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ORIGINAL ARTICLE

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Extensive and saturable accumulation of paclitaxel by the human platelet

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Abstract Little is known about the cellular distribution of paclitaxel in humans. In the present study we examined the distribution of [^{3}H]-paclitaxel in human blood. When 1 μM paclitaxel was incubated with fresh blood at 37°C, the platelet/plasma concentration ratio was 240 ± 17 (mean ± SEM), whereas the red blood cell (RBC)/plasma concentration ratio was only 0.59 ± 0.05. In kinetics experiments using platelet-rich plasma, we observed that the platelet accumulation of paclitaxel was highly temperature- and concentration-dependent. Scatchard analysis of the 37° C uptake data demonstrated a dissociation constant (K_{app}) of 0.80 ± 0.10 μM and a maximal binding capacity of 672 ± 102 pmol/10^9 platelets. It is proposed that the platelet accumulation of paclitaxel reflects binding to microtubules and may serve as a useful model for binding to less accessible cellular sites.

Key word: Taxol · Paclitaxel · Platelets

Introduction

The antitumor activity of the microtubule-interactive natural product paclitaxel (Taxol) has been quite promising since its introduction in humans a few years ago; thus, paclitaxel shows clear effectiveness against breast, lung, and head and neck cancers as well as advanced platinum-resistant ovarian carcinomas [15]. To optimize therapy, a thorough understanding of the disposition of paclitaxel is necessary. The pharmacokinetics of paclitaxel has been examined in a number of studies and is characterized by a clearance of about 400 ml/min and an elimination half-life of about 5 h [15]. The clearance of paclitaxel involves both metabolism and biliary elimination; urinary excretion is low. Studies of human metabolism of paclitaxel have resulted in identification of 6α-hydroxytaxol as the main hepatic metabolite [6, 12], which is produced by specific cytochrome P450 isoforms [3, 13].

Paclitaxel binding to plasma proteins is more than 90% [11, 19], involving hydrophobic interactions with both albumin and α_1-acid glycoprotein [11]. Considering this high degree of plasma binding, the volume of distribution is quite high and surprisingly variable, ranging from 30 to 240 l/m^2 [15]. Interestingly, in a recent study in children it was shown that the volume of distribution of paclitaxel decreased with increasing doses [18]. Similar observations have been made in several preliminary studies in adults [1, 8]. This unusual dose dependency is likely the result of saturable tissue binding.

In an effort to understand this phenomenon better, we determined the uptake of paclitaxel by human platelets as a potential in vivo model of tissue binding. A high degree of platelet accumulation has previously been shown to occur with other tubulin/microtubule-interactive drugs, i.e., the vinca alkaloids [4, 9, 16]. Our findings in the present study demonstrate a very large accumulation of low concentrations of paclitaxel by the platelet, an accumulation that is saturable within the therapeutic plasma concentration range.

Materials and methods

Paclitaxel was obtained from Calbiochem (La Jolla, Calif.) and [^{3}H]-paclitaxel (23 Ci/mmol, generally labeled) from Moravek Biochemicals (Brea, Calif.). Stock solutions of paclitaxel (2 mg/ml in dimethylsulfoxide, DMSO) were stored at −20° C and diluted with phosphate-buffered saline prior to use. Whole blood (50 ml) obtained from normal volunteers (men and women aged 22–55 years) was anticoagulated with ethylenediaminetetraacetic acid (EDTA). [^{3}H]-Paclitaxel is chemically stable. Metabolism occurs in the liver

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[13] but not in whole blood as determined by high-pressure liquid chromatography.

Platelet-rich plasma (PRP) with very little contamination from erythrocytes and leukocytes was prepared by centrifugation at 200 *g* for 20 min at room temperature [10]. Aliquots (1.7 ml) of PRP were placed at 37° C or in ice water for 10 min prior to the addition of varying concentrations of unlabeled paclitaxel and 0.5 μCi of [^{3}H]-paclitaxel, where [^{3}H]-paclitaxel contributes 19 ng/sample, included in the total paclitaxel concentration. Preliminary experiments using incubation periods of 5, 15, 30, and 60 min ($n = 3$; 0.5 μ*M* paclitaxel) established that the time required for maximal paclitaxel uptake from PRP into platelets was 15 min, at both 37° and 0° C and that the amount of DMSO used (0.025%) had no effect on the results. Subsequent incubations were thus carried out for 15 min. The incubations were terminated by transferring 1.5 ml PRP to Eppendorf tubes and centrifuging at 10,000 *g* for 1 min. The platelet-poor plasma (PPP) was removed and an aliquot was counted by liquid scintillation spectrometry. The inside of the tube was wiped dry, the platelets were resuspended in 1 ml of methanol, and an aliquot was counted.

