EFFECT OF TRANSDERMIC ACETYLSALICYLIC ACID ON HEMOSTASIS IN HEALTHY VOLUNTEERS

Adriana B. Martínez[1], Esteban Funosas[1], Lorella Maestri[1], Perla Hermida Lucena[2]

[1] Department of Pharmacology and [2] Department of Microbiology, Faculty of Dentistry, National University of Rosario, Argentina

ABSTRACT

Acetylsalicylic acid (ASA) exerts an antiaggregatory effect on platelets by irreversible inhibition of the enzyme thrombocyte cyclooxigenase when it is administered orally at doses above 80mg/day. For several years ASA has been available as a solution that can be topically applied on the skin. It is widely used by athletes and individuals with chronic rheumatic disorders. However, it has not been established to date whether the plasma levels that result from these doses of ASA affect hemostasis during odontological procedures that involve bleeding, causing platelet dysfunction. The aim of the present study was to evaluate whether topical application is capable of affecting hemostasis. Three studies were conducted: A, B y C. Each of the 3 groups included 12 healthy volunteers of both sexes. The aim of study A was to evaluate if the formulation for topical application resulted in plasma levels of ASA that resembled those observed for the oral formulation and affect hemostasis. In experiment A, plasma levels of salicylic acid (SA) were assessed for each volunteer at 30 minutes, 60 minutes, 6 hours, 12 hours and 24 hours after oral administration of a dose of 500 mg ASA. Experiment B was identical to experiment A except for the fact that ASA was topically applied employing a commercial preparation Aspirub ® in a predetermined area at a rate of 2 ml/day over a period of 15 days. Experiment C was designed in the same way as experiment B, for a higher dose and a longer period of time (4 ml/day over a period of 30 days). One of the volunteers exhibited detectable salicylemia that could affect hemostasis as occurs with the oral formulation. The following two studies (C1 and C2) employed doses of Aspirub ® of 8 and 16 ml/day respectively, over a period of 30 days. We measured biochemical parameters associated to platelet function. The dose of 8ml/day induced moderate alterations in all the parameters related to platelet function and the daily dose of 16 ml inhibited platelet aggregation in all the volunteers involved.

Key words: salicylemia, platelet antiaggregation agent, salicylic acid, route of administration.

EFECTO DEL ÁCIDO ACETILSALICÍLICO APLICADO POR VÍA TRANSCUTÁNEA SOBRE LA HEMOSTASIA EN VOLUNTARIOS SANOS

RESUMEN

El ácido acetilsalicílico (AAS) es un fármaco que posee actividad antiagregante plaquetaria por la inhibición irreversible de la enzima ciclooxigenasa (COX) trombocitaria cuando se administra por vía oral en dosis superiores a 80 mg/día. Desde hace algunos años el AAS está disponible en una solución que se aplica tópicamente y es ampliamente utilizada por deportistas y por personas afectadas por patología reumática durante períodos de tiempo muy prolongados. No se sabe si los niveles plasmáticos que alcanza esas dosis de AAS afectan la hemostasia en las maniobras odontológicas que involucran sangrado produciendo trastornos a nivel del trabajo plaquetario. El objetivo de nuestro trabajo fue saber si este tipo de administración de AAS es capaz de afectar la hemostasia. Para ello se llevaron a cabo tres estudios: A, B y C. Participaron 12 voluntarios sanos de ambos sexos en cada una de ellas. El objetivo del primero fue saber si la utilización de la forma farmacéutica tópica permite alcanzar niveles plasmáticos de AAS semejantes a los conocidos para la vía oral que sean capaces de afectar la hemostasia. En el experimento A se obtuvieron los niveles plasmáticos de ácido salicílico (AS) de cada voluntario a los 30 minutos, 60 minutos, 6 horas, 12 horas y 24 horas posteriores a la administración oral de una dosis de 500 mg de AAS. El experimento B fue idéntico al A excepto que el AAS fue aplicado tópicamente a partir de una solución comercial Aspirub ® en un área predeterminada a razón de 2 ml/día durante 15 días. El C tuvo el mismo diseño que B con variaciones en las dosis aplicadas y la duración de los mismos (4 ml/día durante 30 días). Uno de los voluntarios registró salicilemia detectable que podría afectar la hemostasia a semejanza de la administración por la vía oral. Los dos estudios posteriores (C1 y C2) fueron realizados aplicando una dosis de Aspirub ® de 8 y de 16 ml/día respectivamente durante 30 días. En estos trabajos se midieron parámetros bioquímicos relacionados con la actividad plaquetaria. A la dosis de 8ml/día se encontraron moderadamente alterados todos los parámetros de la función plaquetaria y con una dosis diaria de 16 ml la agregación plaquetaria en todos los voluntarios estudiados.

