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THE SYNERGISM OF DILTIAZEM AND NIFEDIPINE IN THEIR ANTIHYPERTENSIVE FUNCTIONS IN THE ANIMAL MODEL AND THE CASE OF COADMINISTRATION OF KETOTIFEN FUMERATE AND POTASSIUM NITRATE WITH THEM

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Abstract

An *in vivo* study has been carried out to evaluate the synergism of diltiazem and nifedipine on the antihypertensive activity and the effect of ketotifen fumerate and potassium nitrate on this phenomenon of synergism in rabbit. It has been found that the lowering of blood pressure by concurrent administration of diltiazem and nifedipine was not equal to the sum of lowering of blood pressure by individual drug. Concurrent administration of ketotifen fumerate and potassium nitrate with diltiazem or with nifedipine did not make a significant change on the antihypertensive activity of diltiazem or nifedipine. Further considerations and monitoring are to be practiced during concurrent therapy of diltiazem and nifedipine to avoid untoward pharmacological and therapeutic actions related to drug-interactions but coadministration of ketotifen fumerate and potassium nitrate with either diltiazem or nifedipine might be regarded as safe and effective.

Keywords: *In vivo*, antihypertensive activity, nifedipine, diltiazem, ketotifen fumerate, potassium nitrate, coadministration.

Introduction

Both diltiazem and nifedipine are the calcium channel blockers, which have gained increasing acceptance in the treatment of angina pectoris, hypertension and cardiac arrhythmia (Carlsted 1990, Martin 1987). Ketotifen fumerate is an antihistaminic drug used in the treatment of allergic conditions and prophylactic control of asthma (Martindale 1993, Barbuto *et al.*1998). Potassium ion (as nitrate salt) is the predominant intracellular cation. It plays a vital role in the maintenance of electrical excitability of nerve and muscle. K^+ ion also plays an important role in the genesis and correction of imbalances of acid-base metabolism. Potassium salts are thus important therapeutic agents but they are extremely dangerous if use improperly (Gilbert and Irwin 1991).

Clarke (1986) studied the serious drug-drug interaction secondary to high doses of diltiazem used in the treatment of pulmonary hypertension. Changes in pharmacokinetic and pharmacodynamic properties of digoxin and phenytoin were caused by high dose of diltiazem. Glaser *et al.* (1994) studied the safety and compatibility of betoxolol combined with diltiazem therapy in stable angina pectoris. Betoxolol influences the resting systolic blood pressure, heart rate and the rate pressure product. Hamman *et al.* (1987) studied the actions of combined diltiazem and propranolol in dogs and found that diltiazem has no influence on the action of propranolol. Neustein *et al.* (1998) studied the cardiovascular consequences of the concomitant administration of nifedipine and magnesium sulfate in pigs and found that nifedipine alone decreased peripheral vascular resistance and mean arterial pressure. Magnesium sulfate alone decreased the first derivative of left ventricular pressure (LVdP/dt) and increased left ventricular end-diastolic pressure (LVEDP). Magnesium sulfate also decreased peripheral vascular resistance and mean atrial pressure. The concomitant administration of nifedipine and magnesium sulfate led to a further decrease in myocardial

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contractility, as evidenced by a decrease in LVdP/dt and increase in LVEDP. They concluded that depressive effects of nifedipine and magnesium sulfate on the cardiovascular system are potentiated when administered concomitantly.

Weir *et al.* (1998) studied the steady-state pharmacokinetics of diltiazem and hydrochlorothiazide administered alone and in combination. They found that coadministered hydrochlorothiazide did not significantly alter diltiazem (alone versus combination) steady-state maximum plasma concentration, time to maximum plasma concentration, area under the plasma concentration-time curve, oral clearance, or elimination half-life. Similarly, administration of diltiazem did not significantly influence hydrochlorothiazide (alone versus combination) above parameters. They concluded that a clinically significant pharmacokinetic interaction between diltiazem and hydrochlorothiazide does not exist. Pessina *et al.* (2001) studied the efficacy, tolerability and influence on "quality of life" of nifedipine versus amlodipine in elderly patients with mild to moderate hypertension and found that overall mean diastolic blood pressure were 87.5 mmHg for nifedipine and 86.7 mmHg for amlodipine. It was concluded that nifedipine (30-60 mg) was shown to be as efficacious and safe as amlodipine (5-10 mg) in elderly patients with mild-moderate hypertension. Quality of life improved compared to baseline with no significant difference between the two drugs, thus confirming a positive class effect for calcium antagonists.

