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ASSESSMENT OF HEMOSTATIC EFFECTS OF OXYTOCIN AND MISOPROSTOL IN MICE

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ABSTRACT

Oxytocin and Misoprostol are uterotonic drugs, used to prevent postpartum hemorrhage. This work was undertaken to evaluate the effects of oxytocin and misoprostol on blood coagulation, to demonstrate their ability to stop bleeding. Twenty four (24) mice of both sexes were used to determine the bleeding time. Split into four groups of six animals, mice were treated intramuscularly. The control group received saline solution (NaCl 0.9 %) while the Groups II and III were treated with Oxytocin (20 mg/kg) and misoprostol (20 mg/kg) respectively. Pytomenandione (20 mg/kg) used as a reference drug was administered to the last group. After a period of 20 minutes, bleeding time was measured by tail hemorrhage model. Activated partial thromboplastin time and prothrombin time were determined by a coagulometer. Misoprostol, oxytocin and phytomenandione shortened the bleeding time from 270 ± 60s to 90±5s, from 270 ± 60 s to 195±75s, and from 270 ± 60 s to 165±75 s respectively. The effects of misoprostol were significant ($p<0.01$). Misoprostol, oxytocin and phytomenandione reduced, in a concentration dependent manner, activated partial thromboplastin time. At the unique concentration of 1 mg/ml, these drugs shortened significantly activated partial thromboplatin time ($p<0.1$). Misoprostol and oxytocin possess hemostatic effects and could reduce bleedings in the management of postpartum hemorrhage.

KEYWORDS: Bleeding disorders, Misoprostol, Oxytocin, Postpartum hemorrhage.

INTRODUCTION

Maternal health includes women's health during pregnancy, delivery and the post-partum period. Each step should be normal to make sure that women and their babies achieve their full potential for health and well-being.[1] The postpartum period, defined as the six weeks after delivery, is a crucial time for women's health due to haemorrhage. Postpartum haemorrhage (PPH) refers to a total haemorrhage of over 500 ml within 24 hours of delivery of the fetus.[2] This is an insecure condition that results in maternal morbidity and mortality.[3] The most common cause of primary PPH is uterine atony. Oxytocin and misoprostol were used to prevent PPH related to uterine atony. The World Health Organization recommends the use of oxytocin as a pre-eminent prophylactic drug as an uterotonic.[2] A recent study, showed that carbetocin was superior to oxytocin and misoprostol in preventing atonic HPP in patients at high risk of elective cesarean delivery.[4] The association of misoprostol and oxytocin in the prevention of PPH in this low-resource setting improved the obstetrical outcome by reducing the risk and the amount of blood loss during delivery.[5] Misoprostol has been well documented to be an effective uterotonic agent for the treatment of HPP.[6] One cause for postpartum hemorrhage is difficult clotting. Increased levels of oxytocin infusion at 30 IU/h have also been shown to increase coagulability in full-term deliveries.[7] However, there is limited information on the effects of oxytocin and misoprostol on blood clotting.

The aim of our study was to assess *in vitro* and *in vivo* effects of oxytocin and misoprostol on blood coagulation in mice, in order to reveal properties of these medications in managing post-partum hemorrhage associated with bleeding disorders.

MATERIAL AND METHODS

Animals and ethics

Mice (*Mus musculus)* and rabbits (*Orytologus cunuculus*) weighting 25 ± 7 g and 1.5 ± 0.5 kg respectively, were used in this experiment. Experimental procedures and protocols used in this study were approved by ethical committee of Health Sciences, University Felix Houphouet-Boigny of Cocody-Abidjan. These guidelines were in accordance with the internationally accepted principles for laboratory use and care.[8] These animals were obtained from the Animal House of the Laboratory of Biology and Health of UFR Biosciences at Cocody University in Abidjan (Côte d'Ivoire). They were housed in a constant temperature rooms with a light/dark cycle of 14/10 hours. All animals were fed and given water ad libitum until use.

