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# MODIFICATION OF THE ANTIHYPERTENSIVE EFFECT OF INDAPAMIDE BY INDOMETHACIN

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**Abstract :** Oral treatment with indapamide was found to reduce blood pressure of hypertensive rats but not of normotensive rats. Chronic indomethacin treatment had no effect on blood pressure of untreated normotensive and hypertensive rats. Also indomethacin did not modify the antihypertensive effect of indapamide excluding the direct involvement of PGs in the antihypertensive effect of indapamide. Vascular reactivity to pressor agents NA, ADR and ANG was significantly increased after indomethacin treatment. This may be due to the blockade of the actions of PG in modifying vascular reactivity to vasoconstrictor agents or may be a direct effect of indomethacin on calcium fluxes. Indapamide reduced the reactivity to NA and ANG in the presence of indomethacin suggesting that the antihypertensive effect of indapamide may be through a decrease in reactivity to pressor agents which is independent of increase in the synthesis of vasodilator PGs.

**Key words :** hypertensive rats, indapamide, vascular reactivity, indomethacin, prostaglandins, blood pressure

## INTRODUCTION

Indapamide is a new antihypertensive agent which has been shown to exert effective control of hypertension in animals (1) and in man (2). The nature of the antihypertensive effect of indapamide is still debatable. Chemically it is analogous with chlorthalidone (3). Several studies on hypertensive patients have shown indapamide to be unique among diuretics with regards to its ability to induce a significant reduction in arterial pressure at doses which produce little or no diuresis (4, 5). Therefore, it has been hypothesized that the antihypertensive action of indapamide may be due to some alternative mechanism.

Indapamide has been shown to be a potent stimulator of vaso-depressor $PGI_2$ *in vitro* (6, 7). LeBel *et al.* (7) have reported in 11 of 19 patients with essential hypertension that indapamide (2.5 mg daily for 6 weeks) increased urinary $PGE_2$ excretion probably via stimulation of renal production, however there was no correlation between the percentage difference in $PGE_2$ excretion and the mean changes in blood pressure, plasma renin activity or plasma aldosterone concentration.

The present study was undertaken to study the antihypertensive effect of indapamide and modification of the same after chronic treatment with indomethacin (PG antagonist) in DOCA/saline hypertensive rats.

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## METHODS

Male rats (Wistar strain) weighing 200-250 g were made hypertensive by removing the left kidney under ether anaesthesia and by giving DOCA subcutaneously 10 mg/kg weekly for 6 weeks along with water containing 1% NaCl to drink *ad libitum*. Ampicillin (20 mg) was administered im for 5 days. Groups of normotensive and hypertensive rats were treated with the following drugs: indapamide (dissolved in alkaline solution) 10 mg/kg/day orally for 10 days (8), indomethacin 4 mg/kg, ip twice/day for 14 days (9), indomethacin (4 mg/kg twice/day) for 14 days along with indapamide 10 mg/kg for last 10 days.

Blood pressure was recorded 24 hr after the last dose of indapamide and 18 hr after the last dose of indomethacin directly through common carotid artery in pentobarbitone (40 mg/kg) anaesthetized rats by Statham Pressure Transducer (P 23 AA) filled with heparinised saline on calibrated Sanborn Twin-Viso Recorder (160-1300 B). Pressor responses to various doses of NA and adrenaline (ADR) 0.5, 1 and 2 $\mu$g/kg, of ANG (0.025, 0.050 and 0.1 $\mu$g/kg) administered iv were recorded. Pressor responses to the same doses of agonists in normotensive and hypertensive rats were compared before and after chronic treatment with various drugs and increase or decrease in pressor response was considered as change in vascular reactivity.

*Drugs used were :* Indapamide (USV laboratories, New York, USA), indomethacin (Merck Sharpe Dohme, USA), l-adrenaline (Sigma Chemical Co., St. Louis, USA), l-noradrenaline bitartrate (Sigma Chemical Co., St. Louis, USA) and angiotensin (Ciba Geigy, Switzerland).

*Statistical analysis :* Student's 't' test (paired for the same group and unpaired for two different groups) was applied to determine the level of significance. $P<0.05$ was considered as statistically significant.

