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The effects of 1 mT and 5 mT static magnetic fields on platelet aggregation
1 mT ve 5 mT statik manyetik alanların trombosit agregasyonuna etkisi

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Summary

Aim: Numerous studies have been conducted on the biological effects of static magnetic fields, reporting effects on various cell types and functions. The aim of this study is to investigate the biological effects of 1 mT and 5 mT static magnetic fields using platelet aggregation.

Materials and Methods: Blood samples taken from 18 healthy volunteers were anti-coagulated with citrate or with heparin. The platelet rich plasma obtained from the anti-coagulated whole blood was divided into four equal parts. Two were exposed to 1 mT or 5 mT static magnetic fields produced by a Helmholtz coil for 1 hour; the remaining two were assigned as control groups. Both groups were simultaneously studied with a 4-channel optical aggregometer. ADP (2-4 μM), Collagen (38-95 μg/ml) and Epinephrine (10 μM) were used to induce platelet aggregation. The slopes, maximum values, and lag times for the aggregation curves for the control samples and the exposure groups were compared.

Results: No statistically significant differences were observed for any of the four groups formed: 1 mT-citrate (n=5), 5 mT-citrate (n=5), 1 mT-heparin (n=5), 5 mT-heparin (n=5).

Conclusion: 1-hour, 1 mT or 5 mT static magnetic field exposures have no effect on the platelet aggregation.

Keywords: static magnetic field; platelet; optical aggregometer.

Özet

Giriş: Statik manyetik alanların biyolojik etkileri, birçok çalışmada farklı hücreler ve fonksiyonlar üzerinden gösterilmiştir. Bizim bu çalışmadaki amacımız, 1 mT ve 5 mT statik manyetik alanların trombosit agregasyonu üzerine etkisini araştırmaktır.

Gereç ve Yöntem: 18 sağlıklı gönüllüden alınan kan örnekleri sitratla veya heparinle antikoagüle edildi. Antikoagüle tam kandan elde edilen trombositten zengin plazma örnekleri 4'e bölündü. İkisine Helmholtz bobinde 1 saat, 1 veya 5 mT statik manyetik alan uygulanırken diğer ikisi kontrol grubu oldu. Manyetik alan ve kontrol grubu trombositten zengin plazma örnekleri aynı anda 4 kanallı optik agregometrede çalışıldı. Trombosit agregasyonunu uyarmak için ADP (2–4 μM), Kollajen (38–95 μg/ml) ve Epinefrin (10 μM) kullanıldı. Agregasyon eğrilerinin eğimleri, maksimum değerleri ve lag zamanları, kontrol ve manyetik alan grupları arasında karşılaştırıldı.

Bulgular: Oluşturulan 4 grupta [1 mT sitrat (n=5), 5 mT sitrat (n=5), 1 mT heparin (n=5), 5 mT heparin (n=5)] manyetik alan ve kontrol grupları arasında istatistiksel olarak anlamlı bir farklılığa rastlanmadı.

Sonuç: 1 saat uygulanan, 1 mT veya 5 mT statik manyetik alanların trombosit agregasyonu üzerine etkisi gözlenmedi.

Anahtar Kelimeler: statik manyetik alan; trombosit; optik agregometre.

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Introduction

Platelets are blood cells that play a central role in hemostasis and thrombosis. They are the cytoplasmic particles of the largest cells in bone marrow, the megakaryocytes. Platelet receptors that bind the stimulating agents are mostly receptors that are linked with G proteins. Depending on the specific receptor, different subunits of the G proteins are stimulated. With the stimulation of Gq protein, phospholipase C (PLC) is activated, which activates in turn two different, but synergistic pathways. These two pathways, one calcium-dependent, the other protein kinase-C (PKC) dependent, are the main stimulators of platelet aggregation (1,2).

Plasma membrane is suggested as the primary site where magnetic fields exert their effects (3,4). Structural and biophysical changes in the plasma membrane, in turn, affect receptor binding or activation and thereby affect cell function in general (5,6). Various studies conducted with magnetic fields link the effects reported to changes in transmembrane Ca^{++} fluxes (7-13). Ca^{++} ions are mediators of intracellular signaling and play a crucial role in many cellular processes, such as apoptosis, proliferation and changes in cell morphology. For platelets, it is known that a rise in intracellular Ca^{++} concentrations is a crucial step in platelet activation.

