黄连生物碱在大鼠体内的代谢转化及分布

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[摘要] **目的**:测定黄连生物碱在大鼠体内的药代动力学、组织分布,及药根碱、黄连碱、巴马汀、小檗碱 4 种生物碱在大鼠体内的转化。**方法**:大鼠分别灌胃黄连总碱、小檗碱,采用反相高效液相色谱法测定大鼠的血浆、组织、胃肠道中黄连 4 种生物碱的含量。**结果**:小檗碱在大鼠体内的血药浓度出现 2 个峰值,达峰时间分别 2,5 h,其中血药浓度分别为 C_{max} 3.7,2.8 mg·L^{-1};小檗碱在大鼠血液中可以转化为药根碱;给大鼠灌胃黄连总生物碱后,小檗碱在大鼠胃中的浓度单调下降,而黄连碱、巴马汀和药根碱逐渐增加,表明在大鼠的胃中小檗碱可转化为药根碱;小檗碱和巴马汀主要分布在动物的肺部,其次分布在肝脏中,而药根碱和黄连碱主要分布在动物的肝脏中,其次分布在肺部。**结论**:小檗碱可以转化为药根碱,根据胃肠道推进实验部分解释了小檗碱在血液中出现 2 次峰值的原因。

[关键词] 黄连总生物碱;高效液相色谱法;药代动力学;分布;转化

黄连为毛茛科多年生草本植物黄连 *Coptis chinensis* Franch 的根茎[1],其活性成分为黄连生物碱。石柱黄连(味连)含小檗碱 50% 左右,而小檗碱、药根碱、巴马汀和黄连碱等 4 个生物碱之和占总生物碱的 90% 左右[2]。有关小檗碱的药代动力学研究较多[3-4],其他黄连生物碱的药代动力学研究相对较少[5]。中药成分在动物体内的代谢与作用网络具有"网通虹势"规律[6-7]:中药材成分群往往是同一母核的多种衍生物, 药理作用相似;同一母核的有效成分衍生物群在动物内可以相互抑制、加速及转化, 其代谢网络相容相通;动物内存在巨大的与自然界类似的以 CYP450 酶为主导的代谢酶网络系统,按 CYP450 进化树的关系对中药及复方成分组群进行代谢和产生药效。黄连生物碱的结构类似,均是异喹啉类生物碱的衍生物。这些生物碱之间在动物体内是否存在相互之间的转化,目前尚未见报道。

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1 材料

1.1 动物 SD 大鼠,体重(200 ± 20) g,雌雄各半,由重庆医科大学提供,合格证号 SCXK(渝)2007-0001。

1.2 仪器与试剂 LC-6AD 高效液相色谱仪(日本岛津);KQ5200DA 型数控超声波清洗器;TG16-W 微量高速离心机,组织匀浆器。

巴马汀(批号 080125)、黄连碱(批号 080213)、药根碱(批号 080215)、小檗碱(批号 080322)等均购自成都曼思特生物科技有限公司,含量 98% 以上。黄连总生物碱由实验室自制,小檗碱、巴马汀、药根碱和黄连碱 4 个生物碱之和为 90%。甲醇和乙腈为色谱纯,其他试剂为分析纯。

2 方法

2.1 色谱条件 色谱柱 Hypersil C_{18}(4.6 mm × 200 mm,5 μm),流动相乙腈-磷酸二氢钾(28∶72),流速 1.0 mL·min^{-1},柱温 30 ℃,检测波长 345 nm[8]。流动相的配制:由乙腈与醋酸缓冲体系(7.7 g 醋酸铵,12 mL 冰醋酸,定容于 500 mL 蒸馏水中)按 30∶70 组成,流速 1 mL·min^{-1};采用二极管阵列检测器,检测波长 345 nm;色谱柱 ODS-C_{18}(4.6 mm × 250 mm,5 μm);进样量 20 μL。

对照品溶液的配制:精密称取小檗碱、药根碱、巴马汀、黄连碱对照品各 10 mg,置于量瓶中,加甲醇溶解并定容至 100 mL,摇匀,配得质量浓度为 100 mg·

L^{-1}的对照品溶液;再取 0.1 mL 对照品溶液,定容到 100 mL,得质量浓度为 100 μg·L^{-1}的对照品溶液。

2.2 动物分组 SD 大鼠,平衡喂养 3 d 后,随机分成 4 组:其中前 3 组每组 4 只,雌雄各半;第 4 组 12 只,雌雄各半。第 1 组灌胃黄连总生物碱进行血药浓度试验;第 2 组灌胃小檗碱进行血药浓度试验;第 3 组灌胃总生物碱进行组织分布试验;第 4 组灌胃总生物碱进行胃肠道推进试验。