When whole-blood distribution of paclitaxel was determined, paclitaxel with [^{3}H]-paclitaxel was added to whole blood and put in a shaking 37° C water bath for 30 min prior to fractionation by centrifugation at 200 and 10,000 *g*. Platelet and PPP counts were determined as described above. RBC paclitaxel content was calculated as the difference between the added radioactivity and that recovered in platelets and PPP. Platelet paclitaxel concentrations were calculated assuming that the platelets in each milliliter of blood occupy a volume of 4.2 μl [2, 5].

The platelet paclitaxel uptake at increasing paclitaxel concentrations was subjected to Scatchard analysis [17], i.e., plots of bound/free versus bound paclitaxel. To facilitate interpretation of the graphs, the bound concentration was expressed as picomoles per 10^9 platelets on both axes, whereas the free (unbound) concentration was expressed as a micromolar value. The binding capacity (B_{max}) can then be obtained from the x-axis intercept and the dissociation constant (K_{app}), from the negative inverse slope of the line. In analysis of the same data according to the Michaelis-Menten equation [17], K_{app} and B_{max} were obtained from the x-axis and y-axis intercepts of the double reciprocal plots of uptake (picomoles per 10^9 platelets) versus paclitaxel concentration (micromolar).

Results

The distribution of [^{3}H]-paclitaxel in whole blood was determined in five healthy volunteers. To blood samples obtained from these volunteers was added 1 μ*M* [^{3}H]-paclitaxel (0.5 μCi) and, after equilibrium was achieved, fractionation of the blood into RBCs, platelets, and plasma was done by centrifugation. As can be seen in Table 1, the platelets, despite the minuscule volume that they occupy, accumulated more paclitaxel than did the RBCs. This is best reflected in the cell/plasma concentration ratio, which was 240 ± 17 (mean ± SEM) for the platelets and only 0.59 ± 0.05 for the RBCs. The statistically significant ($P < 0.01$) inverse relationship between the platelet and RBC contents of paclitaxel should also be noted. The plasma binding of [^{3}H]-paclitaxel in these volunteers, at a 1-μ*M* concentration, was 94.6% ± 1.2%, very similar to that obtained in a previous study [11].

The extensive accumulation of [^{3}H]-paclitaxel by the platelets was investigated further using PRP obtained from another five healthy volunteers. Preliminary experiments indicated that equilibrium was reached after 15 min of incubation and that saturation of [^{3}H]-paclitaxel accumulation occurred at PRP concentrations of less than 5 μ*M*. Platelet accumulation of [^{3}H]-paclitaxel over the concentration range of 0.125–2 μ*M* in one of the volunteers is shown in Fig. 1 after both 37° and 0° C incubations. A dramatic temperature as well as concentration dependency is apparent. A Scatchard plot of the data from Fig. 1 is shown

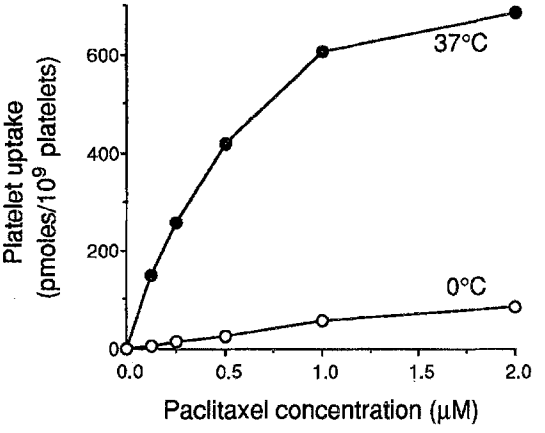

Fig. 1 Effects of paclitaxel concentration and incubation temperature on paclitaxel accumulation by platelets from one subject

Table 1 Distribution of paclitaxel in whole blood from five healthy subjects

Subject number	Percentage of paclitaxel in[a]			RBC/plasma concentration ratio[b]	Platelet/plasma concentration ratio[c]
	Platelets	Plasma	RBCs		
1	34.2	40.3	25.5	0.70	201
2	35.0	40.5	24.5	0.66	206
3	40.2	37.7	22.1	0.61	254
4	41.7	39.5	18.9	0.54	251
5	46.9	38.4	14.7	0.43	290
Mean (± SEM)	39.6 (2.3)	39.3 (0.5)	21.1 (2.0)	0.59 (0.05)	240 (17)

[a]Fresh blood was spiked with 1 μ*M* [^{3}H]-paclitaxel (0.5 μCi), incubated for 30 min at 37° C, and fractionated by centrifugation

