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The Effect of Cancer Chemotherapy Drugs on Platelet Aggregation*

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The importance of the platelet in the hemostatic mechanism is well recognized. In recent years turbidimetric techniques have been developed for the study of platelet function related to platelet aggregation in vitro.

Disorders of platelet aggregation are found in rare congenital diseases, and can occur following the administration of such anti-inflammatory agents as aspirin,[1, 2, 3, 4] phenylbutazone,[5] colchicine,[6] certain antihistamines,[7] presantin,[8] dextran,[9] adrenaline[10] and vinca alkaloids[6] (vincristine and vincaleukoblastine). Patients with defective hemostatis may receive a variety of chemotherapeutic agents. Since cancer chemotherapy drug effect on platelet function is not known, we have studied in vitro and in vivo, the effect of these agents on platelet aggregation to determine whether or not the in vitro effect of chemotherapeutic drugs correlate with findings in vivo.

Material and Method

The in vitro effect of chemotherapeutic drugs was studied using platelets from male sprague Dawley rats and platelets from blood bank donors who had not received any medication, not even aspirin, for one week. For the in vitro study, each drug was incubated with a sample of platelet rich plasma (PRP) at 37°C for 30 minutes. In studies on rats, drugs were tested at two dosage levels, two times and ten times that of the calculated plasma concentration from a single intravenous (I. V.) dosage commonly used in clinical studies. In studies with humans, the concentrations were one and two times that of therapeutic dosages.

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The in vivo effect of chemotherapeutic drugs was studied only in rats. Each drug was given intravenously at two dosage levels as it is used in the in vitro study: two and ten times the usual single intravenous dose body weight. Control animals were given an equivalent volume of saline.

Blood samples were obtained 2 and 24 hours after injection of the drug. Platelet rich plasma was prepared from venous blood anticoagulated with 1: 10 volume of 3.8% sodium citrate collected in a plastic tube. It was centrifuged at room temperature for 20 minutes at 600 rpm. Platelet poor plasma (PPP) was obtained by centrifuging PRP for 10 minutes at 15,000 rpm. Platelet counts were performed by phase contrast microscopy using the Brecker-Cronkite method.[11] Platelet counts in the PRP samples were adjusted to 400,000/cmm with autologous PPP.

Platelet aggregation was studied spectrophotometrically using a platelet aggregometer (Chrono-Log Corp., Bromall, Pa.). Changes in light transmission were recorded continously on a model 702 strip chart recorder. With this method increasing platelet aggregation is reflected by an increase in light transmission through the platelet suspension.

Agents used to induce platelet aggregation were ADP, collagen and epinephrine. Adenosine-5-diphosphate (Sigma Chemical Co., St. Louis, Mo.) was dissolved in Tyrode's solution to the required concentrations. Collagen suspension (Sigma Chemical Co.) was prepared as previously described.[5] Agueous epinephrine was diluted with saline in concentration of 10 γ /ml.

In this study the maximum response of test samples and controls to each aggregating agent were used. The data are expressed as per cent difference from control values. For the in vitro study, platelet aggregation in normal donor and rat PRP samples which had no drug effects, were used as controls.

Results

Results of the in vitro study of rat platelets is shown in Table 1. Epinephrine did not induce platelet aggregation in rat platelets even at high concentration, so only ADP and collagen were used. 6-Azauridine, daunomycin, cyclophosphamide and hydrocortisone caused a 25% or greater decrease in platelet aggregation in response to one or both agents. 6-Mercaptopurine, L. asparaginase, methotrexate and vincristine caused at least 25% increase in aggregation above control values with one or both agents. Dactinomycin and cytosar had no significant effect on platelet aggregation.

