Effects of oral administration of ticagrelor on the plasma level of adrenaline, histamine, serotonin, and acetylcholine in rat

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Abstract

Background: Ticagrelor as a reversible P2Y12 receptor antagonist which plays an important role in the treatment of acute coronary syndrome (ACS). Dyspnea is one of the main adverse reactions of ticagrelor, however the mechanism is not clearly now. The aim of this study was to assess the possible relationship between ticagrelor-related dyspnea and some neurotransmitter in plasma which can contract the bronchial smooth muscle.

Methods: The effects of ticagrelor on the plasma level of adrenaline, histamine, serotonin, and acetylcholine was studied in rats. Ticagrelor was administered at a loading dose of 24 mg/kg for the first time, and then maintenance dose 12 mg/kg, twice a day for 6 days. The plasma level of adrenaline, histamine, serotonin, and acetylcholine was determined by LC-MS/MS.

Results: The plasma level of serotonin increased after ticagrelor administrating, especially at 1.5h ($p < 0.05$) reach the level of statistical significance. The level of serotonin in plasma was consistent with ticagrelor blood concentration. Meanwhile, ticagrelor can cause a decrease in the plasma concentration of histamine, and the change was statistically significant at time points of 1.5h, 3.5h and 10.5h respectively. The concentration of the adrenaline and acetylcholine had no change.

Conclusions: The results of this study reveal that ticagrelor can increases blood serotonin levels and this may be a cause of dyspnea ticagrelor-related.

Background

Ticagrelor is an oral reversible adenosine diphosphate (ADP) receptor antagonist[1]. Compare with clopidogrel, the effect of ticagrelor is more effective in prevention of myocardial infarction and death after acute coronary syndrome[1]. So guidelines recommend priority use of ticagrelor over clopidogrel in combination with aspirin as dual antiplatelet therapy in the treatment of patients with ACS, regardless of plans for invasive management[2,3].

However, the dyspnea caused by ticagrelor is a relatively frequent side effect. In PLATO trial, 13.8% of patients receiving ticagrelor reported dyspnea. In PEGASUS-TIMI54, reported that 18.93 and 15.84% of patients receiving 180 and 120 mg, respectively of ticagrelor experienced dyspnea. The reported incidence of ticagrelor-related dyspnoea in PHILO trial and in SOCRATES was 5.7% and 6.2%, respectively[4]. A real-world study showed that the incidence of patient-reported dyspnoea with ticagrelor was 56.0% at 3 week[5]. In recent years, some case reports and retrospective studies have shown that dyspnea associated with ticagrelor occurs not only in the early stages of drug treatment, but also may cause delayed dyspnea and even more dangerous Cheyne-Stokes (CSR)[6,7,8]. Because of this adverse event, the rate of discontinuation from 0.9–6.5%[9,10], and one in 20 outpatients receiving a stabilized ticagrelor treatment might develop a dyspnea leading to an emergency room visit[11].

The exact mechanism of ticagrelor-induced dyspnea remains unclear[11,13]. There are several hypotheses (i)ticagrelor inhibits adenosine reuptake by red blood cell via inhibiting the sodium-independent equilibrative nucleoside transporter-1 (ENT-1). Elevated extracellular adenosine levels may stimulate the A1 and A2A receptors on vagal C fibers of pulmonary, causing dyspnea; (ii) ticagrelor inhibits P2Y12 receptors located on C fibres of sensory neurons[12]; (iii) ticagrelor inhibits P2Y12 receptor of microglia in the central nervous system, with potential purinergic stimulation of the chemoreflex system[13]. However, A study by Ortega-Paz L et al.[14], demonstrated that Plasma adenosine did not differ in patients with or without ticagrelor-related dyspnea after loading, before or after maintenance of ticagrelor. It is reasonable to suspect that some endogenous compounds level in blood increased after ticagrelor administration, and these endogenous compounds causing dyspnea by binding to receptors on the trachea and bronchi. Consequently in the present study, we evaluated the effect of administration of ticagrelor on the plasma level of adrenaline, histamine, serotonin, and acetylcholine. The plasma of experimental rats, like humans, also has several of the above-mentioned neurotransmitters. These neurotransmitters can also cause respiratory-related symptoms in rats. Therefore, we chose rats as the basis for this experiment. Human plasma experiments will be performed in the future.