Palabras clave: salicilemia, antiagregante plaquetario, ácido acetilsalicílico, vía de administración.

INTRODUCTION

Since the inhibitory effect of aspirin on platelet aggregation was discovered, several studies have established that aspirin can reduce by half the risk of acute coronary syndrome (1-3). This antithrombotic effect can be attributed to the irreversible acetylation of the enzyme cyclooxigenase type I (COX_1) (4) that catalyzes the biotransformation of arachidonic acid into highly unstable byproducts termed cyclic endoperoxides. Only when this process has been completed, the formation of prostacyclin (PGI_2) in vascular cells (5), thromboxane A_2 (TXA_2) in platelets (6) and of prostaglandins takes place. TXA_2 is produced by the platelets and is a potent agreggatory and vasoconstrictor agent at a physiological level (7). Numerous pharmacological studies demonstrated that a dose of 30-80 mg/day of aspirin administered orally (8-9) exerts an antithrombotic effect (10-12). This dose is much lower than the usual dose of aspirin (500 mg every 4-6 hours) (13-14) employed to achieve analgesic, anti-inflammatory and antipyretic effects. Keimovitz et al. (15) examined topical application as a new route to administer acetylsalicylic acid employing an aspirin preparation. The skin has been shown to act as a reservoir of the drug, absorbing a maximum of 10-15% of the drug 48 hours after a single application. This route would allow for greater selectivity in terms of platelet inhibition and would reduce gastrointestinal toxicity. These preparations are employed indiscriminately by a large population of patients affected by inflammatory disorders of the articulations and by athletes, who could in turn experience platelet dysfunction.

The aim of the present study was to determine whether the topical formulation leads to plasma levels of acetylsalicylic acid that resemble those reported for the oral formulation and might affect hemostasis.

MATERIALS AND METHODS

Subjects: 12 adult, healthy volunteers of both sexes (6 women, 6 men), 29-56 years of age, were treated according to protocols A, B and C described below. They were not allowed to take aspirin or any medication for at least 6 months prior to the onset of the study. Prior to recruitment, all the participants signed informed consent forms in keeping with the Bioethics Regulations of the Faculty of Medicine, National University of Rosario.

Drugs: Aspirin tablets from Bayer Laboratory, Arachidonic Acid, Ristocetin, Collagen and Adenosine Di-phosphate (ADP) from Sigma-Aldrich Chemical Company (USA). Aspirin solution: We prepared an aspirin solution, commercially available as Aspirub ®, as follows: for every 100 ml of solution, Acetylsalicylic acid 2.5 g; castor oil 39 ml; turpentine 25.6 ml; camphor 5.4 ml; isopropylic acid 25% to a final volume of 100 ml. The solution was prepared at the Pharmacology Department, School of Dentistry, National University of Rosario, Argentina. All drugs used were prepared in the corresponding vehicles to obtain the final concentrations used throughout the paper.

