DIFFERENTIAL EFFECTS OF PACLITAXEL AND DERIVATIVES ON GUINEA PIG ISOLATED HEART AND PAPILLARY MUSCLE

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ABSTRACT

Pacitaxel (Taxol) is an anticancer agent with clinical activity against various human cancer types. Pacitaxel blocks cell division by stabilizing microtubules, a mechanism that also underlies its major side effects (neutropenia and neurotoxicity). Pacitaxel can also alter cardiac function, and to elucidate the mechanism of this activity, we tested the mechanical and electrical effects of pacitaxel and a series of analogs (docetaxel, taxol B, taxol C and N-methyltaxol C; 5–20 μM) on two different cardiac preparations, the isolated coronary perfused heart and the papillary muscle of the guinea pig. Pacitaxel and N-methyltaxol C induced conduction arrhythmias and reduced coronary flow and left ventricular systolic pressure in the isolated heart, whereas the other taxol derivatives tested had no significant effect. Moreover, paclitaxel blocked the vasodilator effect of bradykinin in the isolated heart. Paclitaxel and N-methyltaxol C produced a positive inotropic effect in papillary muscle without alterations in the action potential. In the latter preparation, no significant variations were observed after treatment with the other taxol derivatives. The in vitro cardiodepressant and arrhythmogenic activity of paclitaxel is similar to that reported after its clinical administration and might be due to coronary vasoconstriction. The precise role of microtubules as modulators of intracellular calcium in cardiac and smooth muscle cells is at present unclear, because docetaxel and other taxol analogs, though they exhibited similar activity on tubulin, lacked cardiac effects.

Taxols are N-acyl(N-alkyl)phenylisoserine esters of bacca
tin III-type taxane diterpenoids, and more than 20 compounds of this class have been isolated from various species and cultivars of the yew tree (Appendino, 1995a, 1995b). The most important taxol is taxol A (paclitaxel), a drug commercialized for the treatment of breast and ovarian cancer under the trade name of Taxol. Paclitaxel is considered the most effective anticancer agent discovered in the last decade, and it is active against a wide range of solid tumors. Even so, it is not an optimal chemotherapeutic agent; its clinical side effects are severe. P-glycoprotein-mediated resistance can occur rapidly, and P-glycoprotein-mediated resistance is directly related to its cytotoxicity or to the disruption of tubulin-mediated transport (Spencer and Faulds, 1994). However, the mechanisms underlying other adverse reactions remain elusive and may not be linked to cytotoxicity. The administration of paclitaxel is accompanied by a high incidence (30–40%) of cardiac brady- and tachyarythmias, and more serious complications, including myocardial ischemia and infarction, have also been reported (Rowinsky et al., 1991; Spencer and Faulds, 1994). This is hardly surprising, because many taxoids are powerful heart poisons (Alloatti et al., 1996). However, at least for

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ABBREVIATIONS: LVSP, left ventricular systolic pressure; LVDP, left ventricular diastolic pressure; DMSO, dimethylsulfoxide; CF, coronary flow; HR, sinus heart rate; A-V, atrioventricular conduction time; Q-T, Q-T interval; QRS, duration of QRS complex; T max, peak tension; +dT/dt, maximal rate of increase of developed tension; −dT/dt, maximal rate of decrease of developed tension; VPB, ventricular premature beat; VT, ventricular tachycardia; AVR, accelerated ventricular rhythm.

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taxine-type alkaloids, this activity is not linked to cytotoxicity (Appendino, 1996) and probably involves interaction with a different target (voltage-gated ion channels) (Alloatti et al., 1996). A better understanding of the mechanism of paclitaxel cardiotoxicity might have important clinical relevance, paving the way to the development of noncardiotoxic analogs.