Methods

Materials and reagents

Ticagrelor tablets (90mg,Belinda, AstraZeneca AB) .Ticagrelor-d7 (Toronto Research Chemicals lnc, product number 282022). Epinephrine (product number 189652);histamine (product number 168525);serotonin (product number 182552) purchased from China National Institute for Food and Drug Control; acetylcholine (purity 98.5%, product number 195455) purchased from Beijing Standards Subsidiary; Pentobarbital sodium(product number: F20020694)purchased from China Pharmaceutical (Group) Shanghai Chemical Reagent Company. All other chemicals were of LC-MS grade.

Drug administration

Healthy male Sprague-Dawley rats(n=12, clean grade, weighed between 180 and 220g, 6 to 8 week old), purchased from the Experimental Animal Center of Hebei Medical University, license number SCXK (Ji) 2018-004, certificate number: 1907228. All procedures were performed in accordance to the Hebei Medical University's Animal Care and Use Committee Guidelines. The rats were acclimatized for two weeks before the experiment. Animals were fasted for 12h prior to dosing with free access to water. All 12 rats received intraperitoneal injection of pentobarbital sodium (150 mg/kg) for euthanasia after this experiment.The ticagrelor suspensions were prepared using power of ticagrelor tablets with 3‰ Carboxymethyl Cellulose Sodium(90mg:10ml). The suspensions were fresh prepared, no earlier than an hour before administration, and were homogenized with vortex right before administration. Treatment modelled human medication intake. Right before the treatment, the body weights of the animals were measured individually in order to precisely calculate the amount of suspension to be given(Loading dose 24 mg/kg, Maintenance dose 12 mg/kg each rat). On the first day of the experiment, 3‰ Carboxymethyl

Cellulose Sodium adout 0.6ml were given at 8:00am. From the second day, ticagrelor suspension was administered at a loading dose of 24 mg/kg for the first time, and then Maintenance dose 12 mg/kg, every 12h for 6 days.

Blood sampling

Blood samples 0.5ml were collected at 9am and 4pm on the first day as blood samples before administration，each single animal is used as its own control．On the second day of the experiment, after Loading dose of ticagrelor 40 min, 1.5 h, 3.5 h,10.5 h，and on the 3rd , 6th days blood was collected from the venous plexus of the fundus of the rats.Blood samples put into the heparin sodium anticoagulant centrifuge tube (containing 20μL heparin sodium). It was immediately vortex mixed for 1 min, centrifuged for 5 min at 6500×g, and stored in the freezer at -40℃ until LC-MS/MS analysis. During our whole experimental period(7days), every rats were collected blood 8 times, about 0.5mL each time.

Quantification of adrenaline, histamine, serotonin, and acetylcholine in plasma samples

The preparation of samples was conducted as following: Plasma sample was thawed at room temperature, added 150 μL of the plasma into a 1.5 mL centrifuge tube, then added 400 μL (containing 50 ng / mL internal standard Ticagrelor-d7, 0.1% formic acid) acetonitrile solution, vortexed for 1 min and then centrifuged for 5 min at 12000g at 4℃, 10 μL supernatant was taken and injected directly into the LC-MS/MS apparatus.

The concentration of adrenaline, histamine, serotonin, and acetylcholine was determined using an LC-MS/MS on a liquid chromatograph system (ABSciex Shimadzu) ,which was equipped with an C18 column (150mm × 4.6mm, 3.5μm) maintained at 35°C. The mobile phase was composed of an aqueous solution containing 0.1% formic acid (A) and acetonitrile (B) at a flow rate of 0.6 mL/min. The gradient elution program was applied with the following program: 0.5min-5% B; 2.0min-20% B; 5.0min-100% B;6.5min-100% B; 6.6min-5% B; and then 5% B to equilibrate the column until 9min.

