Effect of Metronidazole on Clopidogrel Bisulphate Efficacy in Male Albino Rats

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Abstract

The data about this combination effect on platelet function and hematological parameters are sparse, but there is only mention in many medical science websites such as Medscape, there are possible interaction between it, in which metronidazole cause inhibition to cytochrome CYP3A4 which responsible on clopidogrel metabolism, this may put the patients that on clopidogrel bisulphate therapy at risk of clot formation and may be progress, this may occur because clopidogrel is at inactive form and need to activation by cytochrome mention above, so this study was performed in order to achieve the following aims: studying the efficacy of clopidogrel bisulphate on coagulation process by measuring bleeding time when giving clopidogrel alone and in combination with metronidazole and studying the possible effect of two drug alone and in combination on hematological parameters in male albino rats.

Keywords: *Clopidogrel bisulphate, metronidazole, cytochrome CYP3A4, bleeding time*

Introduction

Clopidogrel, a thienopyridine derivative, binds precisely also permanently to the platelet P2RY12 purinergic receptor, constraining ADP-mediated platelet motivation and aggregation [(1)]. Next oral taking of clopidogrel is promptly absorbed owned to more metabolism, clopidogrel is one of prodrug that is absorbed in the intestine and activated in the liver, then it converted to its active metabolite needs dual sequential oxidative steps, at the first time result in formation of 2-oxo-clopidogrel, followed by the conversion of 2-oxoclopidogrel to the active metabolite. CYP2C9 and CYP3A4/5 are connected to cytochrome P450 enzymes involved in the metabolism of clopidogrel [(2)]**.** CYP2C9 is most important hepatic enzymes involved in the formation of clopidogrel's active metabolite. Genetic polymorphisms of *CYP2C9* are associated with impaired clopidogrel metabolism in healthy volunteers and also in patients [(3)]. Metronidazole is antibiotic and antiprotozoal medication. it is one type for a first event of mild-to-moderate Clostridium difficile colitis if vancomycin or fidaxomicin is unavailable [(4)]**.** It has been suggested possible inhibition of cytochrome P450 especially CYP2C9 and CY3A4, this enzyme is responsible for several drugs metabolism such as S-warfarin.*etc.*[5] **.**And this may appear in revers effect when combined with clopidogrel when compared with warfarin because the difference between two drugs chemically (warfarin is active drug and this combination cause abnormal bleeding).

Materials and Mthod

Experimental animals: A total number of twenty male Albino Wistar rats weighed (200-250 g) were utilized in this research. Their ages ranged between 4-5 months. Rats in all time of the experiment were housed in plastic cages in conditioned room (22-25°C) in the animal house of College of pharmacy – University Of Anbar with manually controlled lightening supplying daily light of 12 hours (7.00 AM to 19.00 PM) and 12 hours night cycle. They were left for 7 days for adaptation with the research conditions. Rats had free access to water and diet along the experimental period.

Experimental Design: Rats were randomly divided into four equal groups (5rat/group) and administered orally for 28 days by using special gavage needle as the following: C group rats that received ordinary distilled water and served as control, T1 group rats were treated

1.25 mg/kg B.W of clopidogril bisulphate, T2 group Rats were treated 35 mg/kg B.W of metronidazole and T3 group rats were treated 1.25 mg/kg B.W of clopidogril bisulphate + 35mg/kg B.W of metronidazole.

Bleeding time test performance: Bleeding time was measured via rat tail transection method according to the prescription of The tail of the rat was warmed for 60 seconds in water at 40°C then dried [6] . A slight cut was made in the mid of the tail with a blade. Account started when the 1st drop touched the circular filter paper. It was tested at 30 s intervals till bleeding cosseted

*Total number of spot on filter paper/2= BT /minute.

Hematological Analysis: All animals in experiment comparing with the normal values of blood parameters as control and zero time. The blood collected from heart puncture after general anesthesia by using (Ketamine and xylazine). By using Uto-analyzer (setting on vet mode) Platelet count, RBC, WBC, and HB level were estimated. The procedure done according to manufactured instruction.