Chemicals and reagents

Prothrombin, cephalin-kaolin and Calcium chloride ($CaCl_2$ 0.025 M) were obtained from Cypress Diagnostics (Belgium). All other chemicals and reagents used, were of analytical grade.

Hemostatic activity

Preparation of rabbit platelet-poor plasma

Platelet-poor plasma was obtained according the method described by the professional order of medical technologists of Quebec.[9] Platelet-poor plasma (PPP) was separated from citrated rabbit whole blood. At first, the whole blood was centrifuged at 2500 rpm for 15 min. The supernatant obtained, was removed without disturbing the pellet. To be sure that the plasma was devoided of platelet, a second centrifugation was operated at 2500 rpm for 10 min. The new plasma was taken without cellular debris and was stored at −20°C until used.

Activated partial thromboplastin time (aPPT) assay

Activated partial thromboplastin time and prothrombin time assay were performed according to the method of[10], with slight modifications**.** Plasma (43 µl) was pipetted into clotting tubes and warmed for 2–3 min at 37°C. Then, 7 µl of distilled water (for control), and of plant extracts (10-1- 1 mg/ml) for the test were added. Cephalin-koalin reagent (50 µl) and calcium chloride (50 µl) were added to the mixture respectively. Cephalin-koalin reagent and calcium chloride were pre-warmed at 37°C for 2-3 min. Phytomenadione (10^{-1}- 1 mg/ml) was used as reference drug The coagulation time was recorded with a coagulometer (CyanCoag, Belgium).

Prothrombin Time (PT) assay

To investigate the extrinsic pathway of coagulation, 43 µl of plasma was pipetted into clotting tubes and incubated for 2–3 min at 37 °C. Then, 7 µl of distilled water (for control), and of plant extracts (10^{-1}- 1 mg/ml) for the test, were added to clotting tubes. Prothrombin reagent (100 µl) pre-warmed at 37°C for 2-3 min was added to the mixture. Phytomenadione (10^{-1}- 1 mg/ml) was used as reference drug. The coagulation time was recorded with a coagulometer (CyanCoag, Belgium).

Bleeding time activity

The bleeding time was realized according to the method of[11]**,** with few modifications. Twenty four (24) mice were used for this experiment. Animals were divided into four groups

of six mice. The control group received saline solution (NaCl 0.9 %) by intramuscular route. Oxytocin (20 mg/kg) and Misoprostol (20mg/kg) were administered to group II and group III respectively. The last group received phytomenadione (20 mg/kg), used as positive control and all groups of animal were anesthetized by ketamine (100 mg/kg,). After 20 minutes, a 3-mm segment of the tail tip was cut off with a scalpel. The bleeding from the tail was monitored by gently absorbing the cord with filter paper without touching the wound site. When no blood was observed on the paper after 30-second intervals, bleeding was determined to have ceased.[11,12] The experiment was stopped after 20 minutes.

Statistics

Statistical analysis were realized using Graph-pad Prism 5 (Graph-pad Software Inc., USA). The results were expressed as mean ± SEM of four independent measurements. Statistical analysis was determined by using One-way Analysis of Variance (ANOVA), and Turkey's multiple comparison test was also applied. The results were indicated as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of oxytocin and misoprostol on activated partial thromboplastin time

The hemostatic activity of oxytocin and misoprostol was tested with platelet-poor rabbit plasma in comparison with phytomendione (coagulant drug). Oxytocin and misoprostol have shown coagulant effects, in a concentration dependent manner. For concentrations ranged from 10^{-3} to 1 mg/ml, the aPPT sample / aPPT control ratio declined from 0. 89 ± 0.037 to 0. 54 ± 0.056 and from 0.86 ± 0.05 to 0.44 ± 0.045 for oxytocin and misoprostol respectively. This decrease was significant at a concentration of 1 mg/ml, in comparison with that of phytomenadione (*$p < 0.1$). The outcomes are summarized in **Figure 1.**

Effects of oxytocin and misoprostol on prothrombin time

Oxytocin and phytomenadione did not affect the prothrombin time while misoprostol decreased it. Misoprostol (10^{-3} -1 mg /ml) reduced the prothrombin time to 0.93 ± 0.04 to 0.50 ± 0.80. This decrease was significant at a concentration of 1 mg/ml (*$p < 0.1$) (**Figure 2**).