Mass spectrometric detection conducted on a Triple quadrupole mass spectrometer (model API4000 +, serial number BH20361205, ABSciex Shimadzu). The following parameter settings were used:Ionization method: electrospray ionization (ESI); monitoring method: positive ion monitoring; scanning method: multiple reaction monitoring (MRM); scanning time: 100ms; ion source spray voltage (ionspray voltage, IS) 5500 V; ion source temperature (TEM): 600 ° C; air curtain gas (CUR) pressure: 30 psi; atomizing gas (GS1, N2) pressure: 55 psi; auxiliary gas (GS2, N2) pressure: 60 psi ; Collision gas (CAD) pressure: 8 psi; The declustering potential (DP) and collision energy (CE) values of epinephrine, histamine, 5-hydroxytryptamine, acetylcholine and internal ticagrelor-d7 were 50eV and 20eV, respectively. Ev 50, 20 ev; 30 ev, 12 ev; 47 ev, 18 ev. The mass-to-charge ratio (m/z) of the ion formula used for quantitative analysis were: 184.2→166(adrenaline), 112→95 (histamine), 177→160.1 (serotonin), 146→87 (acetylcholine).

Statistical analysis

Results were expressed as mean ± standard deviation. The paired Student's t-test for comparison between the concentration of before and after ticagrelor administration. A *P* value of < 0.05 was considered statistically significant. All calculations were performed with the SPSS22.0 software.

Results

Changes of adrenaline, histamine, serotonin, and acetylcholine level are shown in table1-4, respectively. The concentration of the 4 substance have no statistical significance at 9am and 4pm on the first day. Compared with before ticagrelor administrating, the concentration difference of adrenaline and acetylcholine did not reach the level of statistical significance at all time points. Ticagrelor caused a decrease in the plasma concentration of the histamine to 22.85±10.1 ng/ml (p<0.05) at 1.5h, 22.04±9.95 ng/ml(p<0.05) at 3.5h, 20.58±12.3 ng/ml(p<0.05) at 10.5h, compared to the ticagrelor received before(31.97±9.99 ng/ml). The plasma level of serotonin incrased after ticagrelor administrating, especially at 1.5h and the third day(p<0.05) reach the level of statistical significance.

Table 1 Plasma level of adrenaline in rats（n=12）

Number	C/ng/ml						
Time	before administration1	before administration2	40min	1.5h	3.5h	10.5h	The third day	The sixth day
NO.1	30	29.8	31.4	29.7	26.3	23.2	29.4	29.8
NO.2	33.9	39	24.6	30.5	29.7	17.4	27.6	23.7
NO.3	28.4	26.5	35.1	26.0	23.3	24.4	24.8	24.1
NO.4	23.3	24.3	23.3	20.2	32.9	22.5	24.7	22.3
NO.5	29.3	23.1	29.8	23.9	35.8	21.9	30.1	28.9
NO.6	28.8	26.7	25.4	35.1	24.7	27.3	33.5	38.7
NO.7	18.9	23.1	27.8	24.5	26.3	17.9	17.5	20.3
NO.8	30.3	29.1	34	27.2	-	32.3	25.8	-
NO.9	21.9	18.7	22.5	31.2	19.7	18.8	22.5	23.7
NO.10	28	22.8	28.3	29.4	27.3	32.9	29.8	27.8
NO.11	32.1	31.6	30.1	34	28.5	36.1	38.8	34.8
NO.12	22.4	24.3	29.8	27.7	27	27.1	29.8	23.3

