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Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil®): possible role in hypersensitivity reactions

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Background: Pegylated liposomal doxorubicin (Doxil®) has been reported to cause immediate hypersensitivity reactions (HSRs) that cannot be explained as IgE-mediated (type I) allergy. Previous *in vitro* and animal studies indicated that activation of the complement (C) system might play a causal role in the process, a proposal that has not been tested in humans to date.

Patients and methods: Patients with solid tumors ($n = 29$) treated for the first time with Doxil were evaluated for HSRs and concurrent C activation. HSRs were classified from mild to severe, while C activation was estimated by serial measurement of plasma C terminal complex (SC5b-9) levels. Increases in SC5b-9 were compared in patients with or without reactions, and were correlated with Doxil dose rate.

Results: Moderate to severe HSRs occurred in 45% of patients. Plasma SC5b-9 at 10 min after infusion was significantly elevated in 92% of reactor patients versus 56% in the non-reactor group, and the rise was greater in reactors than in non-reactors. We found significant association between C activation and HSRs, both showing direct correlation with the initial Doxil dose rate.

Conclusions: C activation may play a key role in HSRs to Doxil. However, low-level C activation does not necessarily entail clinical symptoms, highlighting the probable involvement of further, as yet unidentified, amplification factors.

Key words: allergy, anaphylatoxins, cancer chemotherapy, doxorubicin, liposomes, hypersensitivity reactions

Introduction

Doxil® (Alza Corp., Mountain View, CA) is a pegylated liposome formulation of doxorubicin used in the treatment of Kaposi's sarcoma [1] and metastatic ovarian cancer [2]. It was recently approved for the treatment of metastatic breast cancer in Europe, and its role in various other malignancies is being investigated in ongoing clinical trials [3–6]. By substantially extending the circulation time and possibly increasing tumor uptake, this new formulation provides significant improvement of the therapeutic index of doxorubicin [2–10].

One unsolved problem with Doxil is that the treatment is often associated with 'infusion' or hypersensitivity reactions (HSRs) despite pretreatment of patients with corticosteroids and antihistamines. The reported frequency of HSRs to Doxil varies between 0% and 25%, with average and median values of 8% and 5%, respectively (Table 1). The symptoms include facial flushing, dyspnea, tachypnea, facial swelling, headache, chills, hypotension or hypertension, chest pain and back pain [1, 9, 17, 18]. Unlike IgE-mediated (type I) allergy, these reactions occur mostly at the first exposure to the drug without prior sensitization.

Although most patients with an initial reaction are able to complete the first infusion after interruption and resumption at a slower infusion rate, and often tolerate further infusions without any complications, some HSRs cannot be controlled and exclude the patient from Doxil therapy. Since an HSR is not known to occur with standard doxorubicin, it has been postulated that the reaction is caused by the liposome vehicle [19]. Nevertheless, its pathogenesis has not been elucidated to date.

We have previously reported that Doxil can activate the complement (C) system *in vitro* in normal human sera, and that minute (milligram) amounts of Doxil can induce severe cardiopulmonary changes in pigs [20]. Because liposome-induced hemodynamic changes in pigs had been previously demonstrated to be due to activation of the C system [21, 22], we proposed that the underlying cause of HSRs to Doxil in humans might be the same process, i.e. C activation.

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Table 1. Reports on hypersensitivity reactions to Doxil

Reference	Dose (mg/m^2)	Premedication	No. of patients	Reactions (%)
Gabizon et al. [8]	25–50	None	16	25
Uziely et al. [9]	20–80	Ondansetron	56	11
Northfelt et al. [11]	20	None	53	2
Muggia et al. [12]	40–50	Hydrocortisone, diphenhydramine, cimetidine	65	3
Koukouraski et al. [13]	10–25	Tropisetron	30	13
Gordon et al. [14]	50	None	89	4
Hubert et al. [15]	45–60	None	15	0
Lyass et al. [16]	35–70	None	45	2

Table 2. Grading criteria of hypersensitivity reactions to Doxil

Symptoms	Strength	Grade
No reaction	None	0
Asymptomatic, with only incidental findings	Mild	1
Symptomatic but no intervention required	Moderate	2
Anti-allergic medication and cessation of drug infusion required	Severe	3
Ventilatory or pressor support required	Life threatening	4

In order to test the hypothesis that C activation plays an important role in HSRs to Doxil, in the present study we measured C activation in patients following Doxil infusion and analyzed the relationship between C changes and clinical symptoms of hypersensitivity. We also evaluated the correlation of C activation and HSRs with Doxil dose rate.