2.3 血药浓度 黄连总生物碱:大鼠按照 800 mg·kg^{-1}体重灌胃黄连总生物碱。争取在 5 min 内灌完,以减少误差。灌胃后分别于 1,2,3,4,5,6,7 h 后眼眶取血 0.8 mL,置于肝素化的离心管中,3 000 ×*g*离心 15 min,得血清 0.2 mL,添加 0.6 mL 甲醇,漩涡 1 min,离心,上清液 70 ℃下烘干,再用 0.2 mL 甲醇超声溶解,离心,微孔滤膜过滤。按照 **2.1** 色谱条件测定黄连生物碱含量。

小檗碱:大鼠按照 400 mg·kg^{-1}体重灌胃小檗碱。争取在 5 min 内灌完,以减少误差。灌胃后分别于 0.5,1,1.5,2,2.5,3 h 后眼眶取血 0.8 mL,置于肝素化的离心管中,3 000 ×*g* 离心 15 min,得到血清 0.2 mL,添加 0.6 mL 甲醇,漩涡 1 min,离心,上清液 70 ℃下烘干,再用 0.2 mL 甲醇超声溶解,离心,微孔滤膜过滤。按照 **2.1** 色谱条件测定黄连生物碱含量。

2.4 组织分布 大鼠按照 800 mg·kg^{-1}体重灌胃黄连总生物碱。灌胃后 2 h,宰杀大鼠,分取肝脏、心脏、肺、肾脏、脾脏、脑、血液。脏器用生理盐水冲洗表面血迹,滤纸吸干,称重,磨碎。用 20 mL 甲醇超声提取,取上清液 1 mL,烘干,再用 0.2 mL 甲醇超声提取,离心,微孔滤膜过滤。按照 **2.1** 色谱条件测定黄连生物碱含量。

2.5 肠胃推进 大鼠按照 800 mg·kg^{-1}体重灌胃黄连总生物碱。分别在灌胃 1,2,3,4,5,6 h 处死,分别取胃、小肠、大肠,将器官剪开,外翻,20 mL 甲醇超声提取,离心取上清液 1 mL,用甲醇稀释至 10 mL,微孔滤膜过滤。按照 **2.1** 色谱条件测定黄连生物碱含量。

3 结果

3.1 黄连总生物碱在大鼠体内的药代动力学 对大鼠灌胃黄连总生物碱后,总生物碱在体内的血药浓度变化见图 1。大鼠灌胃黄连总生物碱后 2 h 左右,小檗碱在大鼠血液中的含量出现 1 个极大值(3.7 mg·L^{-1});然后血药浓度下降,到 5 h 左右出现第 2 个极大值(2.8 mg·L^{-1})。黄连碱、巴马汀和药根碱在血液中的浓度含量较低(总生物碱中黄连碱、巴马汀和黄连碱的含量本身也很低),达峰时间分别为 2,5 h。与文献报道结果类似[5]。

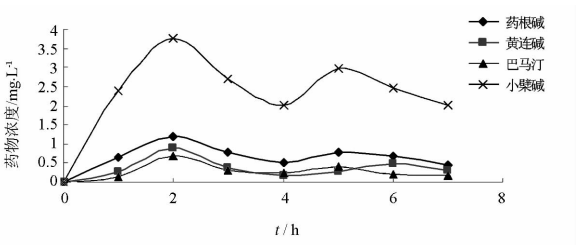

图 1 黄连总碱血药浓度曲线

3.2 小檗碱在大鼠体内的药代动力学 对大鼠单独灌胃小檗碱后,小檗碱在体内的血药浓度变化见表 1。随着时间的增加,小檗碱在血液中的含量不断增加,达峰时间为 2 h 左右。同时,根据 HPLC 分析结果,从大鼠血液中不仅检测到小檗碱,还检测到药根碱。在 0.5 h 时,药根碱在血液中的质量浓度达到(0.44 ±0.02) μg·L^{-1},随着时间延长,药根碱的含量逐渐增加,1.5 h 达到最大血药浓度,然后逐渐下降。表明部分小檗碱在血液中可能转化为药根碱。

