

In vitro and *In vivo* Interactions of Diltiazem with Ibuprofen and Naproxen in Aqueous Medium and Rabbits

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ABSTRACT: A common practice in the medical science is the prescription of multiple drugs at a time, which may sometimes be neither safe nor effective and may be deleterious. The present study was aimed to evaluate such a combination of diltiazem with ibuprofen and naproxen. The *invitro* interaction of diltiazem with ibuprofen and naproxen has been studied at room temperature and at different pH in the aqueous medium by spectroscopic and conductometric methods, and the *invivo* study was done in the rabbit by measuring blood pressure using a mercury manometer. It has been found that diltiazem formed stable 1 : 1 complexes with ibuprofen and naproxen along with some intermediates. The Ardon's spectrophotometric method was employed to confirm the formation of 1 : 1 complex and for the calculation of the stability constants. The *invivo* study was carried out to evaluate the influence of ibuprofen and naproxen on the antihypertensive activity of diltiazem in the rabbit. Concurrent administration of ibuprofen and naproxen with diltiazem did not make any significant change in the antihypertensive activity of diltiazem. It is thus inferred that co-administration of diltiazem with ibuprofen and naproxen may be considered as safe and effective.

Key words: Drug interaction, complexation, co-administration, diltiazem, naproxen, ibuprofen, stability constant

INTRODUCTION

Diltiazem hydrochloride is an important calcium channel blocker. Clinically it is used to treat cardiovascular diseases such as angina pectoris, hypertension and cardiac arrhythmias. Ibuprofen and naproxen are nonsteroidal anti-inflammatory agents. Clinically they are used for the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, acute gout, acute musculoskeletal pains, mild to moderate pain such as dysmenorrhea and migraine. Hamman *et al.*¹ studied the cardiodepressant actions of diltiazem with propranolol in dogs. They observed that when given alone diltiazem increased cardiac output and PR

interval while decreasing mean arterial pressure, heart rate and systemic vascular resistance. Propranolol alone decreased cardiac output and heart rate while increasing vascular resistance. But a combination therapy resulted in a depression upto levels similar to those achieved with propranolol alone and a decrease in mean arterial pressure upto levels achieved with diltiazem alone. PR interval increased beyond the duration produced by either drug given alone. It was thus inferred that there was no apparent interaction between diltiazem and propranolol. Saseen *et al.*² studied the effect of nifedipine on diltiazem/verapamil and showed that combination therapy exhibited a greater antihypertensive activity than nifedipine alone.

The aim of the present study was to evaluate the *invitro* and *invivo* effects of ibuprofen and naproxen on the physicochemical and therapeutic activity of

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diltiazem and thus to infer about the combination therapy.

MATERIALS AND METHODS

The experimental animals were purchased from the animal branch of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Mohakhali, Dhaka. A digital pH meter (Jenway) was used to adjust the pH of the buffer solutions. A Pye unicum SP-800 UV/VIS spectrophotometer was used for the measurement of absorbances of the samples. A digital conductivity meter (Hanna Instruments, USA) was used in the conductometric titration. The instrument had an immersion conductivity cell with a constant of 0.959 cm^{-1} with platinum electrode.

Chemicals and drugs. Ibuprofen, naproxen and diltiazem HCl were kind gift from Beximco Pharmaceuticals Ltd., Dhaka. All other chemicals were purchased from BDH (Dhaka, Bangladesh) and were of reagent grade.

The *in vitro* interaction studies^{3,4} were performed by observing absorption spectra, conductometric data and Ardon's spectrophotometric curves. In spectroscopic method, complexation was detected by comparing the absorption spectra of pure diltiazem ($5 \times 10^{-5} \text{ M}$) and its 1 : 1 molar mixtures with ibuprofen and naproxen ($5 \times 10^{-5} \text{ M}$ for each) in the solutions using buffers^{5,6} of pH 0.4, 1.4, 3.4, 5.4 and 7.4. The Conductometric method was applied to detect complexation as well as possible molar ratios of the drug and other interacting agents in the complexes.⁷ A fixed amount of diltiazem solution ($2.5 \times 10^{-5} \text{ M}$, 20 ml), was titrated with gradual addition of ibuprofen or naproxen ($10 \times 10^{-4} \text{ M}$) from a burette. A reverse titration was carried out in all cases under similar conditions. A plot of molar ratios of diltiazem to ibuprofen or naproxen against conductance was prepared to locate points of possible interaction. In the Ardon's method⁸, the drug concentration was kept constant while the concentration of interacting molecules were varied. Absorbance (A) of free drug solutions and mixtures were measured at 240 nm and a plot of $1/[D-\epsilon_A C]$ versus $1/[B]$ was made using the

Ardon equation $1/[D-\epsilon_A C]=1/KC(\epsilon_{\text{com}}-\epsilon_A)[B]+1/C(\epsilon_{\text{com}}-\epsilon_A)$. The values of stability constants (K) were calculated from the [intercept]/[slope] of the straight lines obtained. In the above equation D is the absorbance of the mixture, C is the molar concentration of the interacting molecules, [B] is the molar concentration of the drug, ϵ_{com} is the molar extinction co-efficient of the complex and ϵ_A is the molar extinction co-efficient of the interacting molecules.