Patients and methods

Patients

This study was conducted after obtaining Institutional Review Board approval. Twenty-nine patients treated with Doxil for the first time were included. These patients were enrolled in a phase I Doxil protocol for a variety of tumors (head/neck, pancreas, breast, esophagus, thyroid, neuroendocrine, melanoma, tubal carcinoma), and were not premedicated with steroids or antihistamines. All patients gave informed consent for use of their blood for research purposes. Three patients were excluded from the final analysis because of missing information and ambiguity of their reaction. In three reactive patients baseline samples were not available. Their inclusion in the C analysis is explained in the text.

Treatment and assessment of HSRs

In accordance with the administration guidelines of Doxil and the underlying treatment protocol, the drug was dissolved in 250 ml 5% dextrose injection (USP) before administration, and was infused over 1 h at an initial rate of one-fifth of the final rate. Following the start of infusion, patients were observed for 30 min for the presence of any of the following symptoms: skin reactions (urticaria, erythema, facial edema, facial rash, pruritus, eruptions), hypotension or hypertension, respiratory problems (laryngospasm, laryngeal edema, bronchospasm, dyspnea), pain (joint pain, back pain, abdominal pain, chest pain) or other manifestations of hypersensitivity (fever, chills, rigors, diaphoresis, nausea, vomiting, neurological changes). The symptoms were graded as specified in Table 2. The clinical observations were blinded to the laboratory findings for the initial 19 patients.

Sample collection and assay of complement activation

Blood samples were collected within 15 min before Doxil infusion (pre-infusion '0 min' sample), and at 10, 30 and 120 min after infusion, using EDTA-containing sterile tubes. Samples were centrifuged to collect plasma, which was stored at –70°C until shipping periodically on dry ice from New York University to the Walter Reed Army Institute of Research, where the C assays were carried out. C activation was assessed by measuring plasma levels of protein S (vitronectin)-bound C terminal complex (SC5b-9) [23, 24] using an enzyme-linked immunosorbent assay (ELISA) kit (Quidel Co., San Diego, CA, USA). Plasma aliquots were diluted four- to five-fold in the sample diluent of the kit and 100 µl aliquots from this mixture were put into the wells of the ELISA plate, and measured in triplicate. As an exception the samples in the last 10 patients were processed with only one determination. These samples were repeated later in triplicate after at least one freezing and thawing. In order to validate such 're-assaying', we carried out repetitive measurement of randomly selected samples at different times with >1 year separation, using different batches of the SC5b-9 kit, and blinding the assayer(s) with regard to the identity of samples. In agreement with the manufacturer's specification of inter-assay variation and warning against repetitive freezing and thawing of samples, these experiments revealed ~10–20% inter-assay variation in SC5b-9 readings with a tendency for increased baseline SC5b-9 values and reduced peak-to-baseline ratios in post-infusion samples that were tested following two or three freeze–thaw cycles. Our final analysis utilized the original readings for patients 1–19 and only the readings in plasma that were thawed not more than twice for the subsequent patients.

Table 3. Patient characteristics and Doxil dose according to reaction status

	Non-reactors (n = 16)	Grade 2 or 3 reactors (n = 13)
Age (years)		
Median	57.5	57
Range	41–73	38–77
Number missing	2	2
No. of males	7	6
Total Doxil dose (mg)		
Mean	70.1	151.8[a]
Standard error of mean	7.7	26.6
Median	60.0	138
Range	40–146	40–306
n	16	13
Initial dose rate (mg/min)		
Mean	0.23	0.51[a]
Standard error of mean	0.03	0.09
Median	0.2	0.46
Range	0.13–0.49	0.13–1.02
n	16	13

[a]P <0.05 (P = 0.011) by Mann–Whitney test, two-tailed.

Statistical analysis

Patients were divided into two groups according to the presence or absence of HSRs. Those who developed any type of reaction, regardless of severity, were referred to as 'clinical reactors', whereas those without symptoms were called 'clinical non-reactors'. Comparisons of Doxil doses and initial dose rates in clinical reactors and non-reactors were carried out with the non-parametric Mann–Whitney test.

