Advances in Brief

Paclitaxel and Docetaxel Enhance the Metabolism of Doxorubicin to Toxic Species in Human Myocardium[1]

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Abstract

Doxorubicin cardiotoxicity is a multifactorial process in which the alcohol metabolite doxorubicinol mediates the transition from reversible to irreversible damage. We investigated whether the tubulin-active taxane paclitaxel increases conversion of doxorubicin to doxorubicinol, thus explaining the high incidence of congestive heart failure when doxorubicin is used with paclitaxel. Specimens of human myocardium from patients undergoing bypass surgery were processed to obtain cytosolic fractions in which doxorubicin was converted to doxorubicinol by NADPH-dependent aldo/keto or carbonyl reductases. In this model, clinically relevant concentrations of paclitaxel (1–2.5 μM) increased doxorubicinol formation by mechanisms consistent with allosteric modulation of the reductases. Stimulation was observed over a broad range of basal enzymatic activity, and was accompanied by a similar pattern of enhanced formation of doxorubicinol aglycone, a metabolite potentially involved in the reversible phase of cardiotoxicity. The closely related analogue docetaxel had effects similar to paclitaxel, but increased doxorubicinol formation over a narrower range of enzymatic activity. The unrelated tubulin-active alkaloid vinorelbine had no effect. These results demonstrate that taxanes have a unique potential for enhancing doxorubicin metabolism to toxic species in human myocardium. The effects on doxorubicinol formation provide clues to explain the clinical pattern of doxorubicin-paclitaxel cardiotoxicity and also caution against the potential toxicity of combining docetaxel with high cumulative doses of doxorubicin.

Introduction

The anthracycline doxorubicin has outstanding activity in several human tumors, but its use is limited by the risk of developing cardiomyopathy and congestive heart failure when patients receive a cumulative dose above ~500–550 mg/m^2 (1). Trials conducted in women with breast cancer have shown that congestive heart failure may occur at lower cumulative doses when doxorubicin is combined with the tubulin-active taxane paclitaxel (2, 3). On the basis of these findings, it has been recommended that the cumulative dose of doxorubicin administered with paclitaxel should not exceed a threshold of 360 mg/m^2 (4, 5). The enhanced cardiotoxicity of doxorubicin-paclitaxel combinations has been attributed to pharmacokinetic interactions between the two drugs, resulting in a reduced elimination of the anthracycline (6). Here we examined whether paclitaxel may act also by facilitating conversion of doxorubicin to the toxic secondary alcohol metabolite doxorubicinol, which is formed by cytoplasmic aldo/keto or carbonyl reductases that shunt electrons from NADPH to the carbonyl group in the side chain of the anthracycline. The study was conducted by determining whether paclitaxel enhanced doxorubicin-to-doxorubicinol conversion in cytosolic fractions derived from human myocardial samples. The specificity of such an effect was assessed by comparing paclitaxel to its analogue docetaxel and to the structurally unrelated, tubulin-active *Vinca* alkaloid vinorelbine, inasmuch as neither docetaxel (7, 8) nor vinorelbine (9) have been reported to increase the incidence of congestive heart failure when combined with doxorubicin.

Materials and Methods

Drugs. We used doxorubicin and (S-R) doxorubicinol (Pharmacia-Upjohn, Milan, Italy); doxorubicin and doxorubicinol aglycones (purified by us after thermoacid hydrolysis of doxorubicin or doxorubicinol; Ref. 10); lyophilized paclitaxel (Bristol Myers Squibb, Wallingford, CT) and docetaxel (Rhône-Poulenc Rorer, Vitry-sur-Seine Cedex, France); and vinorelbine (formulated as Navelbine; Aquitaine Pharm International, Bizanos, France).

Human Myocardium. Small samples (~0.1 g) of normothermic beating myocardium were obtained from patients undergoing aorto-coronary bypass grafting, and were stored at −80°C until used. All specimens derived from the lateral aspect of excluded right atrium and were routinely disposed of by the surgeons during cannulation procedures for cardio-pulmonary bypass (10). Therefore, patients were not subjected to any unjustified or ethically unacceptable loss of tissue.