表 1 大鼠灌胃小檗碱后血液中生物碱的含量变化

μg·L^{-1}

t/h	小檗碱	药根碱
0	0	0
0.5	2.05 ±0.05	0.34 ±0.02
1	3.71 ±0.04	0.56 ±0.02
1.5	7.99 ±0.04	1.19 ±0.03
2	9.02 ±0.06	0.49 ±0.05
2.5	6.78 ±0.05	0.42 ±0.04
3	3.99 ±0.04	0.30 ±0.03

3.3 黄连总生物碱在大鼠体内的分布 文献有关小檗碱组织分布取点时间为 0.25,0.75,6 h[9],但未对血药浓度达高峰时间检测,根据实验,小檗碱达峰值在 2.0 h 左右,因此选择在 2.0 h 进行组织分布实验。黄连生物碱在大鼠各器官中的分布见表 2。小檗碱主要分布在大鼠的肺中,与文献报道一致[9]。巴马汀的分布与小檗碱完全类似,主要分布在肺中,其次分布在肝脏中。而黄连碱和药根碱的分布与小檗碱和巴马汀不同,主要分布在肝脏中,其次分布在肺中。

表 2　黄连总生物碱在大鼠组织中的分布

器官	质量/g	药根碱/μg·kg^{-1}	黄连碱/μg·kg^{-1}	巴马汀/μg·kg^{-1}	小檗碱/μg·kg^{-1}	总生物碱/μg·kg^{-1}
肺	2.033 ±0.145 6	274.2 ±4.64	312.2 ±6.18	376.2 ±5.50	1257.5 ±7.59	2220.2 ±21.07
肝	9.956 ±0.043 5	338.7 ±3.86	535.2 ±9.74	288.0 ±4.52	702.2 ±5.90	1863.2 ±10.75
脾	0.478 ±0.072 3	96.2 ±0.76	126.0 ±6.83	75.1 ±0.74	375.2 ±7.02	673.0 ±10.81
肾	2.342 ±0.109 8	162.7 ±5.37	163.7 ±6.23	41.5 ±0.99	228.5 ±5.97	596.5 ±6.67
心	1.058 ±0.068 4	72.4 ±0.73	44.2 ±1.14	57.4 ±1.08	95.5 ±1.67	269.5 ±2.75
脑	2.033 ±0.094 3	22.4 ±0.81	109.2 ±2.63	8.07 ±0.09	36.3 ±1.22	176.0 ±4.44

3.4　黄连总生物碱在胃肠道中的推进试验　大鼠灌胃黄连总生物碱后，黄连总生物碱在胃中的含量变化见图 2。大鼠灌胃黄连总生物碱后，在 3 h 以前，小檗碱浓度变化很小；3 h 以后小檗碱含量明显下降，4 h 左右达到很低的水平。药根碱、巴马汀、黄连碱的含量在 0 ~4 h 含量逐步增加，4 h 达到最大值，分别为 36.64，20.31，77.72 mg·L^{-1}，然后开始下降，形成 1 个峰值。大鼠灌胃黄连总生物碱后，黄连总生物碱在小肠中的含量变化见图 3。随着时间的增加，小肠中生物碱的含量均单调下降。大鼠灌胃黄连总生物碱后，黄连总生物碱在大肠中的含量变化见图 4。大肠是接近代谢的终端，在大肠中小檗碱出现了 2 个峰值，2 次代谢高峰；黄连碱、巴马汀和药根碱也均出现 2 个峰值，只是它们的浓度明显低于小檗碱。

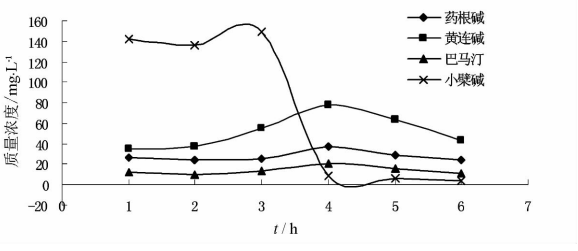

图 2　黄连总碱在胃中分布曲线

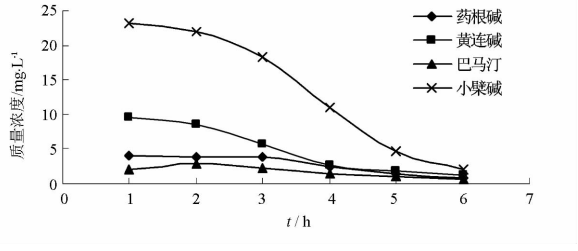

图 3　黄连总碱在小肠中分布曲线

4　讨论

大鼠灌胃小檗碱后，在血液中检测到药根碱，表明小檗碱在大鼠体内部分转化为药根碱，该实验现象尚属首次发现。朱志勇[10]给大鼠灌胃小檗碱后，从大鼠的尿液中发现了大量的转化产物（次生代谢产物）。这些研究均表明小檗碱在动物体内可以迅速转化。小檗碱和药根碱均为来自于黄连的具有相同母核的类似活性成分，根据“网通虹势”理论，小檗碱转化为药根碱甚至于其他黄连生物碱是“正常现象”。