SC5b-9 values were expressed as means ± standard deviation (SD) of triplicate determinations, and the statistical significance of the differences between baseline and 10-, 30- and 120-min samples in each patients was established using analysis of variance followed by pairwise comparisons using the Student–Neumann–Keuls test. Some patients had triplicate measurements only at baseline and 10 min, which were compared by Student's unpaired t-test. Patients who displayed significant (P <0.05) increase of 10-min SC5b-9 values relative to baseline were called 'laboratory reactors'.

Doxil-induced SC5b-9 formation in clinical reactor and non-reactor patients was compared by the Mann–Whitney test. The relationship between C activation and clinical reactions was analyzed by Fisher's exact test and Cohen's κ statistics [25]. Categorization of laboratory and clinical observations in a standardized 2 × 2 contingency table is described in the text (see Table 4).

The sensitivity, specificity and positive and negative predictive value of 10-min SC5b-9 measurements, taken as a screening test for HSRs, were computed according to Bayes' rule [25]. These computations were done with the laboratory reactors stratified to three subgroups according to the extent of C activation, as detailed in the text (see Table 5).

The correlation between SC5b-9 formation at 10 min and Doxil dose rate was established by regression analysis. The probability of developing an HSR at different Doxil dose rates was quantified by the odds ratios as described in the text (see Figure 3). The above statistical calculations were done using the SPSS 10.1 program package.

Table 4. Relationship between *in vivo* complement activation and hypersensitivity reactions to Doxil: cross-tabulation of laboratory and clinical reactions

Laboratory reaction	Clinical reaction		Total
	+	−	
+	12	9	21
−	1	7	8
Total	13	16	29

Fisher's exact P, two-tailed: 0.04.
Cohen's κ: 0.34, P ~0.03.
The standardized 2 × 2 contingency table was constructed from the data shown in Figure 2A and B. Positive (+) and negative (−) mean the presence or absence of reaction. Fisher's exact P gives the probability that the cell frequencies observed are due to chance, while Cohen's κ quantifies the agreement between qualifications of patients as clinical reactors and as laboratory reactors. A κ value of 1 indicates that the agreement is perfect, while 0 indicates that the agreement is no better than chance. P <0.05 indicates significant agreement. Other details of these statistical analyses are given in Patients and methods.

Results

HSRs to Doxil: frequency, patient characteristics and dose-dependency

Forty-five per cent (13 of 29) of patients showed grade 2 or 3 HRS to Doxil (Table 3). Reactions occurred in men and women in approximately equal proportion, and were not related to the age of patients. Importantly, the total Doxil dose, and, hence, the initial dose rate, were significantly higher in the reactor group than in non-reactors (P <0.05, Mann–Whitney test), showing that HSRs were dependent on Doxil dose (rate).

Complement activation by Doxil *in vivo*

Doxil caused C activation in 21 of 29 patients (72%) as reflected by significant elevations of plasma SC5b-9 levels following infusion of the drug. As illustrated in Figure 1A–C, the time course of changes showed substantial individual variation, such as rapid elevation of SC5b-9 until 10 min with gradual return to near baseline within 2 h (Figure 1A), rapid elevation without return within 2 h (Figure 1B), and moderately rapid elevation of SC5b-9 until about 30 min, followed by partial return to baseline during 2 h (Figure 1C). The lack of SC5b-9 response is demonstrated in Figure 1D.

Since the majority (76%) of SC5b-9 elevations were immediate and transient (Figure 1A), and because the slower and sustained responses also produced statistically significant elevations of SC5b-9 at 10 min after infusion, we used the 10-min SC5b-9 readings to compare C activation in clinical reactors and non-reactors. Nine of ten reactors showed significant increases of 10-min SC5b-9 levels over baseline following infusion of Doxil, qualifying them as laboratory reactors (Figure 2A). Even though three clinical reactors had no baseline determinations, their SC5b-9 readings at 10 min exceeded the average baseline in that series (0.17 μg/ml)

Table 5. Characteristics of the complement (C) assay in diagnosing hypersensitivity reactions (HSRs) to Doxil