Preparation of Cytosol and Reconstitution of Doxorubicin Metabolism. Pools of 10–15 anonymous myocardial specimens were processed for cytosol preparation by homoge-

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Table 1 Paclitaxel and docetaxel stimulate doxorubicin and doxorubicin aglycone metabolism by dialysis-inhibitable interactions with human cardiac reductases

Cytosol (0.3 mg prot./ml) was incubated for 1 h with 1 μM taxanes or equivalent volume of taxane-free ethanol in 0.3 M NaCl (pH 7.0) at 4°C. Subsequently, cytosol was dialyzed overnight against 0.3 M NaCl (pH 7.0) at 4°C and eventually reconstituted with 100 μM NADPH and 25 μM doxorubicin or doxorubicinol aglycone to assess the formation of doxorubicinol or doxorubicinol aglycone, respectively. Where indicated, 1 μM paclitaxel or docetaxel was reintroduced in the incubations containing dialyzed cytosol, NADPH, and the anthracycline. Values are taken from representative experiments in a cytosol that metabolized doxorubicin or doxorubicin aglycone with basal activities of 0.33 nmol doxorubicinol/mg protein/4 h or 0.73 nmol doxorubicinol aglycone/mg prot./4 h, respectively.

Experimental condition	Doxorubicinol	Doxorubicinol aglycone
	(nmol/mg protein/4 h)	
Ethanol → Dialysis	0.35	0.69
Paclitaxel → Dialysis	0.39	0.71
Docetaxel → Dialysis	0.32	0.60
Paclitaxel → Dialysis + Paclitaxel	0.59	1.1
Docetaxel → Dialysis + Docetaxel	0.62	1.2

nization, ultracentrifugation, and 65% ammonium sulfate precipitation of 105,000 g of supernatants (10). Next, cytosolic proteins were treated with 100 mM DTT (pH 8.9), gel filtered on Sepharose 6B minicolumns and reprecipitated with 65% ammonium sulfate, precisely as described (11). Anthracycline metabolism was reconstituted in 0.5-ml incubations containing proteins (0.15 mg), NADPH (100 μM), and doxorubicin (25 μM), in 0.3 M NaCl which had been chromatographed previously on Chelex 100 to remove trace metals and adjusted to pH 7.0. Where indicated, 1–10 μl of 0.5 mM ethanolic stocks of paclitaxel, docetaxel, or vinorelbine were included in the incubations to achieve a 1–10 μM range. To permit direct comparisons, taxane-free ethanol was included as appropriate to adjust its final volume to 10 μl in all incubations. Because the vinorelbine formulation used in this study contained ditartrate, aliquots of vinorelbine-free ditartrate were also included as appropriate to adjust its concentration to 25 μM in all incubations. After 4 h of incubation at 37°C, the reaction mixtures were analyzed for doxorubicinol and doxorubicinol aglycone by previously validated two-dimensional TLC (10, 11). After identification against authentic standards, the metabolites were eluted and quantified fluorometrically (excitation 470 nm; emission 547 nm). Other conditions are indicated in the legends to figures and Table 1.