As part of an investigation on the cardiac toxicity of taxoids, we have compared the in vitro cardiotoxicity of paclitaxel, of its three most important “natural” analogs (taxol B, taxol C and N-methyltaxol C) (Appendino, 1995a; 1995b) and of the clinically useful semisynthetic analog docetaxel. This study was further prompted by the discovery that subclinical doses of paclitaxel can prevent restenosis after angioplasty via microtubule-mediated inhibition of neointimal fibroproliferation (Shaw et al., 1995; Sollott et al., 1995). Noncardiotoxic analogs of paclitaxel that retain tubulin activity would obviously be better drugs in this clinical context.

Materials and Methods

The effects of paclitaxel and derivatives were tested on two different cardiac preparations: 1) the isolated, self-paced Langendorff heart and 2) the isolated papillary muscle electrically driven at constant frequency.

Experiments were performed as detailed previously (Alloatti et al., 1996). Adult guinea pigs were anesthetized with ether and killed by stunning and cervical dislocation, and the heart was explanted. Isolated hearts were perfused at constant pressure (80 cm H2O) with Tyrode’s solution (composition, in mM: 154 NaCl, 4 KCl, 2 CaCl2, 1 MgCl2, 5.5 glucose, 5 HEPES; pH adjusted to 7.35 with NaOH and solution. The range of concentrations used (5, 10 and 20 m), they were dissolved in DMSO and then added to the Tyrode’s solution (Milan, Italy). Because of the low water solubility of these compounds, they were isolated or synthesized by Indena S.p.A. Research Laboratories. CF was measured by means of an electronic drop counter.

Isolated hearts were perfused at constant pressure (80 cm H2O) with Tyrode’s solution containing paclitaxel (from 5 to 20 µM) in vitro. The effects of paclitaxel and derivatives were tested on two different cardiac preparations: 1) the isolated, self-paced Langendorff heart and 2) the isolated papillary muscle electrically driven at constant frequency.

Papillary muscles were perfused at 37°C with Tyrode’s solution and electrically driven at a rate of 120/min. Isometric twitches and derivatives were tested after a period of equilibration (30–40 min); each treatment lasted about 20 min, and then the perfusion was switched to control Tyrode’s solution. As a control, five isolated hearts and five papillary muscles were treated with 1 µM/ml DMSO (the concentration of DMSO used as vehicle) for 20 min.

In preliminary experiments, mechanical and electrical activities of the isolated heart and papillary muscle were stable for more than 1 h after the initial equilibration period. To study the electromechanical effects of partial ischemia, we perfused five isolated hearts at constant flow with a peristaltic pump for 40 min and then reduced the flow rate to 60% of the control value for 30 min.

In additional experiments, the vasodilator effect induced by bradykinin (100 nM, in the presence of 1 µM/ml DMSO, for 20 min) in isolated, control hearts was compared with that obtained in hearts perfused with paclitaxel or docetaxel. Isolated hearts were exposed to paclitaxel (5 µM) or docetaxel (5 µM) for 30 min before and during the challenge with bradykinin; the same hearts, after a 60-min perfusion with control, drug-free Tyrode’s solution, were stimulated again with bradykinin to see whether the effects of paclitaxel and docetaxel were reversible.

The data are expressed as the mean ± S.E. The experimental groups were compared using one-way analysis of variance. If a significant F resulted from the analysis of variance (P < .05), then the Newman-Keuls’ multiple range test was applied to determine where differences were located among the groups.