The in vivo study on rats showed that all agents had some effect on platelet aggregation two hours after injection of the chemotherapeutic agent (Table I). 6-Azauridine, L. asparaginase, dactinomycin, methotrexate, vincristine and cytoxan caused a 25% or greater decrease in platelet aggregation response to one or two agents. 6-Mercaptopurine and hydrocortisone caused a 25% or greater increase over that of controls. The only effect seen 24 hours after injection was with 6-Mercaptopurine and 6-Azauridine. The in vivo effect was correlated with the in vitro effects of the following drugs: 6-azauridine, 6-mercaptopurine, cytosar dactinomycin and cyclophosphamide. Dactinomycin and cytosar had no significant effect on platelet aggregation either in vivo or in vitro. On the contrary, the effect of some agents (6-azauridine and cyclophosphamide) decreased, another (6-mercaptopurine) increased ADP and collagen induced aggregation at least 25% over control values for both in vivo and in vitro studies. The in vivo and in vitro effects of hydrocortisone, vincristine and methotrexate do not correlate and may be opposite, as is seen from Table I. The degree of effect investigated for most of these drugs was correlated to the dosage used both in vitro and in vivo.

Results of the in vitro study of human platelets is shown in Table II. All agents tested, except hydrocortisone, reduced the degree of aggregation response to ADP, collagen and epinephrine by at least 25% below that of controls. All of these drugs, except dactinomycin, inhibted the secondary wave of epinephrine-induced aggregation. Inhibition of ADP-induced aggregation appeared to be directly related to the dosage of the drugs. At twice the usual treatment dosage, aggregation usually was affected while the one fold dosage did so only with 6-azauridine and dactinomycnin. Inhibition of collagen and epinephrine-induced aggregation occured at both dosage levels. 6-Azauridine and cyclophosphamide had an inhibitory effect on platelet aggregation in vitro in both animals and human platelet study. Throughout this study inhibition of ADP induced platelet aggregation occured at 10^{-5} molar, the usual concentration, but not at 10^{-4}.

Conclusions

Qualitative platelet defects have been described in a number of bleeding disorders. Platelet defects may be found following administration of pharmacologic agents, but aspirin is the only agent which may cause clinical bleeding, particularly in patients having underlying coagulation disorders.[12, 13]

TABLE I

EFFECT OF CHEMOTHERAPEUTİC DRUGS ON RAT PLATELETS

	ADP–Induced Aggregation				Collagen-Induced Aggregation			
	In Vitro		In Vivo (After 2 hrs)		In Vitro		In Vivo (After 2 hrs)	
Drugs	2X+/ ml PRP	10X++/ ml PRP	2X/100g	10X/100g	2X/ml PRP	10X/ml PRP	2X/100g	10X/100g
6 Azauridine	↓ (30 mg)	↓ (150 mg)	↓ (100 mg)	○(↓)+++ (500 mg)	↓	↓	↓	○(↓)+++
6-Mercaptopurine	↑ (.15 mg)	↑ (.75 mg)	↑ (.5 mg)	○(↑) (2.5 mg)	↑	↑	○(↑)	○(↑)
L-Asparaginase	↑ (15 u)	↑ (75 u)	↓ (40 u)	○ (200 u)	○	↑	↓	○
Daunomycin	○ (.07 mg)	↓ (.3 mg)	○ (.2 mg)	○ (1 mg)	↓	↓	○	○
Dactinomycin	— (.0007 mg)	○ (.003 mg)	○ (.002 mg)	↓ (.01 mg)	—	—	○	○
Methotrexate	↑ (.05 mg)	↑ (.25 mg)	○ (.14 mg)	↓ (.6 mg)	—	○	↓	↓
Cytosar	○ (.2 mg)	— (1 mg)	○ (.6 mg)	○ (3 mg)	—	○	○	○
Vincristine	↑ (.003 mg)	○ (.015 mg)	○ (.01 mg)	↓ (.05)mg	○	○	○	↓
Cyclophosphamide	○ (.5 mg)	↓ (2.5 mg)	↓ (1.4 mg)	↓ (6 mg)	↓	↓	↓	↓
Hydrocortisone	↓ (.3 mg)	↓ (1.5 mg)	↑ (.1 mg)	○ (.5 mg)	↓	↓	↑	○