Results

In vitro measurements of the conversion of doxorubicin to doxorubicinol are made difficult by the ease with which doxorubicinol recycles to doxorubicin after reaction with the iron-sulfur cluster of cytoplasmic aconitase (11). Other artifacts may be caused by nonspecific reaction of the anthracycline molecule with laboratory buffers and adventitious iron possibly present therein (12, 13). These pitfalls were avoided by disassembling the iron-sulfur clusters of human cardiac cytosol with DTT (pH 8.9) and by reconstituting doxorubicin metabolism in 0.3 M NaCl subjected to ion exchange chromatography on Chelex 100. These procedures prevent the recycling of doxorubicinol to doxorubicin and allow for assessment of the net formation of doxorubicinol (11, 13). In the present study, the addition of NADPH and doxorubicin to human cardiac cytosol resulted in the formation and detection of sizable amounts of doxorubicinol. Each cytosol had a different level of activity, but omission of NADPH or cytosol always blunted the detection of doxorubicinol, indicating that the metabolite was formed through the NADPH-dependent activity of aldo/keto or carbonyl reductases present in the cytosol (data not shown). After these characterizations, doxorubicin metabolism was studied in the presence of concentrations of paclitaxel or docetaxel that were in the range of those measured in plasma after standard doses of either taxane to humans (6, 14). As illustrated in Fig. 1, doxorubicin metabolism was influenced by paclitaxel according to a bell-shaped pattern in which 1–2.5 μM paclitaxel increased formation of doxorubicinol, whereas higher concentrations progressively decreased it to the basal levels. Such a response was not observed by replacing paclitaxel with vinorelbine (Fig. 1). Docetaxel had the same bell-shaped effect as paclitaxel and, similarly to paclitaxel, it elicited maximal or near-to-maximal stimulation at 1 μM (Fig. 1). However, the effects of paclitaxel and docetaxel on doxorubicinol formation were influenced in a different fashion by the basal activity of the cytosol. Such a difference was revealed by plotting the basal levels of doxorubicinol formation in a given preparation against the net stimulation elicited by 1 μM paclitaxel or docetaxel in that particular preparation. As shown in the inset of Fig. 1, stimulation by paclitaxel increased and reached a plateau with increasing the basal activity of the cytosol. In contrast, the effect of docetaxel reached a maximum in the preparations with intermediate activity, but decreased in those with higher activity. These results suggested that paclitaxel stimulated doxorubicinol formation over a broader range of enzymatic activity than docetaxel.

Human cardiac cytosol also can form doxorubicinol aglycone. The reaction is mediated by as-yet uncharacterized glycosidases that remove the aminosugar of doxorubicin, releasing a doxorubicin aglycone that goes undetected because of its rapid conversion to doxorubicinol aglycone by carbonyl or aldo/keto reductases (13). Whereas vinorelbine had no effect on doxorubicinol aglycone, paclitaxel and docetaxel modulated its formation through the same concentration-dependent, bell-shaped pattern as that described for doxorubicinol, with both taxanes eliciting maximal or near-to-maximal stimulation at 1 μM (Fig. 2). However, contrary to what was observed in the case of doxorubicinol, paclitaxel and docetaxel exhibited the same range of action in stimulating formation of doxorubicinol aglycone over the basal levels of the different cytosol samples (Fig. 2, *inset*). To obtain additional information in this setting, we monitored the formation of doxorubicinol aglycone in incubations in which doxorubicin was replaced by purified doxorubicin aglycone. As shown in Fig. 3 and *inset*, doxorubicin aglycone was an excellent substrate for aldo/keto or carbonyl reductases, yielding very high amounts of doxorubicinol aglycone. The reaction was modulated by paclitaxel and docetaxel by the usual bell-shaped pattern, maximal stimulation occurring at 1 μM of either taxane. Moreover, both paclitaxel and do-

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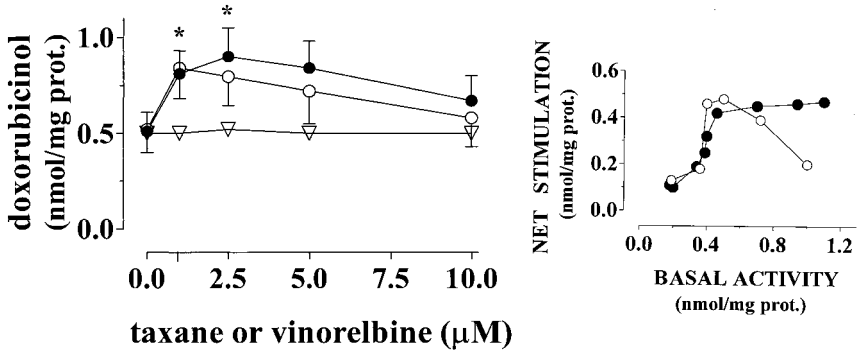

Fig. 1 Paclitaxel and docetaxel, but not vinorelbine, stimulate conversion of doxorubicin to doxorubicinol in human cardiac cytosol. Doxorubicinol formation was measured in 0.5-ml incubations containing human cardiac cytosol (0.15 mg of protein), NADPH (100 μM), and doxorubicin (25 μM) in the absence or presence of paclitaxel (●), docetaxel (○), or vinorelbine (▽), as described in "Materials and Methods." Values are those determined at 4 h and are means ± SE of six to nine experiments for docetaxel or paclitaxel, respectively. Vinorelbine data are taken from a control experiment in a cytosol with a basal activity of 0.48 nmol doxorubicinol/mg protein/4 h. The inset shows net stimulation by 1 μM paclitaxel (●) or docetaxel (○) as a function of the basal activity in doxorubicinol formation of the different cytosolic fractions. ∗, $P < 0.02$ *versus* controls (paired, one-tailed Student's *t* test).