Protocols: **A:** The aim of experiment A was to compare the plasma levels of acetylsalicylic acid at baseline and following oral administration. Postfasting, the volunteers were given 500 mg aspirin orally. Blood samples were taken pre-administration of ASA (control) and 30', 60', 6h, 12h and 24h post administration. Each sample was centrifuged and the plasma was used for evaluation by direct fluorescence in alkaline medium (16). **B:** This experiment was carried out 14 days after experiment A. Kinetic studies were performed following topical application of a solution of Aspirub®. An area of 20 cm x 20 cm was outlined with an ink pencil on both forearms of each of the volunteers. One ml of Aspirub® was applied on each forearm over a period of 15 days. The method of application was identical to that employed in the first experiment. Blood samples were taken to perform biochemical assays, i.e prior to the first topical application (control), at t0, one week and 2 weeks after the first application. The samples were processed and evaluated as previously described. **C:** Experiment C involved 2 groups: **Group C1:** 12 adult, healthy volunteers of both sexes (6 men and 6 women), 29-56 years of age, topical application of the Aspirub® solution on a pre-determined area of 20 cm x 20 cm on the skin of the forearms, 2 ml on each forearm (4 ml/day) over a period of 30 days; **Group C2:** 12 adult, healthy volunteers of both sexes (6 men, 6 women), 29-56 years of age, topical application of the Aspirub® solution on a pre-determined area of 20 cm x 20 cm on the skin of the forearms, 4 ml on each forearm (8 ml/day), over a period of 30 days. Blood samples were taken from the individuals in Groups C1 and C2 at the same times as in protocol

B, in addition to a blood sample taken 30 days after the first application. The samples were processed and evaluated as described for protocol A.

Blood and serum determinations: All the blood samples from the healthy volunteers were obtained by puncture of the median basilic vein, prior to the experiments as control in each of the groups, on day 15 (protocols B and C) and on day 30 (protocol C) after the onset of the experiment (17). The end-points evaluated were: bleeding time (Ivy's method) and platelet count in whole blood by standard laboratory techniques. A platelet count was also performed in platelet rich plasma and agonists (ADP, collagen, Arachidonic Acid and ristocetin) were evaluated by platelet aggregometry.

Platelet Rich Plasma preparations: The blood was aspirated with a 21 G needle. A 10 ml syringe pre-loaded with 1.3 ml of Anticoagulant Citrate Dextrose (ACD) solution was used to avoid coagulation. One millimeter was set apart for cell counting. Each blood sample was centrifuged for 15 minutes at 72 g at 4°C resulting in the three following layers: the inferior layer composed of red cells, the intermediate layer composed of white cells and the superior layer made up of plasma. The 6 ml plasma layer was centrifuged for another 5 minutes at 100 g in order to obtain a two-part plasma: the upper part consisting of 5.5 ml of poor-platelet plasma (PPP) and the lower part consisting of 0.5 ml of platelet-rich plasma (PRP). The PPP was aspirated first to avoid it mixing with the PRP. The PRP was then gently aspirated with another pipette and placed in a sterile tube. The PRP was prepared for activation with calcium chloride (CaCl), which inhibits the blood-thinning effect of ACD. After activation, PRP turned into a gel-like solution with adhesive properties and ready for use.

Statistical Analysis: All the experimental results were analyzed by Student's "t" test. Statistical significance was set at $p \leq 0.05$.

