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THE EFFECT OF GINKGO FORMULATIONS ON PLATELET AGGREGATION IN RATS

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Summary

Ginkgo biloba is an herbal remedy can be found as an ingredient of many dietary supplements. Ginkgo contains different biologically active compounds that can inhibit platelet aggregation. Prolonged platelet aggregation can have a positive effect on the cardiovascular system in cancer patients undergoing chemotherapy, but in oncological surgery (in particular major abdominal, gynecological, and operations with reconstructions) it can increase risk of bleeding. Consequently in these patients there is a need for perioperative assess of the impact of ginkgo on platelet aggregation. In this study we investigated the effect of different supplements on platelet aggregation available on the free market which by declaration are containing ginkgo extract alone or in combination with other plants. The study was conducted on 24 male rats Y59, divided into 4 groups, depending on the herbal food supplements which was used for treatment. Platelet aggregation was determined on Multiplate analyzer, impedance aggregometry. Prolonged platelet aggregation was measured in groups b ginkgo (P=0.025), c vulkan (P=0.004), d GOI (P=0.035) compared to control group when it was used ADP as agonist. Platelet aggregation where it was used collagen as agonist showed no statistically significant difference between groups.

KEY WORDS: *ginkgo, platelet aggregation*

UČINAK PRIPRAVAKA GINKA NA AGREGACIJU TROMBOCITA U ŠTAKORA

Sažetak

Ekstrakt biljke *Ginkgo biloba* može se naći kao sastojak brojnih dodataka prehrani te imamo još registrirane biljne lijekove na bazi Ginka. Ginko sadrži različite biološki aktivne tvari koje mogu djelovati na inhibiciju agregacije trombocita što može pozitivno ali i negativno djelovati na kardiovaskularni sustav u onkoloških bolesnika na kemoterapiji, a kod onkoloških operacija (naročito većih abdominalnih, ginekoloških, te operacija s rekonstrukcijom) povećan je rizik od krvarenja, te je kod takvih bolesnika potrebno perioperativno procijeniti utjecaj na agregaciju u slučaju korištenja pripravaka ginka. U ovom radu istražili smo učinak ginka i raznih suplemenata koji sadrže ginkgo na agregaciju trobocita. Ispitivanje je vršeno na 4 skupine po 6 muških štakora soja Y59, od kojih je jedna bila kontrola, a ostale tri su dobivale ginko i ginko s različitim biljnim dodacima. Agregacija trombocita mjerena je na Multiplate analizatoru, impedancijskom agregometru. Agregacija trombocita bila je produžena gdje smo u testiranju koristili ADP kao agonist u sve tri tretirane skupine u odnosu na kontrolu, skupini b ginkgo (P=0.025), c vulkan (P=0.004) i d GOI (P=0,035), dok agregacija s kolagenom kao agonistom nije bila statistički značajno produžena.

KLJUČNE RIJEČI: *ginkgo, agregacija trombocita*

INTRODUCTION

Herbal remedies, including ginkgo formulations have many components that can affect the metabolism of the cell (1). Commercially available herbal remedies of ginkgo are used to improve circulation and cognitive function (2,3). Extract of ginkgo is one of the most popular plants used for medicinal purposes in the EU (4,5,6).

Extract of ginkgo (EGb 761) is recommended at dosages of 120 mg to a maximum dose of 240 mg in humans. General opinion is that the ginkgo is well tolerated (7,8), although there has been reported a series of unwanted side effects: headache, dizziness, bleeding and gastrointestinal disturbances (3,9,8).

Oncology patients during treatment often use dietary supplements, and some of them can contain ginkgo which can affect perioperativ bleeding during major oncologic surgeries. Ginkgo contains active compounds that can affect the coagulation hemostasis (10). Several review articles (11,12) have described an increased risk of bleeding when taking ginkgo formulations.