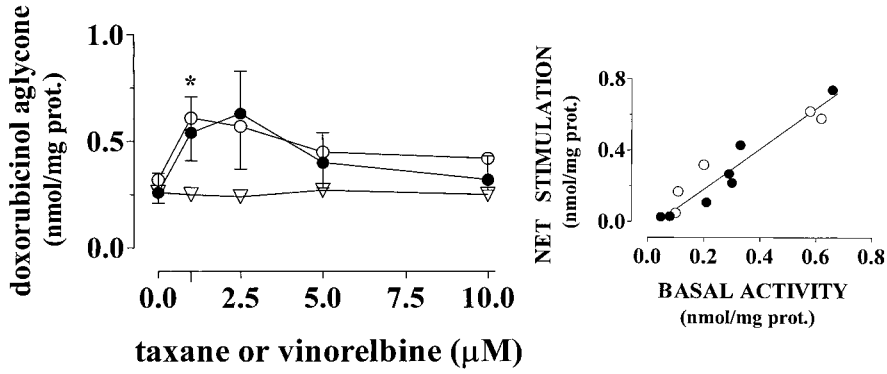

Fig. 2 Paclitaxel and docetaxel, but not vinorelbine, stimulate conversion of doxorubicin to doxorubicinol aglycone in human cardiac cytosol. Experimental conditions were as described in the legend to Fig. 1, except that incubations were assayed for doxorubicinol aglycone. Values are means ± SE of five to seven experiments for docetaxel (○) or paclitaxel (●), respectively. Vinorelbine data (▽) are taken from a control experiment in a cytosol with a basal activity of 0.23 nmol doxorubicinol aglycone/mg protein/4 h. The inset shows net stimulation by 1 μM paclitaxel (●) or docetaxel (○) *versus* the basal activity in doxorubicinol aglycone formation of the different cytosolic fractions. ∗, $P < 0.05$ *versus* controls.

cetaxel were able to stimulate over the whole range of the basal activity of the different preparations. These results showed that paclitaxel and docetaxel were fully identical in stimulating the reduction of doxorubicin aglycone to doxorubicinol aglycone, which explains how they shared a similar range of action when doxorubicin aglycone was released by doxorubicin glycosidases present in the cytosol.

In a final set of experiments, the anthracycline metabolism was studied by adding NADPH and doxorubicin or doxorubicin aglycone to cytosolic fractions that had been preincubated with taxanes or taxane-free ethanol and then subjected to dialysis. These experiments were aimed at establishing whether taxanes enhanced doxorubicin metabolism through an irreversible binding to cytosolic reductases or through reversible, dialysis-inhibitable interactions with these enzymes. As shown in Table 1, cytosol exposed to the sequence taxane-dialysis did not produce more doxorubicinol or doxorubicinol aglycone than did cytosol exposed to the sequence ethanol-dialysis. However, reintroduction of 1 μM paclitaxel or docetaxel in the cytosol exposed to the taxane-dialysis sequence produced the usual enhanced conversion of doxorubicin and doxorubicin aglycone to doxorubicinol and doxorubicinol aglycone, respectively. These results indicated that paclitaxel and docetaxel acted through reversible interactions with cytosolic reductases.