Recently, there is an increase in the number of studies investigating the biological effects of magnetic fields at the cellular level. This is mainly due to the fact that the daily exposure to magnetic fields constantly increases with the increasing dependence on electricity in our daily lives. Although many studies have been conducted on the effects of magnetic fields on blood cells such as macrophages, neutrophils and lymphocytes (7,8,12,14), no studies investigating the effects of magnetic fields on platelets were found. The aim of this study is to investigate the effects of 1 mT and 5 mT static magnetic fields on platelet function.

Materials and Methods

Subjects

Experiments were conducted using blood from 18 healthy volunteers (8/10, ♀/♂) in the age range of 20 to 52. The procedures followed complied with the "Ege University Medical Faculty Research Ethics Committee" protocols. The subjects and their families were screened for a history of bleeding conditions or diseases, and it was made certain that the volunteers did not smoke or take any medication known to have an effect on platelets on the previous 10 days.

Collection and the preparation of blood samples

Fasting blood samples were obtained from the subjects between 8.30-9:30 AM. Only one subject was studied per day. Following the induction of a mild venous stasis in the upper arm, 30 milliliters of blood sample was drawn from the antecubital vein into test tubes containing anticoagulant. They were then centrifuged at 200g (gravitational acceleration) for 10 minutes to obtain platelet-rich plasmas (PRP). The PRPs were then transferred into a single test tube, which was then mixed by turning the tube upside down. The mixed plasma was portioned into 4 tubes equally. The portion of the blood that remained after platelet removal was centrifuged at 2000g for 15 minutes to obtain platelet-poor plasma (PPP).

Exposure to the magnetic field

Two of the four tubes containing PRP, the exposure samples, were exposed to 1 mT or 5 mT static magnetic fields for 1 hour, while the other two tubes were left to stay under ambient conditions as the control samples. Maximum ambient magnetic fields recorded were 0.15 mT DC and 0.21 mT AC and minimum ambient magnetic fields recorded were 0.05 mT DC and 0.12 mT AC. Static magnetic fields were created using a Helmholtz coil configuration with a diameter of 50 cm with 350 turns per coil. A 1 mT or a 5 mT static magnetic field was created at the center of the coils, which were connected in series to a direct current source. The magnetic flux density was measured using a F.W. BELL 5180® Hall Effect gaussmeter at the beginning and at the end of the exposure step. The temperatures at the center of the coil around the exposure group and around the control group were constantly monitored. Ambient temperatures recorded were around $22\pm2^{o}C$ and the temperature difference between the control and the test samples did not exceed $1^{o}C$.

Experimental groups

Sodium citrate and heparin were used as anti-coagulants. Sodium citrate blocks coagulation by binding the free calcium in blood. Heparin, on the other hand, activates

Antithrombin III and blocks the formation of thrombin and some coagulation factors, thus displaying an anti-coagulant effect (15). The blood samples were anti-coagulated using tubes containing 0.106 M sodium citrate or 10 U/ml heparin. Subjects were divided into 4 groups of 5 subjects each, on the basis of the anti-coagulant used and the strength of the magnetic field applied. Two of the 18 subjects were included in both anti-coagulant groups.

Measurement and evaluation of aggregation

Aggregation measurements were performed using an optical aggregometer, Platelet Aggregation Profiler® PAP-4CD (BIO/DATA CORPORATION). Optical aggregometers are modified spectrophotometers. They work on the principle of detecting the change in the amount of light transmitted through platelet rich plasma, stirred at a certain speed, where aggregats form by adding an aggregation inducing agent (16).

ADP, collagen and epinephrine were used as inducing agents (BIO/DATA CORPORATION). The final concentrations of the inducing agents used were adjusted to be 2 μM for ADP (4 μM, if secondary aggregation was not observed at 2 μM), 95 μg/ml for the collagen-citrate group, 38 μg/ml for the collagen-heparin group and 10 μM for epinephrine.

