Differential Effects of Antihypertensive Agents on Proliferation of Vascular Smooth Muscle Cells from Spontaneously Hypertensive Rats

Satoru KURIYAMA, M.D., Koji NAKAMURA, M.D., Yoshihiko KAGUCHI, M.D., Haruo TOMONARI, M.D., Goro TOKUTOME, M.D., Takao HASHIMOTO, M.D., and Osamu SAKAI, M.D.

SUMMARY

We have previously shown that Ca-antagonists and α-blockers substantially inhibit the cellular proliferation of cultured rat vascular smooth muscle cells (VSMC). This study explored whether these inhibitory effects on cellular proliferation differ between cultured VSMC from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY).

SHR VSMC proliferated much faster than WKY VSMC in 10% FCS. Cellular proliferation, determined by both cell number count and 3H-thymidine incorporation, was significantly blunted in the presence of either nifedipine (Nif) or bunazosin (Bun). The magnitude of these inhibitory effects was more pronounced for SHR cells than WKY cells (% reduction of 3H-thymidine uptake with Nif: 62.1±7.8% for SHR vs 75.3±10.2% for WKY, n=6, p<0.05, and with Bun: 70.2±7.8% for SHR vs 82.1±9.9% for WKY, n=6, p<0.05). In contrast, the intracellular water volume was unaffected by these antihypertensive agents based on equilibrium distribution of 3-O-methyl-D-glucose14C.

It is concluded that SHR VSMC grow much faster than WKY VSMC and that this abnormality is innate to the SHR cells. It is also concluded that both Ca-antagonists and α-blockers exerted a substantial inhibitory effect on cellular proliferation of the cultured VSMC of either SHR or WKY. Furthermore, the greater inhibition of proliferation in the SHR VSMC suggests that Ca mediated- and/or α-receptor mediated processes of cellular proliferation of SHR could differ from that of WKY and that these abnormalities may contribute to the hyperproliferative changes of VSMC in this model.

Key Words:
Nifedipine  Bunazosin  Vascular smooth muscle cells  SHR

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From the Second Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan.

Correspondence to: Satoru Kuriyama, M.D., Second Department of Internal Medicine, Jikei University School of Medicine, 3-25-8, Nishi-shinbashii, Minato-ku, Tokyo 105, Japan.

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INCREASED peripheral vascular resistance is a common characteristic of patients with hypertension. Hyperproliferative changes of vascular smooth muscle cells (VSMC) of spontaneously hypertensive rats (SHR) has been postulated by many investigators as an explanation for abnormal increased vascular resistance in this animal model. The increase in wall thickness is due to greater vascular smooth muscle cell mass and may be accounted for by cellular hypertrophy or proliferation. In either instance, abnormal cellular proliferation may be a factor in hypertension.

We have previously shown that proliferation of VSMC is inhibited by both Ca-antagonists and alpha-blockers. These two drugs are widely used in the treatment of hypertension. Unlike other vasodilators, Ca-antagonists decrease blood pressure in hypertensive much more than in normotensive rats and humans. It is intriguing to explore the potential of differential responses to antihypertensive agents in VSMC of hypertensive humans or experimental models.

On the one hand, cultured cells are a useful tool to explore the direct effect of pharmacological agents on cellular proliferation, because they are isolated from mechanical and neurohumoral influences.

This study assessed the proliferation of cultured VSMC from SHR and WKY in response to antihypertensive agents. These experiments indicated that VSMC from SHR proliferate more rapidly than VSMC from WKY and that the proliferation was inhibited by the antihypertensive agents. It is suggested that an increased proliferation capacity of VSMC contributes to hypertension in SHR.

**Materials and Methods**

Cultured VSMC were derived from thoracic arteries of 12-week-old male SHR and WKY. The respective systolic blood pressure values of SHR and WKY were 170±20 mmHg (n=6) and 118±15 mmHg (n=6). The methods of tissue processing, enrichment, propagation and degree of purity have been published previously.

