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Plasma Concentration Detection of Aminophylline with Multi-Walled Carbon Nanotube-Modified Electrodes in the Treatment of Ticagrelor-Related Dyspnea

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ABSTRACT

It was aimed at the plasma concentration detection of aminophylline under multi-walled carbon nanotube (MWNT) modified electrodes and the curative effect of aminophylline for ticagrelor-related dyspnea (TRD). 120 patients with TRD and coronary atherosclerotic heart disease (CAHD) in Wuhan Sixth Hospital Hospital were randomly divided into an intervention group and a control group, with 60 cases in each. The novel chitosan/gold nanoparticles-MWNTs (CTS/Au NPs-MWNTs) composite was prepared for studying the electrochemical behaviors of aminophylline on the electrodes. The clinical curative effect of aminophylline, as well as the levels of peripheral blood T lymphocyte subsets and inflammatory cytokines, was observed in the treatment of TRD. The actual plasma molar concentration of aminophylline was 23.6-47.8 μmol/L. After treatment, the total effective rate in the intervention group was higher than that in the control group significantly (95% VS 65%) (P<0.05). The forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) of the intervention group were better than those of the control group remarkably (P<0.05). The intervention group showed lower levels of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 as well as the higher IL-10 significantly in serum compared with the control group (P<0.05). After follow-up, there was no cardiovascular death in either group. The CTS/Au NPs-MWNTs could be used for detecting the plasma concentration of aminophylline in blood samples. Aminophylline could notably relieve the clinical symptoms, reduce the inflammatory response, and regulate the immunity of patients with good safety.

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Introduction

Coronary atherosclerotic heart disease (CAHD), referred to as coronary heart disease, is stenosis of ≥50% of the lumen caused by atherosclerotic lesions found anywhere in the coronary arteries (1,2). In recent years, percutaneous coronary intervention (PCI) has been important gradually in the treatment, resulting in a significant decrease in the mortality of CAHD; the application of antiplatelet drugs after the intervention is still essential (3-5). Ticagrelor is a new type of antiplatelet drug with the advantages of fast onset and strong effect and has become the first-line drug for preventing thrombosis after PCI gradually. However, patients are prone to dyspnea after using ticagrelor, which limits its clinical application. Therefore, it is urgent to find effective drugs to relieve the symptoms of ticagrelor-related dyspnea (TRD) timely in patients with CAHD. Thereby, the compliance of patients can be improved and their suffering can be relieved (6). Aminophylline, a methyl purine drug, has the effects of strengthening the heart, dilating coronary arteries, and relaxing bronchial smooth muscle, to relieve symptoms of dyspnea (7-9). Aminophylline requires a narrow therapeutic window; the therapeutic dose is very close to the toxic dose, so a variety of adverse reactions occur when the concentration is slightly higher. Therefore, monitoring the plasma concentration of aminophylline is necessary for clinical treatment.

At present, high-performance liquid chromatography and fluorescence polarization immunoassay are common for the detection of the plasma concentration of aminophylline clinically. Salajegheh et al. (2019) (10) reported the determination of aminophylline using Nafion/lead-ruthenium oxide pyrochlore modified electrodes chemically. Koçak et al. (2018) (11) reported that aminophylline content in cosmetics and pharmaceuticals was determined using glassy carbon electrodes, which verified the feasibility of using

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aminophylline's electrochemical behavior for the determination of the concentration. Gold nanoparticles (Au NPs) are widely used in electrochemical sensors due to their special optoelectronic properties, good stability and surface effect, high catalytic activity, as well as unique bio-affinity. Multi-walled carbon nanotubes (MWNTs) are formed by convolution of multilayer graphite sheets and have potential value in sensors for their excellent electrical and mechanical properties (12-14).

In this research, 120 patients suffering from CAHD with TRD, who had been treated in the Department of Cardiology of Wuhan Sixth Hospital in the past two years, were selected and divided into an intervention group and a control group by random number method. The plasma concentration of aminophylline was detected by screen-printed working electrodes modified with the composite material chitosan (CTS)/Au NPs-MWNTs (CTS/Au NPs-MWNTs). It was aimed to investigate the safety and effectiveness of aminophylline in the treatment of TRD in CAHD patients.

Materials and methods

Research objects

120 CAHD patients with TRD were included, as they visited Wuhan Sixth Hospital from March 2020 to December 2021. The selected patients were randomly divided into the intervention group and the control group by the random number table method, with 60 cases in each. No significant difference was shown in clinical data such as gender, age, body mass index, and the number of implanted stents of patients between the two groups (P>0.05). thus, there was comparability as shown in Table 1. This research was approved by the Ethics Committee of Wuhan Sixth Hospital and informed consents were signed by patients or guardians.

Inclusion criteria were as follows. (1) The patients met the diagnostic criteria for TRD (15); they developed dyspnea after using ticagrelor and had no similar symptoms before. (2) The patients had clear consciousness and normal understanding could fully comply with the doctor's orders and could cooperate with the research. (3) Not an abnormality was found in the examination results of the patient's cardiac ultrasound, electrocardiogram, and thoracic X-ray. (4) The patients had no serious adverse reactions to aminophylline.

The exclusion criteria following were also obeyed. (1) Patients were complicated with severe hepatic and renal impairment. (2) Patients went with cardiac function grade II or above depending on the grading of the New York Heart Association. (3) Those gave up treatment midway due to intolerable adverse reactions. (4) Patients went with a previous complicated history of bronchitis, asthma, or pulmonary diseases like chronic obstructive pulmonary disease and respiratory failure. (5) They could not complete the follow-up or could not provide the clinical data completely.

Table 1. Clinical data of patients in two groups ($\bar{x}\pm s$).