Fukui *et al.* (2001) studied the effect of magnesium stearate or calcium stearate as additives on dissolution profiles of diltiazem hydrochloride from press-coated tablets with hydroxypropylmethylcellulose acetate succinate (HPMCAS) in the outer shell, and found that some physicochemical interaction occur between magnesium stearate and HPMCAS in tablets with HPMCAS and magnesium stearate and the uptake increased markedly in each dissolution medium. Takara *et al.* (2002) studied the interaction of digoxin with antihypertensive drugs (diltiazem and nifedipine) via multidrug transporter (MDR1-P-glycoprotein)-mediated interaction and found that diltiazem and nifedipine didn't show inhibitory effects on transcellular transport of [3H]digoxin.

Schug *et al.* (2002) studied the effect of food on the pharmacokinetics of nifedipine and found that the extent of bioavailability of nifedipine as characterized by area under the curve (AUC) was slightly lower for Slofedipine compared with Adalat OROS with a point estimate of 82.3% primarily resulting from pronounced differences in nifedipine concentrations during the first 15 h after administration. Bailey and coworker (2004) studied the interactions between grapefruit juice and cardiovascular drugs (nifedipine) and found that inhibition of organic anion transporting polypeptides by grapefruit juice was observed in vitro. Numerous medications used in the prevention or treatment of coronary artery disease and its complications have been observed or are predicted to interact with grapefruit juice. Such interactions might cause excessive vasodilatation when hypertension is managed with the nifedipine.

Barbier *et al.* (2004) examined the acute study of interaction among cadmium, calcium, and zinc transport along the rat nephron in vivo. Acute Cd^{2+} injection resulted in renal losses of Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, PO_4^{2+}, and water, but the glomerular filtration rate remained stable. ^{45}Ca microinjections showed that Ca^{2+} permeability was strongly inhibited by Cd^{2+} (20 mM), and La^{3+} (1 mM), whereas nifedipine (20 mM) had no effect. Cazzola *et al.* (2004) studied the comparative effects of a two-week treatment with nebivolol and nifedipine in hypertensive patients suffering from chronic obstructive pulmonary disease (COPD) and observed that similar and significant reductions in systolic and diastolic blood pressure were observed with both treatments. It was concluded that the use of nebivolol in hypertensive patients with stable mild to moderate COPD was safe during a 2-week trial.

Odou *et al.* (2005) studied the grapefruit juice-nifedipine interaction and possible involvement of several mechanisms. They found that grapefruit juice increased the bioavailability of nifedipine, but did not

significantly reduce the drug's metabolism as shown by the approximately constant metabolite to parent drug AUC ratio. They conclude that grapefruit juice interferes with the metabolism of nifedipine by inhibiting nifedipine metabolism and slowing down the rate of gastric emptying.

Choi *et al.* (2006) studied the pharmacokinetic interaction between fluvastatin and diltiazem in rats. Pharmacokinetic parameters of diltiazem were determined following an oral administration of diltiazem (15 mg/kg) in the presence and absence of fluvastatin (0.6 and 2.0 mg/kg). They concluded that, the concurrent use of fluvastatin significantly enhanced the oral exposure of diltiazem in rats.

To the best of our knowledge, there was no report on synergism of diltiazem and nifedipine and effect of ketotifen fumerate and potassium nitrate on the antihypertensive activity of diltiazem and nifedipine. The present study, therefore, was to evaluate the synergism of diltiazem and nifedipine, and the effect of ketotifen fumerate and potassium nitrate on hypotensive activity of diltiazem and nifedipine and thus to infer about the fate of combined drug therapy of these drugs.

Materials and Methods

Apparatus

A mercury manometer was used for the measurement of blood pressure of the rabbit. Sterile and disposable plastic syringes were used to inject the drugs in the solution form.

Chemicals

Diltiazem HCl, ketotifen fumerate were obtained from Beximco Pharmaceuticals Ltd., (Bangladesh). ACME Laboratories Ltd. (Bangladesh), kindly supplied nifedipine. Potassium nitrate was of analytical grade (BDH, England) and Heparin was purchased from Leo Pharmaceutical products, Denmark. Phenobarbital injection was purchased from Jayson Pharmaceuticals Ltd., (Bangladesh).