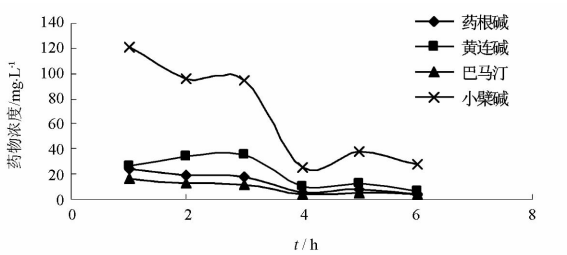

图 4　黄连总碱在大肠中分布曲线

给大鼠灌胃黄连生物碱后，在 3 h 以前，小檗碱缓慢下降，3 ~4 h 迅速下降；大鼠灌胃后，2 h 左右开始进食，食物推动胃中的小檗碱向小肠和大肠推进，是导致 3 ~4 h 大鼠胃中小檗碱迅速下降的原因。与小檗碱的变化规律不同，黄连碱、巴马汀和药根碱在胃中的浓度首先是增加，并于 4 h 左右达到最大值，然后下降，在 4 h 左右形成 1 个峰值。按照“网通虹势”理论中药活性成分相互转化和平衡的观点，胃中的酶系丰富，高浓度的小檗碱转化成为低浓度的黄连碱、巴马汀和药根碱是很有可能的过程。有关结论及机制需进一步证明。

有文献[12]报导，小檗碱经胃肠道吸收后，在体内分布快而广，在肺中含量最高。本实验测定的结果表明，黄连中的不同生物碱在组织中分布的并不相同：小檗碱和巴马汀主要分布在肺中，其次分布在肝脏中，这与文献报道结果类似[11]；与小檗碱的分布不同，黄连碱和药根碱主要分布在肝脏中，其次分布在肺中。由于存在脑屏障，小檗碱等药物在脑的

分布较少[11],本实验的研究也发现小檗碱在大脑中的含量很低;但是,黄连碱和药根碱在大脑中的含量却稍高,提示黄连碱和药根碱有穿过血脑屏障的能力,其原因有待进一步研究。

有文献[3,5]报道,给大鼠灌胃小檗碱(或黄连生物碱)后,在血液中会出现了 2 个峰值。有文献[12]报道,大鼠灌胃黄连 1 h,就可以看到黑便排出,黄连在体内排泄很快,因此,1 h 后,就在大肠中检测到了高浓度的小檗碱。在大肠中,小檗碱又出现了 2 次峰,可能是药物在体内存在肝肠循环或其他循环过程(胃肠循环、肠肠循环)等。从组织分布结果来看,小檗碱在肝脏中的含量很高,肝肠循环可能是小檗碱在血液中会出现 2 次吸收峰[3,5,13]的原因。

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Metabolism, transformation and distribution of *Coptis chinensis* total alkaloids in rat

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[**Abstract**] **Objective**: To determine the pharmacokinetics, distribution and mutual transformation of the total alkaloids, jatrorrhizine, coptisine, berberine and palmatine from *Coptis chinensis* in rats. **Method**: After the total alkaloids and berberine were fed into rats, their contents in plasma, tissues and gastrointestinal tract were determined by reversed-phase HPLC. **Result**: The peak times of berberine in blood were 2.0 h (C_{max} 3.7 mg · L^{-1}) and 5.0 h (C_{max} 2.8 mg · L^{-1}), respectively. Berberine in rat blood can be transformed into jatrorrhizine. After the rats were fed with the total alkaloids by gavage, the content of berberine was decreased monotonously, while coptisine, palmatine and jatrorrhizine contents were increased gradually in the stomach, it speculated that berberine may be transformed into jatrorrhizine in the stomach. Animal experiments showed that berberine and palmatine were mainly distributed in the lungs of animals, followed by the distribution in the liver, while jatrorrhizine and coptisine was mainly in the liver, then in the lungs. **Conclusion**: Berberine could transform into jatrorrhizine. The mechanism on the appearance of two maximum blood concentration of berberine in blood could be explained with the propulsion of the gastrointestinal tract partly.

