ISSN:2790-1688 Volume-10-(2024)

The Synergistic Effect of Panax Notoginseng Saponins and Nicotinamide Mononucleotides on Anticoagulant

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Abstract. Panax notoginseng is a traditional Chinese herbal medicine with rich medicinal value and health care efficacy. Its main active ingredient, panax notoginseng saponins, has pharmacological effects on improving blood circulation and inhibiting platelet aggregation. Nicotinamide mononucleotide has the effect of reducing collagen deposition in blood vessels and reducing vascular obstruction. Method: Compare the thrombolytic efficiency of panax notoginseng saponins and nicotinamide mononucleotide alone and in combination, as well as their effect on prolonging coagulation time, using in vitro anticoagulant experiments and in vitro thrombolysis experiments, to explore the efficacy of panax notoginseng saponins and nicotinamide mononucleotide in combination. The results showed that when combined with a compound drug concentration ratio of 3:1 (PNS: NMN), the coagulation time was 1.2 times that of the PNS liposome single dose group and 1.7 times that of the NMN single dose group. And the optimal dosage for combination therapy is only 50% of the single dose. Conclusion: The combination of PNS and NMN has significant synergistic advantages and saves the dosage, which provides a new drug combination for the development of safe and low-toxic anticoagulant and thrombolytic drugs. It provides a reliable and detailed theoretical basis for the combination of panax notoginseng saponins and nicotinamide mononucleotide.

Keywords: Panax notoginseng saponins; niacinamide mononucleotide; synergistic effect; in vitro anticoagulation assay; in vitro thrombolytic assay.

1. Introdution

Retinal vein occlusion caused by "blood stasis"[1,2], also known as flying mosquito disease, is a common ophthalmic disease that seriously affects patients' daily lives. At present, drugs such as aspirin[3,4], warfarin[5], statins, and other interventional surgeries are commonly used in clinical practice to treat such diseases. However, there are some problems such as large side effects of oral drugs and complications caused by surgical treatment. It has been found that Panax notoginseng saponins, the main efficacy component of the traditional Chinese medicine Panax ginseng in China[6], possess various pharmacological activities such as inhibiting platelet aggregation and promoting blood circulation[7-9]. It was also found that Nicotinamide mononucleotide (NMN), an important precursor of nicotinamide adenine dinucleotide (NAD +), can prevent or treat retinal dysfunction mediated by cardiovascular and cerebrovascular diseases[10]. If combined with medication, it is expected to achieve the goal of promoting blood circulation and eliminating blood stasis. However, the oral drug Panax ginseng saponin has the defect of large dosage to reach the effective concentration, and nicotinamide mononucleotide has the side effect of causing gastrointestinal discomfort and affecting the quality of sleep. Combining the characteristics of Panax notoginseng saponins promoting blood circulation and nicotinamide mononucleotides reducing collagen deposition in blood vessels and reducing vascular obstruction, the two were made into external preparations with oral effective blood concentration as the effective dose, which overcame the first-pass effect of oral preparations, and studied the synergistic effect of the two composite preparations.

2. Materials and Methods

2.1 Materials

New Zealand male experimental rabbits, provided by Da Ren Fu Cheng Company.

Panax notoginseng saponins (≥90%) were purchased from Guangzhou Haoxiang Fine Chemical Co., Ltd. Niacinamide mononucleotide were obtained from Henan Honest Biotechnology Co., Ltd. Carbopol 980 were purchased from Shanghai Changwei Technology Co., Ltd. Sodium polyacrylate, glycerine, and aluminum chloride were acquired from Sinopharm Chemical Reagent Co., Ltd.

2.2 Prescription design and Preparation process

Prescription: 0.3% Carbomer solution concentration of 43.75%, Sodium polyacrylate concentration of 4.69%, Propanetriol concentration of 18.75% and Aluminum chloride concentration of 1.56%； The total saponin content of Panax ginseng was 0.53±0.16 mg/mL, and the nicotinamide mononucleotide content was 1.61±0.38 mg/mL.

