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Synergistic Effect of Ginger and Nifedipine on Human Platelet Aggregation: A Study in Hypertensive Patients and Normal Volunteers

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Abstract: In this study, we evaluated the synergistic effect of ginger and nifedipine on anti-platelet aggregation in normal human volunteers and hypertensive patients. The results showed that the percentage of platelet aggregation induced by collagen, ADP and epinephrine in hypertensive patients was larger than that in normal volunteers. Either aspirin or ginger could potentiate the anti-platelet aggregation effect of nifedipine in normal volunteer and hypertensive patients. These results suggested that ginger and nifedipine possessed synergistic effect on anti-platelet aggregation. A combination of 1 g ginger with 10 mg nifedipine per day could be valuable for cardiovascular and cerebrovascular complication due to platelet aggregation.

Keywords: Ginger; Platelet Aggregation; ADP; Collagen; Epinephrine.

Introduction

Platelets participate in fatal and nonfatal myocardial infarction due to coronary thrombosis. They also contribute to the development and progression of coronary artery atherosclerosis (Fuster *et al*., 1994). Inhibition of platelet activity by aspirin significantly reduces the incidence of first myocardial infarction, recurrent infarction and vascular death among patients with cardiovascular disease (Hennekens *et al*., 1997). The antiplatelet therapeutic (mainly aspirin) reduces the risk of nonfatal stroke, nonfatal myocardial infarction and vascular death by 25–32% in patents with high-risk factors. However, there are reports of adverse effects of aspirin, mainly associated with gastric ulcers and gastrointestinal bleeding (Lloyd, 1996). Thus, the use of aspirin may be confined to elderly patients whose benefits

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are likely to outweigh any increased risk of gastrointestinal ulcers or bleeding (Silagy, 1996). Even though low-dose aspirin (75–150 mg) is used to minimize the incidence of adverse effects, longitudinal studies show that a dose of 75 mg daily can still cause a small but significant increase in gastrointestinal bleeding (Hirsh, 1992). Thus, research and development of new antiplatelet drugs with least adverse effect profile are required.

Ginger is the dried rhizome of *Zingiber officinale* Roscoe (Zingiberaceae). Zingiber comes from the Greek "zingiberis" and Arabic "zindschebil" or the root of zindschi (India), which is interpreted as "known already to the ancients". Its common name, ginger, is derived from the Sanskrit "gringa" or horn and "vera" meaning body, in references to the shape of the root. Gingiberis rhizome contains two classes of constituents (i) the essential oils that give the aroma, and (ii) the main pungent principles: gingerols, shogaols. Ginger contains 1–2% volatile oil, 5–8% resinous matter, starch and mucilage. The oil of ginger is itself a mixture of over 24 constituents, consisting of monoterpenes (phellandrene, carnphene, cineolc, citral, and borneol) and sesquiterpenes (zingiberine and hisabolene) etc. (Conell and Suther, 1969). The major substances present in the ginger extracts were zingiberine, gingerols and shogaols (Kelly, 2002). The pungent elements of [6]-gingerol have been shown to possess analgesic and anti-inflammatory activities (Young *et al*., 2005). It has been used extensively in traditional oriental medicine for alleviating symptoms of asthma, shortness of breath, water retention, earache, diarrhea, nausea, and vomiting. [6]-Gingerol has been reported to reduce inflammation and inhibit platelet activation (Sifton, 1999). Gingerols and related analogues, the active components of ginger, represent a potential new class of inhibitors of platelet activators (Koo *et al.*, 2001). [6]-Gingerol and [6]-shogaol have been found to possess a variety of interesting pharmacological effects, for example, antipyretic and cardio tonic effects, and the inhibition of spontaneous motor activity and prostaglandin biosynthesis (Suekawa *et al.*, 1984). [6]-Gingerol has been used as a marker substance of ginger. However, little information is available on the antiplatelet effects of raw ginger in humans. The purpose of the present study, therefore, is to examine the synergistic effects of ginger and nifedipine on human platelet aggregation by using the Born's method (Born, 1962).

