3.0[a]
DOPAC	7	45.5±14.9	12.9±5.9[a]	3.6±1.0[a]	6.4±1.2[a]
	14	31.1±6.4	17.8±3.5[a]	4.7±1.1[a]	6.1±0.8[a]
	28	34.7±8.1	13.9±2.2[a]	2.6±0.7[*a]	6.4±1.01[a]
HVA	7	3.6±2.8	2.9±2.1	3.3±2.3	5.9±3.1
	14	4.9±4.1	2.9±2.6	2.9±2.1	6.2±1.9
	28	4.9±1.2	2.1±1.5[a]	2.9±1.6	6.3±1.3

[a]$P < 0.01$, *vs. Zhichan* decoction group at the same time point. Data are expressed as mean ± SD of four rats at each time point in each group. Differences between groups were compared using one-way analysis of variance and least significant difference tests. DOPAC: Dihydroxyphenylacetic acid; HVA: homovanillic acid.

$$y = 0.1247 \times 10^{-4}x^4 - 0.2616 \times 10^{-2}x^3 + 0.1438x^2 + 0.789x - 107.8348 \quad (1)$$

Using the genetic algorithm and dopamine = 65.24, maximal dihydroxyphenylacetic acid levels are 55.15 nmol/g.

In the *Zhichan* decoction group, the fitting equation between dopamine and homovanillic acid is:

$$y = 0.2777 \times 10^{-4}x^4 - 0.7798 \times 10^{-2}x^3 - 0.7961x^2 + 34.9285x - 552.0093 \quad (2)$$

Using the genetic algorithm and dopamine = 91.65, maximal homovanillic acid levels are 6.09 nmol/g.

In the neural stem cell transplantation group, the fitting equation between dopamine and dihydroxyphenylacetic acid is:

$$y = 0.1478 \times 10^{-2}x^4 + 0.2699x^3 - 18.3222x^2 + 547.7365x - 6{,}072.0989 \quad (3)$$

Using the genetic algorithm and dopamine = 53.51, maximal dihydroxyphenylacetic acid levels are 18.41 nmol/g.

In the neural stem cell transplantation group, the fitting equation between dopamine and homovanillic acid is:

$$y = 0.4249 \times 10^{-3}x^4 - 0.0759x^3 + 5.0044x^2 - 144.0305x + 1{,}528.4161 \quad (4)$$

Using the genetic algorithm and dopamine = 44.99, maximal homovanillic acid levels are 4.53 nmol/g.

In the model group, the fitting equation between dopamine and dihydroxyphenylacetic acid is:

$$y = 0.7612 \times 10^{-3}x^4 - 0.0959x^3 + 4.4890x^2 - 92.1514x + 703.1392 \quad (5)$$

Using the genetic algorithm and dopamine = 22.17, maximal dihydroxyphenylacetic acid levels are 4.33 nmol/g.

In the model group, the fitting equation between dopamine and homovanillic acid is:

$$y = 0.6609 \times 10^{-3}x^4 - 0.0819x^3 + 3.7553x^2 - 74.9645x + 550.6835 \quad (6)$$

Using the genetic algorithm and dopamine = 39.61, maximal homovanillic acid levels are 4.25 nmol/g.

In the control group, the fitting equation between dopamine and dihydroxyphenylacetic acid is:

$$y = 0.5819 \times 10^{-2}x^4 - 1.3904x^3 + 124.4835x^2 - 4{,}949.5149x + 73{,}743.2991 \quad (7)$$

Using the genetic algorithm and dopamine = 63.96, maximal dihydroxyphenylacetic acid levels are 6.70 nmol/g.

In the control group, the fitting equation between dopamine and homovanillic acid is:

$$y = 0.9448 \times 10^{-2}x^4 + 2.2073x^3 - 193.0269x^2 + 7{,}487.3203x - 108{,}678.6567 \quad (8)$$

Using the genetic algorithm and dopamine = 55.46, maximal homovanillic acid levels are 9.35 nmol/g.