RESULTS

The data corresponding to the 36 samples obtained in experiment A are shown in Fig. 1. Eleven of the 12 healthy volunteers exhibited very similar kinetic curves. The remaining individual showed a plateau (not a peak), indicating that plasma levels remained constant up to 12 hours post-administration and fell thereafter. Topical administration resulted in fluorescence intensity values of 0.016 mg/ml (4 ppm of salicylate in the original sample). These values are below the detection limit of this method. In the case of experiment B, free salicylate was not detected either, despite the increase in dose and length of the experimental period. The data corresponding to experiment C revealed the presence of free salicylate in the serum of one of the healthy volunteers at 30 days. Oral intake of 500 mg of acetylsalicylic acid resulted in the following salicylemia values: 1.80 mg/% at 30 minutes post-administration; 9 mg/% at 2 hours and 0.5 mg/% at 24 hours post-administration (Fig. 1). At the doses and time-points evaluated, only 1 volunteer (Nr. 5) exhibited a peak in plasma levels 30 days after topical application of ASPIRUB at a dose of 4 ml/application, twice a day. The value was 1.02 mg/% (Table 1). Interestingly, this volunteer had a thinner skin than the rest of the participants.
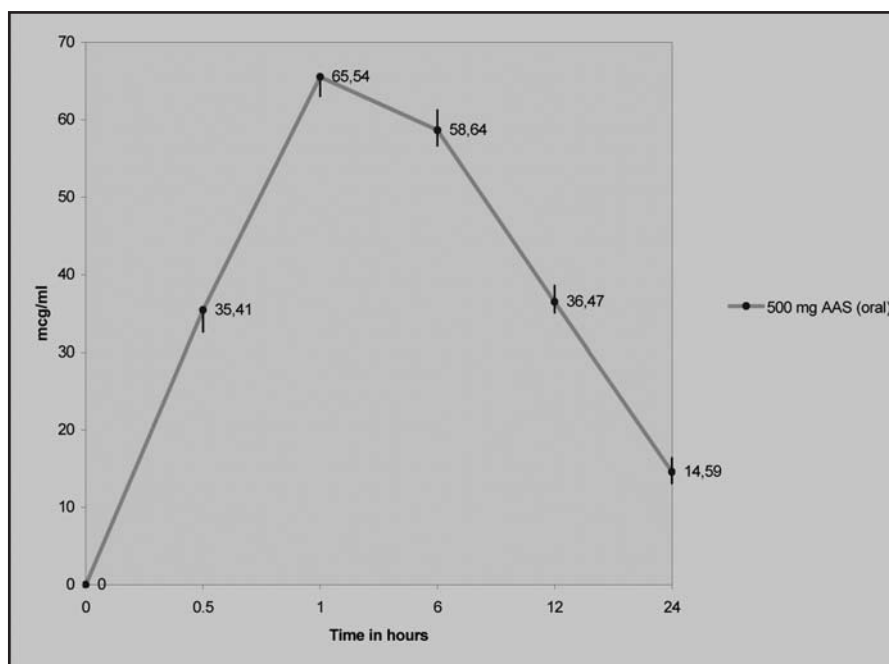

Figure 1. Kinetic curve for oral administration of ASA.

TABLE 1. Salicylemia values in Experiment C		
	Salicylemia on day 15 in mg/%	Salicylemia on day 30 in mg/%
Volunteer 1	0.075	0.55
Volunteer 2	0.098	0.46
Volunteer 3	0.105	0.68
Volunteer 4	0.088	0.56
Volunteer 5	0.258	1.02
Volunteer 6	0.064	0.72
Volunteer 7	0.154	0.63
Volunteer 8	0.075	0.59
Volunteer 9	0.125	0.72
Volunteer 10	0.077	0.65
Volunteer 11	0.113	0.59
Volunteer 12	0.134	0.77

