

Vancomycin Skin Tests and Prediction of "Red Man Syndrome" in Healthy Volunteers

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The purpose of the present study was to assess the cutaneous response to intradermally administered vancomycin in healthy adults and to determine whether the magnitude of the cutaneous response correlated to the severity of "red man syndrome" (RMS) following intravenous administration of vancomycin to the same subjects. Eleven healthy males were skin tested with intradermally administered histamine and saline controls and intradermally administered vancomycin at different concentrations. Vancomycin caused a dose-dependent area of flare in all subjects. The sigmoidal maximal flare model was used to fit each dose-response curve, and cutaneous responsiveness to vancomycin was quantified by various methods, including the flare area at each dose, maximum flare area (maximal flare), dose required to produce 50% of maximum flare, dose required to produce a flare area of 400 mm², and the slope of the dose-response curve. One week after skin testing, subjects received an infusion of vancomycin, 15 mg/kg of body weight over 60 min. For the assessment of the severity of RMS, we used previously described methods. Although all subjects experienced erythema from the intravenously administered vancomycin and 10 subjects had pruritus, there was no significant correlation between vancomycin skin test results and the severity of RMS. We conclude that vancomycin skin tests do not predict the severity of RMS. In addition, vancomycin skin tests may be of no benefit for assessing immunoglobulin E-mediated allergy to vancomycin, since all subjects had a positive reaction at concentrations of ≥ 10 $\mu\text{g/ml}$.

The most common adverse effect of vancomycin is "red man syndrome" (RMS), which is characterized by flushing and itching, especially of the upper torso (23). In severe cases, hypotension may occur. These signs and symptoms result from the nonimmunologic release of histamine, and the severity is proportional to the amount of histamine released (24). The source of histamine is believed to be primarily cutaneous mast cells, and vancomycin has been shown to result in mast cell degranulation *in vitro* (15). The frequency of RMS is largely determined by dose and infusion time. In healthy volunteers who receive 1 g as a 60-min infusion, the frequency is 80 to 90% (23). The frequency of RMS in infected patients who receive vancomycin (1 g over 60 min) varies from <10 to 50% in prospective studies (25, 28). RMS may be prevented in most patients by a reduction in the dose or the rate of infusion or by premedication with antihistamines (23).

In normal volunteers, the severity of RMS varies widely, despite administration of identical doses (24). The reason for this large difference in susceptibility to vancomycin toxicity is unknown, but it probably reflects between-subject differences in histamine release. We reasoned that if vancomycin causes a direct release of histamine by degranulation of cutaneous mast cells, then intracutaneous administration of vancomycin should produce a dose-related weal-and-flare

response. Ideally, a single intradermal dose that would elicit a wide range in weal or flare sizes and that would discriminate between those who release histamine easily from those who do not could be found. Furthermore, the size of the dermal response should predict those who are more likely to exhibit RMS when vancomycin is administered intravenously. The purpose of the present study was twofold: to evaluate the cutaneous responses to various doses of intradermal vancomycin and to determine whether a correlation exists between skin test results and the severity of RMS following intravenous administration of vancomycin.

(A report of this study was presented at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 29 September to 2 October 1991 [22a].)

MATERIALS AND METHODS

Pilot study. The study protocol was approved by the Committee on the Conduct of Human Research at Virginia Commonwealth University and Medical College of Virginia hospitals, and written informed consent was obtained from the study subjects. Four healthy adults (two males, two females) without prior exposure to vancomycin participated in a pilot study to evaluate the cutaneous response to skin testing with vancomycin and to identify the appropriate concentrations for subsequent testing. Vancomycin in 500-mg vials (Vancocin; lot 36228AF; Eli Lilly & Co., Indianapolis, Ind.) was reconstituted according to the manufacturer's recommendations with 20 ml of sterile water for injection to yield a concentration of 25 mg/ml. This concentration was further diluted with sterile water for injection to yield dilutions of 1,000, 100, 50, 10, and 1 $\mu\text{g/ml}$. Skin prick