Statistical Analysis: The data was accomplished by using SAS (Statistical Analysis System - version 9.1). One-way ANOVA, two-way ANOVA and Least significant differences (LSD) post hoc test were complete to assess significant differences among means. $P < 0.05$ was considered statistically significant.

Results

Before beginning of dosage of experiment (at zero time), Bleeding time and other hematological parameters was determined and there was no significance different ($P < 0.05$) in all groups.

1. Bleeding time: A statistical analysis indicated that the mean value of bleeding time was non-significantly ($P<0.05$) differed in all experimental groups at zero time when compared to each other. The final results showed significant ($P>0.05$) increased after oral intubation of clopidogrel bisulphate, metronidazole and combination in T1, T2 and T3 groups comparing to the control group and zero time. The T1 group that receive 75 mg/Kg/day of clopidogrel bisulphate was more potent in increasing of bleeding time comparing to 35 mg/kg/day of metronidazole and in combination treated group (T2, T3) respectively.

Table1: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on bleeding time (by tail method as prescribed previously) of treated adult male rats. (min)

Group	Day of experiment	
	Zero time	End of experiment
Control	A2.40±0.18a	A2.30±0.12d
T1	B2.50±0.22a	A13.90±0.18a
T2	B2.60±0.18a	A3.80±0.12c
T3	B2.50±0.15a	A7.20±0.12b
LSD	0.4831	

- Different Capital letters denote differences between groups, ($P<0.05$).

- Different Small letters denote differences within group, ($P< 0.05$).

2. Platelet count: The effect of oral intubation of 75 mg/Kg/day of clopidogrel bisulphate (T1), 35 mg/Kg/day metronidazole (T2) and combination dose of both of them T3 for 28 day on treated rats RBCs count are clarified in table 2**.** There were none considerable ($P<0.05$) differences in platelet count between empirical groups in zero time, at the finish of research there was a significant ($P<0.05$) reduction in platelet count in T1 and T3 groups respectively compared with control group, zero time and T2 group. A high significant decreases ($P<0.05$) were recorded in animal of T1 group followed by T3 (group of combination treatment).

Table 2: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on platelet count of treated adult male rats ($X10^9$/µl).

Group	Day of experiment	
	Zero time	End of experiment
Control	A200.40±0.50ab	A200.20±0.37a
T1	A201.80±0.58a	C178.00±0.44c
T2	A201.20±0.86a	A200.20±0.37a
T3	A199.80±0.37b	B186.80±0.80b
LSD	1.6797	

- Different Capital letters denote differences between groups, ($P<0.05$).

- Different Small letters denote differences within group, (P< 0.05).

3. Red blood cells count: A statistical analysis indicated that the mean value of RBCs count was non-significantly (P<0.05) differed in all experimental groups at zero time when compared to each other. At the end of experimental RBCs count was significantly (P>0.05) decreased after oral intubation of clopidogrel bisulphate, metronidazole and combination (clopidogrel bisulphate plus metronidazole) in T1, T2 and T3 groups comparing to the control group. The extent of decrement was higher in T1 group, where as in T3 group (combination therapy) the change was at little degree when compared with T1 group.

Table 3: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on RBC count of treated adult male rats ($X10^6$/µl).

Group	Day of experiment	
	Zero time	End of experiment
Control	A8.84±0.62a	A8.44±0.01a
T1	A8.33±0.09a	C5.26±0.04c
T2	A8.35±0.09a	A7.26±0.04b
T3	A8.26±0.10a	B6.09±0.02c
LSD	0.6602	

- Different Capital letters denote differences between groups, (P<0.05).

- Different Small letters denote differences within group, (P< 0.05).