Preparation of ginkgo in combination with ticlopidine (platelet ADP receptor antagonist, antiaggregating effect) compared to ticlopidine alone showed greater efficacy in mice in protection from thromboembolic events (13). In now days it is increasingly probable that patient may have cancer and cardiovascular disease. Many different cytotoxic agents used to treat cancer, classic chemotherapy, monoclonal antibodies that target tyrosine kinase receptors, antiangiogenic drugs and chemoprevention agents such as COX-2 inhibitors can have influence on the cardiovascular system. It is desirable to find potential protective agents that could be administered to patients with occult or overt risk for cardiovascular complications (14).

In this study we investigated the effect of different supplements on platelet aggregation available on the free market which by declaration are containing ginkgo extract alone or in combination with other plants.

MATERIALS AND METHODS

The study was conducted on 24 male rats Y59, divided into 4 groups, depending on the herbal food supplements which was used for treatment. Animals were 4-6 months old, weighing 350-450 g from the cultivation of the Institute of Animal Physiology, Faculty of Science in Zagreb. The preparations used in the study were Ginkocel (Preparation of ginkgo), Vulcan (mixture ginkgo, hop, lemon balm and oregano) and GinkAlert (mixture ginkgo, blueberries and Gotu Cola). Group a was the control group, group b – treated with Ginkocel, group c – with Vulcan, group d – with GinkAlertom.

Animals were treated once daily trough intragastric tube with recommended daily dosage of ginkgo formulations for 14 days. Platelet aggregation was determined on Multiplate analyzer, impedance aggregometry. Impedance aggregometry is based on the principle that the platelets are not thrombogenic when not activated, with activation they express receptors on the surface and allow the attachment to the damaged endothelium or artificial surface. Platelets adhere to the sensing electrodes, enhanced electrical resistance between them is being measured. Each Multiplate measuring well is contains two independent sensor units made of two copper electrodes. The instrument detects impedance change beatween two electrodes. Impedance change is expressed as AU (eng. aggregation units). Coefficient of correlation between two measurements is automatically calculated. In the study we used two tests to assess platelet function, COL test (collagen) and ADP test (with adenosinediphosphate) (Roche diagnostics reagents, according to the manufacturer's instructions). Collagen activates collagen receptors and the ADP activates platelets via the ADP receptors. 300 mL of 0.9% saline is placed in measuring well, then 300 mL of whole blood and then incubated for 3 min. After incubation, we added 20 ml of a suitable reagent (collagen or ADP). Measuring time takes 6 minutes and the result is obtained by calculating the area under the curve. Statistical analysis was performed using the program Statistica. We used data obtained by the analysis of variants and gained insight into the correlation and differences of the measured parameters between the groups. The differences were confirmed with the LSD *post hoc* test.

RESULTS

Prolonged platelet aggregation was measured in groups b ginko (P=0.025), c vulkan (P=0.004),

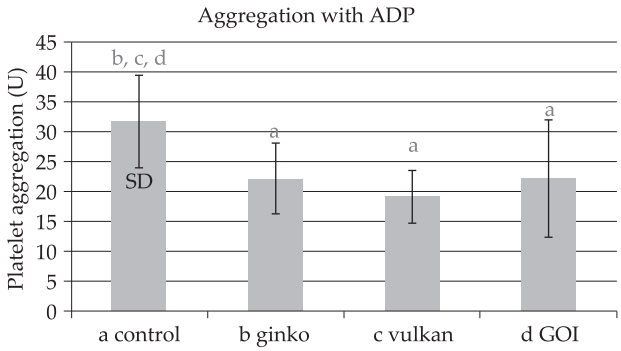

Figure 1. The effect of herbal suplements on platelet aggregation with ADP as agonist of aggregation. Letters a, b, c, d above the columns denote statistically significant differences (P≤0.05) between groups for platelet aggregation with ADP(a control grup, b Ginkocel treated group, c Vulkan treated group, d Ginkalert treated group). SD-standard deviation.