10-min C terminal complex (SC5b-9), μg/ml	Sensitivity [tp/(tp + fn)]	Specificity [tn/(fp + tn)]	Positive predictive value [tp/(tp + f p)]	Negative predictive value [tn/(fn + tn)]
Significant increase[a] (SC5b-9, no limit)	0.92	0.44	0.57	0.88
Significant increase[a], SC5b-9 ≤0.98	0.83	0.54	0.45	0.88
0.98 ≤SC5b-9 ≤1.96 (≥2×, ≤4× normal)	0.80	0.70	0.57	0.88
SC5b-9 ≥ 1.96 (≥4× normal)	0.75	1.00	1.00	0.88

Based on the data in Figure 2, patients were classified into four groups according to the concurrent presence (+) or absence (−) of HSR and C reactivity, as follows: true positive (tp: HSR+, C+), false positive (fp: HSR−, C+), true negative (tn: HSR−, C−) and false negative (fn: HSR+, C−). In addition, laboratory reactors were stratified into three categories on based on 10-min SC5b-9 values, as specified in column 1. The 0.98 and 1.96 μg/ml cut-off values represent 2× and 4× the upper limit of normal SC5b-9 levels (0.49 μg/ml [24]), respectively, and were chosen arbitrarily. The sensitivity, specificity, and positive and predictive values of the SC5b-9 assay with regard to HSRs were computed according to Bayes' rule [25], as defined in the respective columns.
[a]Significant increase refers to significant (P <0.05) increase of 10-min SC5b-9 relative to baseline.

by 13- to 15-fold, providing a basis to include these patients among the laboratory reactors. Thus, 92% of clinical reactors were also laboratory reactors.

The normal plasma level of SC5b-9, reported for 50 healthy humans, is 0.25 ± 0.12 μg/ml (mean ± SD) [24]. The lines in Figure 2A and B demarcate the normal range (0.01–0.49 μg/ml), considered to be within 2 SD of the normal mean [24]. Based on this information, the Doxil-induced (significant) rise of SC5b-9 was below and above 2× upper limit of normal (i.e. <0.98 μg/ml) in five and four patients, respectively, while the 10-min rise of SC5b-9 exceeded the upper limit of normal by four-fold (i.e. were >1.96 μg/ml) in three patients. Figure 2B shows that nine of 16 (56%) of the non-reactors also qualified as laboratory reactors, of whom only four patients had SC5b-9 readings >2× normal and none had readings exceeding 4 × normal.

Relationship between C activation and HSRs

We subjected the above data to statistical analysis addressing the following questions: (i) was C activation greater in clinical reactors than in non-reactors? (ii) was there association between C activation and HSR? (iii) was there agreement between two surveys identifying patients as reactors by clinical and laboratory criteria? and (iv) how reliable were serial SC5b-9 determinations in diagnosing HSRs?

For comparing C activation in clinical reactors and non-reactors we considered that the SC5b-9 values at 10 min were not normally distributed and that the sample sizes were small. Thus, we used the non-parametric Mann–Whitney test to compare the ranks of SC5b-9 values at 10 min in the reactor and non-reactor groups, as well as the ranks of another measure of C activation, the 10-min to baseline SC5b-9 ratios. While the former analysis resulted in borderline significance (P = 0.051), comparison of the ranks of $SC5b\text{-}9_{10min}/SC5b\text{-}9_{baseline}$ ratios indicated statistically significant (P <0.05) increase in C activation in the reactor group.

To assess the relationship between C activation and HSRs we used two methods of qualitative data analysis: Fisher's exact test and Cohen's κ statistics. They address different questions regarding the relationship between categorical variables; in our case Fisher's method tests the degree of association between laboratory and clinical reactions, while κ quantifies the agreement (or reproducibility) of two surveys identifying patients as reactors by clinical and laboratory criteria. Table 4 presents the 2 × 2 contingency table used for these analyses, which indicated a significant (P <0.05) relationship between C activation and HSR by both statistical methods. Hence, our questions regarding the association between C activation and HSRs, and also about the agreement between two surveys identifying patients as reactors by clinical and laboratory criteria, can be affirmatively answered.