[**Key words**] *Coptis chinensis* total alkaloids; reversed-phase HPLC; pharmacokinetics; distribution; transformation

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Protective effect of effective part of *Acanthopanacis senticosus* on damage of PC12 cells induced by MPP^+

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[**Abstract**] The purpose of our research was to evaluate the protective effect of the effective part of *Acanthopanacis senticosus* (AS) on the damage of PC12 cells induced by MPP^+, an *in vitro* cell model for Parkinson's disease. Cell viability and apoptosis of PC12 cells induced by MPP^+ were assayed by MTT and flow cytometry respectively in the prescence or absence of the effective part of AS. The contents of lactate dehydrogenase (LDH), nitric oxide (NO), nitric oxide synthase (NOS) and malondialdehyde (MDA) were determined by UV spectrophotometer. Our study showed that the survival rate of PC12 cells was markedly increased while cell apoptosis was decreased in the range of 200 to 400 mg · L^{-1} of the effective part of AS. The contents of LDH, NO, NOS, MDA were reduced. Our experimental results indicated that the effective part of AS had the protective effect on the damage of PC12 cells induced by MPP^+. The underlying mechanisms might be the combination of reduction of LDH leakage and decreases in the contents of NO, NOS and MDA, and finally prevent the apoptosis in PC12 cells and increase the cell survival rate.

[**Key words**] *Acanthopanacis senticosus*; PC12 cells; Parkinson's disease

Parkinson's disease (PD) is a common neurodegenerative disease of the central nervous system, which has seriously affected the patient's mobility and quality of life. Although PD has been studied for many years, but there is no effective treatment so far. Traditional Chinese medicines contain a variety of chemical compositions which could function at multiple targets of disease. Thus the traditional Chinese medicines may have unique advantages in the treatment of PD which is a multifactorial disease. A large number of clinical and experimental data showed that Chinese medicine had an obvious therapeutic effect, such as, delaying the process of PD, improving the efficacy of foreign medicine, reducing the toxic side-effects, controlling nonmotile symptoms of PD, and protecting nerve cells, etc [1].

Acanthopanacis senticosus (Rupr. Et Maxim), belongs to Araliaceae, is normally used in medicine for its dry root and rhizome which contains a variety of glycosides. It functions well in nourishment of Qi by invigorating spleen and tonifying kidney and relieving mental strain . This medicine has been used to cure symptoms such as ache and faint in waist and knee, weakness in body, insomnia, multiple-dream, inappetence and so on. The medicine has also been used to accommodate blood pressure, treat coronary artery disease, nervous prostration, ect [2]. The effective part of AS has been selected through pharmacodynamics experiments. The aqueous extract of the crude drug was firstly eluted with macroporous resin column to obtain 50% ethanol eluate, then a gel silica column chromatography was used to collect the eluate when the ratio of methylene bichloride and methyl alcohol reach to 10∶1. The protective effect of the effective part of AS on the damage of PC12 cells induced by MPP^+, an *in vitro* PD cell model, was investigated.

1 Experimental apparatus and materials

Inverted microscope, philippines Co. (USA); ELIASA, Mofeishier equipment Ltd. Co. Shanghai China; Flow cytometer, BD Co. (USA). PC12 cells

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were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences; 1-methyl-4-phenylpyridine ion (MPP^+), MTT and tetramethyl azo azole were purchased from Sigma Co. (USA); DMEM medium was purchased from GIBCO corporation; Normal blood serum (NBS) was purchased from Hangzhou Sijiqing company.

2 Experimental methods

2.1 The culture of cell line

PC12 cells were frozen and stored in liquid nitrogen, and recovered before use. Cells were primarily cultured in DMEM supplemented with 10% NBS, 1% mycillin mixed liquor and 1% *L*-gln-glutamine, and maintained at 37 ℃ in a humidified 5% CO_2-95% air incubator. Cells were passaged every 2 or 3 days, and cells used in all the experiments were in the logarithmic phase of growth.

2.2 Cell viability assay

2.2.1 The relationship between different concentrations of MPP^+ and PC12 cells′ survival rate Cell viability was assessed using MTT assay in 5×10^3 PC12 cells seeded in a 96-well plate. After 24 h incubation, media was changed and MPP^+ was added to the final concentrations of 100, 300, 500, 700 μmol · L^{-1}. Further culture of those cells was done at shown time points. At the end, MTT (20 μL of 5 g · L^{-1}) was added and the cells were incubator for 4 h in the tissue culture incubator. Crystal was dissolved by adding 150 μL DMSO after removal of culture medium, and vibrating for 10 min. Absorbance of the samples was measured at 490 nm and the reference wavelength was set at 630 nm. The cell survival rate was calculated using the equation: Cell survival rate = Light absorption value of the treatment group / Light absorption value of the control group × 100%.