First, every channel of the 4-channel aggregometer was set to 100% transmittance using the PPP. Then, the two tubes that have been exposed to the static magnetic field and the two tubes set aside as the control group were placed in the four channels. Each inducing agent was studied individually using all four channels simultaneously. The transmittance of PRP is accepted as 0% by the aggregometer. When the inducing agent is added to the PRP, platelets are activated and start to aggregate. The aggregation of platelets causes the light transmittance of the PRP to increase. In order to mix the minute amounts of the stimulating agent (5–25 μl) efficiently into the PRP (475–495 μl) and also to assure that platelets contact each other, a magnetic stirring bar was used and its speed was set to 800 rpm. The experiment was terminated once maximum aggregation was reached and no further change took place in the level of aggregation for a full minute.

The results of the experiments were evaluated over the parameters shown in Figure-1.

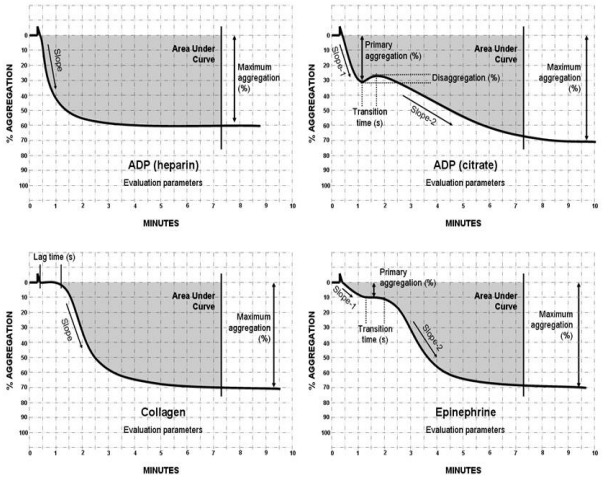


Figure-1. The parameters used to evaluate the aggregation curves plotted by the optical aggregometer.

All parameters were evaluated using the software bundled with the aggregometer. Area under curve (AUC) was evaluated for the first seven minutes upon the introduction of the inducing agent. PRP was prepared within 30 minutes of obtaining the blood from the volunteer. The PRP was exposed to the magnetic field for 1 hour. Aggregation measurements were made within the 90th and the 150th minutes. All procedures were completed within 2.5 hours.

Statistical analyses

The final parametric values were obtained for both groups by averaging the values measured from the two tubes in the group. The values from the exposure group were compared with the respective values from the control group using the paired t-test. p values lower than 0.05 were regarded as statistically significant.

Results

No statistically significant difference was found between the exposure (E) and the control (C) values of any aggregation parameter for any experimental group (1 mT citrate, 5 mT citrate, 1 mT heparin or 5 mT heparin). A comparative chart is presented for the composite parameter AUC in Figure-2.

