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**THE ANTI-TUMOR AGENT, TAXOL, ATTENUATES CONTRACTILE  
ACTIVITY IN RAT AORTIC SMOOTH MUSCLE**

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**Abstract:** Using 16-20 week old female spontaneously hypertensive rats (SHRs), the effects of the antitumor agent, taxol, on vascular reactivity were examined. Taxol significantly inhibited contraction induced by phenylephrine, angiotensin II, phorbol 12,13-dibutyrate and increasing concentrations of calcium. The data suggest that taxol does not augment hypersensitivity of the vascular, but instead attenuates contractile activity and may have important implications with respect to treatment of women with both cancer and cardiovascular disease.

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*Key Words:* taxol, vascular smooth muscle, anti-cancer agents, hypertension, muscle

### Introduction

The dramatic increase in the incidence of breast cancer has brought concern among health care professionals as well as the general public. An intense interest in taxol has evolved due to its antitumor activity in ovarian and breast carcinomas and malignant melanomas (reviewed in 1). Taxol is a diterpene extracted from the bark of the Western Yew, *Taxus brevifolia*. Its antitumor activity is due to an inhibition of G2 phase of the cell cycle. More specifically, taxol induces reorganization of the microtubule cytoskeleton resulting in nonfunctional microtubules, a loss of mitotic spindle polarity and chromosome breakage (2).

Microtubules are involved in a number of cellular activities, including mitosis, cell integrity, motility, transport, modulation of growth factor receptors and subsequent cell signaling (reviewed in 3). Any disruption of microtubule equilibrium results in disruption of mitosis and related activities. Because of its unique mode of action and cytotoxicity, taxol is a prototype for a new class of chemotherapeutic agents. Toxic or side effects of taxol include hematopoietic, lymphatic, gastrointestinal and reproductive disturbances (3), neuropathy, alopecia, hypotension and hypertension (4).

Since the incidence of both breast cancer and cardiovascular disease is increasing in women, it would be of great value to examine the effects of this chemotherapeutic agent on the cardiovascular system so that treatment of one disease will not exacerbate symptoms of the other. This study examined the *in vitro* effects of taxol on vascular reactivity in female spontaneously hypertensive rats (SHR), the most thoroughly studied model of human essential hypertension.

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## **Materials and Methods**

### **Tissue preparation**

Sixteen to twenty week old female SHR's were used in this study. Blood pressures were taken using the tail cuff method. Rats were anesthetized with an i.p. injection of sodium pentobarbital (100 mg/kg body weight) and exsanguinated. Thoracic aortae were removed and bathed in room temperature Krebs' buffer ([mM]: EDTA, 0.027; NaCl, 136; KCl, 5.6; NaHCO<sub>3</sub>, 20; MgSO<sub>4</sub>-H<sub>2</sub>O, 1.2; Na<sub>2</sub>PO<sub>4</sub>, 1.2 and glucose, 5.0) containing 1.5 mM CaCl<sub>2</sub> and aerated with 95% O<sub>2</sub>/5%CO<sub>2</sub>.

After removing extraneous fascia and endothelium, helical strips measuring 3 to 4 mm by 12 to 15 mm were cut and mounted in water-jacketed, tissue chambers containing Krebs' plus 1.5 mM CaCl<sub>2</sub> maintained at 37° C and aerated with 95% O<sub>2</sub>/5%CO<sub>2</sub>. Tissues were mounted isometrically to Grass FT03 force displacement transducers and were left to equilibrate for 1.5 to 2 hrs. with a 1,500 mg preload, which had been previously determined to allow for optimal contractile activity (5). Solutions in each bath were changed every 20 min.

### **Pharmacodynamic studies**

For concentration-response curves, tissues were contracted with cumulative concentrations of either phenylephrine (PE) (10<sup>-10</sup>-10<sup>-5</sup>M), angiotensin II (10<sup>-10</sup>-10<sup>-6</sup>M) or phorbol 12,13-dibutyrate (PDBu) (10<sup>-9</sup>-10<sup>-5</sup>M). Each concentration was added when the prior concentration produced a steady-state or plateau response. Tissues were then rinsed several times, incubated for 10 min. with taxol (1 uM) or vehicle control (0.1% DMSO) and the contractile response to each concentration of drug repeated. We have previously shown that DMSO at this concentration has no significant effects on contractile activity (6). Periodically, tissues were rinsed again several times and rechallenged with the original contractile agent in the absence of taxol to ensure that inhibition of contractile activity by taxol was not merely due to a loss of tissue reactivity over time or to receptor down-regulation. Contractions were calculated as percentage of maximal mg of tension produced by each specific agent.