2.2.2 Determination of the effective part of AS on the damage of PC12 cells induced by MPP^+ The effective part of AS at 25, 50, 100, 200, 400, 800 mg · L^{-1} were supplemented into the media along with 300 mg · L^{-1} MPP^+. Cell morphology was observed and recorded using inverted microscope at 48 h time point, and cell survival rate was calculated.

2.3 Analysis of apoptosis by flow cytometry

2×10^5 PC12 cells were cultured in flask in 1 mL volume. After 24 h incubation, the old media was replaced with fresh media contained 300 μmol · L^{-1} MPP^+ and 400 μmol · L^{-1} of the effective part of AS. Cells were detached by trypsin at the end of 48 h further culture, and cells were collected by centrifugating at 1 500 r · min^{-1} for 5 min. After twice washing with PBS, 5 μL Annexin V-FITC (20 mg · L^{-1}) and 10 μL PI staining solution (5 mg · L^{-1}) were added to 500 μL cell suspension and stained for 5 min at ambient temperature and protected from light prior to flow cytometery assay. The obtained data was analyzed by CellQuest software.

2.4 Assessment of the contents of LDH, NO, NOS, MDA

0.5×10^5 PC12 cells were seeded in 6-well plate in a volume of 1 mL. After 24 h culture cells were co-treated with 300 μmol · L^{-1} MPP^+ and 400 mg · L^{-1} of the effective part of AS. At the end of 48 h further culture, the supernatant of culture medium was collected and divided into 4 parts which were used for the measurements of the contents and activities of LDH, NO, NOS and MDA by UV spectrometer.

2.5 Statistical treatment

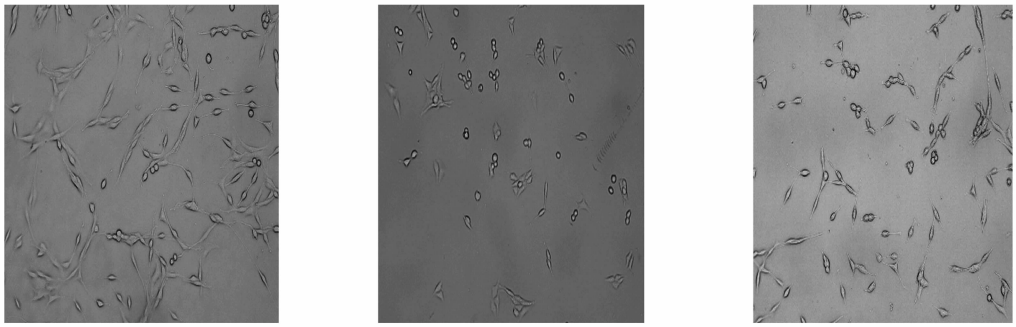
The data of all arrays were expressed by $\bar{x} \pm s$, we adopted SPSS software to carry out t test between every two groups. Differences with $P < 0.05$ were considered statistically significant.

3 Results

3.1 The effect of MPP^+ and the effective part of AS on PC12 cells′ morphology

The control group, the MPP^+ model group and the treatment group were all observed under light microscopy. PC12 cells in the control group were shuttle-type shape and distributed evenly which indicated cells were in good condition. The PC12 cells induced by MPP^+ had morphology changes in the model group. The number of shuttle-type cells decreased, while spherical or oval-shaped cells began to appear, as the action time prolonged and the dose increased, the quantity of spherical or oval-shaped cells was in-

creased. These morphology changes indicated that MPP^+ had the injury effect on PC12 cells. In the effective part of AS treatment group, PC12 cells were also in good condition, shuttle-type cells were obviously to see, spherical or oval-shaped cells were rarely seen, the cells were relatively well-distributed (Figure 1).