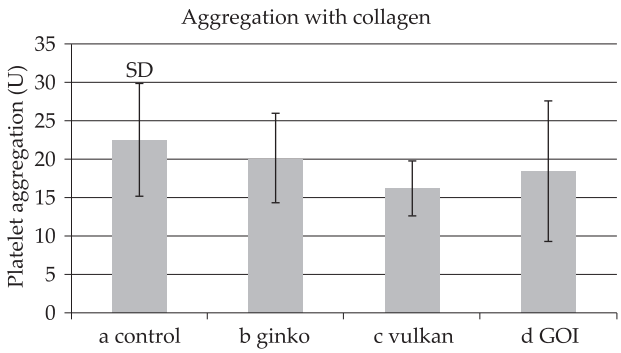

Figure 2. Effect of herbal supplements on platelet aggregation with collagen as agonist of aggregation. There was no statistically significant differences between groups of experimental animals (a control grup, b Ginkocel treated group, c Vulkan treated group, d Ginkalert treated group). SD-standard deviation.

d GOI (P=0.035) compared to control group when it was used ADP as agonist. (Figure 1)

Platelet aggregation where it was used collagen as agonist showed no statistically significant difference between groups. (Figure 2)

DISSCUSION

Earlier studies cited possible mechanisms of ginkgo effect on coagulation homeostasis through interaction with platelet factor 4 (PF4) and collagen which leads to prolongation in platelet aggregation (15,16). Studies showed that the active compound of the gingko inhibit platelet aggregation by increasing the concentration of endothelial thrombolytic agents, such as nitric oxide (NO) and prostacyclins (17,18).

Ginkgolide B directly inhibits binding of platelet factor 4 (PF4) to receptors on the platelet membrane (15). Other researchers believe that ginkgo primarily affects reaction between platelets and collagen, but not on the platelet-activating factor (16). Cho and Nam (2007) in the study found that ginkgo inhibits platelet aggregation via ginkgolide B (GB). They found that the different concentrations of GB significantly reduce platelet aggregation stimulated by collagen, induces metalloproteinase-9 (MMP-9) which inhibits collagen-stimulated platelet aggregation. They also found that GB directly acts on adenylate cyclase activity and cAMP-dependent phosphodiesterase (PDE) guanylate cyclase and cGMP dependant PDE. Increased levels of cAMP and cGMP indirectly lead to activation of the enzymes protein kinase A and protein kinase G which phosphorylate their target proteins leading to the negative regulation of platelet aggregation (19,20).

In this study, platelet aggregation was inhibited in the groups b ginkgo, c Vulkan and d GOI where it was used ADP as an agonist (P=0.02, P=0.004, P=0.03). Possible mechanisms of action of Ginkgo on hemostasis is based on the inhibition of platelet activating factor (eng. Platelet activating factor, PAF) by ginkgolides B (21). Possible effect of ginkgo flavonoids on platelet aggregability by inhibition of various protein kinases could led lower concentration of PLC (phospholipase C) (22). Bojić et al (2011) in vitro showed that all analyzed flavonoids, including quercetin and isorhamnetin (flavonoides in ginkgo suplements used in this study) showed antiplatelet effect. Much lower concentrations of flavonoids are needed to inhibit aggregation when ADP was used in aggregation assays (0.119 to 122 microns), in contrast to collagen-induced aggregation (15-244 uM) (23).

Since there is no data on the elimination halflife of ginkgo, a recommendation to discontinue ginkgo supplementation prior to surgery lacks empirical evidence. However, with the known implications of ginkgo on hemostasis, it would be prudent to discontinue supplements a minimum of 5 to 7 days prior to major oncologic surgery (24).

CONCLUSION

In our opinion, it is necessary to carry out further research on a larger number of samples

with prolonged treatment and with the individual active compounds in order to clarify the molecular mechanisms of effect on endothelium and hemostasis.

With these findings it is necessary to assess the risk and benefit ratio of ginkgo use in cancer patients during active treatment.

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