Groups	Cases	Gender		Age (years old)	Weight (kg)	Number of stents (pieces)
		Male	Female			
Control group	60	27	33	56.78±8.67	73.62±6.38	1-4
Intervention group	60	28	32	61.52±7.85	74.62±6.49	2-4

Treatment plan

Patients in both groups were given 20 mg of atorvastatin tablets. Those in the control group were given with placebo (Guangzhou Baiyunshan Mingxing Pharmaceutical Co., Ltd., China). Those in the intervention group were given 0.25 g of aminophylline injection (Anhui Chengshi Pharmaceutical Co., Ltd., China; the specification: 2 mL, 0.5 g; approval number: H34022983). The aminophylline injection was fully diluted with 100 mL of 5% glucose injection, with an infusion rate of 30-40 drops/min, once a day for 1 week.

Observation indicators

The clinical treatment effect was evaluated using CR-10 grading (16) for evaluating the severity of dyspnea in the two groups before treatment and 7 days after treatment. If it was markedly effective, the CR-10 grade was downgraded by ≥ 2 grades after treatment, the symptoms of dyspnea were relieved significantly, and the clinical symptoms disappeared. If it was effective, the CR-10 grade was downgraded by 1 grade after treatment, the dyspnea symptoms were also relieved, and the treatment could be continued. If it was ineffective, The CR-10 grade did not change or even increased after treatment, and the

symptoms of dyspnea did not relieve or even further aggravated. Equation [1] below was for calculating the total effective rate (%).

$$Total\ effective\ rate = \frac{markedly\ effective + effective}{total} \times 100\% \quad [1]$$

Pulmonary function was measured using the SpiroUSB, a CareFusion spirometer made in the United States. The pulmonary functions of patients in both groups were measured before treatment and also 7 days after treatment. The major indicators included forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1).

The content of T lymphocyte subsets in peripheral blood was measured before treatment as well as 7 days after treatment. 6.0 mL of fasting peripheral blood was collected from patients in the two groups, and flow cytometry was adopted for detecting the proportions of a cluster of differentiation (CD)4+ and CD8+ in T lymphocyte subsets in peripheral blood. Then, CD4+/CD8+ was calculated.

The level of inflammatory cytokines was detected in the remaining peripheral blood after the above items. After being placed at room temperature for 30 minutes, the serum was separated. The levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) were determined under the enzyme-linked immunosorbent assay.

The incidence of major adverse cardiovascular events (MACE) was investigated in the telephone follow-up during the 6th month of the treatment. The MASE included myocardial infarction, cardiac death, unstable angina pectoris, stent thrombosis, and so on. The number of MACE cases in the two groups was counted, and the incidences of MASEs were calculated.

Preparation of Au NPs-MWNTs complex

Au NPs were prepared at first. 1 mL of 1% chloroauric acid solution was mixed with 100 mL of distilled water to boil, and then 2.5 mL of 1% sodium citrate was added quickly to the reflux mixed solution of chloroauric acid. When the solution changed from light yellow to dark red, it meant that the chloroauric acid had been reduced to Au NPs.

100 mg of MWNTs was added to 80 mL of a mixed solution of concentrated sulfuric acid and concentrated nitric acid (volume ratio 3:1). The mixture was ultrasonically shaken for 4 h, then rinsed with distilled water, and centrifuged at 10000 r/min for 15 min. As the solution was tested with pH=7, the carboxylated MWNTs were obtained. In a water bath at 80 ℃, Au NPs colloidal solution was added dropwise and slowly according to the volume ratio 2:1 of MWNTs: Au NPs colloidal solution. After ultrasonic shaking for 2 h, centrifugation at 10000 r/min for 15 min, and washing, Au NPs-MWNTs complex was obtained.

Construction of CTS/Au NPs-MWNTs modified working electrode

0.5% CTS solution was prepared, as 0.1 g of CTS was dissolved in 20 mL of 1.0% glacial acetic acid and magnetically stirred at room temperature for 2 h. As shown in Figure 1, the screen-printed electrode was mainly composed of a carbon working electrode modified by MWNT composites, a carbon auxiliary electrode, and a silver (Ag)/silver chloride (AgCl) reference electrode. 3 μL of Au NPs-MWNTs composite was added dropwise to the treated screen-printed working electrode. After drying at room temperature for 30 min, 1 μL of CTS was added to the Au NPs-MWNTs modified working electrode. CTS/Au NPs-MWCNTs working electrode was well prepared finally after drying at room temperature again.

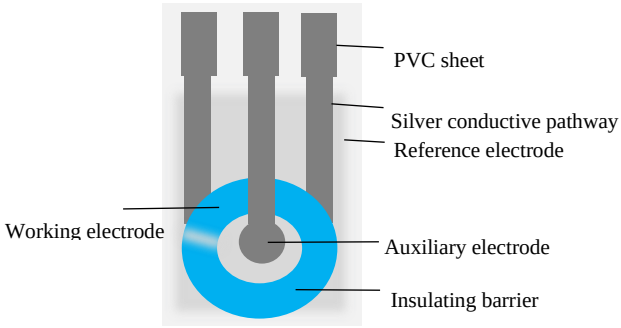

Figure 1. Screen-printed electrode.

Electrochemical methods

1 mL of blood samples to be tested were taken from each group, diluted to 5 mL with 0.1 mol/L phosphate buffer (pH 7.5). The measurement was performed in a range of 0-1.3 V in the three-electrode screen-printed electrode system with Ag/AgCl as the reference electrode, through cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), respectively. The scanning

speed was 100 mV/s, and the enrichment time was 5 min. It was measured 3 times to get the average value for each group, and the measurement process should be completed at room temperature.

Statistical methods

In this work, SPSS 20.0 was applied as the statistical software for data processing and statistical analysis. One-way analysis of variance was adopted for comparison among multiple groups, measurement data were expressed as $\bar{x}\pm s$, and enumeration data were analyzed by χ^2 test. The results were significant statistically at $P<0.05$.