Discussion

Doxorubicin-induced chronic cardiomyopathy has been attributed to such different mechanisms as: (*a*) free radical reactions; (*b*) alterations of calcium release/sequestration in mitochondria or in the sarcoplasmic reticulum; and (*c*) reduced expression of energy metabolism enzymes and myofibrillar or sarcoplasmic reticulum proteins (reviewed in Ref. 15). However, pharmacological and structure-activity considerations suggest that none of the above mechanisms may exceed the threshold of acute, reversible toxicity unless doxorubicin were

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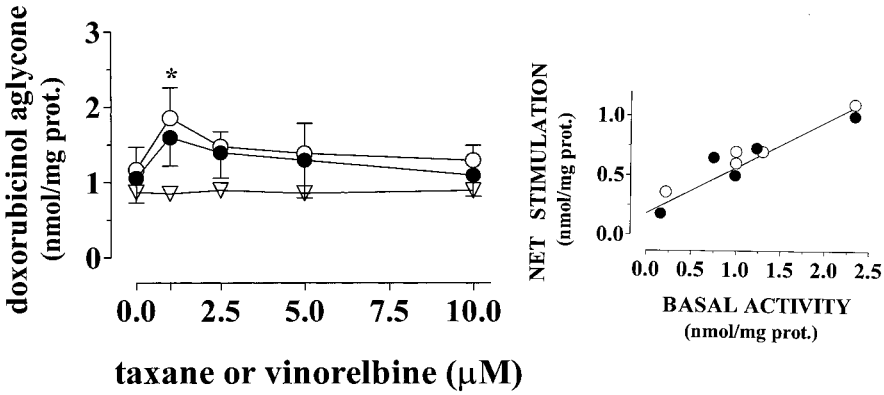

Fig. 3 Paclitaxel and docetaxel, but not vinorelbine, stimulate conversion of doxorubicin aglycone to doxorubicinol aglycone in human cardiac cytosol. Doxorubicinol aglycone was measured in incubations in which 25 μM doxorubicin was replaced by equimolar, purified doxorubicin aglycone, usually dissolved 10–15 μl of ethanol. Values are means ± SE of five experiments for paclitaxel (●) and docetaxel (○). Vinorelbine data (▽) are taken from a control experiment in a cytosol with a basal activity of 0.87 nmol doxorubicinol aglycone/mg protein/4 h. The inset shows net stimulation by 1 μM paclitaxel (●) or docetaxel (○) *versus* the basal activity in doxorubicinol aglycone formation of the different cytosolic fractions. *, $P < 0.02$ *versus* controls.

converted to doxorubicinol (15, 16). This metabolite inhibits several membrane ion pumps (16) and irreversibly inactivates cytoplasmic aconitase/iron regulatory protein-1, a posttranscriptional regulator of iron uptake and storage proteins, of mitochondrial aconitase, and perhaps of succinate dehydrogenase (11, 15). Because of its broad effects on cardiac homeostasis, doxorubicinol has the potential for synergizing other concomitant mechanisms of toxicity beyond the threshold of reversible damage, setting the stage for the development of irreversible cardiomyopathy and congestive heart failure (15). In this study, we probed the hypothesis that paclitaxel increases the incidence or accelerates the course of the cardiomyopathy related to the cumulative dose of doxorubicin by facilitating its conversion to doxorubicinol in the heart. For this purpose we exploited a human model of cardiac tissue which avoids interpretation problems posed by the high variability of doxorubicin metabolism in laboratory animals (11, 13, 15). The results demonstrate that paclitaxel enhances the enzymatic conversion of doxorubicin to doxorubicinol, presumably through an allosteric modulation of carbonyl or aldo/keto reductases. The allosteric effect is suggested by the bell-shaped response of doxorubicinol to increasing concentrations of paclitaxel, and by the fact that paclitaxel stimulates doxorubicinol formation through reversible, dialysis-inhibitable mechanisms rather than through irreversible, covalent-type binding to the cytosol. Similar mechanisms enable paclitaxel to also enhance the reduction of doxorubicin aglycone to doxorubicinol aglycone, a metabolite that may contribute to the reversible phases of cardiotoxicity by partitioning from cytosol into mitochondria and by increasing oxygen radical formation by the respiratory chain (13). These findings show that paclitaxel has broad effects on doxorubicin metabolism, resulting in increased formation not only of doxorubicinol but also of metabolites—such as doxorubicinol aglycone—which may act synergically with doxorubicinol in a unified pathway leading to chronic cardiomyopathy. Importantly, such metabolic changes were not observed by replacing paclitaxel with vinorelbine, which does not increase the cardiotoxicity of doxorubicin. This shows that the effects of paclitaxel are specific and lends weight to their relevance for explaining the cardiotoxicity observed with doxorubicin-paclitaxel combinations. Purification and structure-activity characterizations of the carbonyl reductases of human myocardium will help to elucidate further the unique influence of paclitaxel on the metabolism of doxorubicin to toxic species.