4. WBC count: Table 4 illustrates the mean value of WBC count in the control and treated group throughout the experimental period. It can be seen that WBC count were in the normal in all groups at the zero time, after animals received clopidogrel bisulphate, metronidazole and combination of them a significant fall (p<0.05) in WBC count., a significant (P<0.05) decrease in WBC count was observed in all treated groups (T1, T2, T3) comparing to the pretreated period but at a different extent more potent in T1 follwed by T3 and T2 group.

Table 4: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on WBC count of treated adult male rats ($X10^3$/µl).

Group	Day of experiment	
	Zero time	End of experiment
Control	A9.56±0.10a	A9.26±0.02a
T1	A9.53±0.07a	D6.10±0.03d
T2	A9.55±0.12a	B8.31±0.05b
T3	A9.51±0.06a	C7.23±0.04c
LSD	0.2184	

- Different Capital letters denote differences between groups, (P<0.05).

- Different Small letters denote differences within group, (P< 0.05).

5. Hemoglobin concentration: Table 5 illustrates the mean value for Hb concentration in the control and treated group throughout the experimental period. It can be seen that hemoglobin concentrations were in the normal in all groups at the zero time, after animals received clopidogrel bisulphate, metronidazole and combination of them a considerable fall (p<0.05) in hemoglobin concentration after 28 days of the treatment as compared with pretreated period and control group, this decrease is coincided with the decrease of RBCs in all groups of experiment.

Table 5: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on Hemoglobin concentration of treated adult male rats (mg/dl).

Group	Day of experiment	
	Zero time	End of experiment
Control	A14.76±0.15a	A14.30±0.12a
T1	A15.05±0.11a	D9.51±0.14d
T2	A14.84±0.12a	B13.59±0.05d
T3	A15.03±0.15a	C11.97±0.04d
LSD	0.3522	

- Different Capital letters denote differences between groups, (P<0.05).

- Different Small letters denote differences within group, (P< 0.05).

Figure (1): showed the steps of experiment performance starting from housing of animals (A), dosing of the drugs (B), (C) anesthetizing animals, (D) bleeding time test, and (E&F) blood collection for hematological test parameters.

Discuusion

It was reported that clopidogrel bisulphate cause a significant change in bleeding time in 36 human volunteer after giving a 75 mg of Plavix [7]. Also another study indicated that clopidogrel bisulphate associated with significantly increased risk for postoperative wound complications especially increasing of bleeding time in tested patient [8]. These normally cause increasing of bleeding in T1 group. In T2 group treated with metronidazole alone, it was reported a partial inhibition platelet aggregation with dose and time dependent in guinea pig and human platelet-rich plasma and this may cause a mild increasing in bleeding time according to dose that we give in comparison with previous study [9].

In T3 the combination of clopidogrel bisulphate and metronidazole which sharing same metabolic enzyme or cytochrome that may lead to inhibition or slowing the process of clopidogrel bisulphate converting to its active form [10] ,this mention why the combination of two drugs causing less bleeding time than clopidogrel bisulphate alone treated group. Many of case report indicates that in addition to the increased risk of bleeding with the use of Clopidogrel, some of its additional serious side effects include agranulocytosis, aplastic anemia/pancytopenia, thrombotic thrombocytopenic purpura, acquired hemophilia A, and neutropenia, neutrophils make up

a significant portion of the WBCs [11]**.** With regard to metronidazole there was reported that metronidazole can cause bone marrow depression [12]. And normally this can affect all type of blood cells especially in chronic use

Conclusion

The efficacy of clopidogrel bisulphate as blood thinner agent may be reduced when combined with metronidazole despite of the second have characteristic of antiplatelet agent, this were obvious in bleeding time test results and other hematological parameters, may be due to the inhibition of metronidazole to cytochrome that responsible for clopidogrel metabolism, in addition to possibility of compete the two drugs and/or inhibition of each other. This still need to more clearing by check the platelet activity in presence of this combination by more specific tests and apparatus. more attention should be paid when use the metronidazole in patient who take clopidogrel bisulphate especially critical cases by putting the patient under close monitoring or use alternative drugs.

Conflict of Interest: personal relationship

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Ethical Clearance: institutional

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