Results and discussion

Characterization of the CTS/Au NPs-MWNTs modified working electrodes

Characterization of the screen-printed working electrode was a very important factor affecting the electrode response. The characterization of the screen-printed electrodes with different modifications was determined by scanning electron microscopy. The MWNTs were bound to the electrode compared with the bare electrodes, which could be observed in Figure 2-A. It was also observed from Figure 2-B that Au NPs-MWNTs composite formed a thin film on the surface of the working electrode. In Figure 2-C, CTS was bound to the surface of the Au NPs-MWNTs modified screen-printed electrode successfully. The disorder of CTS/Au NPs-MWNTs modified working electrode made it have a larger surface area, which provided a good basic condition for the enrichment of detection substances. The ultraviolet spectrum of Au NPs showed that the ultraviolet absorption peak of Au NPs was around 510 nm, which was displayed in Figure 3.

CV characteristics of aminophylline on the working electrode of the screen-printed electrodes

In 0.1 mol/L phosphate buffer at pH 7.5, as presented in Figure 4, the volumetric curves of 100 μmol/L aminophylline were drawn by CV, DPV, and SWV with unmodified screen-printed electrodes, respectively. Aminophylline had a sensitive oxidation peak at 100 mV. In comparison with Figure 4-A, the measured value of the SWV method was greater than that of CV and DPV, and the differences were of statistical significance ($P<0.05$). The comparison of Figures 4-B, 4-C and 4-D suggested that SWV was the best on the measurement effect.

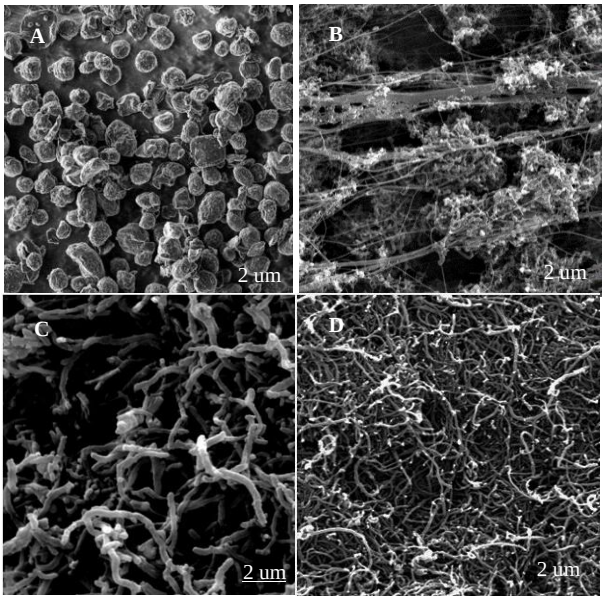

Figure 2. Electron microscope scanning image of silk-printed working electrodes. A: Bare electrode; B: MWNTs modified electrode; C: Au NPs-MWNTs modified electrode; and D: CTS/Au NPs-MWNTs modified electrode.

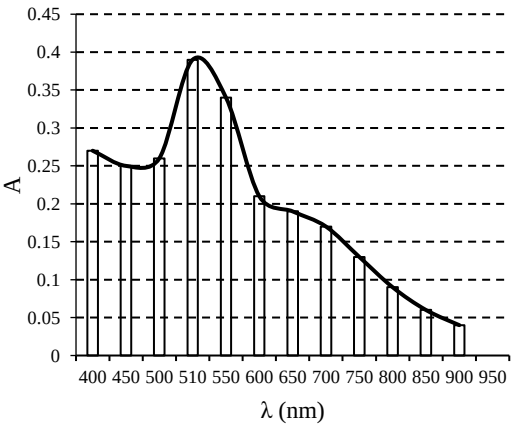

Figure 3. Ultraviolet spectrum of Au NPs colloidal solution.

The standard curve and the detection limit

Under the best experimental conditions, the CV curves of different concentrations of aminophylline standard solutions were determined in 0.1 mol/L phosphate buffer (pH 7.5). It maintained a good linear relationship with the peak current in the concentration range of 1.0×10^{-5}-2.0×10^{-4} mol/L, as shown in Figure 5. The detection limit was 1.0 μmol/L, and the regression equation was the equation [2].

$$Ip(\mu A) = 0.042c(\mu mol/L) + 0.631,\ R = 0.995 \quad [2]$$

The CV curve of aminophylline standard solution with different concentrations in the blood samples of patients with dyspnea was determined. As presented in Figure 6, the concentration kept a good linear relationship with the peak current in the range of 2.0×10^{-5}-2.0×10^{-4} mol/L. The detection limit was 5.0 μmol/L, and its regression equation was as equation (3).

$$Ip(\mu A) = 0.006c(\mu mol/L) + 0.115,\ R = 0.999 \quad [3]$$

The relative molecular mass of aminophylline was 420 g/mol. Since the plasma concentration of aminophylline was 10-20 mg/L, the actual plasma molar concentration was 23.6-47.8 μmol/L. Since the toxic concentration was 20-40 mg/L, the molar concentration was calculated to be 47.4-95.6 μmol/L. Under the optimal conditions of this method, the linear range of the determination satisfied 1.0×10^{-5}-2.0×10^{-4} mol/L, which could reach the plasma concentration ranging from half the therapeutic dose to twice the toxic dose.