We showed that in the *Zhichan* decoction group, with dopamine = 65.24, maximal dihydroxyphenylacetic acid levels are 55.15 nmol/g, and with dopamine = 91.65, maximal homovanillic acid levels are 6.09 nmol/g. In the neural stem cell transplantation group, with dopamine = 53.51, maximal dihydroxyphenylacetic acid levels are 18.41 nmol/g, and with dopamine = 44.99, maximal homovanillic acid levels are 4.53 nmol/g. In the model group, with dopamine = 22.17, maximal dihydroxyphenylacetic acid levels are 4.33 nmol/g, and with dopamine = 39.61, maximal homovanillic acid levels are 4.25 nmol/g. In the control group, with dopamine = 63.96, maximal dihydroxyphenylacetic acid levels are 6.70 nmol/g, and with dopamine = 55.46, maximal homovanillic acid levels are 9.35 nmol/g (**Tables 3, 4**).

Therefore, in the brain of Parkinson's disease rat models neural stem cell transplantation + *Zhichan* decoction can potentially increase dihydroxyphenylacetic acid levels up to 10 times, while neural stem cell transplantation alone, only 3 times. Homovanillic acid levels were not increased signifi-

Table 3 The fitting equation between dopamine and DOPAC, maximal DOPAC content (nmol/g) in each group

Group	The fitting equation between dopamine and DOPAC	Maximal DOPAC content
Zhichan decoction	y=0.1247×10$^{-4}x^4$–0.2616×10$^{-2}x^3$+0.1438x^2+ 0.7896x–107.8348	55.15
NSC transplantation	y=0.1478×10$^{-2}x^4$+0.2699x^3–18.3222x^2+ 547.7365x–6,072.0989	18.41
Model	y=0.7612×10$^{-3}x^4$–0.0959x^3+4.4890x^2 –92.1514x–703.1392	4.33
Control	y=0.5819×10$^{-2}x^4$–1.3904x^3+124.4835x^2 –4,949.5149x–73,743.2991	6.70

DOPAC: Dihydroxyphenylacetic acid; NSC: neural stem cell.

Table 4 The fitting equation between dopamine and HVA, maximal HVA content (nmol/g) in each group

Group	The fitting equation between dopamine and HVA	Maximal HVA content
Zhichan decoction	y=0.2777×10$^{-4}x^4$+0.7798×10$^{-2}x^3$–0.7961x^2+ 34.9285x–552.0093	6.09
NSC transplantation	y=0.4249×10$^{-3}x^4$–0.0759x^3+5.0044x^2– 144.0305x–1,528.4161	4.53
Model	y=0.6609×10$^{-3}x^4$–0.0891x^3+3.7553x^2 –74.9645x+550.6835	4.25
Control	y=0.5819×10$^{-2}x^4$–1.3904x^3+124.4835x^2 –4,949.5149x–73,743.2991	9.35

HVA: Homovanillic acid; NSC: neural stem cell.

cantly after treatment with neural stem cell transplantation + *Zhichan* decoction.

Discussion

Directed neural stem cell differentiation into dopaminergic neurons and long-term survival after transplantation, remain a fundamental problem of neural stem cell transplantation in Parkinson's disease treatment. Some Chinese herbs induce stem cell differentiation and promote stem cell proliferation (Yang et al., 2008). Chinese medicine compound recipes are characterized by a holistic, multi-target treatment strategy, and may influence genes and cytokines in the brain microenvironment through multiple channels, thereby contributing to survival and differentiation of transplanted neural stem cells (Li et al., 2001). However, it is still unknown if these Chinese herbs induce neural stem cell differentiation into dopaminergic neurons, promote survival of transplanted neural stem cells or dopaminergic neurons, and/or affect levels of dopamine and its metabolites.

Growing evidence shows that *Zhichan* decoction improves the brain microenvironment and neuronal metabolism in Parkinson's disease patients and animals, likely favoring survival of transplanted neural stem cells and promotion of neuronal differentiation (Shi et al., 2011). Nevertheless, neural stem cell differentiation and integration in the host is affected by many factors. We found that raw dopamine, dihydroxyphenylacetic acid, and homovanillic acid content was nonlinear during the experimental period. A correlation algorithm has previously been adopted to examine the relationship between *Zhichan* decoction and dopamine and its metabolites, in the Parkinson's disease rat brain after neural stem cell transplantation, although the results showed that changes in dopamine, dihydroxyphenylacetic acid, and homovanillic acid follow a non-linear relationship (Shi et al., 2012).