Number in Parenthesis indicates that the drug concentration
\+ two times the usual single I.V. dosage
++ ten times the usual single I.V. dosage
+++ drug which is given intraperitoneally

↓ at least 25% below controls
↑ at least 25% above controls
○∓ 25% of controls ( ) after 24 hrs
— no difference from controls

TABLE II

THE EFFECT OF CHEMOTHERAPEUTIC DRUGS OF HUMAN PLATELET AGGREGATION IN VITRO

Drugs	ADP-5 Induced Agg. 1X+/ml PRP	ADP-5 Induced Agg. 2X++/ml PRP	Collagen-Induced Agg. 1X/ml–PRP	Collagen-Induced Agg. 2X/ml PRP	Epinephrine-Induced Agg. 1X/ml PRP	Epinephrine-Induced Agg. 2X/ml PRP
6-Azauridine	↓ (10 mg)	↓ (20 mg)	—	↓	↓	↓
6-Mercaptopurine	O (.05 mg)	↓ (.1 mg)	↓	↓	O	O
L-Asparaginase	O (4 u)	↓ (8 u)	↓	↓	O	↓
Daunomycin	O (.02 mg)	↓ (.04 mg)	↓	↓	O	↓
Dactinomycin	↓ (.0002 mg)	↓ (.0004 mg)	O	O	O	O
Methotrexate	— (.012 mg)	— (.024 mg)	↓	↓	O	↓
Cytosar	— (.06 mg)	— (.12 mg)	O	↓	O	O
Vincristine	O (.001 mg)	O (.002 mg)	↓	O	↓	O
Cyclophosphamide	O (.15 mg)	O (.30 mg)	O	O	↓	↓
Hydrocortisone	O (.1 mg)	O (.2 mg)	O	O	O	O

Number in parenthesis indicates that the drug concentration in 1 ml PRP sample.

\+ one time the usual single I.V. dosage

++ two times the usual single I.V. dosage

↓ at least 25% below controls

↑ at least 25% above controls

O∓ 25% of controls

— no difference from controls

From our study, some of the commonly used cancer chemotherapy drugs have been shown to inbibit platelet aggregation. (Tables I and II). Platelet aggregation tests of this type have been academically of great interest, but have not necessarily correlated with clinical syndromes. Since there are no studies of the effect of these agents in vivo on platelet aggregation in humans, it is difficult to say whether the inhibition seen in vitro would also occur in vivo or would correlate with clinical bleeding in humans. Although there are no in vitro assay methods that accurately predict in vivo results, results from the animal study suggest that some of the drug effects on aggregation were similar in vivo to those in vitro; therefore, some degree of correlation might exist for human platelets. Until it is shown that these agents have no clinically significant effect on platelet function, it would be prudent to keep the possibility in mind, particularly in the patient with a compromised coagulation status.

In this study 6-azauridine and cyclophosphamide are more likely to impair platelet function, perhaps due to their greater molar concentration. Cytosar is least likely to effect platelet function because it showed little effect on platelet function in all platelet aggregation studies with rat and human platelets. Although we cannot explain the pathogenesis of these drug effects, our findings from the in vivo study suggest that the altered platelet function was probably due to a transitory defect on the platelet because abnormal platelet function usually was seen 2 hours after injection of these drugs, but not at 24 hours.

Summary

In this study the in vitro and in vivo effects of cancer chemotherapy drugs on platelet aggregation were studied. All of the drugs tested, except hydrocortisone, inhibit the human platelet aggregation induced with ADP, epinephrine and collagen in the in vitro test. Studies on rats indicated that the effect of some of the drugs on platelet function correlated in vivo and in vitro. This also might be true for human platelet function.

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