A. control group; B. model group; C. treatment group.

Figure 1 Effect of MPP^+ and effective part of AS on PC12 cells' morphology

3.2 The effect of MPP^+ on survival rate of PC12 cells

PC12 cells were interfered by MPP^+ at different concentrations. Incubated for 12 h, the cell survival rate decreased gradually, but there was no statistical significance. After the treatment time has been prolonged for another 12 h, the cell survival rate decreased significantly. When the concentrations of MPP^+ were 100, 300 $\mu mol \cdot L^{-1}$, the differences with $P < 0.05$ were considered to be statistically significant. 500, 700 $\mu mol \cdot L^{-1}$ groups had dramatically significant difference ($P < 0.01$). After being interfered by MPP^+ for 48 h, cell survival rate decreased more obviously, All groups of MPP^+ at different concentrations had exceedingly significant differences ($P < 0.001$). This showed that MPP^+ could decrease PC12 cells' survival rate, with the final concentration ranging from 100 $\mu mol \cdot L^{-1}$ to 700 $\mu mol \cdot L^{-1}$. The decrease of P12 cell survival by MPP^+ is dose- and time- dependent. As many relative factors had been taken into consideration, we chose 300 $\mu mol \cdot L^{-1}$ as the final concentration of MPP^+, and 48 h as the interaction time to be the modeling condition in our follow-up experiments(Table 1).

Table 1 The effect of MPP^+ on the survival rate of PC12 cells and absorbability ($\bar{x} \pm s, n = 12$)

MPP^+ /$\mu mol \cdot L^{-1}$	Time					
	12 h		24 h		48 h	
	Absorbance	Survival rate/%	Absorbance	Survival rate/%	Absorbance	Survival rate/%
Control	0.346 ± 0.07	100	0.366 ± 0.06	100	0.720 ± 0.06	100
100	0.312 ± 0.03	90.2	0.319 ± 0.03[1)]	87.2	0.379 ± 0.02[3)]	52.6
300	0.303 ± 0.07	87.6	0.308 ± 0.03[1)]	84.2	0.376 ± 0.05[3)]	52.2
500	0.301 ± 0.06	87.0	0.303 ± 0.03[2)]	82.8	0.349 ± 0.04[3)]	48.5
700	0.298 ± 0.05	86.1	0.293 ± 0.02[2)]	80.1	0.345 ± 0.04[3)]	47.9

Note: As compared with the control group, [1)] $P < 0.05$, [2)] $P < 0.01$, [3)] $P < 0.001$.

3.3 The effect of the effective part of AS on the damage of PC12 cells induced by MPP^+

After treatments by the effective part of AS at various, PC12 cell survival rate was gradually rescued when compared with the model group. When the final concentration reached 200 $mg \cdot L^{-1}$ or 400 $mg \cdot L^{-1}$, there was statistical significant difference with $P < 0.05$ (Table 2, 3). This showed that the effective part of AS had the

protective effect on the damage of PC12 cells induced by MPP^+, and according to these studies, 400 mg · L^{-1} was chosen as the final work concentration of the effective part of AS in our further research.

Table 2 The effect of different concentrations of the effective part of AS on the damage of PC12 cells induced by MPP^+ ($\bar{x} \pm s, n = 12$)

Group	Concentration	Absorbance	Survival rate/%
Control group	–	0.41 ± 0.08	100
Model group	MPP^+ 300 μmol · L^{-1}	0.22 ± 0.04	54
Treatment group	25 mg · L^{-1} + MPP^+ 300 μmol · L^{-1}	0.24 ± 0.06	59
	50 mg · L^{-1} + MPP^+ 300 μmol · L^{-1}	0.25 ± 0.02	61
	100 mg · L^{-1} + MPP^+ 300 μmol · L^{-1}	0.26 ± 0.04	63

Table 3 The effect of different concentrations of the effective part of AS on the damage of PC12 cells induced by MPP^+ ($\bar{x} \pm s, n = 3$)

Group	Concentration	Absorbance	Survival rate/%
Control group	–	0.41 ± 0.08	100
Model group	MPP^+ 300 μmol · L^{-1}	0.22 ± 0.03	54
Treatment group	25 mg · L^{-1} + MPP^+ 300 μmol · L^{-1}	0.24 ± 0.06	59
	50 mg · L^{-1} + MPP^+ 300 μmol · L^{-1}	0.25 ± 0.02	61
	100 mg · L^{-1} + MPP^+ 300 μmol · L^{-1}	0.26 ± 0.04	63

3.4 The effect of the effective part of AS on apoptosis rate of PC12 cells induced by MPP^+

In the model group when cells treated with MPP^+ the apoptosis rate was (40.9 ± 3.9)% which is a significant difference ($P < 0.001$) when compared with the control group (14.8 ± 1.8)%. This result demonstrated that MPP^+ was able to induce apoptosis in PC12 cells under the experimental condition. When PC12 cells were co-treated with MPP^+ and the effective part of AS the apoptosis rate lowed to (26.7 ± 3.9)% in the treatment group which was a transparantly statistical difference with $P < 0.05$. This indicated that the effective part of AS could inhibit the apoptosis in PC12 cells induced by MPP^+ (Table 4).