Effects of oxytocin and misoprostol on bleeding time

At the end of this experiment, it appears that misoprostol and oxytocin tested, induced coagulant effects. The control group treated with saline solution (0.9 %) induced a bleeding

time of 270 ± 60 s. The two groups of mice who received oxytocin and misoprostol have shown a bleeding time of 195±75 s and 90±5 s respectively. The group of animal treated with phytomenadione, shortened the bleeding time from 270 ± 60 s to 165±75 s. There is a strong activity of misoprostol on coagulation time. This decrease induced by the misoprostol was significant compared to that of the saline solution (0.9 %) (*p < 0.1). **Figure 3** showed the effects of drugs on bleeding time.

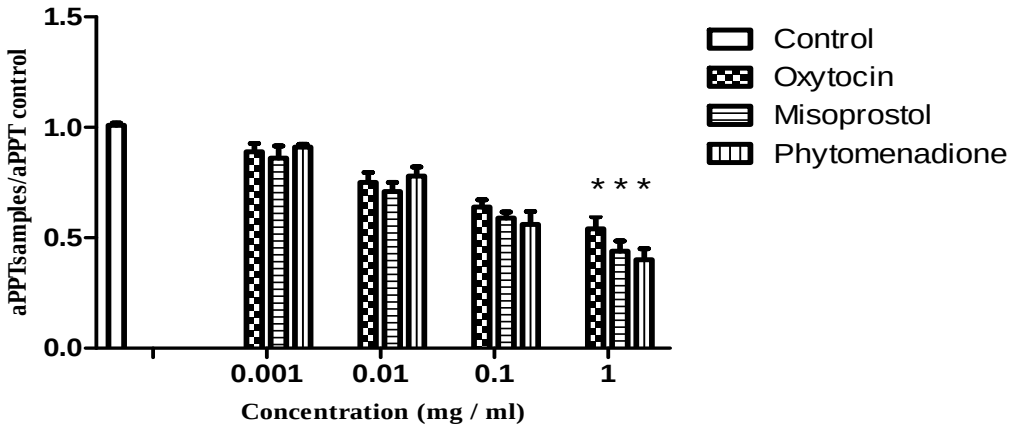

Fig 1: Effects of oxytocin, misoprostol and phytomenadione on actived partial prothrombin Time (aPPT) *in vitro*. Oxytocin, misoprostol and phytomenadione decreased coagulation time in a concentration-dependent manner by intrinsic pathway. This decrease was significant at concentration of 1mg/ml (* p < 0.1, n = 4).