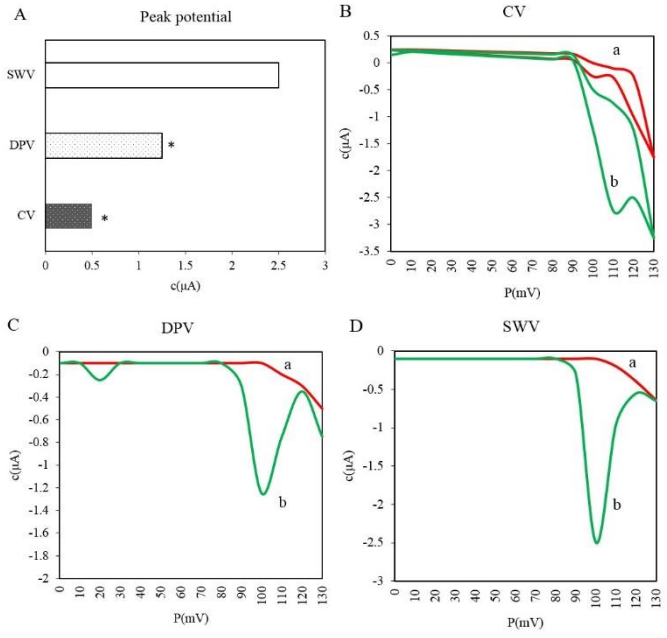

Figure 4. CV characteristics of aminophylline on the screen-printed working electrode. Figures A, B, C, and D were the peak potential diagram under different measurement methods, that under CV, that under DPV, and that under SWV. Notes: a meant blank buffer solution, while b meant 100 μmol/L aminophylline buffer. * marked the statistical and significant difference compared with SWV, $P<0.05$.

Comparison of clinical efficacy and pulmonary function between two groups of patients

As shown in Table 2 after treatment, the total effective rate of the intervention group was higher significantly than that of the control group (95% VS 65%), showing a difference statistically significant ($P<0.05$).

Before treatment, there was no significant difference in FVC and FEV1 between the two groups ($P>0.05$). The FVC and FEV1 in both groups of patients were improved significantly after treatment compared with those before treatment, as the differences were significant statistically ($P<0.05$). After treatment, the FVC and FEV1 of the intervention group were better remarkably than those of the control group, and the significant differences statistically were presented ($P<0.05$) in Figure 7.

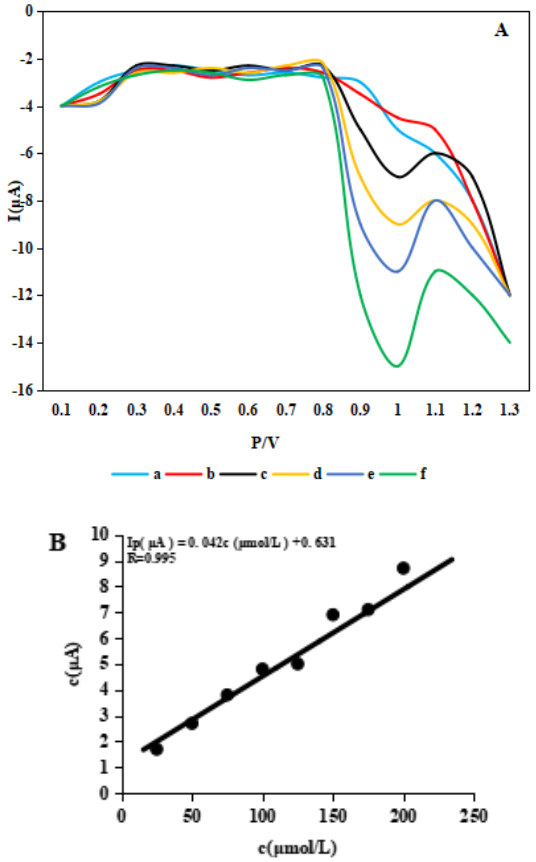

Figure 5. Standard Curves of the peak current-aminophylline concentration in the buffer. Notes: A showed the standard curves of peak current-aminophylline concentration in the buffer; B displayed the linear equation of the phosphate buffer concentration and the peak current. Curves a-f represented that with the concentrations c=10 μmol/L, 20 μmol/L, 40 μmol/L, 80 μmol/L, 100 μmol/L, and 200 μmol/L, respectively.

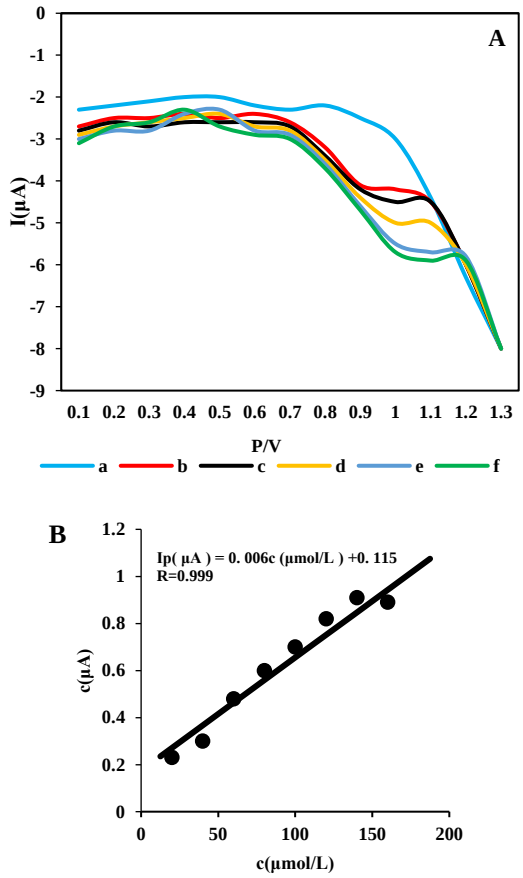

Figure 6. Peak current-aminophylline concentration standard curves in blood samples. Notes: A showed the standard curves of peak current-aminophylline concentration in a blood sample, while B represented the linear equation between the peak current and the concentration of aminophylline in blood samples. Curves a-f stood for those with the concentrations of c=10 μmol/L, 20 μmol/L, 40 μmol/L, 80 μmol/L, 100 μmol/L, and 200 μmol/L, respectively.