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tests were initially performed by placing a drop of the most concentrated solution (1,000 $\mu\text{g/ml}$) on the volar aspect of the forearm. A needle was passed through the drop and the skin was tented, creating a small break in the epidermis and allowing a small amount of drug to enter (3, 11). None of the participants developed a cutaneous reaction to the skin prick test. Each subject then received an intradermal injection (0.02 ml) of each dilution into the volar aspect of the forearm, beginning with the highest concentration below the elbow crease. In the opposite forearm, 0.02 ml of saline and histamine (1 mg/ml) were administered intradermally as negative and positive controls, respectively. Intradermal injections were administered with a 1-ml tuberculin syringe and a 27-gauge needle (bevel up) at approximately a 10° angle. All injections were spaced at least 5 cm apart. All subjects had a positive weal and flare to histamine, and there was no reaction to saline. A weal-and-flare response, accompanied by local pruritus, resulted in all subjects from vancomycin concentrations of $\geq 10 \mu\text{g/ml}$; there were no reactions to 1 $\mu\text{g/ml}$. The maximum area of flare occurred 10 to 15 min after injection. A concentration of 50 $\mu\text{g/ml}$ appeared to produce the greatest range in the area of flare, but since the optimal dose of vancomycin for skin testing was unknown, concentrations of 10, 25, 50, 100, 500, and 1,000 $\mu\text{g/ml}$ were selected for subsequent skin testing.

Volunteers in main study. Twelve subjects were enrolled in the open study described here. Inclusion criteria included the following: healthy males aged 18 years or older with a normal medical history and examination. Exclusion criteria included known hypersensitivity to any drug, previous exposure to vancomycin, and receipt within the preceding 96 h of any medication likely to interfere with study endpoints, such as antihistamines. Subjects were instructed to avoid all medications including alcohol and caffeine throughout the study period.

Drug administration and sample collection. (i) **Skin testing.** Vancomycin hydrochloride in 500-mg vials (Vancocin; lot 36228AF; Eli Lilly & Co.) was used to prepare fresh skin testing solutions as described above for the pilot study. Intradermal skin tests were administered as described above, including the use of saline and histamine controls. The maximum weal-and-flare area for each dose was outlined with a pen by an investigator. Clear tape was applied to each test area to transfer the image, and the tape was then placed on graph paper (1 by 1 mm) to provide a permanent record. The area of flare was measured by counting squares as described previously (9). To determine whether skin tests were reproducible, subjects were retested with 50 $\mu\text{g/ml}$ 1 week after the infusion of vancomycin. The flare areas did not differ significantly, the mean coefficient of variation for the replicate flare areas was 13%, and there was a significant linear relationship between the two results ($r = 0.73$; $P < 0.01$). These data indicate that skin testing is reproducible and is not subject to a period effect.

(ii) **Vancomycin infusion.** One week after initial skin testing, vancomycin in 500-mg vials (Vancocin; lot 36228AF; Eli Lilly & Co.) for infusion was aseptically reconstituted according to the manufacturer's directions. The dose of vancomycin infusion (15 mg/kg of total body weight) was weight-normalized in order to produce similar concentrations in the sera of all subjects. The dose was diluted with 5% glucose, resulting in a final volume of approximately 300 ml. Vancomycin was administered over 1 h as a continuous infusion with a precalibrated volumetric infusion device (Travenol 8000 volumetric pump; Travenol Laboratories, Morton Grove, Ill.). Serial blood samples for vancomycin

concentration determinations were taken at the following times: -5 min (prior to infusion), then 15, 30, 45, and 60 min (during infusion), and 15, 30, 45 min and 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, and 24 h. Sera were separated by centrifugation at 3,500 rpm (Marathon 21K/BR; Fisher Scientific) for 10 min, and serum samples were stored at -70°C until the time of assay.

Vancomycin assay. Serum vancomycin concentrations were determined by fluorescent polarization immunoassay procedure (TDX; Abbott Diagnostic Division, Irving, Tex.) (22). The assay has a sensitivity limit of 0.6 $\mu\text{g/ml}$, and within-day and between-day coefficients of variation in the concentration range 7 to 80 $\mu\text{g/ml}$ were between 2 and 4%.

Analysis of cutaneous response. A dose-response curve for each subject was generated from a plot of the logarithm of the vancomycin concentrations versus flare areas. Different methods were used to assess and quantify the cutaneous response to intradermally administered vancomycin and are identified below. A representative log dose-response curve for one subject illustrates these measures of skin test responsiveness (Fig. 1). Model-independent measures of response included (i) the flare area from each skin test concentration, (ii) the maximum flare area, and (iii) the interpolated skin test dose from log-linear regression which produced a flare area of 400 mm^2 . Model-dependent parameters were determined by fitting the sigmoidal maximal flare model (Hill equation) by using a nonlinear least-squares minimization program (MINSQ) to each set of dose-response data (19). Fitted parameters included (iv) the dose that produced 50% of maximal flare, (v) the maximal flare, and (vi) the parameter n , representing the steepness of the dose-response curve. MINSQ includes a goodness-of-fit analysis called the model selection criterion (MSC) which is a modification of the Akaike information criterion (1). A higher MSC indicates an improved fit, and an MSC of at least 2 was required for the parameter estimates to be accepted into the statistical analysis.