For the cell number determination, aliquots of 1×10^6 cells/ml were inoculated into each well of Nunc-12-Well clusters (Passage 6–9) in the presence and absence (control) of bunazosin (Bun) or nifedipine (Nif) at 37°C in 5% CO₂ and 95% air. Preliminary experiments showed that the effect of either Nif or Bun was in a dose dependent fashion in the range of 0.1 to 100 µM thus, 100 µM of the agent was used. Cultures were maintained in Dulbecco’s modified Eagle Medium (DMEM, Na 145 mEq/L and K 5 mEq/L, Gibco Oriental, Tokyo, Japan) with 10% fetal calf serum.
(FCS) (Gibco Oriental). On the day of the experiments, the growth medium was aspirated and the cells were washed twice with DMEM. Thereafter, 0.25% of trypsin was added to the wells for 30 min. The cells were suspended in isotonate and subsequently the cells were counted with a coulter counter.

Three days prior to ³H-thymidine incorporation experiments, an aliquot of 1x10⁵ cells/ml was inoculated into each well without the probes. On the day of the experiment, the growth medium was removed and the cells were placed in the same wells in quiescence for 24-48 hrs. Thereafter, 1 μCi/ml of ³H-thymidine with or without the agents was added to the wells for 24 hr. The cells were rapidly washed 5 times with ice-cold DMEM. They were then extracted with 1N-NaOH for 20 min. The incorporation of the tracer was measured with a scintillation counter.

The intracellular water volume (ICWV) was obtained from the equilibrium distribution of 3-O-methyl-D-glucose¹⁴C following a 120 min incubation with 10 μCi/ml of the radioactive tracer and rapid washing (4 times) with ice-cold MgCl₂. The probes were included in the medium. Preliminary experiments showed that the radioactivity in the cells reached steady state within the indicated incubation period. The cells were extracted with 5% trichloracetic acid, and the radioactivity was assessed with a beta counter. The ICWV was calculated based on the ratio of the radioactivity between the medium and inside the cells.

³H-thymidine and 3-O-methyl-D-glucose¹⁴C were purchased from New England Nuclear. Nifedipine was donated by Bayer Pharmaceutical Co. Ltd., Japan and bunazosin by Eisai Pharmaceutical Co. Ltd., Japan.

The statistical method employed was the unpaired t-test. Data are presented as mean±SD, unless otherwise indicated.

**Results**

Figures 1 and 2 illustrate the effects of 100 μM Nif and 100 μM Bun on the number of cultured VSMC. In the absence of either agent, the VSMC number was greater in SHR than that in WKY (5.0±0.6 for WKY VSMC vs 7.1±0.6 (x10⁵ cells/well) for SHR VSMC, n=8, p<0.01 in Fig. 1), indicating an innately greater proliferative capacity of SHR VSMC cells. In addition, Nif substantially reduced the cell number of either WKY VSMC (5.0±0.6 for control (C) vs 2.2±0.5 (x10⁵ cells/well) for Nif, n=8, p<0.01) or SHR VSMC (7.1±0.6 for C vs 1.8±0.4 (x10⁵ cells/well) for Nif, n=8, p<0.01). The same holds true with Bun for either WKY VSMC (5.5±0.6 for C vs 4.5±0.5 (x10⁵ cells/well) for Bun, n=8, p<0.05) or SHR VSMC
Fig. 1. The inhibitory effects of nifedipine on the proliferation of cultured VSMC from SHR and WKY.

a: \( p < 0.01 \) compared to the WKY VSMC control (C).

*: \( p < 0.01 \) compared to corresponding C.

Fig. 2. The inhibitory effects of bunazosin on the proliferation of cultured VSMC from SHR and WKY.

a: \( p < 0.01 \) compared to the WKY VSMC control (C).

*: \( p < 0.05 \) compared to corresponding C.

**: \( p < 0.01 \) compared to corresponding C.

Table I. 3H-Thymidine Incorporation of the Cultured VSMC from SHR and WKY in Response to Either Nifedipine or Bunazosin

<table>
<thead>
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<th>3H-Thymidine uptake (%)</th>
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<tbody>
<tr>
<td>Nif (100μM)</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>62.1±7.8*(^{(n=6)})</td>
</tr>
<tr>
<td>WKY</td>
<td>75.3±10.2(^{(n=6)})</td>
</tr>
<tr>
<td>Bun (100μM)</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>70.2±7.8*(^{(n=6)})</td>
</tr>
<tr>
<td>WKY</td>
<td>82.1±9.9(^{(n=6)})</td>
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*: \( p < 0.05 \) compared to WKY VSMC

(6.9±0.5 for C vs 4.6±0.5 \( \times 10^5 \) cells/well) for Bun, \( n = 8, p < 0.01 \). Table I shows the effect of the two antihypertensive agents on 3H-thymidine incorporation in cultured VSMC from SHR and WKY. The
Table II. Intracellular Water Volume Determination Based on 3-O-methyl-D-glucose $^{14}$C in the Presence of Nifedipine or Bunazosin in Cultured VSMC from SHR and WKY