Materials and Methods

Materials and Instruments

Dried ginger was purchased from Taiping, Taichung, and was identified as *Zingiber officinale* Roscoe by Dr. Chung-Chuan Chen (Professor of Pharmacognosy, China Medical University). Platelet Lumi-aggregmeter (Cronolog 560) and Centrifuge (Kokusan-700F) were provided by the Paochien Hospital.

Chemicals

Nifedipine, aspirin (abbrev. as ASA), collagen, epinephrine and ADP were obtained from SIGMA (St. Louis, MO 63178, USA).

Patient Population

The study was carried out in 2 phases, one comprised of normal volunteers and the other is hypertensive patients. The Institutional Review Board at the Paochien Hospital approved the protocol and subjects signed informed consent prior to participation.

Phase I: The subjects were normal volunteers. Number of subjects = 10 of either sex; Age group = 25–60 years

The subjects refrained from ingesting ethanol, aspirin, or other medications known to alter platelet function such as tea, wine, beer, citrus fruits, fruit juices, grape products and alcohol for the 2-week period of enrolment. They were non-alcoholic, non-smokers and had no history of exposure to ASA, and/or non-steroid anti-inflammatory drugs (NSAIDs) for at least the previous 2 weeks. Normal subjects with a history of hypersensitivity to aspirin or other NSAIDs and those with abnormalities in organ functions were excluded from the study.

Phase II: The subjects were having high blood pressure patients. Number of subjects = 10 of either sex; Age group = 35–60 years; Blood pressure range: Systolic = 150–180 mmHg; Diastolic = 96–120 mmHg

These were freshly detected uncomplicated essential hypertensive patients. Patients with a history of secondary, transient ischemic attacks, peptic ulcer disease, hypersensitivity to aspirin or receiving any other NSAIDs were excluded from the study.

Procedure of the Regiments of Medications in both Phases

As shown in Fig. 1, after 75 mg of ASA was administered for one week, platelet aggregation was carried out by the method of Born before, 2 and 24 hours after administration of ASA of the last dosage of ASA. After a wash out period of 7 days, 1 g of ginger was administered to the volunteers on day-8 for one week. Platelet aggregation was carried out 1 hour after administration of the last dosage of ginger.

In the 3rd part, after a washout period of 10 days, nifedipine 10 mg was administered twice daily for one week. On the 7th day, platelet aggregation was carried out 1 hour after administration of the last dosage of nifedipine.

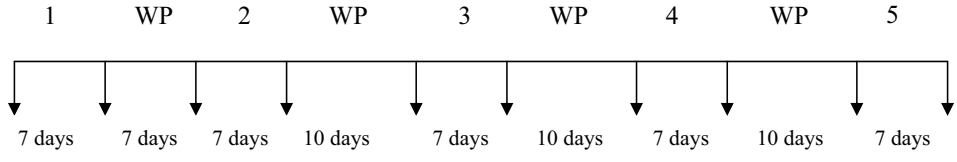

Figure 1. Schematic diagram of the procedure of regiments of oral medications in the volunteers (Phase I) and the hypertensive patients (Phase II). 1: 75 mg aspirin (ASA), 2: 1 g ginger, 3: 10 mg nifedipine, 4: 1 + 2, 5: 2 + 3, WP: washout period.

In the 4th part, after a washout period of 10 days, both nifedipine 10 mg and 1 g ginger, were administered orally and platelet aggregation was carried out 1 hour after administration of the last dosage of the combined drugs.

In the 5th part, after a washout period of 10 days, both nifedipine 10 mg and 75 mg ASA, were administered orally and platelet aggregation was carried out 1 hour after administration of the last dosage of the combined drugs.