As for the diagnostic value of the C test with regard to HSR, Table 5 specifies the sensitivity, specificity and positive and negative predictive values of 10-min SC5b-9 readings. In our case, sensitivity gives the proportion of clinical reactors correctly identified by increased C activation, specificity is the proportion of clinical non-reactors correctly identified by a lack of C activation, positive predictive value is the proportion of laboratory reactors correctly diagnosed as clinical reactors, while the negative predictive value is the proportion of laboratory non-reactors correctly diagnosed as clinical non-reactors. We computed these indices for all laboratory reactors (row 1), as well as for laboratory reactor subgroups differentiated by the extent of C activation (rows 2–4). It is seen in Table 5 that the C assay was highly sensitive in predicting HSRs, but the specificity and positive predictive value of the test was relatively low, particularly in patients in whom the rise of SC5b-9 at 10 min remained below 2× upper limit of normal SC5b-9 (row 2). However, when we restricted the criteria for laboratory reactivity to 10-min SC5b-9 values exceeding two-fold or four-fold the upper threshold of normal, the specificity and positive predictive value of the C assay remarkably increased with relatively less decrease in sensitivity. Thus, the extent of SC5b-9

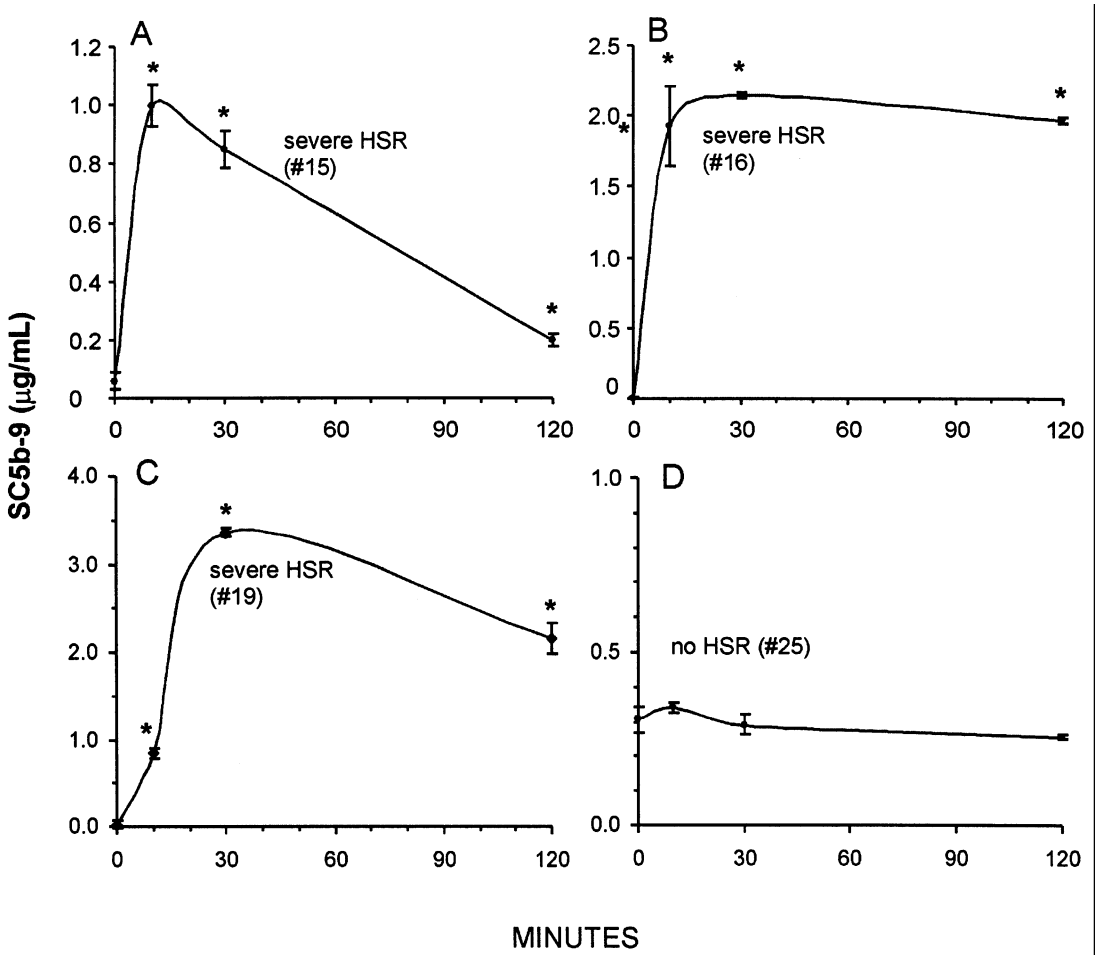

Figure 1. Time course of Doxil-induced changes in plasma complement terminal complex (SC5b-9) in cancer patients and its individual variation. Panels **A**–**D** show data from four subjects displaying different patterns of response (# indicates patient identification number, as also used in Figure 2). Data are means ± standard deviation for triplicate determinations. *Significantly different from baseline, as determined by analysis of variance followed by the Student–Neumann–Keuls test. HSR, hypersensitivity reaction.