## RESULTS

*Blood pressure* (Fig. 1): The blood pressure of normotensive rats (136±6 mmHg) was not modified by indapamide treatment (147±5 mmHg) but that of hypertensive rats (177±9 mmHg) was significantly ($P<0.05$) reduced (136±7 mmHg).

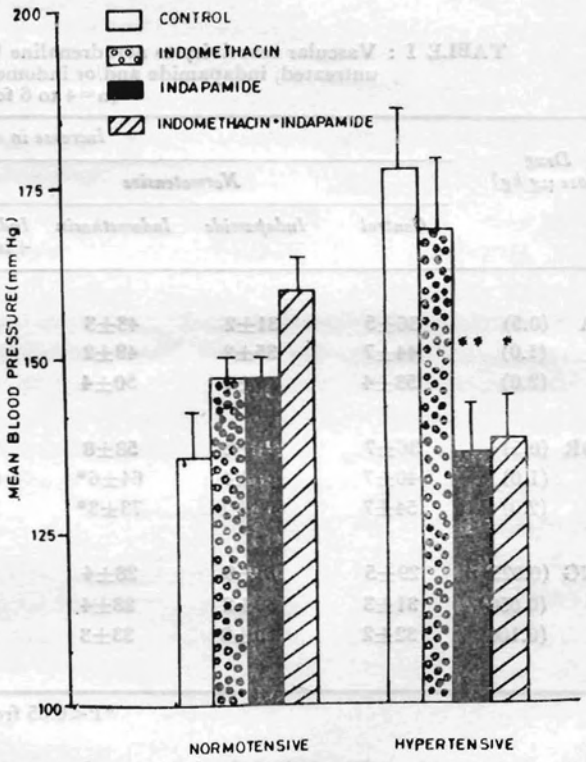


Fig. 1: Effect of oral treatment with indapamide and indomethacin on the blood pressure of normotensive (left hand panel) and hypertensive rats (right hand panel). Vertical lines denote S.E.M. (n=5 to 6 observation). The level of significance is indicated by asterisks. **($P<0.01$) in relation to hypertensive control and *($P<0.05$) in relation to indomethacin treated hypertensive rats.

Chronic indomethacin treatment did not modify the blood pressure of normotensive, indapamide treated normotensive and hypertensive rats. Also indomethacin treatment did not reverse or potentiate the antihypertensive effect of indapamide.

*Vascular reactivity :*

(1) *Indapamide treated animals :* NA, ADR (0.5, 1 and 2 μg/kg) and ANG (0.025, 0.050 and 0.1 μg/kg) produced dose related pressor responses in untreated and indapamide treated normotensive and hypertensive rats. There was no difference in reactivity to pressor agents in these groups of rats (Table-I).

*Reactivity to ANG :* Pressor responses to ANG were not modified after indomethacin treatment in normotensive rats but those in hypertensive rats were potentiated after indomethacin treatment. The potentiating effect of indomethacin was blocked after indapamide treatment (Table-I).

TABLE I : Vascular reactivity to noradrenaline (NA), adrenaline (ADR) and angiotensin (ANG) in untreated, indapamide and/or indomethacin treated normotensive and hypertensive rats. (n=4 to 6 for each observation)

| Drug (dose μg/kg) | Increase in mean blood pressure mmHg±SEM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Normotensive | | | | Hypertensive | | | |
| | Control | Indapamide | Indomethacin | Indomethacin +indapamide | Control | Indapamide | Indomethacin | Indomethacin +indapamide |
| NA (0.5) | 36±5 | 31±2 | 43±3 | 47±4 | 26±1 | 30±1 | 52±8* | 32±6 |
| (1.0) | 44±7 | 35±2 | 49±2 | 60±6 | 35±4 | 31±4 | 67±6* | 43±4 |
| (2.0) | 53±4 | 48±1 | 50±4 | 64±5 | 42±4 | 45±4 | 68±4* | 54±7 |
| ADR (0.5) | 36±7 | 42±2 | 58±8 | 51±7 | 40±5 | 39±3 | 61±8* | 46±5 |
| (1.0) | 40±7 | 54±4 | 64±6* | 60±5* | 44±4 | 48±5 | 68±8* | 64∓2* |
| (2.0) | 54±7 | 66±7 | 73±3* | 75±6* | 63±4 | 51±6 | 78±4* | 78±1* |
| ANG (0.025) | 29±5 | 18±3 | 28±4 | 29±3 | 18±2 | 17±1 | 30±2* | 25±5 |
| (0.050) | 31±3 | 30±2 | 28±4 | 36±5 | 25±2 | 26±3 | 42±6* | 25±6 |
| (0.100) | 32±2 | 35±8 | 33±3 | 41±6 | 31±5 | 37±9 | 50±5* | 35±6 |