Platelet aggregation was measured by using collagen, ADP and epinephrine as the agonists, percent inhibition of aggregation was calculated as follows:

$$\% \text{ Inhibition of aggregation} = \frac{\text{Platelet aggregation of induced drugs} - \text{Platelet aggregation of test drugs}}{\text{Platelet aggregation of induced drugs}} \times 100$$

Induced drugs: collagen, ADP or epinephrine; Test drugs: nifedipine, aspirin, ginger, combination of ginger and nifedipine or combination of aspirin and nifedipine.

Statistical Analysis

Statistical analysis was carried out by paired-t test. Data are reported as means ± SEM. Differences were considered to be significant when the $p < 0.05$.

Results

Platelet Aggregation in Normal Volunteers and Hypertensive Patients

As shown in Table 1, the percentage of platelet aggregation with collagen, ADP and epinephrine was 44.1%, 44.5% and 42.1% in normal subjects and 64.2%, 67.7% and 62.9% in hypertensive patients, respectively. The platelet aggregations induced by the collagen, ADP or epinephrine in hypertensive patients was higher than that in normal subjects.

Synergistic Effect of Ginger and Nifedipine on Platelet Aggregation in Normal Volunteers

After treatment with nifedipine, the percentages of inhibition of platelet aggregation induced by collagen, ADP and epinephrine were 20.2%, 22.6%, 23.4% respectively (Table 2). After administration of aspirin alone, the values were 37.2%, 39.7%, 34.9% with collagen, ADP and epinephrine respectively. When the volunteers were administered both aspirin and nifedipine in combination, it was observed that the percentage of inhibition of platelet aggregation induced by collagen, ADP and epinephrine were 82.8%, 78.2%, 72.2% respectively. Thus the combination of aspirin and nifedipine appeared to have a synergistic effect on inhibition of platelet aggregation in normal individuals. After administration of ginger alone, the values were 35.3%, 37.8%, 35.9% with collagen, ADP and epinephrine

respectively. When the volunteers were administered both ginger and nifedipine in combination, it was observed that the percentage inhibition of platelet aggregation induced by collagen, ADP and epinephrine were 79.8%, 75.2%, 69.3% respectively.

Synergistic Effect of Ginger and Nifedipine on Anti-Platelet Aggregation in Hypertensive Patients

As shown in Table 3, after treatment with nifedipine, the percentage of inhibition with collagen, ADP and epinephrine were 20.5%, 22.3%, 19.2% respectively. After treatment with a combination of nifedipine and aspirin, the percentage of inhibition values with the three agonist were 65.2%, 64.6%, 62.8% respectively. After the treatment of the combination of nifedipine and ginger, the percentage of inhibition values with the three agonist were 64.2%, 63.8%, 61.1% respectively.

Table 1. Percentage of Platelet Aggregation with Collagen, ADP and Epinephrine in Normal Volunteers and Hypertensive Patients

	Percentage of Aggregation (%)	
	Normal Volunteers	**Hypertensive Patients**
Collagen	44.1 ± 0.2	64.2 ± 0.5
ADP	44.5 ± 0.4	67.7 ± 0.4
Epinephrine	42.1 ± 0.3	62.9 ± 0.7

Data are expressed as mean ± SEM (n = 10).

Table 2. Synergistic Effect of Ginger and Nifedipine on Platelet Aggregation in Normal Volunteers

	Percentage of Inhibition of Platelet Aggregation				
	Nifedipine	**Aspirin**	**Nifedipine + Aspirin**	**Ginger**	**Nifedipine + Ginger**
Collagen	20.2 ± 0.7	37.2 ± 0.7	82.8 ± 8.5***	35.2 ± 0.8	79.8 ± 0.6***
ADP	22.6 ± 0.6	39.7 ± 0.8	78.2 ± 9.15***	37.8 ± 0.8	75.2 ± 0.8***
Epinephrine	23.4 ± 1.0	34.9 ± 0.5	72.2 ± 8.5**	35.9 ± 0.7	69.3 ± 0.5**

Data are expressed as mean ± SEM (n = 10). *p < 0.05, **p < 0.01, ***p < 0.001, as compared to the nifedipine group.