elevation was proportional with the specificity and positive predictive value of the C assay with regards to HSRs. Taken together, our data and statistical analysis indicate that C activation plays a causal role in HSRs, although C activation *per se* does not guarantee HSR. Other pathogenic factor(s) may also be involved that become(s) rate limiting to HSRs in the case of low-level C activation.

Relationships among Doxil dose rate, C activation and HSRs

Figure 3 shows the 10-min SC5b-9 values of clinical reactors and non-reactors plotted against the initial rate of Doxil administration. Regression analysis revealed significant correlation between dose rate and SC5b-9 (P <0.01), indicating that C activation at 10 min was Doxil dose-dependent. Consistent with the correlation between HSRs and Doxil dose (Table 3) and the significant association between C activation and HSRs (Table 4), the upper right quadrant of the plot contained readings obtained exclusively from clinical reactors.

The data in Figure 3 also allowed quantification of the odds in favor of developing HSRs at different dose rates. Stepwise computation of odds ratios at increasing dose rate (legend to Figure 3) indicated significantly increased risk of HSR at and above 0.38 mg/min (dashed line), corresponding to 114 mg Doxil infused over 1 h at an initial rate one-fifth of the final rate.

Discussion

The routine use of premedication (glucocorticoids and antihistamines) and initial slow infusion rate have substantially reduced the risk of HSRs to Doxil. However, reactions occur despite these preventive measures (Table 1) leading to significant morbidity, anxiety, increased hospital expenses and, most importantly, exclusion of some patients from a state-of-art life-prolonging therapy. The mechanism of Doxil reactions is poorly understood. Based on *in vitro* and animal studies we proposed earlier that the phenomenon might represent an unusual allergic reaction called 'C activation-related pseudoallergy' [20–22]. We provided several lines of evidence in support of this proposal, namely: (i) C activa-