In the *in vivo* studies^{9,10}, young healthy rabbits weighing 2-3 kg were used. The rabbits were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). Respiration was maintained by artificial ventilation through the cannula in the trachea to maintain pCO_2 , pO_2 , and pH within the normal range. A polyethylene tube was inserted into the left femoral vein to administer drugs. The common carotid artery was cannulated and connected to a mercury manometer to monitor the blood pressure. After 15 min of stabilization, saline (as vehicle or control, 0.5 ml) was injected and normal blood pressure was recorded. Diltiazem (1 mg/kg body weight), and 1 : 1 mixtures of diltiazem + ibuprofen, and diltiazem + naproxen were administered to the different groups for each dose of the vehicle or drugs through the femoral vein as i.v. bolus injection and the subsequent effect on the blood pressure was recorded. Each animal received only one dose (treatment) of either vehicle or any of the drugs.¹¹

Data analysis and statistics. Data were expressed as mean \pm S.E.M. Differences in mean values between experimental groups were analyzed by unpaired 't' test. A probability value less than 0.05 ($p < 0.05$) was defined to be significant.

RESULTS AND DISCUSSION

The ultra violet (UV) absorption of diltiazem and its 1 : 1 mixtures with ibuprofen and naproxen was measured at 200-400 nm. Diltiazem showed a sharp absorption maximum at 237 nm and another peak at 211 nm. The pattern of the peaks of diltiazem at

various pH has been shown in Figure 1a. The change in absorption intensity at pH 7.4 is shown in Figure 1b and 1c for 1 : 1 mixtures of diltiazem plus ibuprofen and diltiazem plus naproxen. A noticeable

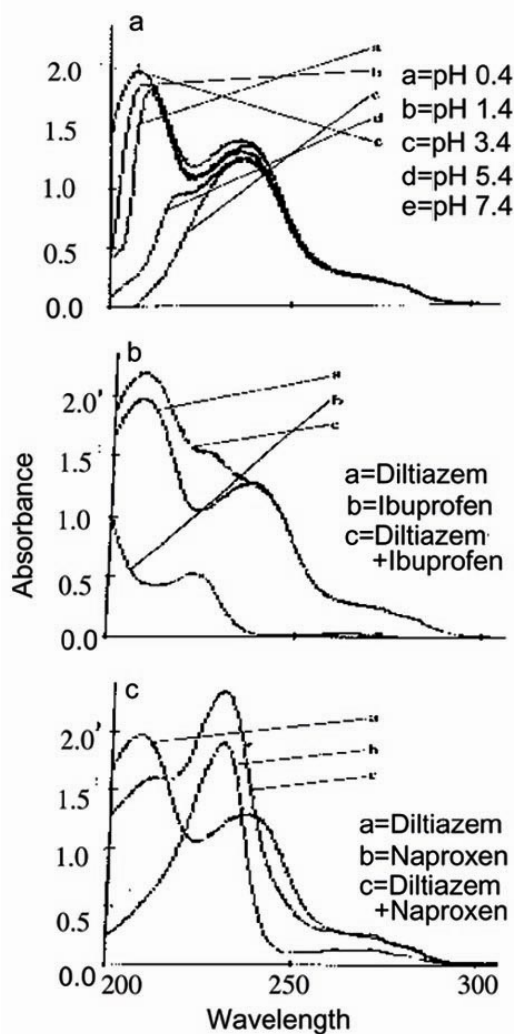


Figure 1. Spectrophotometric data of (a) diltiazem at different pH and its 1:1 mixture with (b) ibuprofen and (c) naproxen at pH 5.4

change in absorption peaks was also observed at pH 3.4 and 5.4 when diltiazem was mixed with ibuprofen and naproxen at 1 : 1 ratio. These changes in the pattern of peaks or absorption intensities indicate that diltiazem formed complexes of varying strength at different pH with ibuprofen and naproxen. Conductometric data (Figure 2) showed that diltiazem formed a stable 1 : 1 complex with some intermediary products. Ardon plot⁸ confirmed the formation of 1:1 complex as shown in Figure 3 that a

straight line was obtained in each case and stability constants of diltiazem-ibuprofen and diltiazem-naproxen complexes were calculated according to Ekschlager (1969)¹² and Gould (1942)¹³ and have been given in the table (Table 1). Stability constants data showed that diltiazem-naproxen system formed relatively very weaker complexes than those of diltiazem-ibuprofen system at all pH conditions.