Table 2 Plasma concentration of histamine in rats（*n*=12）

Number	C/ng/ml							
Time	before administration1	before administration2	40min	1.5h	3.5h	10.5h	The third day	The sixth day
NO.1	33.8	35.2	30.6	22.9	18.9	15.8	38.8	40.1
NO.2	38	39.1	40.9	21.1	21.1	33.2	29.1	39.5
NO.3	10.9	20.1	21.2	10.04	28.8	10.4	11.2	17.8
NO.4	44.9	53.3	32.9	35.4	24.7	41.6	20.4	28.5
NO.5	33.5	37.6	22.4	16.3	9.17	25.7	10.9	23.3
NO.6	41	38.3	32	16	13.4	41.4	29	39.7
NO.7	44.1	38.5	42.6	27.6	36.1	14.3	38.9	29.7
NO.8	38.1	29.9	22	22.1	17.5	10.3	10.9	31.3
NO.9	22.5	22.7	23.1	15.2	22.7	7.14	33.9	25
NO.10	22.2	36.6	44.4	13.5	10.9	9.54	28.2	28.3
NO.11	28.2	21.6	26.7	45.1	42.8	15.2	22.3	29.8
NO.12	16.3	21	29.4	29	18.5	22.4	23.2	10.9

Table 3 Plasma concentration of serotonin in rats (*n*=12)

Number	C/ng/ml							
Time	before administration1	before administration2	40min	1.5h	3.5h	10.5h	The third day	The sixth day
NO.1	70.2	77.3	70.3	79.9	85.3	71.7	88.8	76.8
NO.2	82.2	39.4	80.3	88.7	89.7	66.3	88.7	69.7
NO.3	107	111	132	133	109	131	133	109
NO.4	93.3	90.3	109	107	-	88.8	93.2	108
NO.5	69.6	70.5	55.3	78.8	79.7	80.9	67.7	66.8
NO.6	59.1	66.7	55.8	70.7	-	79.8	77.1	56.6
NO.7	73	73	60.3	94.3	89.7	75.6	88.6	75
NO.8	93.7	98.8	133	105.3	75.4	59.1	110	92.1
NO.9	86.7	88.8	75.3	75.4	59.1	93.3	98.3	80.3
NO.10	108	118	77.6	121	103	93.2	105	118
NO.11	62.1	66.8	36.6	150	68.3	76.6	76.6	79.8
NO.12	43.9	49.9	56.3	59.7	55.5	43.7	43.7	60.1

Table4 Plasma concentration of acetylcholine in rats (n=12)

Number	C/ng/ml							
Time	before administration1	before administration2	40min	1.5h	3.5h	10.5h	The third day	The sixth day
NO.1	2.48	2.1	2.03	1.78	2.55	1.97	1.87	2.2
NO.2	2.43	2.57	2.93	2.52	2.34	2.44	2.03	2.32
NO.3	2.62	2.52	2.9	2.99	2.51	2.65	2.55	2.65
NO.4	1.5	1.61	1.57	1.59	1.42	1.49	1.71	1.42
NO.5	1.57	1.65	1.75	2.18	1.96	1.42	0.93	1.27
NO.6	2.24	1.93	1.23	1.48	2.2	1.98	2.18	1.79
NO.7	1.34	1.54	1.21	1.25	2.03	1.36	1.58	2.05
NO.8	2.06	1.98	1.68	1.98	2.54	-	1.68	1.97
NO.9	1.55	1.55	0.9	1.69	2.34	1.32	0.98	1.5
NO.10	1.91	2.14	2.2	2.04	2.19	2	2.61	2.2
NO.11	2.67	2.29	2.39	2.68	2.35	2.14	2.19	2.53
NO.12	1.65	1.89	3	2.13	2.02	2.04	2.27	2.01

Table 5 Effect of Ticagrelor Tablets on the pharmacokinetics of Neurotransmitter in rats (n=12)