[b]Taking into account each individual subject's hematocrit value

[c]Assuming that the platelets in each milliliter of blood occupy a volume of 4.2 μl [2, 5]

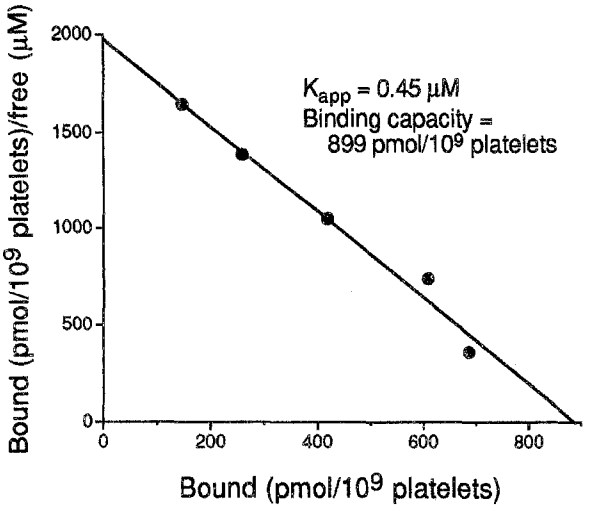

Fig. 2 Scatchard plot of the binding of paclitaxel to platelets at 37° C. The data shown were obtained in the subject in Fig. 1. The binding capacity (pmol/10^9 platelets) is obtained from the *x*-axis intercept, whereas the K_{app} value (μM) is obtained from -1/slope

Table 2 Characteristics of the binding of paclitaxel to platelets from five healthy subjects. The data were obtained as indicated in Fig 1

Subject number	K_{app} (μM)	Binding capacity (pmol/10^9 platelets)
6	0.93	937
7	0.88	588
8	0.71	442
9	1.02	502
10	0.45	889
Mean ($\pm$ SEM)	0.80 (0.10)	672 (102)

in Fig. 2, indicating a single binding process over the therapeutic concentration range studied, with a K_{app} value of 0.45 μM. The K_{app} value for all subjects investigated was 0.80 ± 0.10 μM, and the binding capacity (B_{max}) was 672 ± 102 pmol/10^9 platelets (Table 2). Analysis of the data according to the Michaelis-Menten equation produced very similar data with a K_{app} value of 0.65 ± 0.10 μM and a B_{max} value of 593 ± 90 pmol/10^9 platelets.

Discussion

This study demonstrates very large accumulation of paclitaxel by human platelets, which becomes saturated over the therapeutic blood concentration range for this drug. The 240-fold accumulation of paclitaxel in the platelet as compared with plasma at low concentrations appears to be of a magnitude similar to that found for the vinca alkaloids, previously studied in the rat [4, 9, 16]. As was proposed for the vinca alkaloids, it is likely that the extensive platelet uptake of paclitaxel is also due to binding to the tubulin/microtubule system. The binding constant for paclitaxel to microtubules has been determined to be 0.87 μM [14]. The apparent binding constant for paclitaxel to the platelets in the present study was almost identical, i.e., 0.80 μM. Whereas the platelet saturation value at 37° C was 370 pmol/10^9 platelets for vinblastine in the rat [16], it was even higher (672 pmol/10^9 platelets) for paclitaxel in our study in humans. In a recent study examining the mechanism of mitotic block and inhibition of cell proliferation by paclitaxel, a similar, apparently saturable uptake of paclitaxel by HeLa cells was observed [7].

Under the same conditions there was no accumulation of paclitaxel in the RBCs, which lack the tubulin/microtubule system. It should be noted that paclitaxel is highly plasma protein-bound, which has been shown to limit the RBC uptake [11]. Plasma binding does not, however, seem to affect the platelet uptake. Also, the plasma binding of paclitaxel is not saturable within the concentration range investigated [11].

The significance of the present findings for the in vivo pharmacokinetics of paclitaxel can only be speculated on. The findings of dose-dependent volumes of distribution for paclitaxel in children by Sonnichsen et al. [18] and in adults in both our preliminary investigations [1] and the study by Kearns et al. [8] clearly suggest saturable extravascular binding. An apparent binding constant for this process in vivo was assessed to be about 0.45 μM [18]. This value is similar to our value of 0.80 μM for platelet uptake. The saturable uptake process in the platelet and in extravascular tissue may therefore be the same. Thus, the high interindividual variability in the volume of distribution of paclitaxel in cancer patients [15] may be reflected in the platelet uptake, which likely involves microtubule binding. This aspect deserves further exploration during paclitaxel therapy in cancer patients.

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