Table 3. Synergistic Effect of Ginger, Aspirin and Nifedipine on Platelet Aggregation in Hypertensive Patients

	Percentage of Inhibition of Platelet Aggregation		
	Nifedipine	**Nifedipine + Aspirin**	**Nifedipine + Ginger**
Collagen	20.5 ± 0.7	65.2 ± 0.7***	64.2 ± 0.5***
ADP	22.3 ± 0.7	64.6 ± 1.1***	63.8 ± 0.4***
Epinephrine	19.2 ± 1.0	62.8 ± 1.1***	61.1 ± 0.8***

Data are expressed as mean ± SEM (n = 10). *p < 0.05, **p < 0.01, ***p < 0.001, as compared to the nifedipine group.

Discussion

The results of the present study indicated that the platelet aggregation in hypertensive patients prior to drug therapy was higher than that in normal healthy volunteers. Both groups were in comparable in age. Hence, it could be concluded that platelets were hyperactive in hypertensive patients. It was reported that the platelet function was shown to be altered in hypertensive patients and that these deranged platelet functions were responsible for the enhanced thrombogenesis (Declerk, 1986). However, nifedipine was reported to have a beneficial effect on platelets to a certain extent in hypertensive patients. It not only lowered the blood pressure, but it helped impartially to inhibit the ADP, collagen and epinephrine induced platelet aggregation (Mehta and Mehta, 1981; O'Smialoski *et al*., 1990).

In an *in vitro* study, it was demonstrated that nifedipine and aspirin have a synergistic effect on inhibition of platelet aggregation (Dong and Shi, 1990). The anti-platelet aggregation effect was markedly increased when both nifedipine and aspirin were used. It was reported that aspirin in combination with nifedipine is beneficial in arterial thrombosis. However, there are reports of adverse effects of aspirin, mainly associated with gastric ulcers and gastrointestinal bleeding (Lloyd, 1996). Thus, the use of aspirin may be confined to elderly patients for whom benefits are likely to outweigh any increased risk of gastrointestinal ulcers or bleeding (Silagy, 1996). Even though low-dose aspirin (75–150 mg) is used to minimize the incidence of adverse effects, longitudinal studies show that a dose of 75 mg daily can still cause a small but significant increase in gastrointestinal bleeding (Hirsh, 1992).

The anti-platelet aggregation effect was also markedly increased when both nifedipine and ginger were used. The nifedipine and ginger had a synergistic effect on inhibition of platelet aggregation. Ginger and its relative compounds have been found to possess substantial antioxidative activity as determined by inhibition of phospholipid peroxidation induced by the $FeCl_3$-ascorbate system (Aeschbach *et al.*, 1994). The phenolic compounds of ginger exert an inhibitory effect on xanthine oxidase that is responsible for the generation of reactive oxygen species (Chang *et al.*, 1994), such as superoxide anion. Guh *et al.* (1995) reported [6]-gingerol inhibits arachidonic acid-induced platelet aggregation and formation of thromboxane B_2 and prostaglandin D_2. Gingerol, shogaol and other structurally related substances in ginger inhibit prostaglandin and leukotriene biosynthesis through suppression of 5-lipoxygenase or prostaglandin synthesis (Kiuchi *et al.*, 1982; Flynn *et al.*, 1986).

Calcium is a cation involved in the mediation of platelet aggregation and thromboxane A2 formation. Inhibition of platelet aggregation by nifedipine results in an internal re-distribution of calcium in human platelets and thereby further inhibiting platelet aggregation. The inhibitory effect of ginger on platelet aggregation may be related to the inhibition of platelet cyclooxygenase or redistribution of calcium in the platelet. It needs to be further studied in the future.

In conclusion, both ginger and aspirin could potentiate the anti-platelet effect of nifedipine. A combination of 1 g ginger with 10 mg nifedipine would be valuable in cardiovascular and cerebrovascular complication due to platelet aggregation.

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