Analysis of RMS. During infusion of vancomycin, subjects were evaluated for signs and symptoms consistent with RMS. Blood pressure and heart rate while setting were measured immediately after the collection of each blood sample during and for 2 h following infusion. The methods for assessing erythema, pruritus, and global severity score were the same as in a previous study (24) and are summarized below.

(i) **Erythema.** The area of erythema was estimated for each subject as a percentage of body surface area by using a burn chart. The extent and severity of erythema were scored as follows: erythema area of 1 to 5% of body surface area, mild severity (score of 1); erythema area of 5 to 10% of body surface area, moderate severity (score of 2); and erythema area of $>10\%$ of body surface area, severe (score of 3).

(ii) **Pruritus.** The maximum intensity of itching was determined by the subject and was classified a priori as no reaction (score of 0) or a mild (score of 1), moderate (score of 2), or severe (score of 3) reaction.

(iii) **Global severity.** The global severity score was the sum of severity scores for erythema and pruritus. Global severity was graded by the following criteria: no reaction (total score, 0) or mild (total score, 1 to 2), moderate (total score, 3 to 4), or severe (total score, 5 to 6) reaction.

Analysis of vancomycin concentrations. Vancomycin concentrations were measured primarily to confirm similarities in pharmacokinetic parameters between subjects; detailed pharmacokinetic analysis of vancomycin in normal adults have been described previously (13). The nonlinear least-

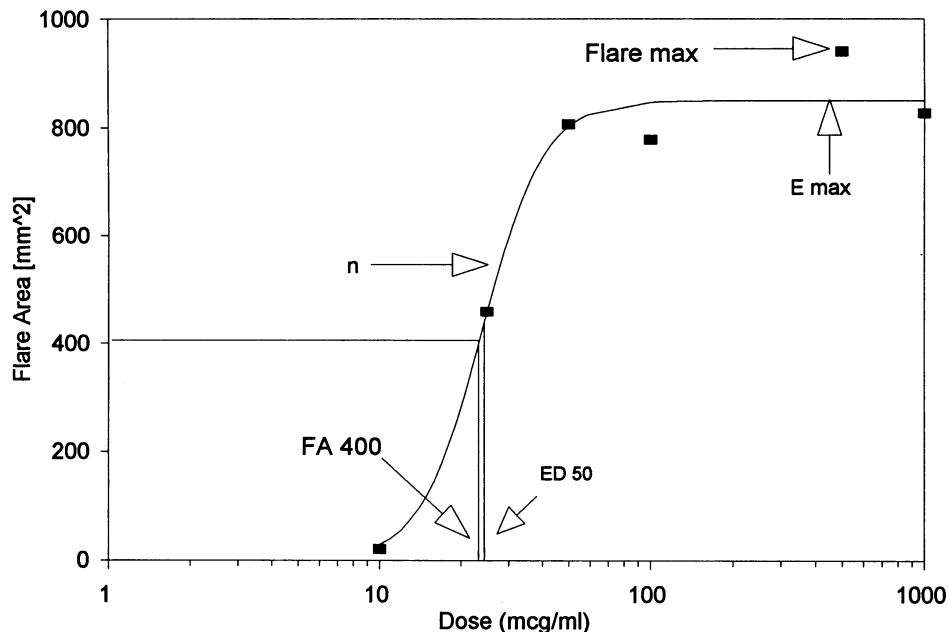


FIG. 1. Log dose-response curve for a representative subject illustrating the fitting to the Hill equation and measures of skin test reactivity. FA_{400} , interpolated skin test dose from log-linear regression which produced a flare area of 400 mm^2 ; $Flare_{max}$, maximum flare area; n , steepness of the dose-response curve; ED_{50} , dose that produced 50% of maximal flare; E_{max} , maximal flare.

squares regression curve-fitting program RSTRIP was used to generate model-dependent parameter estimates including the terminal elimination rate (18). A two-compartment model was found to provide the best fit to the data, assessed by the MSC. The area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) for vancomycin was calculated from postinfusion data by the trapezoidal rule, and the AUC from time zero to infinity ($AUC_{0-\infty}$) was calculated by adding the extrapolated area, determined from the elimination rate and the last measurable datum point, to AUC_{0-24} . Total clearance was calculated as $dose/AUC_{0-\infty}$ (12). The maximum concentration of drug in serum was identified by visual inspection.

Statistical analysis. Descriptive statistics are reported as means and standard deviations. Measures of skin test reactivity (methods described above) were independent variables and were regressed against percent erythema (using linear regression), pruritus, and global severity score (using Spearman's rank correlation). Significance was defined as $P < 0.05$.