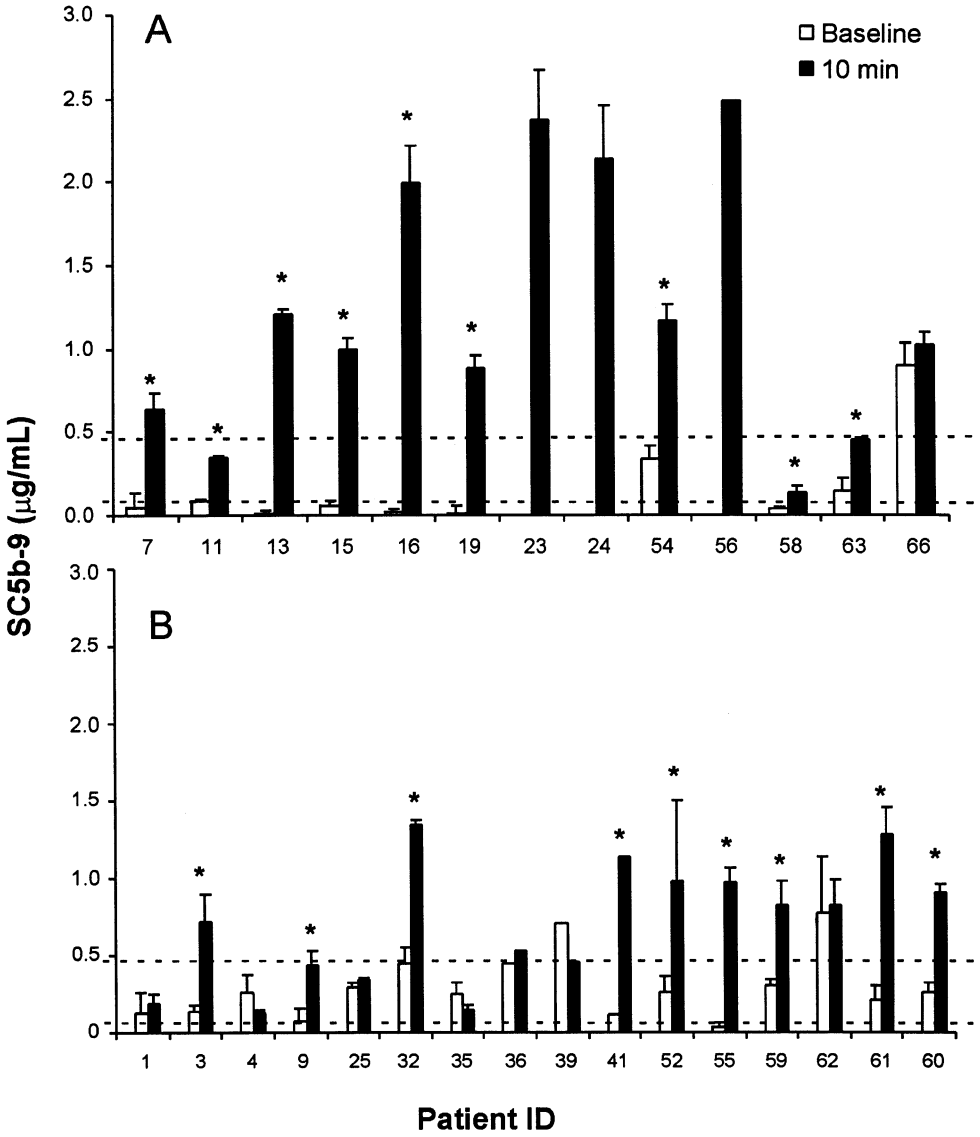

Figure 2. Plasma complement terminal complex (SC5b-9) levels at baseline and at 10 min after infusion of Doxil in cancer patients displaying (**A**) or not displaying (**B**) hypersensitivity reactions to Doxil. Data are means ± standard deviation (SD) for triplicate or duplicate determinations. The dashed lines indicate the normal range of SC5b-9, i.e. the normal mean ± 2 SD [24]. *Significantly different from baseline, as determined by analysis of variance followed by the Student–Neumann–Keuls test, or by two-sample *t*-test. The numbers under the bars are the patient identification (ID) numbers. In addition to the differences described in the text, the figure shows a tendency for lower baseline SC5b-9 levels in reactors (**A**, 0.17 ± 0.09, mean ± standard error of mean) than in non-reactors (**B**, 0.29 ± 0.05). This difference is not significant by two-sample *t*-test and is not addressed further in the paper.

tion explains almost all symptoms [26]; (ii) Doxil activates C in human serum *in vitro* [20]; (iii) some other drugs that contain the C-activating lipid emulsifier vehicle, Cremophor EL (paclitaxel and intravenous ciclosporin), cause similar HSRs [27, 28]; and (iv) the hemodynamic changes of HSRs could be mimicked in a porcine model by injection of C-activating substances and liposomes, including therapeutically relevant doses of Doxil [20–22]. The causal role of C activation in these reactions was also supported by a report by Sculier et al. [29] on the rise of C3d/C3 ratio in the blood of cancer patients infused with liposomes containing the cytostatic agent NSC 251635. This change is characteristic of C activation, just like the decrease in blood of C3, C4 and factor B levels reported recently by Brouwers et al. [30] in a patient who developed a HSR to ^{99m}Tc-labeled pegylated liposomes. A further report by Skubitz and Skubitz [18] provided indirect evidence for a role of C activation in HSR to Doxil, inasmuch as the authors described transient neutropenia only in those patients who developed HSR. Although these changes were not linked to C activation, transient neutropenia is a well-known consequence of *in vivo* C activation [26].

The present report describes the first human study of the role of C activation in the development of HSRs to Doxil. In this study no premedications were routinely used; however, we did follow the recommendation in the Doxil package insert [19] with the warning to infuse the drug at an initial rate of <1 mg/min. The incidence of HSRs (45%) was much higher than reported earlier (Table 1),