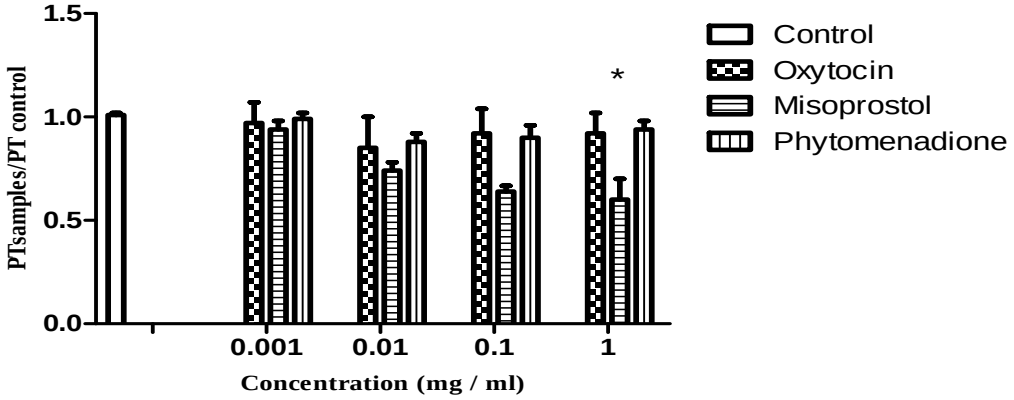

Fig 2: Effects of oxytocin, misoprostol and phytomenadione (vitamin K_1) on prothrombin time *in vitro*. Oxytocin and phytomenadione did not affected coagulation time by extrinsic pathway. However, misoprostol shortened PT significantly at concentration of 1 mg/ml (* p < 0.1, n = 4).

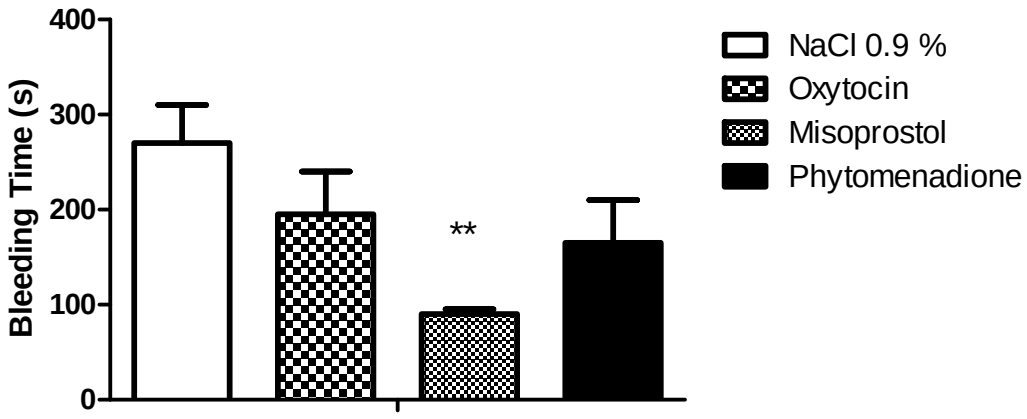

Fig 3: Effects of oxytocin, misoprostol and phytomenadione on Bleeding Time *in vivo*. Clotting time is reduced in mice treated with oxytocin, misoprostol and phytomenadione compared to those treated with saline solution. This decrease was significant with misoprostol (p < 0.01, n = 6).**

In vivo assays in mice demonstrated a reduction in bleeding time in animals treated with misoprostol and oxytocin. *In vitro* tests were realized to account for the hemostatic activities induced by oxytocin and misoprostol. The effects of oxytocin and misoprostol were evaluated on activated thromboplastin time and prothrombin time. Misoprostol shortened activated thromboplastin time and prothrombin time while oxytocin reduced activated thromboplastin time. Clotting factors VIII, IX, XI and XII are thought to play a part in the intrinsic pathway.[13] The extrinsic pathway would involve coagulation factors II, VII and X.[14] Misoprostol caused hemostatic activity which would act by the intrinsic and extrinsic pathway of coagulation. In addition, misoprostol showed a significant hemostatic effect *in vivo*. A possible intervention of misoprostol in the activation of the factors of the two coagulation pathways would explain its strong capacity to reduce clotting time. Oxytocin would act mainly by the intrinsic pathway of coagulation like phytomenadione (vitamin K1). Oxytocin also developed an *in vitro* hemostatic effect on the whole blood of term parturient.[15] This coagulant effect of oxytocin would be due to the increase in factor VIII and the degradation of fibrinogen into fibrin.[16] Oxytocin and misoprostol are uterotonic drugs, used to prevent PPH related to atony. Additionally, the hemostatic effects of oxytocin and misoprostol would argue for their use in the management of PPH associated with bleeding disorders.