*P<0.05 from control

(2) *Indapamide and indomethacin treated rats : Reactivity to NA :* Pressor responses to NA were not modified after indomethacin treatment in normotensive rats but those in hypertensive rats were potentiated after indomethacin treatment. The potentiating effect of indomethacin was blocked after indapamide treatment (Table-I).

*Reactivity to ADR :* Pressor responses to ADR were potentiated after indomethacin treatment in normotensive as well as in hypertensive rats. Indapamide treatment did not modify responses to ADR (Table-I).

## DISCUSSION

In the present study, the antihypertensive action of indapamide was not observed in normotensive rats but the drug was found to reduce the blood pressure of DOCA/saline hypertensive rats suggesting that the drug is only effective in hypertension. The present results are in agreement with those of Finch *et al* (8), Kyncl *et al* (1) and Moore *et al* (10).

Indapamide being a diuretic might lower the blood pressure through increase in the synthesis of vasodilator PG as reported for furosemide (11) and

for thiazides (12). In the present study, indomethacin in doses which block cyclooxygenase did not modify the blood pressure of untreated, normotensive and hypertensive rats. Puddey *et al* (13) reported that PG synthesis inhibition with NSAI drugs increases blood pressure in untreated essential hypertensive and normotensive subjects although these findings are not uniform. The pressor effect of indomethacin was maximal at day 7 of therapy with return towards baseline values by the end of treatment. This may reflect a compensatory activation of depressor mechanisms to counteract a sustained increase in blood pressure and might explain no change in blood pressure after indomethacin treatment in our study.

Also indomethacin treatment did not abrogate the antihypertensive effect of indapamide suggesting that PGs are not directly involved in the antihypertensive action of indapamide.

In the present study, pressor responses to different vasoconstrictor agents were not affected by indapamide treatment in normotensive or hypertensive rats while Finch *et al* (8) have reported reduced reactivity to NA and TYR after indapamide treatment in hypertensive pithed rats. We could observe such effect after indapamide treatment only in indomethacin treated hypertensive rats, possibly because reflex homeostatic mechanism and PGs (mainly vasodilator) synthesized continuously in cardiovascular tissues in response to high blood pressure and increased sympathetic activity may modify response to pressor agents in this form of hypertensive model. De Champlain *et al* (14) reported that activation of the sympathetic system is primary rather than secondary to elevation of blood pressure in DOCA/saline hypertensive rat model as observed by biochemical evidences. Also Nasjlett *et al* (15) have reported increased $PGE_2$ excretion in DOCA/saline hypertensive rats. In SHR enhanced formation of $PGI_2$ by aortic wall has been observed by several researchers (16, 17).

To exclude the role af PGs, pressor reactivity to vasoconstrictor agents was studied after indomethacin treatment. Indomethacin treatment poteniated pressor responses to ADR in normotensive and to NA, ADR and ANG in hypertesive rats. Similar results were observed by others. Ferreira *et al* (18) showed that administration of inhibitor of PG synthesis, indomethacin caused augmentation of responses to NA, ANG II and nerve stimulation. Zimmerman *et al* (19) reported that inhibition of PG synthesis with indomethacin potentiated responses of various vascular beds to adrenergic nerve stimulation and/or to administered catecholamines.

Puddey *et al* (13) have reported that indomethacin in addition to blockade of PGs also affects directly sodium and calcium transport in vascular smooth muscle which has been implicated in the potentiation of vascular smooth muscle responses to vasoconstrictor agents.

Lack of potentiation of pressor responses to NA and ANG after combined treatment with indomethacin and indapamide suggests that indapamide acts by decreasing reactivity to NA and ANG, and decreases blood pressure and this effect is independent of PG synthesis as indomethacin did not modify this effect. Decrease in reactivity to pressor agents by indapamide may be due to action of indapamide on calcium fluxes (20, 21, 22, 23).

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