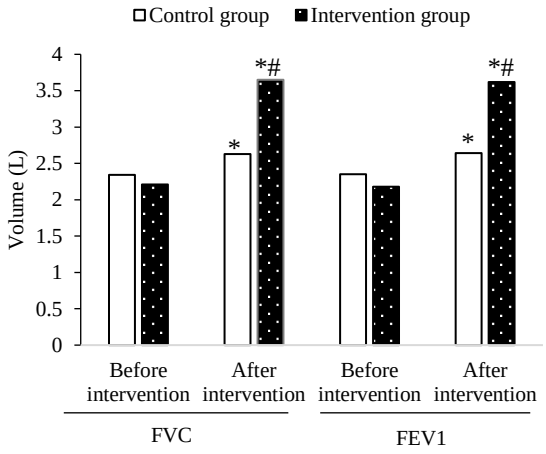

Figure 7. Comparison of pulmonary function indicators before and after treatment. Note: * and # indicated that compared with the corresponding results before treatment and in the control group, respectively, the differences were of statistical significance (P<0.05).

Table 2. Comparison of clinical treatment effect on patients between the two groups (n, %).

Groups	Cases	Markedly effective	Effective	Ineffective	Total effective rate
Control group	60	21(35)	18(30)	21(35)	39(65)
Intervention group	60	38(63.33)	19(31.66)	3(5)	57(95) *
P					<0.05

Note: * indicated the statistically significant difference compared to the control group (P<0.05).

Comparison of the levels of T lymphocyte subsets and inflammatory cytokines in peripheral blood between the two groups before and after treatment

Before treatment, there was not a significant difference in the ratios of CD4+, CD8+, and CD4+/CD8+ in T lymphocyte subsets between the two groups (P>0.05). After treatment, the proportions of CD4+ and CD4+/CD8+ were increased in both groups compared with those before treatment, with the differences statistically significant (P<0.05). The ratios of CD4+ and CD4+/CD8+ were also notably higher in the intervention group than those in the control group, going with the statistically significant differences (P<0.05). No significant change was found in the ratio of CD8+ between the two groups as well as before and after treatment (P>0.05), which were shown in Figures 8-A and 8-B.

Before treatment, the TNF-α, IL-6, and IL-10 levels in serum were not different between the two groups (P>0.05). After treatment, the levels of TNF-α and IL-6 were lower in both groups than those before treatment, while the level of IL-10 was much higher than that before treatment, and the differences were significant statistically (P<0.05). The TNF-α and IL-6 levels in the intervention group were greatly lower than those in the control group, and the IL-10 level in the intervention group was dramatically higher than that in the control group, indicating a difference significant statistically (P<0.05). More were represented in Figure 8-C.

Comparison of the incidence of MACE between the two groups of patients

During the 6-month follow-up, no cardiovascular death occurred in either group. There was 1 case of myocardial infarction in the intervention group, and the incidence of MACEs was 1.67%. In the control group, there was 1 case of unstable angina pectoris, so the incidence of MACEs was 3.33%. No significant

difference was observed in the incidence of MACEs between the two groups (P>0.05).

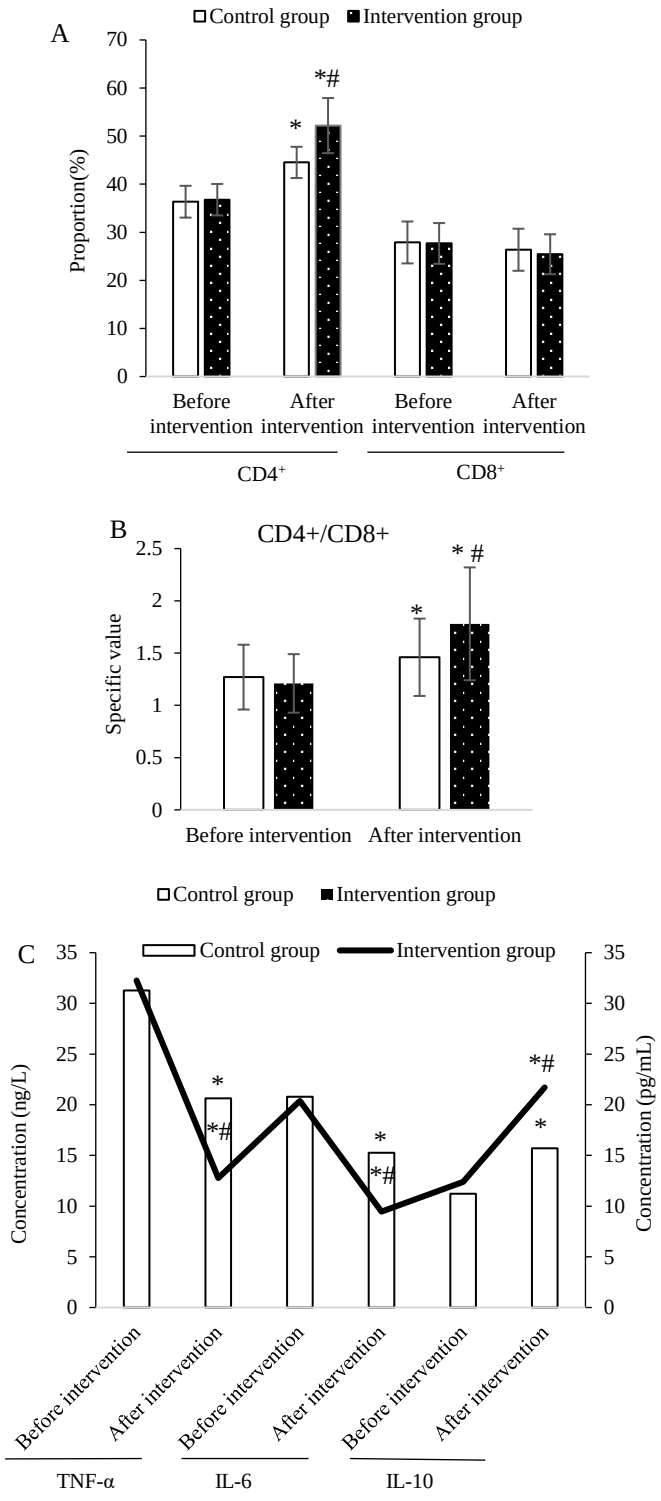

Figure 8. Comparison of the T lymphocyte subsets levels and inflammatory cytokines levels in peripheral blood. A: The levels of CD4+ and CD8+; B: The level of CD4+/CD8+; C: The levels of inflammatory cytokines. Note: * and # indicated that there was a difference statistically significant compared with those before treatment and in the control group, respectively (P<0.05).