### **Statistics**

Data are expressed as the mean ± SEM. Data were normalized by converting mg of tension to a percentage of maximal contraction for each strip. The EC<sub>50</sub> was calculated for each experiment and is expressed as the mean ± SEM (7). Statistical significance between EC<sub>50</sub>s was established using a Student's *t*-test with a *p* value of < 0.05 considered to be significant.

### **Materials**

Female 16 to 20 week old SHR's were purchased from Taconic Farms (Germantown, NY). Taxol and all other drugs and reagents were purchased from Sigma Chemicals (St. Louis, MO).

## **Results**

In Figures 1 through 3 are concentration-response curves to PE, angiotensin II and PDBu in the absence and presence of 1 uM taxol in rat aorta. This concentration is within the range of EC<sub>50</sub>s reported for taxol (0.1-10 uM) in *in vitro* studies (3). For each agent used, taxol caused a significant right shift of the EC<sub>50</sub>. The EC<sub>50</sub>s for PE,

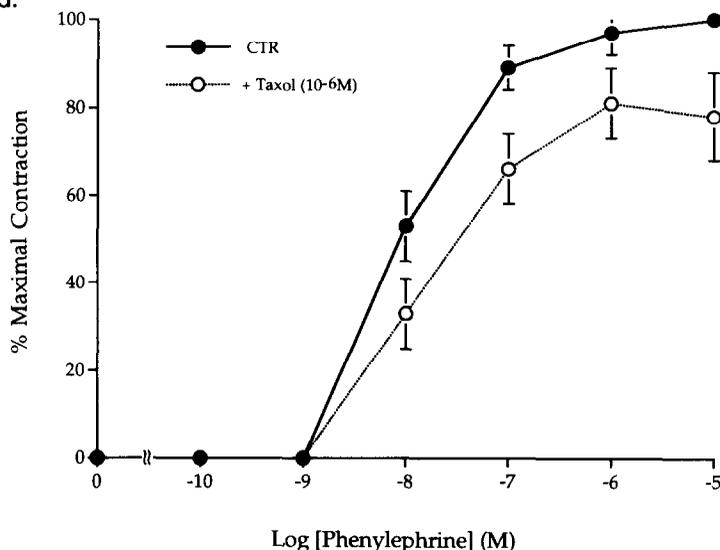
angiotensin II, PDBu and increasing concentrations of calcium in the presence of  $10^{-5}$  M PE (graph not shown) were significantly different in the absence and presence of taxol (Table 1).

**TABLE I**

**EC<sub>50</sub>s for Various Contractile Agents in the Absence and Presence of 1  $\mu$ M Taxol.**

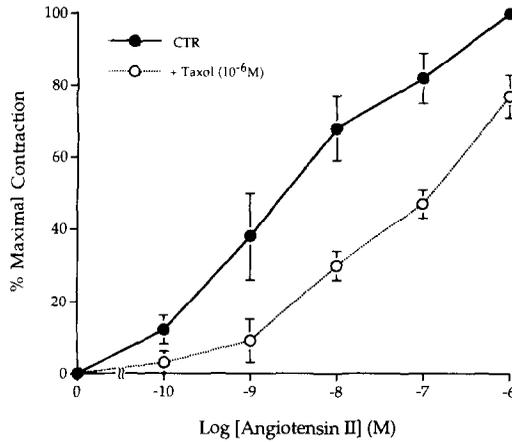
	Control	+ Taxol (1 $\mu$ M)
	EC <sub>50</sub>	
PE (M)	$1.11 \times 10^{-8} \pm 4.74 \times 10^{-9}$	$6.80 \times 10^{-8} \pm 5.79 \times 10^{-9} *$
Ang. II (M)	$9.46 \times 10^{-9} \pm 4.73 \times 10^{-9}$	$1.84 \times 10^{-7} \pm 5.39 \times 10^{-8} *$
PDBu (M)	$2.26 \times 10^{-7} \pm 2.55 \times 10^{-8}$	$1.76 \times 10^{-6} \pm 7.78 \times 10^{-7} *$
CaCl <sub>2</sub> (mM)	$0.61 \pm 0.11$	$0.98 \pm 0.03 *$

EC<sub>50</sub>s were calculated for each separate experiment and are expressed as the mean concentration  $\pm$  SEM for 3 - 5 experiments. Asterisks denote a statistically significant difference between vehicle control and taxol-treated strips ( $p < 0.05$ ) for all agents used.