CONCLUSION

Misoprostol and oxytocin exhibited hemostatic activity and could act mainly to shorten bleeding time through intrinsic blood clotting pathways. This work opens a way to research natural products with both uterotonic and hemostatic properties to manage PPH.

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REFERENCES

1. World Health Organization. WHO recommendations on maternal health: guidelines approved by the WHO Guidelines Review Committee. 2017. https://apps.who.int/iris/handle/10665/259268. Licence: CC BY-NC-SA 3.0 IGO.
2. World Health Organization. Recommandations de l'OMS pour la prévention et le traitement de l'Hémorragie Post-partum (HPP). 2014. www.who.int /reproductivehealth /publications / maternal-perinatal-health / 9789241548502 / en /.
3. Zhang HY, Wang CJ, Jin YS. Effects of calcium supplementation during delivery on postpartum hemorrhage, labor time, and Apgar score in high-risk women [J]. China Matern Child Health Care, 2018; 33(16): 3609-3611.
4. Abd El-Naser AG, Ahmed MN, Hazem H A, Mahmoud IR, Mahmoud B. Carbetocin versus Oxytocin and Misoprostol in prevention of atonic post-partum hemorrhage in high risk patients planed for cesarean delivery. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. Int J Reprod Contracept Obstet Gyneco, 2018; 7(1): 10-14.
5. Numfor E, Fobellah NN, Tochie JN, Njim T, Ndesso SA. Oxytocin Versus Misoprostol Plus Oxytocin in the Prevention of Postpartum Hemorrhage at a Semi-Urban Hospital in sub-Saharan Africa: A Retrospective Cohort Study. Int J Matern Child Health AIDS, 2020; 9(3): 287-296.
6. Prata N and Weidert K. Efficacy of misoprostol for the treatment of postpartum hemorrhage: current knowledge and implications for health care planning. Int J Women's Health, 2016; 8(1): 341-349.
7. Golparvar M, Esterabi M, Talakoub R, Saryazdi HH. Evaluation of *in vivo* effects of Oxytocin on coagulation of parturient during cesarean delivery by thromboelastography .J Res Pharm Pract, 2014; 3(1): 28-33.

8. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization and documentation of herbal medicine. Pure Appl Chem, 2008; 80: 2195-2230.
9. OPTMQ. Guide d'hémostase. 2017. ISBN: 978-2-9816759-1-0.
10. Mao W, Li H, Li Y, Zhang H, Chen Y, Guo S. Chemical characteristic and anticoagulant activity of the sulfated polysaccharide isolated from *Monostroma latissimum* (Chlorophyta). Int J Biol Macromol, 2009; 44: 70-74.
11. Grüner S, Prostredna M, Aktas B, Moers A, Schule V, Krieg T, Offermanns S, Eckes B, and Nieswandt B. Anti–Glycoprotein VI Treatment Severely Compromises Hemostasis in Mice with Reduced α2β1 Levels or Concomitant Aspirin Therapy. Vasc Med, 2004; 110(18): 2946-2951.
12. Carmeliet P, Stassen JM, Schoonjans L. Plasminogen activator inhibitor-1 gene-deficient mice, II: effects on hemostasis, thrombosis, and thrombolysis. J Clin Invest, 1993; 92: 2756–2760.
13. Wheeler AP and Gailani D. The intrinsèque pathway of coagulation as a target for antithrombotic therapy. Hematol Oncol Clin North Am, 2016; 30(5): 1099-1114.
14. Mackman N, Tilley RE and Key NS. Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. Arterioscler Thromb Vasc Biol, 2007; 27: 1687-1693.
15. Butwick A and Harter S. An in vitro investigation of the coagulation effects of exogenous oxytocin using thromboelastography in healthy parturients. Anesth Analg, 2011; 113: 323-326.
16. Neuenschwander S and Pliska V. Effects of neurohypophyseal hormone analogues on blood clotting factor VIII and fibrinolytic activity in sheep. Eur J Pharmacol, 1997; 323: 205–213.