The panax notoginseng saponins liposome was prepared by thin film dispersion method. Sodium polyacrylate and propanetriol were mixed in a mass ratio of 1:4 to form phase I; 0.3% carbomer solution was added to the prescribed amount of panaxoside liposomes and nicotinamide mononucleotide to form phase II; phase I and phase II were mixed in equal amounts and added to phase III cross-linking agent (5% aluminum chloride and EDTA-2Na) solution to form a compound hydrogel agent.

Dose grouping: The preparation of different concentrations of Panax notoginseng saponin liposome suspensions, nicotinamide mononucleotide solutions, and compound drug solutions with different concentration ratios is shown in Table 1.

Table 1. Grouping of low, medium and high doses for each sample

Group/mg/mL	0	I	II	III
PNS	0	6.07	10.88	21.76
NMN	0	3.63	7.26	14.51
PNS-NMN	0	3.63:3.63(1:1)	7.26:3.63(2:1)	10.88:3.63(3:1)

Note: Compound drugs with different concentration ratios (drug concentration 3.63 mg/mL)

2.3 Preparation of sample solution and plasma

Weigh an appropriate amount of aspirin standard solution and prepare an aspirin standard solution with a concentration of 1 mg/mL. Using PBS solution with pH=7.4 as the solvent, different concentrations of Panax notoginseng saponin liposome suspensions, nicotinamide mononucleotide solutions, and compound drug solutions with different concentration ratios were prepared.

New Zealand male experimental rabbits, blood was taken from the heart with 3.8% sodium citrate anticoagulant tubes, centrifuged at 3600 r/min for 15 min, and the supernatant was extracted to obtain the platelet-depleted plasma, which was preserved in vacuum blood collection tubes and refrigerated at 4 ° C for spare use. The experimental animal procedures were approved by the Ethics Committee for Animal Experiments of the University (License No. 2017-1).

2.4 In vitro anticoagulation assay

The plasma to be tested and each sample solution were mixed in 9:1 volume ratio and incubated for 5 min at 37 ° C. Activated partial thromboplastin time (APTT), Prothrombin time (PT) and Thrombin time (TT) were used as in vitro anticoagulant activity assay indexes, aspirin standard solution product was used as a positive control group, and PBS (pH=7.4) solution was used as a

blank control group, and the experimental operation was carried out according to the indicator box, and the measurements were made three times in parallel.

2.5 In vitro thrombolytic assay

Fresh rabbit blood was placed in a petri dish to prepare a thrombus model. PBS (pH = 7.4) solution was used as the blank control group. Total saponins of panax notoginseng, total saponins of panax notoginseng liposomes, and nicotinamide mononucleotide were added to a beaker containing 50 mL PBS solution, respectively. The thrombus model in the petri dish was transferred to the above beaker, and the in vitro thrombolysis test was carried out in a constant temperature and humidity incubator at 37 ° C 150 r / min to investigate the thrombolysis ability of total saponins of panax notoginseng, total saponins of panax notoginseng liposomes and nicotinamide mononucleotide. Consistent with the above experimental operation, the thrombolytic ability of the compound drug was investigated with different concentration ratios of panax notoginseng saponins liposomes and nicotinamide mononucleotide compound drugs (group I 1 : 1, group II 2 : 1, group III 3 : 1, with nicotinamide mononucleotide dose of 3.63 mg / mL as 1 portion) and the optimal drug ratio was determined. The remaining thrombus was weighed at fixed times of 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h, respectively, and the in vitro thrombolysis curves were plotted as the percentage of the remaining mass of thrombus after dissolution versus time.

$$M = \frac{M_i}{M_0} \times 100\% \qquad (1)$$

In the formula, Mi is the residual weight (g) of the thrombus model for the i th weighing ; M0 was the weight of 0 h thrombus model (g).

Note : The dose calculation method is as follows : the equivalent dose ratio of adult (60 kg) to rabbit is calculated according to the body surface area. The effective dose of rabbit is 5.78 mg / kg at low dose, 10.36 mg / kg at middle dose and 20.72 mg / kg at high dose.