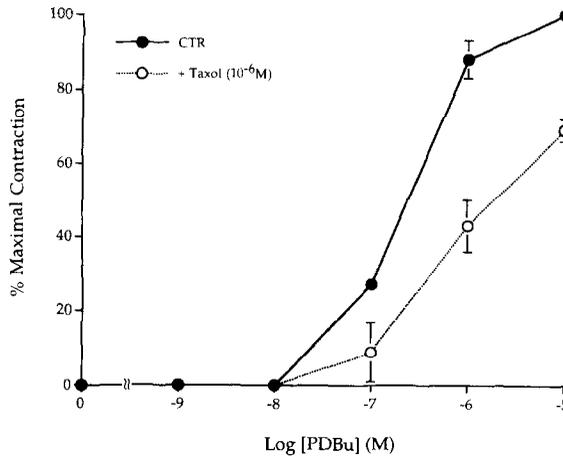
**Fig. 1**

**Effects of Taxol on Phenylephrine-Induced Contractile Activity in SHR Aorta.** Results are expressed as mean  $\pm$  SEM from 3 - 5 experiments. There was a significant shift of the EC<sub>50</sub> in the presence of taxol ( $p < 0.05$ ).



**Fig. 2**

**Effects of Taxol on Angiotensin II-Induced Contractile Activity in SHR Aorta.** Results are expressed as mean  $\pm$  SEM from 3 - 5 experiments. There was a significant shift of the EC<sub>50</sub> in the presence of taxol ( $p < 0.05$ ).



**Fig. 3**

**Effects of Taxol on Phorbol 12,13-Dibutyrate-Induced Contractile Activity in SHR Aorta.** Results are expressed as mean  $\pm$  SEM from 3 - 5 experiments. There was a significant shift of the EC<sub>50</sub> in the presence of taxol ( $p < 0.05$ ).

**Discussion**

It is not entirely surprising that an agent such as taxol, which has effects on cell motility, may also affect contractility. Others have found that taxol inhibited both velocity and extent of sarcomere shortening in isolated cat cardiocytes (8). It has also been reported that many contractile agents are also mitogenic and many mitogens and growth factors cause smooth muscle contraction, suggesting a common pathway for hyperplasia, growth and contraction (9,10). This is the first report to our knowledge, illustrating the effects of taxol on smooth muscle function.

PE is an alpha<sub>1</sub>-adrenoceptor agonist which causes vascular smooth muscle contraction through calcium mobilization and activation of phospholipase C (PLC) and

consequently, protein kinase C (PKC). Phorbol 12,13-dibutyrate (PDBu) is a tumor promoter which contracts smooth muscle through bypassing PLC and directly activating PKC. PKC participates in the maintenance phase of contraction through phosphorylation of smooth muscle contractile proteins. Taxol was able to inhibit contraction induced by both of these agents, suggesting that it may operate in part, through inhibition of PKC or calcium mobilization. While there are no studies on the direct effects of taxol on PKC, others have found that taxol and other agents which act on the cytoskeleton altered calcium channel activity and therefore calcium mobilization in neuronal tissue (11).

Angiotensin II is a polypeptide hormone which raises blood pressure through a multitude of mechanisms including vasoconstriction. It is thought to operate through PKC and also protein tyrosine kinases (PTKs), which have also been shown to be involved in both VSM proliferation and contraction (6,12). Taxol was able to inhibit angiotensin-induced contraction, implicating PTKs as another possible intracellular signaling pathway for taxol. However, activity studies for both PKC and PTKs would be needed to substantiate these findings.

The finding that taxol has direct effects on the vasculature has important implications for women on taxol therapy who are at risk for cardiovascular disease. Since 1950, the incidence of cardiovascular disease has increased in women, but decreased in men (13). And since the early 1900s, cardiovascular disease has been a leading cause of mortality in women (13), although this fact has been largely ignored. If taxol is to be a viable alternative for at-risk women, then it is imperative that it not exacerbate such conditions as hypertension, angina pectoris, myocardial infarctions, atherosclerosis and stroke. It should also be noted that a number of malignancies such as malignant melanomas and lung carcinomas which also afflict males, are also responsive to taxol. While the focus of this particular study was limited to effects in females, for completeness, we should mention that we have seen the same results using male SHR (data not shown). Our data indicate that taxol does not induce hyperresponsiveness to contractile agents *in vitro*. Examining the mechanism by which this new class of chemotherapeutics operates will certainly warrant further study.

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