MWNTs have good physical adsorption and electrical conductivity; CTS has excellent adhesion and anti-interference ability. In combination with Au NPs material, the electron transfer ability and electrocatalysis of electrodes are highly improved eventually (17-19). The CTS/Au NPs-MWNTs composite modified electrode will greatly improve the sensitivity and selectivity of the response in aminophylline concentration determination. The feasibility of the screen-printed electrode method was verified in this work for determining the plasma concentration of aminophylline. The method of determining plasma concentration of aminophylline using the new CTS/Au NPs-MWNTs composite modified electrodes was accurate, simple, and fast, and it satisfied the range of aminophylline plasma concentration that needed to be determined in clinical practice.

Ticagrelor is a cyclopentyl triazole pyrimidine drug. As a third-generation novel P2Y12 receptor inhibitor, it can act on adenosine diphosphate selectively, thereby improving the anti-platelet aggregation activity by blocking the binding of glycoproteins to fibrinogen (20). Ticagrelor is a non-prodrug, which can act directly without activation of liver metabolism, and the onset time is short. In addition, its binding to the P2Y12 adenosine diphosphate receptor is reversible and does not damage platelets; the platelet function can be restored after drug withdrawal. Therefore, it is widely used in cardiovascular-related treatments. With the wide application of ticagrelor, its related dyspnea disease has attracted people's attention gradually. This adverse reaction will lead to poor medication compliance in patients, reduce the treatment effect of antiplatelet therapy, and affect the prognosis seriously (21-23). After the patient takes ticagrelor, the level of adenosine in vivo will increase. Although adenosine has the functions of dilating blood vessels, anti-inflammatory, anti-platelet aggregation, and protecting the heart, it can also excite the C-fibers of the pulmonary vagus nerve and induce dyspnea. Regarding the mechanism of ticagrelor-induced dyspnea, Wu and Shi (2021) (24) believed that ticagrelor might cause dyspnea by increasing the concentration of extracellular adenosine or inhibiting the P2Y12 receptor in neuronal cells directly. Adenosine stimulates C-fibers in the vagus nerve in

the lungs, which can result in dyspnea. Moreover, Adamski et al. (2021) (25) illustrated that ticagrelor could inhibit the uptake of adenosine by erythrocytes and increase the concentration of adenosine in the serum of patients, thereby increasing the incidence of dyspnea.

Aminophylline is a phosphodiesterase inhibitor that relaxes tracheal smooth muscles and relieves dyspnea (26). Firstly, as a purinergic receptor blocker, aminophylline can antagonize airway constriction caused by adenosine, improving dyspnea symptoms effectively. Secondly, aminophylline can dilate bronchi by regulating the concentration of free Ca^{2+} in bronchial smooth muscle cells (27,28). The results of this research suggested that the total effective rate of the intervention group was much higher than that of the control group; the improvement of FVC and FEV1 after treatment in the intervention group was highly better than that of the control group. These indicated that aminophylline treatment could improve the clinical treatment effect and improve the pulmonary functions of patients effectively. After treatment, the CD4+ ratio and CD4+/CD8+ in peripheral blood, as well as serum IL-10 levels, were significantly higher in the intervention group than those in the control group; the serum TNF-α and IL-6 levels were greatly lower than those in the control group. It was indicated that the combined treatment of ticagrelor and aminophylline could reduce inflammation response in vivo significantly, and promote the immunity of the body. Furthermore, during the 6-month follow-up, the incidences of MACEs were not different remarkably between the intervention group and the control group, suggesting that aminophylline treatment did not increase the risk of MACEs and with great safety.

However, due to the small sample size, it was affected by certain subjective factors with the tolerance to dyspnea being different for each patient. In addition, this work was conducted as a short-term application, and the effect was acceptable. But whether it could be used for a long time for people with more stubborn dyspnea, is expected to be further verified through future trials with larger samples.

Conclusions

The novel CTS/Au NPs-MWNTs composite was prepared in this research, and the electrochemical behaviors of aminophylline on the electrodes were investigated. The clinical treatment effect of aminophylline was observed for the treatment of TRD, and its influences on peripheral blood T lymphocyte subsets and inflammatory cytokines were also discussed. The CTS/Au NPs-MWNTs in this work could measure the plasma concentration of aminophylline in blood samples. Aminophylline showed a good effect on the treatment of TRD; it could relieve the clinical symptoms of patients significantly, reduce the inflammatory response in vivo, and regulate the immunity of the body with excellent safety. The limitations of this research lay in the small sample size included. Thus, large-sample and multi-center trials were needed in the later stage in the future. In conclusion, aminophylline could treat TRD safely and effectively and could be taken for clinical treatment.

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Not applicable.

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Interest conflict

The authors declare that they have no conflict of interest.