RESULTS

Eleven of 12 subjects completed the study. The mean age and weight were 25 ± 3 years and 81 ± 20 kg, respectively. One subject was withdrawn when he developed a near syncopal episode with marked sinus bradycardia and left anterior fascicular block during venipuncture immediately before the infusion of vancomycin.

Vancomycin pharmacokinetics. The mean $AUC_{0-\infty}$ of vancomycin was $157 \pm 16.8 \mu\text{g} \cdot \text{h/ml}$. Total clearance was 0.097 ± 0.010 liter/h/kg. The mean peak concentration in serum was $62 \pm 7 \mu\text{g/ml}$. The coefficient of variation for all of these parameters was approximately 10%, indicating that systemic exposure to parenterally administered vancomycin was similar for all subjects. There was no significant correlation

between peak concentration in serum or AUC and any measure of RMS.

Cutaneous reactivity. All subjects exhibited a weal and flare from intradermally administered histamine; there were no reactions to saline. Weal and flare to intradermally administered vancomycin occurred in all subjects at all concentrations tested. The weal areas remained relatively constant, regardless of the vancomycin concentration, whereas the flare area increased in a dose-related manner. A summary of skin test reactivity is given in Table 1. Figure 2 displays the mean log dose-response curve. Because of single outlying values in three subjects, the maximal flare model did not adequately fit the dose-response curves (MSCs for all three subjects were less than 2). These subjects were excluded from further statistical analysis of model-dependent measures of skin test reactivity, although their inclusion after the outlying values were deleted made no difference in outcome (see below).

RMS. All subjects experienced RMS. Erythema was observed in all subjects (moderate in two subjects and severe in nine subjects). The mean area of erythema was $28.6 \pm 25.1\%$ of body surface area. Ten of the 11 subjects (91%) experienced pruritus (mild in 3 subjects, moderate in 4 subjects, and severe in 3 subjects). Global reaction severity was rated as mild in one subject, moderate in four subjects, and severe in six subjects. Five subjects experienced hypotension at the end of the infusion (diastolic pressure, <70 mm Hg with a drop of >9 mm Hg from the baseline value). These five subjects were otherwise classified as having a severe reaction on the basis of the a priori criteria described previously (24). Other adverse reactions included nasal congestion ($n = 1$), tearing of the eyes ($n = 1$), swelling of the hands ($n = 2$), blanching of the hands ($n = 1$), and tightness and tingling of the face ($n = 2$).

Despite the relatively high frequency and intensity of systemic reactions to intravenously administered vancomy-

TABLE 1. Summary statistics for skin test reactivity^a

Subject no.	Flare area (mm ²) versus vancomycin skin tests for the following skin test concn (μg/ml)						FA ₄₀₀ (mm ²)	Flare _{max} (mm ²)	n	ED ₅₀ (μg/ml)	Erythema (%)
	10	25	50	100	500	1,000					
1	261	677	920	870	1,265	987	13.5	1,093	1.49	20	12.9
2 ^b	31	85	372	491	976	349	58.9	650	2.29	49	14
3	20	83	668	774	743	666	36.3	728	6.46	34	46.8
4 ^b	213	354	630	431	607	910	28.2	1,555	0.35	722	90.5
5	25	349	646	645	1,118	919	28.2	1,014	1.4	44	50
6 ^b	28	407	285	390	541	694	24.5	803	0.63	98	19.4
7	25	67	233	238	417	431	426	445	1.26	70	22.4
8	25	33	193	196	357	461	660	546	0.87	186	17.8
9	68	184	899	819	870	972	30.9	981	9.53	29	7
10	21	460	808	779	942	827	21.9	852	3.82	24	5.4
11	100	271	364	344	540	461	158	500	1.11	26	28.4
Mean ± SD	74 ± 85	270 ± 202	547 ± 268	543 ± 244	761 ± 296	698 ± 240					

^a FA₄₀₀, interpolated skin test dose from log-linear regression which produced a flare area of 400 mm²; Flare_{max}, maximum flare area; n, steepness of the dose-response curve; ED₅₀, dose that produced 50% of maximal flare.

^b Subjects with MSC of less than 2 (see Results).

cin and the wide range in skin test reactivities, there was no significant correlation between any measure of skin test results and systemic effects. The most strongly correlated parameter ($r = 0.13$; $P > 0.05$) was observed for the flare area from a dose of 25 μg/ml and percent erythema (Fig. 3).