Results

Isolated heart. Perfusion of the isolated heart with Tyrode’s solution containing paclitaxel (from 5 to 20 µM) induced different, concentration-related responses. Whereas paclitaxel had no significant effect on cardiac function at the lowest concentration of 5 µM, higher concentrations (10 and 20 µM) induced a biphasic response characterized by a transient initial increase in LVSP, which occurred between 0 and 2 min, and reached 115.0 ± 2.1% of the control value at 20 µM, followed by a concentration-related reduction of LVSP and CF (figs. 2 and 3). The reduction of LVSP and CF, which was already present after the infusion of 10 µM paclitaxel and became highly significant at 20 µM, started at 2 min, declined over 8 to 10 min, and was accompanied by a marked increase in LVPD (194.8 ± 25.3% of the control value at 20 µM). Concomitantly with the reduction in contractility, paclitaxel induced conduction arrhythmias, which included VBP, both isolated and in the presence of bigeminy, AVR and VT. The incidence and duration of these alterations were related to the concentration of the drug (table 1). Short-lasting (<10 s), sporadic atrioventricular blocks were observed in paclitaxel-treated hearts (½ and ¾ at 10 and 20 µM, respectively). Paclitaxel, however, did not significantly affect HR, A-V, Q-T or duration of QRS (fig. 2; table 2). Among the taxol derivatives tested, only N-methyltaxol C showed significant effects on myocardial performance at the highest concentration used. This compound induced concentration-related reductions in LVSP and CF (fig. 2) that were
Effects of paclitaxel and docetaxel on bradykinin-induced vasodilatation in isolated heart. It has been suggested that paclitaxel might reduce coronary blood flow by inhibiting the response of endothelial cells to bradykinin (Hamm-Alvarez et al., 1994). We have performed experiments in order to study the interaction between paclitaxel and bradykinin in the modulation of the isolated heart. Further experiments were performed using the paclitaxel derivative docetaxel, which had no coronary vasoconstrictor effect even at the highest concentration used (20 μM). Perfusion of isolated hearts with Tyrode's solution containing 1 μM bradykinin induced a prompt, significant increase in CF (fig. 6) that was fully reversible when perfusion was switched to control Tyrode's solution. The vasodilator effect of bradykinin

comparable to those caused by infusion of paclitaxel. Similarly to paclitaxel, infusion of N-methyltaxol C also induced an increase of LVDP (210.0 ± 17.9%) and the appearance of conduction arrhythmias were similar to those observed after treatment with paclitaxel and N-methyltaxol C.

Perfusion of isolated hearts with 1 μM DMSO had no significant effect on cardiac performance (LVSP = 101 ± 7.1%; HR = 91 ± 4.5%; CF = 90 ± 3.1%; A-V = 91 ± 2.2%; Q-T = 105 ± 7.2%; QRS = 108 ± 1.8% of the control values).

Isolated papillary muscle. Perfusion of isolated papillary muscle with paclitaxel and N-methyltaxol C at the concentration of 5 μM had no significant effect, whereas higher doses (10 and 20 μM) induced a positive inotropic effect characterized by increases in $T_{\text{max}}$ and in $+dT/dt$ and $-dT/dt$ of developed tension (figs. 4 and 5). These effects occurred about 5 min after the beginning of perfusion and lasted during the whole period of treatment. As shown in table 3 and figure 5, no significant variation of the action potential parameters was observed during the inotropic effect of paclitaxel and N-methyltaxol C; similarly, the time to $T_{\text{max}}$ was not modified. The increase in contractility induced by perfusion with paclitaxel and N-methyltaxol C declined to control values after a 20 to 30-min washout. The other taxol derivatives tested in the present study had no effect on the electrical and mechanical properties of the isolated papillary muscle, also at the highest dose (fig. 4; and table 3).

In control experiments, perfusion of isolated papillary muscles with Tyrode's solution containing 1 μM DMSO had no significant effects ($T_{\text{max}} = 81.1 ± 5.7%$; $+dT/dt = 83.4 ± 6.2%$; $-dT/dt = 79.3 ± 7.9%$ of the control value; see also table 3).