References

1. Fioranelli M, Bottaccioli AG, Bottaccioli F, Bianchi M, Rovesti M, Roccia MG. Stress and Inflammation in Coronary Artery Disease: A Review Psychoneuroendocrineimmunology-Based. Front Immunol. 2018 Sep 6;9:2031. doi: 10.3389/fimmu.2018.02031. PMID: 30237802; PMCID: PMC6135895.
2. Faroux L, Guimaraes L, Wintzer-Wehekind J, Junquera L, Ferreira-Neto AN, Del Val D, Muntané-Carol G, Mohammadi S, Paradis JM, Rodés-Cabau J. Coronary Artery Disease and Transcatheter Aortic Valve Replacement: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019 Jul 23;74(3):362-372. doi: 10.1016/j.jacc.2019.06.012. PMID: 31319919.
3. Williams MC, Moss AJ, Dweck M, Adamson PD, Alam S, Hunter A, Shah ASV, Pawade T, Weir-McCall JR, Roditi G, van Beek EJR, Newby DE, Nicol ED. Coronary Artery Plaque Characteristics Associated With Adverse Outcomes in the SCOT-

HEART Study. J Am Coll Cardiol. 2019 Jan 29;73(3):291-301. doi: 10.1016/j.jacc.2018.10.066. PMID: 30678759; PMCID: PMC6342893.

4. Li GM, Zhang CL, Rui RP, Sun B, Guo W. Bioinformatics analysis of common differential genes of coronary artery disease and ischemic cardiomyopathy. Eur Rev Med Pharmacol Sci. 2018 Jun;22(11):3553-3569. doi: 10.26355/eurrev_201806_15182. PMID: 29917210.
5. Zheng J, Huang M, Huang Q, Chen Q, Chen Z. The relationship between fetuin-A and coronary atherosclerotic heart disease (CHD) and CHD-related risk factors: A retrospective study. Medicine (Baltimore). 2021 Oct 29;100(43):e27481. doi: 10.1097/MD.0000000000027481. PMID: 34713826; PMCID: PMC8556024.
6. Bhatt DL, Steg PG, Mehta SR, Leiter LA, Simon T, Fox K, Held C, Andersson M, Himmelmann A, Ridderstråle W, Chen J, Song Y, Diaz R, Goto S, James SK, Ray KK, Parkhomenko AN, Kosiborod MN, McGuire DK, Harrington RA; THEMIS Steering Committee and Investigators. Ticagrelor in patients with diabetes and stable coronary artery disease with a history of previous percutaneous coronary intervention (THEMIS-PCI): a phase 3, placebo-controlled, randomised trial. Lancet. 2019 Sep 28;394(10204):1169-1180. doi: 10.1016/S0140-6736(19)31887-2. Epub 2019 Sep 1. PMID: 31484629.
7. Minner SA, Simone P, Chung BB, Shah AP. Successful Reversal of Bradycardia and Dyspnea With Aminophylline After Ticagrelor Load. J Pharm Pract. 2018 Feb;31(1):112-114. doi: 10.1177/0897190016680978. Epub 2016 Dec 5. PMID: 27920235.
8. Conte L, Pugliese NR, Giannoni A. Reversal of Ticagrelor-Induced Arrhythmias and Cheyne-Stokes Respiration With Aminophylline Infusion. J Cardiovasc Pharmacol. 2017 Nov;70(5):290-292. doi: 10.1097/FJC.0000000000000518. PMID: 28704306.
9. Megaly M, Sandoval Y, Lillyblad MP, Brilakis ES. Aminophylline for Preventing Bradyarrhythmias During Orbital or Rotational Atherectomy of the Right Coronary Artery. J Invasive Cardiol. 2018 May;30(5):186-189. Epub 2018 Feb 15. PMID: 29440624.
10. Salajegheh M, Ansari M, Foroghi MM, Kazemipour M. Computational design as a green approach for facile preparation of molecularly imprinted polyarginine-sodium alginate-multiwalled carbon nanotubes composite film on glassy carbon electrode for theophylline sensing. J Pharm Biomed Anal. 2019 Jan 5;162:215-224. doi: 10.1016/j.jpba.2018.09.032. Epub 2018 Sep 19. PMID: 30265981.
11. Koçak ÇC, Nas A, Kantekin H, Dursun Z. Simultaneous determination of theophylline and caffeine on novel [Tetra-(5-chloroquinolin-8-yloxy) phthalocyanato] manganese(III)-Carbon nanotubes composite electrode. Talanta. 2018 Jul 1;184:452-460. doi: 10.1016/j.talanta.2018.03.029. Epub 2018 Mar 15. PMID: 29674068.
12. Huang H, Wang D, Zhou Y, Wu D, Liao X, Xiong W, Du J, Hong Y. Multiwalled carbon nanotubes modified two dimensional MXene with high antifouling property for sensitive detection of ochratoxin A. Nanotechnology. 2021 Aug 17;32(45). doi: 10.1088/1361-6528/ac1a42. PMID: 34343976.
13. Plekhanova Y, Tarasov S, Bykov A, Prisyazhnaya N, Kolesov V, Sigaev V, Signore MA, Reshetilov A. Multiwalled Carbon Nanotubes and the Electrocatalytic Activity of Gluconobacter oxydans as the Basis of a Biosensor. Biosensors (Basel). 2019 Nov 14;9(4):137. doi: 10.3390/bios9040137. PMID: 31739608; PMCID: PMC6955703.
14. Li J, Liu Y, Tang X, Xu L, Min L, Xue Y, Hu X, Yang Z. Multiwalled carbon nanotubes coated with cobalt(II) sulfide nanoparticles for electrochemical sensing of glucose via direct electron transfer to glucose oxidase. Mikrochim Acta. 2020 Jan 2;187(1):80. doi: 10.1007/s00604-019-4047-8. PMID: 31897753.
15. Furtado RHM, Venkateswaran RV, Nicolau JC, Gurmu Y, Bhatt DL, Storey RF, Steg PG, Magnani G, Goto S, Dellborg M, Kamensky G, Isaza D, Aylward P, Johanson P, Bonaca MP. Caffeinated Beverage Intake, Dyspnea With Ticagrelor, and Cardiovascular Outcomes: Insights From the PEGASUS-TIMI 54 Trial. J Am Heart Assoc. 2020 May 18;9(10):e015785. doi: 10.1161/JAHA.119.015785. Epub 2020 May 15. PMID: 32410485; PMCID: PMC7660882.
16. Lo KH, Donohue J, Judson MA, Wu Y, Barnathan ES, Baughman RP; Sarcoidosis T-48 Investigators. The St. George's Respiratory Questionnaire in Pulmonary Sarcoidosis. Lung. 2020 Dec;198(6):917-924. doi: 10.1007/s00408-020-00394-7. Epub 2020 Sep 26. PMID: 32979072.
17. Nicoletti M, Gambarotti C, Fasoli E. Proteomic exploration of soft and hard biocorona onto PEGylated multiwalled carbon nanotubes. Biotechnol Appl Biochem. 2021 Oct;68(5):1003-1013. doi: 10.1002/bab.2020. Epub 2020 Sep 24. PMID: 32910836.
18. Rey-Raap N, Enterría M, Martins JI, Pereira MFR, Figueiredo JL. Influence of Multiwalled Carbon Nanotubes as Additives in Biomass-Derived Carbons for Supercapacitor Applications. ACS Appl Mater Interfaces. 2019 Feb 13;11(6):6066-6077. doi: 10.1021/acsami.8b19246. Epub 2019 Jan 31. PMID: 30652469.
19. Wei C, Zhu Y, Zhang Y. Effects of multiwalled carbon nanotubes on the dissolved organic matter released by Prorocentrum donghaiense: Results of spectroscopic studies. Ecotoxicol Environ Saf. 2020 May;194:110447. doi: 10.1016/j.ecoenv.2020.110447. Epub 2020 Mar 12. PMID: 32171959.
20. Li J, Sheng Z, Tan Y, Liu C, Zhou P, Zhou J, Chen R, Chen Y, Song L, Zhao H, Yan H. Combined with ticagrelor, 50 mg aspirin daily can reduce bleeding events without increasing ischemic risk compared with