Test Animal

Forty rabbits were used for determination of blood pressure or antihypertensive activity. Body weight of rabbit was 2$\pm$0.1 kg. Animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B)

Method

The antihypertensive activity of diltiazem and nifedipine, and the combinations of ketotifen fumerate and potassium nitrate in rabbits was measured directly by a mercury manometer. Forty adult healthy rabbits were used as experimental animals. They were kept at rest for 7 days with normal diets. Thirty-five rabbits were divided into 7 groups each having 5 (marked as group I-VIII) and the rest five as control (control group). They were fasted overnight before drug administration. Each rabbit was sedated by injecting Phenobarbital sodium (30mg/kg, intraperitonially, i.p.). The common carotid artery was cannulated and connected directly to the mercury manometer. The femoral vein was also cannulated and used for drug administration. Diltiazem HCl (1mg/kg) was injected through the femoral vein to each animal (group I) and the subsequent effect on blood pressure was noted. Then nifedipine was injected in each rabbit at the same dose (1mg/Kg) in the similar manner (group II). The fall in the blood pressure was recorded. The combined doses {(diltiazem+nifedipine (group III), diltiazem+ketotifen fumerate (group IV), diltiazem+ potassium nitrate (group V) and (nifedipine+ketotifen fumerate (groupVI), nifedipine+ potassium nitrate (group VII)} were injected respectively in all groups of animals and the subsequent effects on the blood pressure were recorded. Heparin was injected to prevent *in vivo* blood coagulation.

Data analysis and statistics:

Data are shown as mean ± SEM. The variation in blood pressure was analyzed by students 't' test. A probability value of 0.05 ($p<0.05$) was defined to be significant. Data were analyzed by the software graphpadPrism.

Results and discussion

The blood pressure of the animal of control group was found to be 87 mmHg. After administration of diltiazem the pressure was lowered on the average to 72 mmHg and after administration of nifedipine the pressure was lowered on the average to 66 mmHg. When diltiazem and nifedipine were coadministered then the blood pressure was lowered on the average to 57 mmHg in comparison with the control animal. Thus it was observed that the combined hypotensive effect of diltiazem and nifedipine was higher than either of the drugs (Fig. 1).

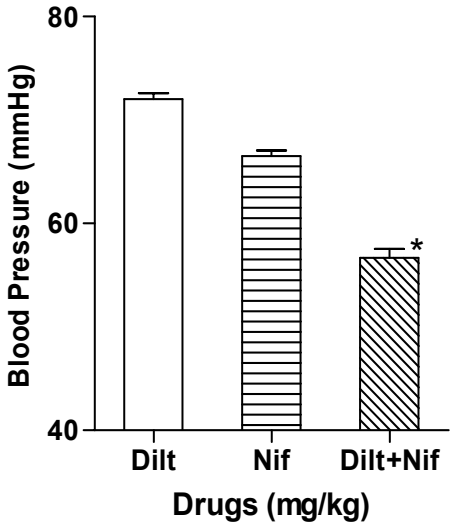

Fig. 1. Comparison of blood pressure in mmHg showed by the animals after the administration of diltiazem HCl alone, nifedipine alone and diltiazem+nifedipine combination. Data are shown as mean ±SEM, (n=6). * indicates significant change in blood pressure ($P<0.0001$) after coadministration of diltiazem and nifedipine in comparison to both diltiazem and nifedipine when given as single therapy. Dilt-diltiazem, Nif-nifedipine, Dilt+Nif-diltiazem+nifedipine.

But the hypotensive activity of diltiazem and nifedipine when coadministered was not additive sum (36 mmHg) of lowering by diltiazem and nifedipine individually (15 mmHg and 21 mmHg). In other words diltiazem lowers the blood pressure on the average 15 mmHg, nifedipine lowers the blood pressure on the average 21 mmHg, average lowering being 18 mmHg. But (diltiazem + nifedipine) lowers the blood pressure on the average 30mmHg. So it may be remarked that a negative synergism occurs in the (diltiazem+nifedipine) combined therapy. This is probably because both the drugs are calcium channel antagonists and a competitive inhibition has occurred.

Combined administration of diltiazem+ketotifen fumerate lowers the blood pressure slightly than that of diltiazem when used alone. The same result was obtained when (nifedipine+ketotifen) was administered concurrently. Ketotifen didn't antagonize the antihypertensive activity either of diltiazem or nifedipine because being a nonsteroidal antihistaminic agent ketotifen does not have any effect on the activity of calcium channel blockers (Fig. 2).