Effects of paclitaxel and docetaxel on bradykinin-induced vasodilatation in isolated heart. It has been suggested that paclitaxel might reduce coronary blood flow by inhibiting the response of endothelial cells to bradykinin (Hamm-Alvarez et al., 1994). We have performed experiments in order to study the interaction between paclitaxel and bradykinin in the modulation of the isolated heart. Further experiments were performed using the paclitaxel derivative docetaxel, which had no coronary vasoconstrictor effect even at the highest concentration used (20 μM). Perfusion of isolated hearts with Tyrode's solution containing 100 nM bradykinin induced a prompt, significant increase in CF (fig. 6) that was fully reversible when perfusion was switched to control Tyrode's solution. The vasodilator effect of bradykinin

Fig. 2. Effects of paclitaxel (n = 8; □), docetaxel (n = 5, ◆), taxol B (n = 6, ▲), taxol C (n = 5, ○) and N-methyltaxol C (n = 5, ■) on contractility, CF and HR in isolated heart. Data are expressed as the mean ± S.E. % of the control, prechallenge value. Statistical analysis (ANOVA followed by Newman-Keuls’ multiple comparison test), was performed to compare the effects of the different paclitaxel derivatives with those of 1 μM DMSO, used as vehicle (* P < .05; ** P < .01). Base-line control values were as follows: LVSP = 58.9 ± 2.3 mm Hg; LVDP = 5.8 ± 0.4 mm Hg; CF = 2.6 ± 0.4 ml/min/g; HR = 174.6 ± 12.2 beats/min.

Fig. 3. Typical tracing showing the effect of 20 μM paclitaxel (the challenge is indicated by the arrow) on left ventricular developed pressure in the isolated heart.
was accompanied by a slight increase in contractility, whereas HR remained constant. A second stimulation with bradykinin, performed on the same heart 60 min after the first challenge, induced a comparable coronary vasodilation (data not shown). No significant changes were observed in CF, LVSP or HR when isolated hearts were perfused with 5 μM paclitaxel. However, paclitaxel significantly reduced the vasodilator response to bradykinin and the related increase in LVSP. As shown in figure 6, docetaxel (5 μM) reduced the effect of bradykinin in a similar fashion. The effects of paclitaxel and docetaxel were partially reversible; a second challenge with bradykinin, performed after 60 min of washing with Tyrode’s solution, induced a reduced but significant increase in CF (115.1 ± 4.4% and 111.3 ± 5.4% of the control value for the hearts previously treated with paclitaxel and those treated with docetaxel, respectively).

**Discussion**

Our experiments show that paclitaxel, at concentrations (10 and 20 μM) comparable to those measured in plasma during its administration to patients (Spencer and Faulds, 1994), causes alterations in the electrical activities of the isolated heart that resemble those reported in patients treated with this drug (Rowinsky et al., 1991; Spencer and Faulds, 1994). In the isolated heart, conduction arrhythmias were accompanied by reduction in both the contractile force and the coronary flow. Interestingly, whereas one of the taxol derivatives (N-methyltaxol C) used in these experiments caused similar dose-dependent alterations, the other compounds had no significant effects on cardiac performance when tested at the same concentrations.

Previous studies indicate that paclitaxel, like other tubulin-binding agents, interferes with cardiac activity. In isolated cardiac cells, paclitaxel reduces the extent and the velocity of shortening after 2 h of exposure (Tsutsi et al., 1993); a more lasting treatment (24 h) produces a negative inotropic effect accompanied by the onset of arrhythmias (Lampidis et al., 1992; Brouty-Boyé et al., 1995).

In the guinea pig isolated heart, we observed that a relatively short (15–20 min) treatment with paclitaxel exerts a cardiodepressant effect characterized by reduced contractility and conduction arrhythmias. Profound cardiac disturbances were observed in patients treated with paclitaxel (Rowinsky et al., 1991; Spencer and Faulds, 1994). In the isolated papillary muscle, which is independent of alterations in CF and beating rate, paclitaxel induces a slight but significant increase in contractile force; this indicates that the ischemic state consequent to the reduction of CF is the main factor responsible for the reduction in LVSP and the arrhythmias observed in the isolated heart after paclitaxel infusion. Indeed, we observed that the reduction in coronary flow to 60% in control hearts perfused via a peristaltic pump induces alterations in contractility and conduction arrhythmias comparable to those caused by 20 μM paclitaxel or N-methyltaxol C. The study by Galli and DeFelice (1994), showing that paclitaxel may influence the kinetics of single calcium channels in embryonic chick ventricular cells, may justify the increase in contractility observed in the isolated papillary muscle. Paclitaxel did not cause significant variations in the action potential parameters, but in particular for the action potential plateau, an increased calcium influx through calcium channels may account for the positive inotropic effect.