Previous attempts to elucidate the mechanisms of enhanced cardiotoxicity by doxorubicin-paclitaxel combinations have focused on pharmacokinetic rather than metabolic interactions between the two drugs. In particular, studies in breast cancer patients have shown that paclitaxel, perhaps in concert with its vehicle Cremophor EL, interferes with the elimination of the anthracycline and consequently increases the plasma levels of doxorubicin and especially of doxorubicinol (6). From a mechanistic point of view, however, an elevation of doxorubicinol in plasma cannot explain a greater incidence of cardiac events, because doxorubicinol is too polar for partitioning from extracellular fluids into cardiomyocytes (16). The increased plasma exposure to doxorubicin caused by paclitaxel may result in a commensurate elevation of the anthracycline in cardiac tissues, but chronic cardiomyopathy would only develop after the conversion of critical amounts of doxorubicin to doxorubicinol by cardiac reductases. Here we have shown that paclitaxel *per se* is able to accelerate such a conversion, an effect that would be very unlikely to be shared by Cremophor EL because of its limited distribution in extravascular compartments (17). Therefore, our findings form the basis to recapitulate paclitaxel-doxorubicin interactions in a more comprehensive sequence of events, according to which the clinical formulation of paclitaxel causes slower elimination of doxorubicin and correspondingly increases its penetration into myocardial tissue, whereas paclitaxel *per se* eventually facilitates the metabolic conversion of doxorubicin to toxic species inside cardiomyocytes.

Recent trials in breast cancer patients suggest that docetaxel does not enhance the cardiotoxicity of doxorubicin (7, 8), presumably because it does not interfere with its elimination

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(18). However, an additional and confounding factor in interpreting these trials may be represented by the lower cumulative dose of doxorubicin administered in combination with docetaxel. As a matter of fact, relatively few patients in the docetaxel-doxorubicin trials were exposed to more than 360–400 mg/m^2 of the anthracycline (7, 8), precluding direct comparison with the paclitaxel-doxorubicin trials in which congestive heart failure occurred after 420–480 mg/m^2 of the anthracycline (6). Here we have shown that docetaxel and paclitaxel share the same effects and range of action over different cytosol samples in stimulating the conversion of doxorubicin to doxorubicinol aglycone, a metabolite of potential relevance to the reversible phases of cardiotoxicity. However, our data also show that docetaxel exhibits a narrower range of action in stimulating doxorubicinol formation. In view of the predominant role of doxorubicinol in advancing the course of cardiomyopathy (19), such a narrower range of action might concur with the lack of pharmacokinetic interactions, and the lower cumulative dose of administered doxorubicin, in determining the reported better cardiac safety of docetaxel-doxorubicin combinations. On all other grounds, docetaxel and paclitaxel have similar effects on the conversion of doxorubicin to toxic species. This supports the concept of intensifying the cardiac surveillance and follow-up of patients receiving doxorubicin in combination with either taxane to assess the actual window of safety for clinical use.

In conclusion, we have shown that paclitaxel and docetaxel, but not the structurally unrelated tubulin-active drug vinorelbine, enhance doxorubicin metabolism to toxic species. These findings were obtained in an ethically acceptable model of human myocardium, provide reliable correlates to interpret the clinical pattern of doxorubicin-paclitaxel cardiotoxicity, and caution against the potential toxicity of combining docetaxel with cumulative doses of doxorubicin higher than 360–400 mg/m^2 that were proved safe in available clinical studies. These results also suggest that the human myocardium model is exploitable for screening novel taxanes. Such studies would promptly identify those new analogues devoid of effects on doxorubicin metabolism and eventually of the potential for enhancing cardiotoxicity when used in combination with the anthracycline.

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