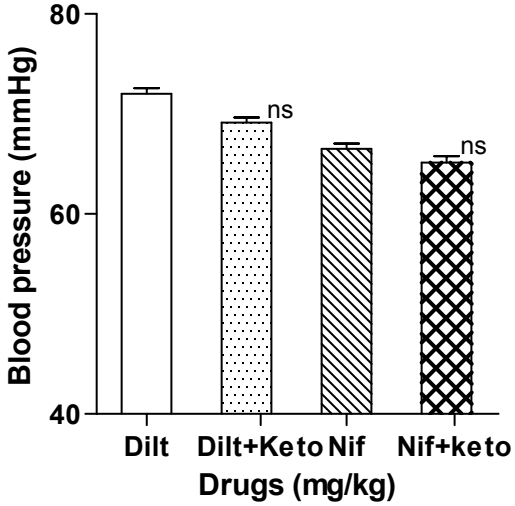

Fig. 2. Comparison of blood pressure in mmHg showed by the animals after the administration diltiazem HCl alone, diltiazem HCl+ketotifen fumerate, nifedipine alone and nifedipine+ketotifen fumerate combination. Data are shown as mean ±SEM, (n=6). ns indicates non-significant change in blood pressure after coadministration of ketotifen fumerate with diltiazem (p=0.1364) and nifedipine (p=0.1572) in comparison to both diltiazem and nifedipine when given as single therapy. Dilt-diltiazem, Nif-nifedipine, Dilt+Keto-diltiazem+ketotifen fumerate, Nif+keto-nifedipine+ ketotifen fumerate.

After coadministration of diltiazem+potassium nitrate the observed blood pressure was found on the average to be 71 mmHg and after coadministration of nifedipine+ potassium nitrate it was observed that potassium nitrate didn't make any significant change on the hypotensive activity when compared to the hypotensive activity of diltiazem or nifedipine alone. These results indicate that coadministration of diltiazem or nifedipine with potassium nitrate will not affect the antihypertensive effect of these two drugs. It has become clear that addition of potassium nitrate salt in presence of calcium channel blockers does not influence the antihypertensive property of this group of drug. Therefore, K^+ ion in blood and in the body fluids will not interfere or antagonize the antihypertensive activity of diltiazem and nifedipine (Fig. 3).

Polypharmacy, that means, 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 coadministration of more than two different classes of drugs may ensue effects that are neither safe nor effective and sometimes may be deleterious. In our *in vitro* study (data not shown in this paper) it was found that diltiazem formed 1:1 complexes with ketotifen fumerate and potassium nitrate 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 our in vivo study in rabbits, it was found that neither ketotifen fumerate nor potassium nitrate had any deleterious effects on the activity of diltiazem. We, thus, conclude that concurrent administration of diltiazem with ketotifen fumerate and potassium nitrate may be safe and effective. Since it has been seen that coadministration of ketotifen

fumerate and potassium nitrate does not interfere much with the antihypertensive properties of either diltiazem or nifedipine, though a very small decrease occurs in blood pressure index of the normal animal in each case. This observation is made also because administration of potassium nitrate becomes sometimes necessary for patients suffering from hypokalemia as well as hypotension and the necessity of ketotifen intake in the presence of antihypertensive drugs may become pertinent in treating patients suffering from allergic conditions.

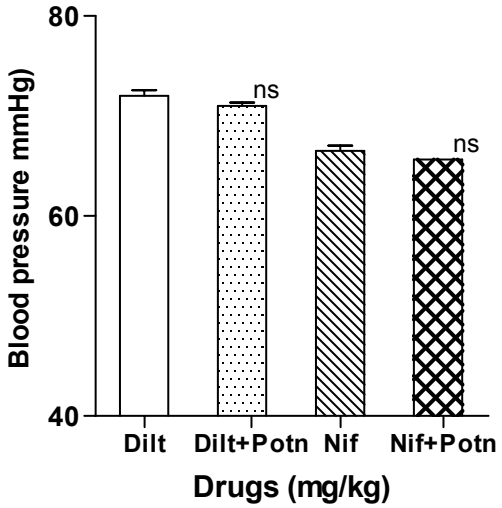

Fig. 3. Comparison of blood pressure in mmHg showed by the animals after the administration diltiazem HCl alone, diltiazem HCl+potassium nitrate, nifedipine alone and nifedipine+potassium nitrate combination. Data are shown as mean ±SEM, (n=6). ns indicates non-significant change in blood pressure after coadministration of potassium nitrate with diltiazem (p= 0.1739) and nifedipine (p= 0.1956) in comparison to both diltiazem and nifedipine when given as single therapy. Dilt-diltiazem, Nif-nifedipine, Dilt+potn-diltiazem+ potassium nitrate, Nif+potn-nifedipine+ potassium nitrate.

It can be inferred that great care and monitoring should be practiced during coadministration of diltiazem with nifedipine. Ketotifen fumerate and potassium nitrate can be coadministered either with diltiazem or nifedipine without fear of any interaction or adverse effects.

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