3. Result

3.1 In vitro anticoagulant assay results

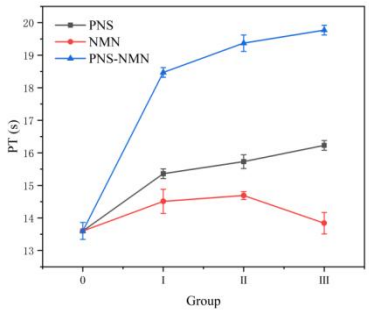

Fig. 1 Effect of different concentrations of each sample on PT in rabbits(n=3)

From Figure 1, it can be seen that the anticoagulant effect in vitro is positively correlated with its dose. Compared with the blank control group, the PT values of panax notoginseng saponins liposome suspension and nicotinamide mononucleotide in different dose groups were significantly prolonged. Notoginsenoside liposome suspension in different dose groups was positively correlated with its dose. The PT value of nicotinamide mononucleotide shows a trend of first prolonging and then shortening with increasing dose. The PT values of compound drugs with different concentration ratios were significantly prolonged, and the optimal concentration ratio was Group III (3:1, with a nicotinamide mononucleotide dose of 3.63 mg/mL as 1 part). At the same dosage, with the prolongation of PT value as the evaluation index, the coagulation time prolonged by the combined drug was 1.2 times that of the single dose of PNS liposome and 1.3 times that of nicotinamide mononucleotide.

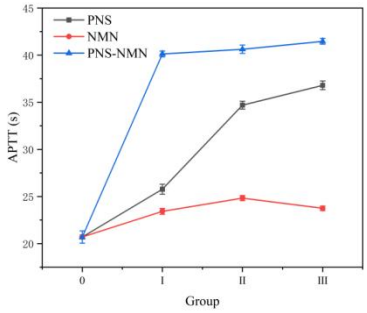

Fig. 2 Effect of different concentrations of each sample on APTT in rabbit(n=3)

From Figure 2, compared with the blank control group, the APTT values of panax notoginseng saponins liposome suspension and nicotinamide mononucleotide in different dose groups were significantly prolonged. There is a positive correlation between the dosage of Panax notoginseng saponin liposome suspensions in different dosage groups. The APTT values of nicotinamide mononucleotides in different dose groups were also prolonged. The APTT values of compound drugs with different concentration ratios were significantly prolonged, and the optimal concentration ratio was Group III (3:1, with a nicotinamide mononucleotide dose of 3.63 mg/mL as 1 part). At the same administered dose, the prolongation of APTT clotting time was evaluated as an index, and the prolongation of clotting time by the combination drug was 1.2 times as much as that of the single-dose group of panax ginseng total saponin liposome, and 1.6 times as much as that of nicotinamide mononucleotide, and the administered dose of the drug in the combination was only 50% of the amount of the drug used alone.

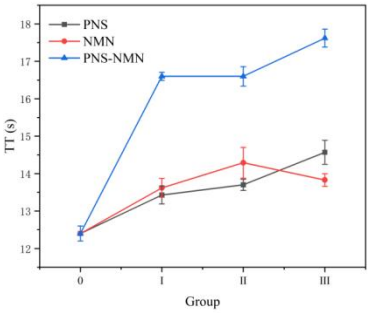

Fig. 3 Effect of different concentrations of each sample on TT in rabbits(n=3)

From Figure 3, compared with the blank control group, the TT values of panax notoginseng saponins liposome suspension and nicotinamide mononucleotide in different dose groups were significantly prolonged. There is a positive correlation between the dosage of Panax notoginseng saponin liposome suspensions in different dosage groups. The TT value of nicotinamide mononucleotide was prolonged first and then shortened with the increase of dose. The TT values of compound drugs with different concentration ratios were significantly prolonged, and the optimal concentration ratio was Group III (3:1, with a nicotinamide mononucleotide dose of 3.63 mg/mL as 1 part). At the same dosage, with the prolongation of TT value as the evaluation index, the coagulation time prolonged by the combined drug was 1.2 times that of the single dose of PNS liposome and 1.2 times that of nicotinamide mononucleotide.