DISCUSSION

The present trial is the first to systematically study skin reactivity to intradermal administration of vancomycin, although Lin (17) described a patient with possible immunoglobulin E-mediated allergy to vancomycin who demonstrated a positive reaction to intradermal injection of vancomycin at concentrations of 50 and 500 μg/ml. The first study hypothesis, that intradermally administered vancomycin would result in a dose-related dermal response, was confirmed. Although it is not possible to be certain of the mechanism of local erythema, vancomycin degranulates

human mast cells in vitro (15), suggesting that histamine is at least in part responsible. A number of other drugs demonstrate a similar dermal response following local application, including narcotics (4, 6, 8), neuromuscular blocking agents (9, 21), and platelet-activating factor and prostaglandin E₂ (27). Most of these agents have been shown to degranulate cutaneous mast cells, and the size of the weal and flare is thought to depend on the amount of histamine released and the amount of drug injected (2, 4, 6, 8, 10, 14, 20, 21). It is not known why these agents cause mast cell degranulation (16).

The lack of a significant relationship between skin test reactivity and severity of anaphylactoid reactions to vancomycin is not completely surprising. Although skin test reactivity is often useful in predicting immunoglobulin E-mediated allergy (5), the predictive value of skin testing for drugs which release histamine by a nonimmunologic mechanism has been inconsistent (3, 6, 7, 26). For example, the dermal response to skin testing with skeletal muscle relaxants failed to correlate with the frequency of their adverse reactions following systemic administration (11). Reasons for the lack of correlation between skin test reactivity and RMS include the possibility that histamine may not be the sole mediator of local or generalized erythema, the subjective nature of most

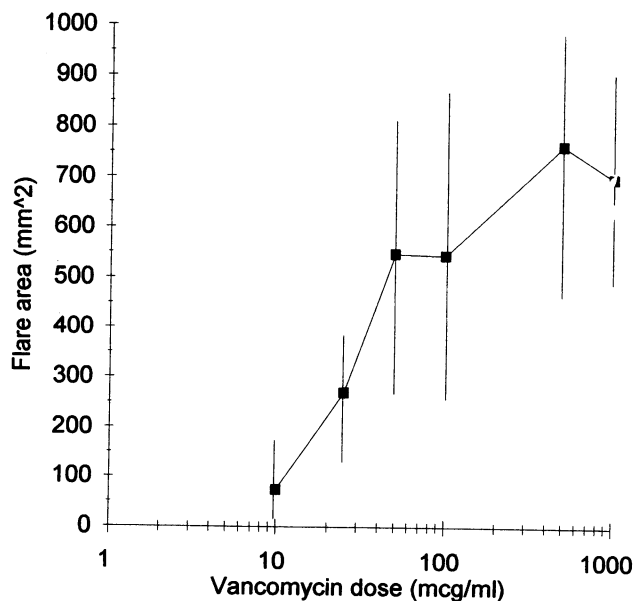


FIG. 2. Mean log dose-response curve (\pm SD) to intradermal vancomycin. mm², mm².

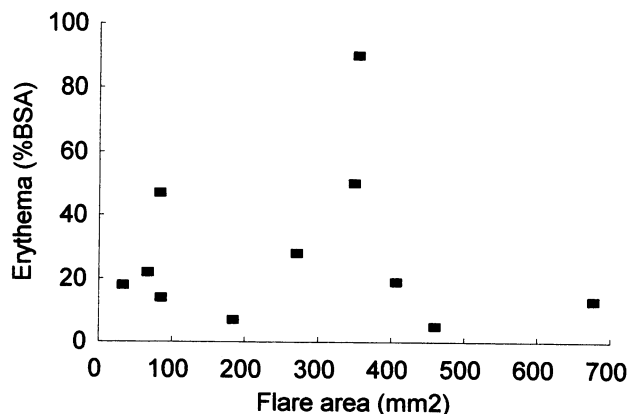


FIG. 3. Correlation between flare area from the 25-μg/ml skin test and percent erythema following parenteral vancomycin. BSA, body surface area. mm², mm².

of the signs and symptoms of RMS, between-subject variability in dermal concentrations of vancomycin following parenteral administration, between-subject variability in distribution of dermal mast cells, and beta error. Inspection of Fig. 3 suggests that the last reason is unlikely or that if a significant relationship does exist in the population, it is clinically unimportant.

The data presented here also indicate that vancomycin skin tests may not be useful for diagnosing immunoglobulin E-mediated allergy to vancomycin since all subjects had a positive reaction at concentrations of $\geq 10 \mu\text{g/ml}$. Whether a lower skin test dose could prove useful in the identification of allergic individuals remains to be determined.

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