TABLE 2. Biochemical parameters: values of Experiment C1							
Volunteer	Bleeding time	Platelet count in whole blood	Platelet count in platelet rich plasma	ADP 1 µg/ml	ADP 2 µg/ml	Arachidonic Acid 0.05 mM	Collagen 2 µg/ml
Volunteer 1	C 3.5 min	C 250.000 mm^3	C 420.000 mm^3	C Normal	C Normal	C Normal	C 90% Aggreg
	E 4.5 min	E 258.000 mm^3	E 420.000 mm^3	E Alt 1st phase	E Alt 2nd phase	E Normal	E 70% Aggreg
Volunteer 2	C 4 min	C 400.000 mm^3	C 580.000 mm^3	C Normal	C Normal	C Normal	C 95% Aggreg
	E 4 min	E 400.000 mm^3	E 580.000 mm^3	E Normal	E Normal	E Normal	E 75% Aggreg
Volunteer 3	C 4.5 min	C 150.000 mm^3	C 380.000 mm^3	C Normal	C Normal	C Normal	C 90% Aggreg
	E 5.5 min	E 150.000 mm^3	E 380.000 mm^3	E Normal	E Normal	E Altered	E 70% Aggreg
Volunteer 4	C 3.5 min	C 370.000 mm^3	C 560.000 mm^3	C Normal	C Normal	C Normal	C 85% Aggreg
	E 5 min	E 370.000 mm^3	E 560.000 mm^3	E Alt 1st phase	E Alt 2nd phase	E Normal	E 60% Aggreg
Volunteer 5	C 4 min	C 410.000 mm^3	C 570.000 mm3	C Normal	C Normal	C Normal	C 90% Aggreg
	E 4.5 min	E 410.000 mm^3	E 570.000 mm^3	E Alt 1st phase	E Alt 2nd phase	E Normal	E 75% Aggreg
Volunteer 6	C 4 min	C 330.000 mm^3	C 475.000 mm^3	C Normal	C Normal	C Normal	C 85% Aggreg
	E 5.5 min	E 330.000 mm^3	E 475.000 mm^3	E Normal	E Normal	E Normal	E 60% Aggreg
Volunteer 7	C 4.5 min	C 275.000 mm^3	C 570.000 mm^3	C Normal	C Normal	C Normal	C 85% Aggreg
	E 5.5 min	E 275.000 mm^3	E 570.000 mm^3	E Normal	E Normal	E Normal	E 55% Aggreg
Volunteer 8	C 3.5 min	C 335.000 mm^3	C 442.000 mm^3	C Normal	C Normal	C Normal	C 95% Aggreg
	E 4.5 min	E 335.000 mm^3	E 442.000 mm^3	E Normal	E Normal	E Normal	E 75% Aggreg
Volunteer 9	C 4 min	C 385.000 mm^3	C 515.000 mm^3	C Normal	C Normal	C Normal	C 90% Aggreg
	E 5.5 min	E 385.000 mm^3	E 515.000 mm^3	E Alt 1st phase	E Alt 2nd phase	E Altered	E 40% Aggreg
Volunteer 10	C 4.5 min	C 455.000 mm^3	C 590.000 mm^3	C Normal	C Normal	C Normal	C 85% Aggreg
	E 7 min	E 455.000 mm^3	E 590.000 mm^3	E Alt 1st phase	E Alt 2nd phase	E Altered	E 45% Aggreg
Volunteer 11	C 4.5 min	C 305.000 mm^3	C 440.000 mm^3	C Normal	C Normal	C Normal	C 90% Aggreg
	E 6.5 min	E 305.000 mm^3	E 440.000 mm^3	E Normal	E Normal	E Normal	E 70% Aggreg
Volunteer 12	C 4 min	C 415.000 mm^3	C 530.000 mm^3	C Normal	C Normal	C Normal	C 85% Aggreg
	E 8 min	E 415.000 mm^3	E 530.000 mm^3	E Alt 1st phase	E Alt 2nd phase	E Altered	E 65% Aggreg
C: control / E: experimental							

The data obtained corresponding to experiment C1 and C2 are shown in Table 2 and Table 3 respectively. The end-points studied in experiment C1 did not reveal statistically significant differences between the control and experimental groups. Conversely, all the parameters related to bleeding time and the study of agonists (ADP, collagen and arachidonic acid) presented in Table 3 and corresponding to