In summary, the anticoagulant effect in vitro is positively correlated with its dosage. The high dose group (21.76 mg/mL) had the best anticoagulant effect in vitro with the total saponin liposome suspension of Panax notoginseng, while the medium dose group (7.26 mg/mL) had the best anticoagulant effect in vitro with the nicotinamide mononucleotide. The optimal ratio for the combination of total saponins of Panax notoginseng and nicotinamide mononucleotides is 3:1, which means that the concentration of total saponins of Panax notoginseng is 10.88mg/mL, and the concentration of nicotinamide mononucleotides is 3.63mg/mL. It is proved that total saponins of Panax notoginseng and nicotinamide mononucleotide have a certain synergistic effect. Compared with individual medication, the dosage of total saponins of Panax notoginseng in combination medication is significantly reduced, and the efficacy of the combination medication is enhanced.

3.2 In vitro thrombolysis experimental results

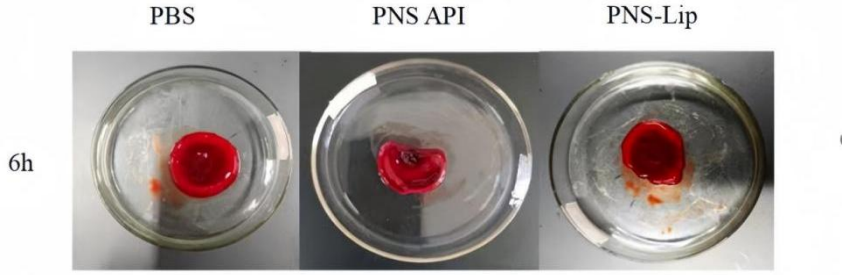

Fig. 4 In vitro thrombolysis effect of PNS API and PNS liposome for 6 hours

It can be seen from Fig.2 that the in vitro thrombolysis effect of PNS API and PNS liposome at 6 h. Compared with the blank control group, the thrombus model with the addition of raw materials of Panax notoginseng saponins and liposomes of Panax notoginseng saponins decreased the remaining mass of thrombus at 6 hours, indicating that Panax notoginseng saponins have a certain thrombolytic ability.

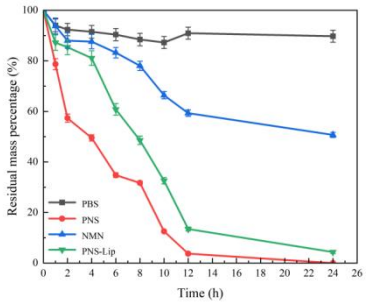

Fig. 5 Single component thrombolytic kinetics curve

According to Figure 3 of the unilateral experimental results, it can be seen that the thrombolysis time of the raw material and liposome suspension of Panax notoginseng saponins has changed. When the thrombolysis effect of Panax notoginseng saponins liposome reaches 50%, it is extended from 4 hours to 12 hours compared to the raw material, and the nearly complete thrombolysis time is extended from 12 hours to 24 hours, indicating that Panax notoginseng saponins liposomes have a certain slow dissolution effect.

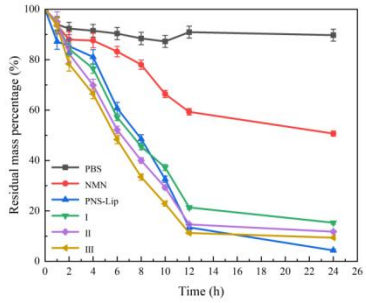

Fig. 6 Thrombolytic kinetics curves of complex components

It can be seen from Fig.4 of the compound thrombolysis experiment that the thrombolytic effects of compound drugs with different concentration ratios are significantly different. Compared with the single solution, the thrombolytic effect of the compound drug was significantly improved, and the thrombolytic effect of the compound drug with different concentration ratios was as follows : group III > group II > group I. It was best when the concentration ratio of total saponin of Panax ginseng to nicotinamide mononucleotide was 3:1.

4. Conclusion

The combination of Panax notoginseng saponins and nicotinamide mononucleotides has a synergistic anticoagulant effect of reducing drug dosage and enhancing drug efficacy, providing a reliable and detailed theoretical basis for the modernization of Panax notoginseng saponins in

traditional Chinese medicine and the application of nicotinamide mononucleotides in topical formulations.

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