To further examine the role of *Zhichan* decoction on dopamine and its metabolites in the Parkinson's disease rat brain after neural stem cell transplantation, we normalized the data and established polynomial fitting equations, with dopamine content as an independent variable, and dihydroxyphenylacetic acid or homovanillic acid levels as dependent variables. Because the correlation equation is nonlinear, there is no exact solution method (Zhong, 2010), and contemporary intelligent optimization algorithms, such as genetic algorithms, are suggested to obtain an optimal solution (Li, 2008; Zhang et al., 2010). We show that in the *Zhichan* decoction group, with dopamine = 65.24, maximal dihydroxyphenylacetic acid levels are 55.15 nmol/g, and with dopamine = 91.65, maximal homovanillic acid levels are 6.09 nmol/g. In the neural stem cell transplantation group, with dopamine = 53.51, maximal dihydroxyphenylacetic acid levels are 18.41 nmol/g, and with dopamine = 44.99, maximal homovanillic acid levels are 4.53 nmol/g. In the model group, with dopamine = 22.17, maximal dihydroxyphenylacetic acid levels are 4.33 nmol/g, and with dopamine = 39.61, maximal homovanillic acid levels are 4.25 nmol/g. In the control group, with dopamine = 63.96, maximal dihydroxyphenylacetic acid levels are 6.70 nmol/g, and with dopamine = 55.46, maximal homovanillic acid levels are 9.35 nmol/g. The highest dihydroxyphenylacetic acid levels were found in the *Zhichan* decoction group, being 10 times as high as the model group. Levels in the neural stem cell transplantation group were lower than the *Zhichan* decoction group, but 3 times as high as the model group. In addition, in the normal group dihydroxyphenylacetic acid peaked at a higher level than the model group, indicating that neural stem cell transplantation increases dihydroxyphenylacetic acid levels and relieves Parkinson's disease symptoms, and *Zhichan* decoction enhances these therapeutic effects by significantly increasing dihydroxyphenylacetic acid levels. Similarly, we also found homovanillic acid levels in the *Zhichan* decoction group were higher than in the neural stem cell transplantation group, and levels in the neural stem cell transplantation group higher than the model group. This suggests that both *Zhichan* decoction and neural stem cell transplantation increase homovanillic acid levels in Parkinson's disease animals, although the increment was limited. The fact that *Zhichan* decoction enhances the therapeutic effect of neural stem cell transplantation for Parkinson's disease, and has an impact on dopamine and its metabolites, provides experimental evidence for *Zhichan* decoction as a potential Parkinson's disease therapy with certain innovations.

In summary, compared with the model group, transplanted neural stem cells increase dopamine content, and *Zhichan* decoction + neural stem cell transplantation increase dopamine content further. This indicates that *Zhichan* decoction may influence genes and cytokines in the brain microenvironment of Parkinson's disease animals through multiple pathways. It is clear that *Zhichan* decoction may also contribute to functional restoration of dopaminergic neurons in the brain and promote differentiation of endogenous neural stem cells into dopaminergic neurons; further studies will focus on these issues. There may be errors with establishing a nonlinear equation using a polynomial fitting method, and even the optimal solution obtained from genetic algorithms is possibly only an approximate optimal solution. The present study demonstrates that *Zhichan* decoction increases the levels of dopamine and its metabolites in the substantia nigra of Parkinson's disease rats after neural stem cell transplantation, and further quantifies its efficacy. Our findings reduce the lagging role of traditional Chinese medicine in information- and digitalized technology-based modern medicine, and more broadly, explore the better combination of Chinese herbal medicine with informatic approaches.

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Author contributions: *Yang XM was responsible for the paper. Shi HF and Yang XM were responsible for the study concept and design, supervising the study, and in charge of the funds. Shi HF, Song J and Yang XM analyzed data, wrote the manuscript, supervised the manuscript, conducted statistical analysis, and provided technical or information support. All authors approved the final version of the manuscript.*

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