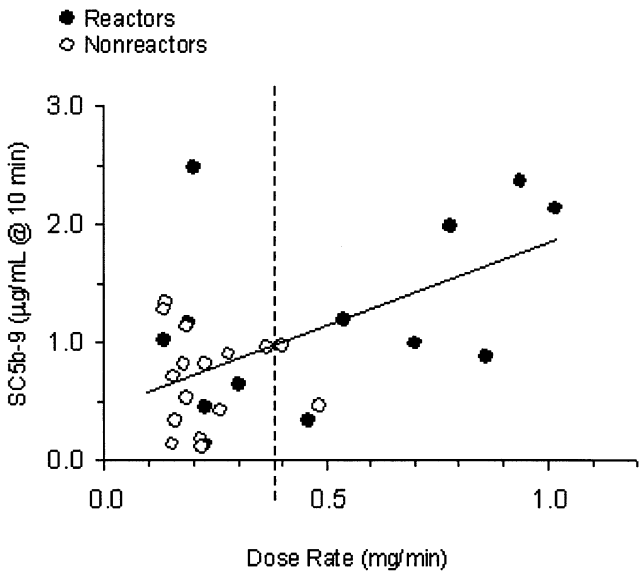

Figure 3. Dependence of complement (C) activation and hypersensitivity reactions (HSRs) on Doxil dose rate. The 10-min C terminal complex (SC5b-9) levels were plotted against the initial rate of Doxil administration (total dose/60 min × 1/5) in cancer patients displaying (filled circles) or not displaying (empty circles) HSRs. The regression line correlates Doxil dose rate with C activation, $R^2 = 0.25$, $n = 29$, $P < 0.01$. The probability of developing HSRs at different dose rates were quantified by serial computation of odds ratios in the 0.2–0.5 mg/min range. These computations were done at 0.02 mg/min steps, using the formula: (Ra × NRb)/(Rb × NRa), where R, NR, a and b denote the number of reactors and non-reactors above and below the tested dose rate, respectively [25]. The odds ratios (and 95% CI) at <0.36, 0.36, 0.38 and 0.40 mg/min were 4.81 (0.8–25.8), 4.81 (0.8–25.8), 11.4 (1.7–78.4) and 24.5 (2.3–262.5), respectively, with corresponding exact P values of 0.11, 0.11, 0.014 and 0.003. Thus, 0.38 mg/min was the lowest dose rate where the odds in favor of HSRs became significant (dashed line).

which could be due to a combination of such circumstances as the relatively high-dose Doxil regimens studied, the greater attention to HSRs and lack of premedication with antihistamines and steroids.

Measuring plasma SC5b-9 levels as an index of C activation [23, 24] we found significant rises in the majority (72%) of patients treated with Doxil for the first time. This reaction rate is very similar to our *in vitro* observations with normal human sera (70%) [20], indicating that *in vitro* C activation simulates the *in vivo* process. We also found that the frequency and the degree of C activation was greater in reactors than in non-reactors, and that there was significant association between C activation and HSRs. These facts, together with data from animal experiments [20–22], strongly suggest that C activation plays a causal role in HSRs to Doxil. However, the fact that not all patients with C activation displayed HSR highlights the involvement of other essential factors in the pathogenesis that can limit the adverse consequences of anaphylatoxin release during C activation. Such mechanisms include the breakdown of anaphylatoxins by carboxypeptidase N, and the coupling of the anaphylatoxin signal to mast cell and/or leukocyte (mainly basophil) release of histamine and other inflammatory mediators. It is possible that patients who develop severe HSR to Doxil are prone to C activation, the anaphylatoxin clearance is slow, and at the same time their mast cells have increased susceptibility to release reactions. Consistent with this hypothesis, atopic constitution, which is characterized by mast-cell hypersensitivity to various stimuli, is a risk factor for liposome reactions as well.

Our data provide some potentially useful guidelines for the prevention of HSRs to Doxil and other liposomal drugs, inasmuch as they show an already increased risk of HSR at 0.38 mg/min initial infusion rate. This value is lower than the manufacturer's recommended 1 mg/min, and is consistent with a study by Gabizon and Muggia [10] reporting HSR in only one of 25 patients when the initial infusion rate was 0.1–0.2 mg/min. The critical role of infusion rate in HSRs to Doxil is clearly in line with the prediction of the C hypothesis that if the rate of anaphylatoxin production exceeds the rate of clearance, the threshold for mast-cell and leukocyte activation may be reached sooner.

In conclusion, the present study confirms and extends former clinical evidence [29, 30] of C activation by parenteral liposomes. Our data indicate that C activation may play a key role in HSRs, although it may not be the only cause or rate-limiting factor, as the presence of other abnormalities also seems to be necessary. The high rate of reactions in this study strengthens the need for slow initial infusion, and is consistent with (although does not prove) the potential usefulness of premedication with steroids and antihistamines.

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