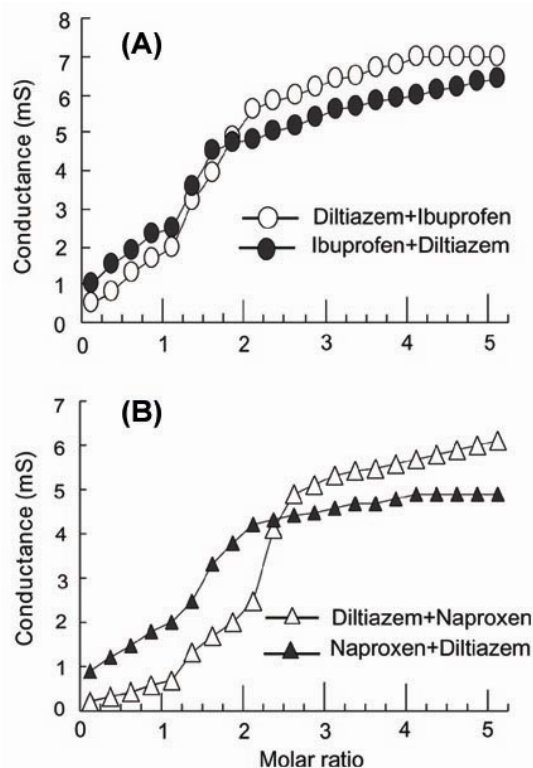


Figure 2. Conductometric plots for (a) diltiazem + ibuprofen and (b) diltiazem + naproxen systems at pH 7.4

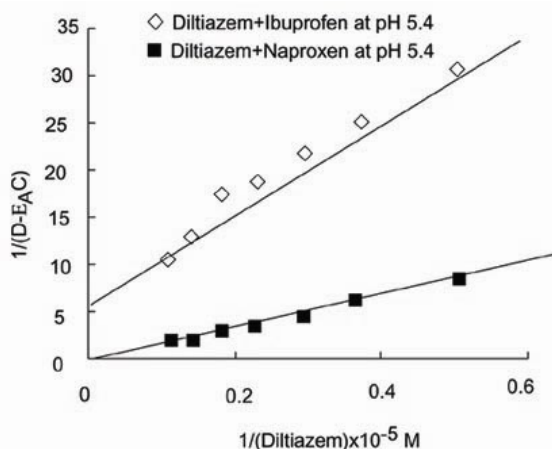


Figure 3. Ardon plot for diltiazem-ibuprofen and diltiazem-naproxen systems

In the *in vivo* study, it was found that neither ibuprofen nor naproxen affected the antihypertensive activity of diltiazem in the dose range studied (Figure 4). In control condition the blood pressure was 87 ± 3 mmHg (mean \pm SEM, $n = 6$). After administration of diltiazem (3 mg/kg) alone blood pressure was decreased to 71 ± 2 mmHg which was statistically significant ($p < 0.0001$). After combination therapy of diltiazem with ibuprofen and diltiazem with naproxen, the blood pressure was decreased to 70 ± 1 mmHg and 69 ± 1 mmHg, respectively.

Table 1. The stability constants for Diltiazem-ibuprofen and Diltiazem-naproxen systems at different pH at room temperature.

| System | Stability constants ($K \times 10^{-5}/\text{mole}$) | | | | |
|---------------------|--|--------|--------|--------|--------|
| | pH 0.4 | pH 1.4 | pH 3.4 | pH 5.4 | pH 7.4 |
| Diltiazem-ibuprofen | 0.66 | 0.15 | 0.63 | 0.36 | 0.66 |
| Diltiazem-naproxen | 0.03 | 0.03 | 0.18 | 0.03 | 0.05 |

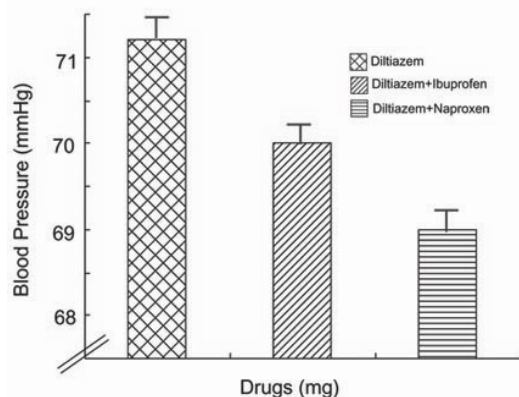


Figure 4. Comparison of effects of diltiazem and its 1 : 1 mixtures with ibuprofen and naproxen on blood pressure in rabbits. Data are shown as mean \pm SEM ($n = 6$ in each case).

Polypharmacy (prescribing many drugs at a time) is a common practice in case of patients undergoing a major operation, hospitalized patients, and also in geriatric patients. Sometimes co-administration of more than two different classes of drugs may ensure effects that are neither safe nor effective and sometimes may be deleterious. In our *in vitro* study it was found that diltiazem formed 1 : 1 complexes with ibuprofen and naproxen along with some intermediary complexes at room temperature and various pH, but the stability constants indicated

that these complexes were not so strong and might be reversible in nature. However, in the *in vivo* study in rabbits, it was found that the decrease in blood pressure after co-administration of diltiazem with ibuprofen and naproxen was statistically insignificant with a p value 0.6723 for diltiazem plus ibuprofen and 0.9076 for diltiazem plus naproxen systems. It was observed that neither ibuprofen nor naproxen had any deleterious effects on the activity of diltiazem. We, thus, conclude that concurrent administration of diltiazem with ibuprofen and naproxen may be safe and effective.

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