<table>
<thead>
<tr>
<th>Nif (100$\mu$M)</th>
<th>Intracellular water volume ($\mu l/10^5$cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>1.5 ± 0.4 (n=6) n.s</td>
</tr>
<tr>
<td>WKY</td>
<td>1.7 ± 0.5 (n=6) n.s</td>
</tr>
<tr>
<td>Bun (100$\mu$M)</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>1.6 ± 0.6 (n=6) n.s</td>
</tr>
<tr>
<td>WKY</td>
<td>1.7 ± 0.8 (n=6) n.s</td>
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</tbody>
</table>

*p < 0.05 compared to the values from WKY VSMC.

uptake of the tracer was reduced substantially by either Nif or Bun.

Basal ICWV values were 1.6 ± 0.7 for SHR VSMC and 1.7 ± 0.9 (μl/10^5 cells) for WKY VSMC. Table II depicts ICWV in the presence of the probes. Neither nifedipine nor bunazosin exerted any effect on ICWV of either SHR VSMC or WKY VSMC. There were no differences between the two strains.

**DISCUSSION**

The present study demonstrated that SHR VSMC proliferate more rapidly than WKY VSMC in vitro. Since these results were obtained from cultured VSMC, they indicate an innate increased proliferative capacity of SHR cells. A growing body of evidence has suggested that VSMC can display an uncontrolled proliferation in hypertension.1)–3) Thus, extrapolation of these results in vivo may imply a linkage between VSMC proliferation, hypertension and atherosclerosis.

We have previously shown that Ca antagonists and α-blockers inhibit the proliferation of VSMC derived from a normotensive rat strain.4) Additionally, it has been shown that altered utilization of Ca may be a significant underlying factor in vascular reactivity changes in SHR.5)–9) From a therapeutic point of view, then, it is of particular interest to explore the differential effects of antihypertensive agents on cell proliferation and blood pressure.

The central finding in this study is a pronounced inhibitory effect of antihypertensive agents on cellular proliferation of VSMC from SHR compared to that from WKY. The mechanism by which Ca-antagonists exert their inhibitory effect on cellular proliferation has been investigated in conjunction with intracellular Ca and Na-H antiport.10) It has been postulated
that intracellular Ca is a key factor in initiating intracellular alkalinization by activating the Na-H antiport.\textsuperscript{11}) In this context, it is rational to speculate that a reduction of intracellular Ca mobilization by Ca-antagonists causes a reduction of cell number and DNA synthesis. Of further interest is the evidence that Na-H antiport activity in SHR cells is higher than that in WKY.\textsuperscript{12–14}) This increase in the activity of Na-H antiport in SHR cells may account for both the increased growth activity of the cells and the augmented response to Ca-antagonists in SHR VSMC. Furthermore, if the present results may be extrapolated to the small resistance vessels, it would explain the higher efficacy of Ca antagonists in reducing blood pressure.\textsuperscript{5,6)}

The basis for the antiproliferative effect of bunazosin is unknown. We are unaware of any in vitro experiments showing an inhibition of cellular proliferation by \( \alpha \)-blockers in VSMC except for our previous one,\textsuperscript{4}) although possible involvement of \( \alpha \)-receptor mediated mechanisms in the process of cellular proliferation has been demonstrated in heart muscle.\textsuperscript{15–17}) However, since \( \alpha \)-receptor blocking action is not specific for bunazosin, one should consider mechanisms other than an \( \alpha \)-receptor blocking action as being involved in bunazosin-sensitive inhibition of VSMC growth.

It is noteworthy that after the treatments with Nif and Bun, the cell number was reduced to almost the same point as controls (in the absence of drugs). This implies that these drugs ameliorate abnormal growth of SHR VSMC and blunted hyperreactivity of SHR cells. In other words, it may possibly suggest that these specific drugs have potential benefit for the prevention of abnormal cellular proliferation in VSMC of patients with hypertension.

The fact that ICWV was not affected by the two agents does not necessarily imply that these drugs reduced the cell number (determined by cell number count) but not cell size (determined by ICWV). We feel that it is still too early to conclude that cellular hypertrophy is not influenced by these two agents.

Acknowledgments

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References