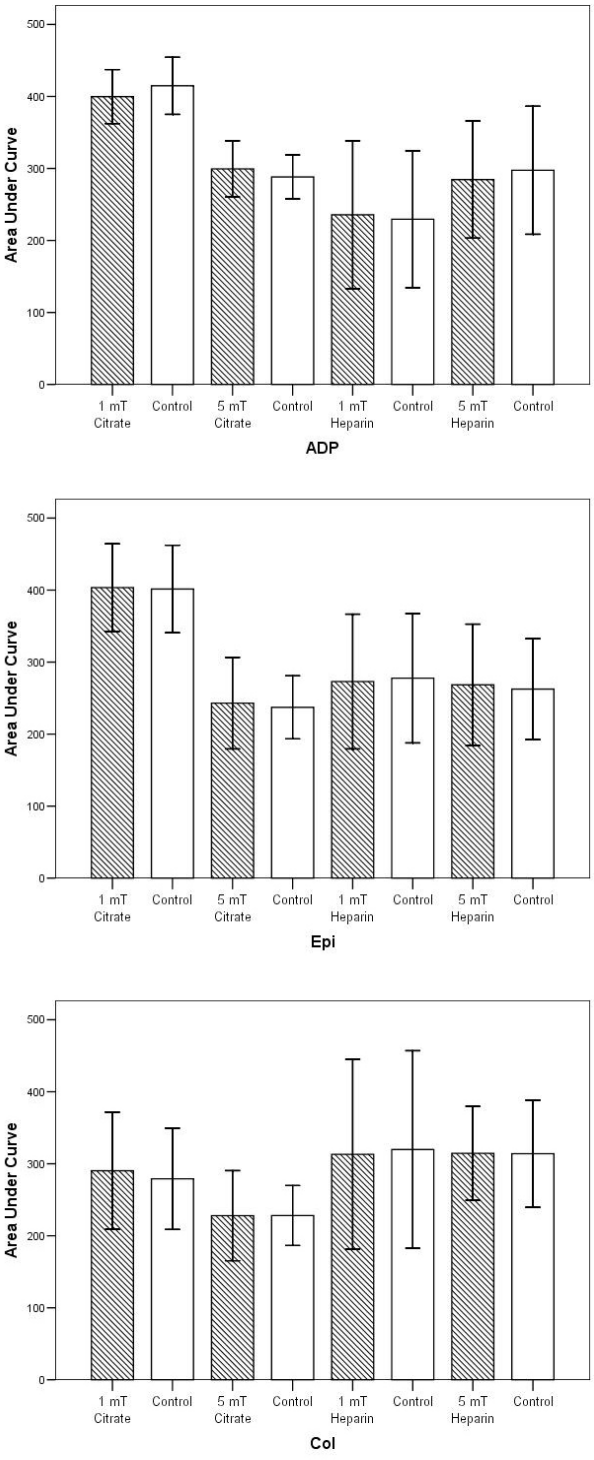
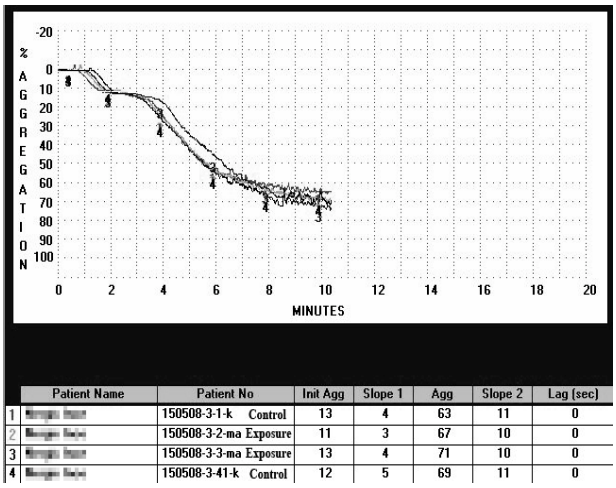


Figure-2. The comparison of the area under curve parameter for the control and exposure groups.

A typical aggregation curve is presented in Figure-3 as an example.



	Patient Name	Patient No	Init Agg	Slope 1	Agg	Slope 2	Lag (sec)
1	[redacted]	150508-3-1-k Control	13	4	63	11	0
2	[redacted]	150508-3-2-ma Exposure	11	3	67	10	0
3	[redacted]	150508-3-3-ma Exposure	13	4	71	10	0
4	[redacted]	150508-3-41-k Control	12	5	69	11	0

Figure-3. A typical aggregation curve: 1 mT heparin group, using Epinephrine as the inducing agent.

A high correlation was seen between the values from the exposed samples and control samples. In 56 (93.3%) of the 60 comparisons made between the exposure and the control samples, the correlation coefficient (r) was found to be higher than 0.800.

Discussion

In this study, it was observed that static magnetic fields of 1 mT or 5 mT had no effect on platelet aggregation stimulated by various activating agents.

There are two different types of ADP receptors on platelet membranes. The $P2_{Y1}$ receptors activate PLC through $Gi_{2\alpha}$. whereas the $P2_{Y12}$ receptors inhibit AC through $Gi_{2\alpha}$. Collagen receptors such as $\alpha_2\beta_1$, glycoprotein IV (GP IV) and GP VI activate PKC via tyrosine kinase and cause an increase in the intracellular Ca^{++} levels through PLCγ Epinephrine α_{2A} adrenergic receptors inhibit AC through Gαz (Figure-4) (2,17).