Table 4 The effect of the effective part of AS on apoptotic rate of PC12 cells induced by MPP^+ ($\bar{x} \pm s, n = 3$) %

Group	LV	VA	NVA	Apoptpsis
Control group	85.1 ± 1.9	5.4 ± 1.2	9.4 ± 1.7	14.8 ± 1.8
Model group	58.8 ± 4.0	11.4 ± 3.7	29.5 ± 4.0	40.9 ± 3.9[1)]
Treatment group	72.9 ± 3.9	7.4 ± 0.8	19.3 ± 4.3	26.7 ± 3.9[2)]

Note: As compared with the control group [1)] $P < 0.001$; As compared with the model group [2)] $P < 0.05$.

3.5 The effect of the effective part of AS on contents of LDA, NO, NOS, MDA in PC12 cells induced by MPP^+

The treatment of MPP^+ on PC12 cells significantly increased the contents of LDH ($P < 0.01$) and NO, NOS and MDA ($P < 0.05$) when compared with the control group (Table 5). The effective part of AS treatment was able to decrease the high contents of LDH ($P < 0.01$) and the contents of NO, NOS, and MDA ($P < 0.05$) in PC12 cells induced by MPP^+ (Table 5).

4 Discussion and conclusion

Studies have suggested that environmental factors might play important role in the occurrence of PD. It had been certified that 1-methyl-4- phenyl-1, 2, 3, 6 tetrahydropyridine (MPTP) is a neurotoxin, which

Table 5 The effect of the effective part of AS on contents of LNA, NO, NOS, MDA in PC12 cells induced by MPP^+ ($\bar{x} \pm s, n = 3$)

Group	LDH/U · L^{-1}	NO/μmol · L^{-1}	NOS/U · mL^{-1}	MDA/nmol · L^{-1}
Control group	248.8 ± 48.9	44.3 ± 8.4	35.1 ± 5.8	9.5 ± 2.2
Model group	569.0 ± 46.4$^{2)}$	74.1 ± 11.5$^{1)}$	57.4 ± 7.5$^{1)}$	17.2 ± 2.8$^{1)}$
Treatment group	311.1 ± 31.9$^{4)}$	48.6 ± 7.5$^{3)}$	37.7 ± 3.2$^{3)}$	10.6 ± 1.8

Note: As compared with the control group, $^{1)}P < 0.05$, $^{2)}P < 0.01$; As compared with the model group, $^{3)}P < 0.05$, $^{4)}P < 0.01$.

has similar molecular structure as some pesticides and herbicides had[3], it wouldn't poison cells unless it had been biotransformed into 1-methyl-4-phenylpyridine ion (MPP^+) which was toxic to cerebral dopaminergic neuron selectively and lead to PD. PC12 cells come from Adren chromaffin tumor, which had the same function as mesocephalon dopaminergic neuron in synthesizing and excreting DA [4-5] (dihydroxyphenyl ethylamine) DA absorption mediated by DA transporter[6-7] and featured like DA recipient[8]. Studies have ascertained that the damage of PC12 cells induced by MPP^+ was by far the better cell model for doing research on PD. Our study confirmed that MPP^+ could inhibit the PC12 cells' survival rate, and this inhibition is dose- and time dependent. We selected 300 μmol · L^{-1} as the final concentration, and 48 h as the action time to be the follow-up experiment modeling condition. MPP^+ could strikingly reduce the cell survival rate and result in cell apoptosis, which was consistent with reports in the literature [9].

After the modeling condition had been finally determined, MTT method had been used to detect the effect of the effective part of AS at different concentrations on the survival rate of PC12 cells induced by MPP^+. we found that the effective part of AS could enhance cell survival rate. Many other methods had been used to study the protective effect of the effective part of AS on the damage of PC12 cells induced by MPP^+. We used flow cytometry method to confirm that the effective part of AS could inhibit apoptosis in PC12 cells induced by MPP^+. In addition, it also has an impact on the contents of NO, NOS, MDA. These results suggested that the effective part of AS had the protective effect on the damage of PC12 cells induced by MPP^+. The underlying mechanisms might be the combination of reduction of LDH leakage and decreases in the contents of NO, NOS and MDA, and finally prevent the apoptosis in PC12 cells and increase the cell survival rate.

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