A transient increase in contractility was also observed in the isolated heart as an early effect of paclitaxel infusion; in this preparation, however, an increase in intracellular calcium may also occur in coronary smooth muscle cells, leading to vasoconstriction, reduced CF and ischemia, thus causing a reduced inotropism that overcomes the positive effect.

A reduced CF can be induced by paclitaxel by another
mechanism. Paclitaxel has been shown to reduce the recycling of bradykinin receptors and the increase in intracellular Ca\(^{++}\) levels induced by bradykinin in endothelial cells (Hamm-Alvarez et al., 1994), thus decreasing the endothelial-dependent vasodilation. In agreement with these results, we observed that paclitaxel, at a relatively low concentration ineffective \textit{per se} on coronary vessels, significantly reduced the vasodilation induced by bradykinin in the isolated heart. However, even if this finding is relevant to \textit{in vivo} situations in which bradykinin plays an important role as a physiological modulator of coronary blood flow, it can hardly explain the vasoconstrictor effect of paclitaxel observed in the isolated heart perfused with Tyrode’s solution without bradykinin. Moreover, docetaxel, which had no vasoconstrictor effect on the isolated heart at any concentration tested, could also block the vasodilator response to bradykinin.

Lampidis et al. (1992) and Brouty-Boye et al. (1995) attribute the cardiotoxic activity and antitumor action to the capability of paclitaxel to bind to tubulin and to stabilize microtubules. Our results do not support their assumption. All compounds tested in this study had an ability similar or even higher than that of paclitaxel to promote the tubulin assembly into microtubules and to inhibit their depolymerization (Ringel and Horwitz, 1991; Gueritte-Voegelein et al., 1991). Among these compounds, however, only paclitaxel and N-methyltaxol C induced a cardiotoxic effect. Consistent with our observations are toxicological studies in mice and dogs showing that the toxicity of docetaxel involves hematological, gastrointestinal and neuromotor systems, whereas no alterations are reported for the cardiac activity (Bissery, 1995; Fulton and Spencer, 1996).

It thus seems that modifications on the side-chain can affect not only the anticancer activity but also the pattern of side effects of anticancer taxoids. In this context, the lack of cardiac effects of taxol B and taxol C is surprising, because these compounds differ from paclitaxel only in the replacement of the N-benzoyl moiety with an aliphatic N-acyl group. On the other hand, the cardiodepressing activity of N-methyltaxol C shows that the cardiac effect does not depend simply on the aliphatic/aromatic nature of the N-acyl group on the side-chain at C-13. It has been observed that in the anticancer taxoids, this aminoacylic moiety can adopt different conformations according to the polarity of the solvent (Vander Velde et al., 1993). The observed differences in car-

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**Fig. 4.** Mechanical effects of paclitaxel (n = 5, ○), docetaxel (n = 5, ●), taxol B (n = 5, ▲), taxol C (n = 5, □) and N-methyltaxol C (n = 5, ■) in isolated papillary muscle. Data are expressed as the mean ± S.E. % of the control, prechallenge value. Statistical analysis was performed to compare the effects of the different paclitaxel derivatives with those of 1 µl/ml DMSO (★ P < .05, ** P < .01). Base-line control values were as follows: T\(_{\text{max}}\) = 230 ± 3.6 mg; +dT/dt = 4.1 ± 0.2 g/s; −dT/dt = 3.3 ± 0.1 g/s.