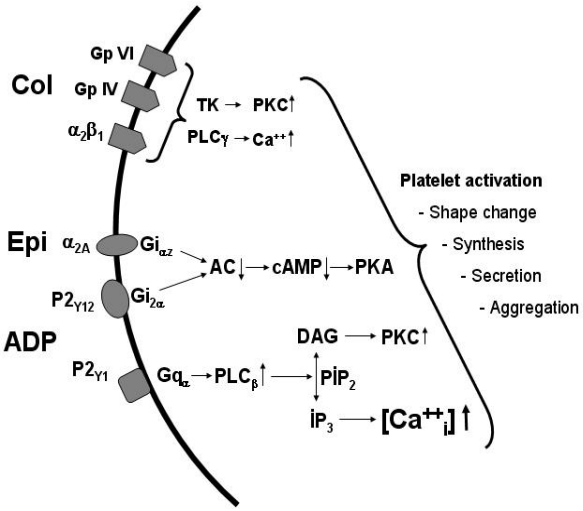


Figure-4. The activation pathways of platelets with ADP, collagen and epinephrine. [Col: Collagen; Epi: Epinephrine; GP (glycoprotein) IV, VI, and α2β1: Collagen receptors; α2a: Epinephrine receptor; $P2_{Y1}$, $P2_{Y12}$: ADP receptors; TK: Tyrosine kinase; PLC: phospholipase C; PKC: protein kinase C; Ca^{++}: Calcium; AK: adenylate kinase; AC: adenylate cyclase; cAMP: cyclic adenosine mono phosphate; PKA: protein kinase A; PiP_2: phosphatidylinositol biphosphate; DAG: diacylglycerol; IP_3: Inositol trisphosphate.]

The stimulating agents added to the PRP initiate platelet activation directly over their respective receptors. Afterwards, with the additive effects of the synthesis and the secretion carried out by the platelets (of PAF, TxA_2, ADP, ATP, Ca^{++}, Thrombin and many active agents in the granules), other stimulating factors also come into play and a complex aggregation process takes place (1). Throughout the platelet activation process calcium plays a crucial role, taking part in the secretion of granules and shape change.

An increase in intracellular Ca^{++} concentrations has been found to take place in many cell types after an exposure to static magnetic fields (5-9). Fanelli et al reported that a 6 mT static magnetic field increased calcium influx in U937 cells, and that this increase took place in the first 100s of exposure (8). Tenuzzo et al also examining the effects of 6 mT static magnetic fields reported an increase in intracellular calcium concentrations in various cells exposed to the magnetic field and also observed that 6 mT static magnetic fields had effects on cell morphology (7). Additionally, Teodori et al showed that a 6 mT static magnetic field increased intracellular calcium concentration in human glioblastoma primary culture cells within the few minutes of exposure (9).

In the current study the effects of a 1 hour exposure to a static magnetic field were evaluated in terms of platelet function. In this way, although no direct observations on intracellular calcium levels were made, the interaction of static magnetic fields with platelets was assessed as a whole. In a complementary manner, both citrate and heparin were used for anti-coagulation in this study. Two alternative anti-coagulants, which block two different pathways, one binding Ca^{++} and one inhibiting thrombin formation, were used in order to isolate calcium related effects from other possible effects.

The limitations of the study mainly are due to reasons inherent to platelet aggregometry. Firstly, the necessity of completing the aggregation tests within four hours of obtaining the blood, during which there is minimal loss of platelet functions, restricts the amount of time the PRP can be exposed to the magnetic field to approximately 1 hour.

Secondly, in the aggregometer, the minute amounts of stimulating agents need to be mixed efficiently with the PRP, and the platelets should come into contact with each other (16). For this purpose, a small magnetic stirring bar is used, which is placed in the PRP and is rotated by another magnet on a DC motor below the testing well. The maximum magnetic flux density at the sides of the cylindrical stir bar is approximately 15 mT, and it is approximately 65 mT at the two ends of the cylinder. This means that the platelets are actually exposed to a magnetic field by a magnet spinning at a speed of 800 rpm in the plasma, while their aggregation is being evaluated. There is a possibility that the parameters of aggregation may have been affected by the magnetic field applied during the aggregation tests.

The aggregometer records the aggregation curves on the computer with special software and these curves were later examined in full detail with the aid of the computer. Whereas aggregation curves are usually evaluated only over the maximum aggregation values; the capability to extract all the parameters of the aggregation process, and not just the maximum value, enabled us to evaluate all the stages of the aggregation process that could be affected by static magnetic field exposure.

Our results show that the 1-hour, 1 mT or 5 mT static magnetic field exposures have no effect on platelet aggregation. Using an alternative system eliminating the use of a magnetic stirring bar and thus eliminating the

exposure during the aggregation process is the next step in our studies. Using a different method for evaluating the effects of magnetic fields on platelet functions are also planned for future projects.

Acknowledgments

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