TABLE 3. Biochemical parameters: values of Experiment C2							
Volunteer	Bleeding time	Platelet count in whole blood	Platelet count in platelet rich plasma	ADP 1 µg/ml	ADP 2 µg/ml	Arachidonic Acid 0.05 mM	Collagen 2 µg/ml
Volunteer 1	C 3.5 min	C 250.000 mm³	C 420.000 mm³	C Normal	C Normal	C Normal	C 90% Aggreg
	E 6 min	E 258.000 mm³	E 420.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 35% Aggreg
Volunteer 2	C 4 min	C 400.000 mm³	C 580.000 mm³	C Normal	C Normal	C Normal	C 95% Aggreg
	E 7.5 min	E 400.000 mm³	E 580.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 45% Aggreg
Volunteer 3	C 4.5 min	C 150.000 mm³	C 380.000 mm³	C Normal	C Normal	C Normal	C 90% Aggreg
	E 8 min	E 150.000 mm³	E 380.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 40% Aggreg
Volunteer 4	C 3.5 min	C 370.000 mm³	C 560.000 mm³	C Normal	C Normal	C Normal	C 85% Aggreg
	E 6.5 min	E 370.000 mm³	E 560.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 40% Aggreg
Volunteer 5	C 4 min	C 410.000 mm³	C 570.000 mm³	C Normal	C Normal	C Normal	C 90% Aggreg
	E 8.5 min	E 410.000 mm³	E 570.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 35% Aggreg
Volunteer 6	C 4 min	C 330.000 mm³	C 475.000 mm³	C Normal	C Normal	C Normal	C 85% Aggreg
	E 9.5 min	E 330.000 mm³	E 475.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 40% Aggreg
Volunteer 7	C 4.5 min	C 275.000 mm³	C 570.000 mm³	C Normal	C Normal	C Normal	C 85% Aggreg
	E 9.5 min	E 275.000 mm³	E 570.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 45% Aggreg
Volunteer 8	C 3.5 min	C 335.000 mm³	C 442.000 mm³	C Normal	C Normal	C Normal	C 95% Aggreg
	E 8.5 min	E 335.000 mm³	E 442.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 35% Aggreg
Volunteer 9	C 4 min	C 385.000 mm³	C 515.000 mm³	C Normal	C Normal	C Normal	C 90% Aggreg
	E 8.5 min	E 385.000 mm³	E 515.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 40% Aggreg
Volunteer 10	C 4.5 min	C 455.000 mm³	C 590.000 mm³	C Normal	C Normal	C Normal	C 85% Aggreg
	E 7.5 min	E 455.000 mm³	E 590.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 35% Aggreg
Volunteer 11	C 4.5 min	C 305.000 mm³	C 440.000 mm³	C Normal	C Normal	C Normal	C 90% Aggreg
	E 9.5 min	E 305.000 mm³	E 440.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 30% Aggreg
Volunteer 12	C 4 min	C 415.000 mm³	C 530.000 mm³	C Normal	C Normal	C Normal	C 85% Aggreg
	E 10 min	E 415.000 mm³	E 530.000 mm³	E Alt 2nd phase	E Alt 2nd phase	E Altered	E 35% Aggreg

C: control
E: experimental

experiment C2 exhibited statistically significant differences between the control and experimental groups (p≤0.05).

DISCUSSION
Our results confirmed our working hypothesis
The topical administration of a commercial solution of ASA, as from a daily dose of 8 ml over a period of 30 days, is capable of altering platelet function in healthy volunteers. The application of a daily dose of 16 ml over the same time period inhibits platelet aggregation. These data are of great relevance in clinical practice in the case of patients who use the topical formulation excessively with analgesic and anti-inflammatory purposes. Athletes who suffer articulation, bone or muscle traumatisms and patients with rheumatic pathologies frequently use this formulation. For many years aspirin has been sold freely in many countries, with no need of a medical prescription. We consider that the professionals who are responsible for prescribing these drugs must educate their patients in terms of the daily safe dose limits that will not affect hemostasis. In this way they will avoid unexpected difficulties during odontological or medical procedures that involve bleeding. Health professionals who treat this type of patient should also draw up a detailed medical history that includes information on the use of this type of topical formulation. The professional should ask explicitly about aspirin because if the patient is asked, "Do you take any medication?" he/she will frequently not consider aspirin as medication.

As we previously mentioned, aspirin is not usually considered medication and the transcutaneous administration of any drug is thought to be harmless for physiological processes at a systemic level.