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**Fig. 5.** Superimposed action potentials (left) and mechanograms (right) recorded before and after (broken lines) treatment with 10 µM paclitaxel in the isolated papillary muscle. Similar effects were observed after challenge with 20 µM paclitaxel.
TABLE 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Er</th>
<th>Os</th>
<th>Apd</th>
<th>Mrd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>80.3 ± 0.7</td>
<td>31.6 ± 0.5</td>
<td>183.9 ± 1.2</td>
<td>116.6 ± 3.0</td>
</tr>
<tr>
<td>20 µM</td>
<td>78.2 ± 0.6</td>
<td>30.9 ± 0.5</td>
<td>175.8 ± 1.5</td>
<td>107.2 ± 2.8</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>84.9 ± 0.8</td>
<td>33.1 ± 0.6</td>
<td>218.7 ± 4.7</td>
<td>142.5 ± 3.5</td>
</tr>
<tr>
<td>20 µM</td>
<td>83.5 ± 1.2</td>
<td>32.7 ± 0.9</td>
<td>207.3 ± 6.0</td>
<td>138.5 ± 4.9</td>
</tr>
<tr>
<td>Taxol B</td>
<td>89.4 ± 0.4</td>
<td>30.2 ± 0.6</td>
<td>202.8 ± 2.8</td>
<td>117.5 ± 3.5</td>
</tr>
<tr>
<td>20 µM</td>
<td>81.9 ± 0.9</td>
<td>31.1 ± 0.6</td>
<td>205.4 ± 3.0</td>
<td>108.8 ± 4.1</td>
</tr>
<tr>
<td>Taxol C</td>
<td>80.1 ± 1.1</td>
<td>29.8 ± 0.6</td>
<td>205.2 ± 3.7</td>
<td>129.4 ± 4.9</td>
</tr>
<tr>
<td>n-Methyltaxol C</td>
<td>82.3 ± 0.5</td>
<td>31.8 ± 0.5</td>
<td>201.1 ± 4.9</td>
<td>144.5 ± 4.4</td>
</tr>
<tr>
<td>20 µM</td>
<td>78.3 ± 0.5</td>
<td>26.7 ± 0.4</td>
<td>215.4 ± 3.0</td>
<td>108.6 ± 5.0</td>
</tr>
<tr>
<td>DMSO</td>
<td>79.4 ± 2.4</td>
<td>28.5 ± 1.2</td>
<td>179.2 ± 5.2</td>
<td>127.1 ± 9.8</td>
</tr>
<tr>
<td>1 µM/micliter</td>
<td>80.3 ± 2.3</td>
<td>29.9 ± 1.0</td>
<td>175.0 ± 5.1</td>
<td>133.7 ± 9.1</td>
</tr>
</tbody>
</table>

Er = resting membrane potential (mV); Os = overshoot (mV); Apd = action potential duration (ms); Mrd = maximum rate of depolarization (V/s). All values were expressed as the mean ± S.E. No statistically significant differences were found among papillary muscles treated with paclitaxel, with its derivatives and with DMSO.

diac activity might thus reflect subtle conformational differences within these compounds.

In conclusion, our study demonstrates that paclitaxel possesses an in vitro cardiodepressant activity related to a coronary constricting effect; however, the precise role of microtubules as modulators of intracellular calcium is not yet completely understood. The finding that docetaxel and other taxol analogs, with similar or even higher tubulin affinity, have no significant effect on cardiac muscle is of particular relevance for the design of second-generation antitumor taxoids and in the context of the nononcological applications of these compounds (e.g., prevention of restenosis) (Shaw et al., 1995; Solott et al., 1995).

References


Solott SJ, Cheng L, Pauly RB, Jenkins GM, Monticone RE, Kuzuya M, Froehlich JP,


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