Substance	Before administration	40min	1.5h	3.5h	10.5h	3day	6day
adrenaline	27.27±4.57	28.5±4.00	28.28±4.27	27.4±4.39	25.15±6.13	27.85±5.44	27.94±5.87
histamine	31.97±9.99	30.68±8.25	22.85±10.1*	22.04±9.95*	20.58±12.3*	24.73±10.11	28.66±8.82
Serotonin	79.13±20.38	78.48±30.86	96.98±27.13*	81.00±16.68	80.01±21.34	89.22±22.43*	82.68±19.97
acetylcholine	1.99±0.41	1.98±0.72	2.02±0.51	2.20±0.31	1.89±0.44	1.88±0.54	1.99±0.43

Note：The paired Student's t-test for comparison between the concentration of before and after ticagrelor administration(loading dose 24mg/kg, maintenance dose 12mg/kg).*P<0.05(mean ± standard deviation,n=12),nonparametric tests-wilcoxon are applied at a few time points where the differences do not conform to the normal distribution; The before administration concentration are the mean of the concentration from the twice blood samples in the first day of this experiment, the results of paired Student's t-test show that there was no significant difference between the twice concentration.

Discussions

The aim of our study was to assess the presumable relationship between some compounds in blood which can contract the bronchial smooth muscle and ticagrelor-related dyspnea.

The results obtained from our study demonstrated that blood concentration of serotonin increased after ticagrelor administration, and the peak blood concentration of serotonin reached at 1.5 h after ticagrelor administration. Our previous study showed that the T_{max} of ticagrelor in rats is 1.3 ± 0.59 h. The data at each time point proved that the increase of serotonin concentration was consistent with the increase of ticagrelor concentration. As the times of doses administration increases, especially on day 6, the blood concentration of serotonin decrease to the baseline values. Our result was agreement with the characterized of ticagrelor-related dyspnea, which occurs early after ticagrelor administration, short-lived, relevance of higher plasma concentration of ticagrelor, disappearing after drug withdrawal[15,16,17].

Serotonin is one of the major bronchoconstrictors[18,19], it causes the respiratory smooth muscle to contract by acting directly on the serotonin receptors located on the respiratory smooth muscle or regulating the release of other neurotransmitters [20]. 95% of serotonin is produced by the enterochromaffin cells of the gastrointestinal tract and only 5% is produced in brain. While serotonin cannot pass across the blood-brain barrier thereby isolating the brain from serotonin generated in the periphery. Approximately 99% of serotonin in blood is stored in platelets, which are released into the blood when they are activated[21]. Platelets cannot synthesize serotonin on their own, due to a lack of enzymes responsible for serotonin synthesis. But circulating platelets can readily take up serotonin from the blood, store it in their δ-granules at high concentration (65 mmol/L) and secrete during their activation. In platelets, serotonin transporters (SERT) mediates the uptake of serotonin from the circulation[19,22]. This is the first time to observe ticagrelor induced serotonin blood concentration increased, further study on the mechanism is needed. According to literatures[21,23], selective serotonin reuptake inhibitors bind to SERT on platelet yet and block the uptake of serotonin into platelets to decrease cellular serotonin, and the reduction leads to impairment of platelet aggregation. In theory, we guess that ticagrelor maybe inhibit SERT function of platelet to inhibit serotonin uptake, thereby increasing blood serotonin levels. So the SERT inhibition may be the mechanism of dyspnea as well as anti-platelet of ticagrelor.

Conclusions

In conclusion, ticagrelor-related dyspnoea possibly be caused by high level of serotonin in plasma after ticagrelor administration, but definitely requires experimental support and much more data to become substantiated.

Abbreviations

ACS acute coronary syndrome

SERT serotonin transporters

Declarations

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Authors' contributions

YXL analyzed and interpreted the relationship between endogenous substances and ticagrelor related dyspnea.GX performed the experiments, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

In vivo experiments were performed in accordance with international guidelines and experimental procedures performed with Animal ethics committee of the second hospital of Hebei medical university.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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