CORRESPONDENCE
Dra. Adriana B. Martínez
Balcarce 1268.
(2000) Rosario, Santa Fe, Argentina
e-mail: adrianabmartinez@yahoo.com.ar

REFERENCES

1. Hennekens CM, Buring JE, Sandercock P, Collins R, Peto R. Aspirin and other antiplatelet agents in the secondary and primary prevention of cardiovascular disease. Circulation 1980; 80: 749-756.
2. Lewis HD, Davis JW, Archibald DG, Steinke WE, Smitherman TC, Doherty JE, Schnaper HW, Le Winter MM, Linares E, Pouget JM, Sabharwal JC, Chester E, De Mots H. Protective effect of aspirin against acute myocardial infarction and death in men with unstable angina. N Engl J Med 1983; 309: 396-403.
3. ISIS-2 (Second International Study Group of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both or neither in 17.187 cases of acute myocardial infarction. ISIS-2 Lancet 1988; 2: 349-360.
4. Roth GJ, Stanford N and Majerus PW. Acetylation of prostaglandin synthase by aspirin. Proc. Natl. Acad. Sci. USA 1975; 72: 3073-3076.
5. Moncada S and Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin. Pharmacol. Rev. 1979; 30: 293-331.
6. Hamberg M, Svensson J, Wakabayashi T and Samuelsson B. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc. Natl. Acad. Sci. USA 1974; 71: 345-349.
7. Granstrom E, Diczfalusy U, Hamberg M, Malmsten C, Samuelsson B. Thromboxane A_2 biosynthesis and effect on platelets. Adv. Prostaglandin Thromboxane Res. 1982; 10: 15-57.
8. Patrignani P, Filabozzi P, Patrono C. Selective, cumulative inhibition of platelet thromboxane production by low dose aspirin in healthy subjects. J. Clin. Invest. 1982; 69: 1366-1372.
9. Kallmann R, Nieuwenhuis HK, de Groot PG, van Gijn J, Sixma JJ. Effects of low dose of aspirin, 10 mg and 30 mg daily, on bleeding time, thromboxane production and 6-keto-PGF_{1-a} excretion in healthy subjects. Thromb. Res. 1987; 45: 355-361.
10. The RISC group. Risk of myocardial infarction and death during treatment with low dose aspirin and intravenous heparin in men with unstable angina. Lancet 1990; 336: 827-830.
11. The Dutch TIA Trial Study Group. (A comparison of two doses of aspirin (30 mg vs. 283 mg a day) in patients after a transient ischemic attack or minor ischemic stroke N Engl J Med 1991; 325: 1261-1304.
12. Wallenburg HCS, Dekker GA, Makovitz JW, Rotmans P. Low-dose aspirin prevents pregnancy- induced hypertension and preeclampsia in angiotensin-sensitive primigravidae. Lancet 1986; 1: 1-3.
13. Wolff HG, Hardy JD, Goodell H. Measurement of the effect on the pain threshold of acetylsalicylic acid, acetanilid, acetophenetidin, aminopyrine, ethyl alcohol, trichloroethylene, a barbiturate, quinine, ergotamine tartrate and caffeine. An analysis of their relation to the pain experience. J Clin Invest 1941; 20: 63.
14. Grollman A and Grollman EF. Pharmacology and Therapeutics 7th Ed. Lea & Febiger, Philadelphia 1970.
15. Keimovitz RM, Pulvermacher G, Mayo G, Fitzgerald DJ. Transdermal modification of platelet function. Circulation 1993; 88: 556-561.
16. Damiani P, Ibáñez G, Olivieri AC. Zero-crossing first and second derivative synchronous fluorescence spectrocopic determination of aspirin metabolites in urine. J Pharm Biomed Anal 1994; 12: 1333-1335.
17. Deenbandhu S, Chokshi MD, Frank J, Hildner MD. Subclavian vein catheterization: A reassessment. Catheteriz Cardiovasc Diagnos 2005; 2: 1-3.