75-100 mg aspirin daily in coronary artery disease patients: insights from the TIFU (Ticagrelor in Fuwai Hospital) study. Platelets. 2020 Aug 17;31(6):788-794. doi: 10.1080/09537104.2019.1680825. Epub 2019 Oct 23. PMID: 31645164.

21. Xi Z, Li J, Qiu H, Guo T, Wang Y, Li Y, Zheng J, Dou K, Xu B, Wu Y, Qiao S, Yang W, Yang Y, Gao R. Ticagrelor vs. Clopidogrel After Complex Percutaneous Coronary Intervention in Patients With Stable Coronary Artery Disease. Front Cardiovasc Med. 2021 Nov 22;8:768190. doi: 10.3389/fcvm.2021.768190. PMID: 34881313; PMCID: PMC8645933.
22. D'Amario D, Restivo A, Leone AM, Vergallo R, Migliaro S, Canonico F, Galli M, Trani C, Burzotta F, Aurigemma C, Niccoli G, Buffon A, Montone RA, Flex A, Franceschi F, Tinelli G, Limbruno U, Francese F, Ceccarelli I, Borovac JA, Porto I, Crea F. Ticagrelor and preconditioning in patients with stable coronary artery disease (TAPER-S): a randomized pilot clinical trial. Trials. 2020 Feb 17;21(1):192. doi: 10.1186/s13063-020-4116-7. PMID: 32066489; PMCID: PMC7027127.
23. Yu D, Ma L, Zhou J, Li L, Yan W, Yu X. Influence of CYP2C19 genotype on antiplatelet treatment outcomes after percutaneous coronary intervention in patients with coronary heart disease. Exp Ther Med. 2020 May;19(5):3411-3418. doi: 10.3892/etm.2020.8592. Epub 2020 Mar 11. PMID: 32266041; PMCID: PMC7132245.
24. Wu B, Shi L. Cost-utility of ticagrelor plus aspirin in diabetic patients with stable coronary artery disease. Eur Heart J Cardiovasc Pharmacother. 2021 Nov 3;7(6):529-538. doi: 10.1093/ehjcvp/pvaa082. PMID: 32645147.
25. Adamski P, Ostrowska M, Navarese EP, Kubica J. Pharmacodynamic and clinical efficacy of reduced ticagrelor maintenance doses in patients with coronary artery disease. Curr Med Res Opin. 2021 Feb;37(2):195-206. doi: 10.1080/03007995.2020.1854207. Epub 2020 Dec 7. PMID: 33211543.
26. Abidov A, Dilsizian V, Doukky R, Duvall WL, Dyke C, Elliott MD, Hage FG, Henzlova MJ, Johnson NP, Schwartz RG, Thomas GS, Einstein AJ. Aminophylline shortage and current recommendations for reversal of vasodilator stress: An ASNC information statement endorsed by SCMR. J Nucl Cardiol. 2019 Jun;26(3):1007-1014. doi: 10.1007/s12350-018-01548-0. Epub 2018 Dec 20. PMID: 30574677; PMCID: PMC6517353.
27. Chen Q, Zhou X, Hou R, Zhou Z, Wang Z, Chen Y, Weng J, Xu J. Aminophylline modulates the permeability of endothelial cells via the Slit2-Robo4 pathway in lipopolysaccharide-induced inflammation. Exp Ther Med. 2021 Sep;22(3):1042. doi: 10.3892/etm.2021.10474. Epub 2021 Jul 21. PMID: 34373728; PMCID: PMC8343459.
28. Peng C, Xiao D, Chen H, Rao L, Xiao H, Li H. Effects of beclomethasone and aminophylline combined with enteral nutrition in chronic obstructive pulmonary disease on nutritional status and immune function in elders. Asia Pac J Clin Nutr. 2021;30(1):60-66. doi: 10.6133/apjcn.202103_30(1).0008. PMID: 33787041.