Induction of Tolerance to Poison Ivy Urushiol in the Guinea Pig by Epicutaneous Application of the Structural Analog 5-Methyl-3-n-pentadecylcatechol

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Previous studies have established that epicutaneous application of 5-methyl-3-n-pentadecylcatechol (5-Me-PDC), a synthetic analog of a poison ivy urushiol component, leads to immune tolerance to 3-n-pentadecylcatechol (PDC) in mice. The induction of tolerance by 5-Me-PDC may be mediated by a protein conjugate formed via selective reaction of thiol nucleophiles present on the carrier macromolecule with the corresponding *o*-quinone derived from the parent catechol. In order to examine further the tolerogenic properties of 5-Me-PDC, we have extended our studies to the guinea pig, the generally accepted experimental species for the study of contact allergy. The results have established that specific immune tolerance to poison ivy urushiol is induced following 2 epicutaneous applications of the PDC analog. Furthermore, we were able to show that the treated animals remained tolerant for at least 6 weeks, a period of time comparable to that observed following the intravenous administration of the O,O-*bis*-acetyl derivative of PDC. The data point to the possibility of developing a therapeutically effective topical tolerogen for poison ivy contact dermatitis. *J Invest Dermatol 86:535–538, 1986*

The allergenic principles of poison ivy (*Toxicodendron radicans*), a plant from the Anacardiaceae family, are a mixture of catechol containing compounds, collectively called urushiol, which bear a 15 carbon straight alk(en)yl side chain at C-3 [1–3]. Human or animal (guinea pig, mouse) sensitization with urushiol or 3-n-pentadecylcatechol (PDC) (Fig 1) leads to a typical delayed type contact hypersensitivity. It is generally accepted that the ability of a chemical contactant to induce sensitization or tolerance is related to its potential to bind covalently with a self-protein to form a complete antigen. Although variation in the catechol ring substituents and the length of the hydrocarbon side chain influence the immunologic response to urushiol [4,5], antigen formation presumably proceeds via the corresponding *o*-quinone intermediates which may function as Michael acceptors.

We have found that the synthetic *o*-quinone derived from PDC reacts with amino and thiol nucleophiles in a regiospecific manner [6]. Amino nucleophiles attack exclusively the 5-position whereas thiol nucleophiles attack the 6-position. The analogous regiospecificity was observed with ring-methylated PDC derivatives including 5-methyl-3-n-pentadecylcatechol (5-Me-PDC) (Fig 1). Since the 5-position on the catechol ring of 5-Me-PDC is blocked, the corresponding quinone is protected from nucleophilic attack by amino groups. Additionally, our studies have established that 5-Me-PDC is a weak sensitizer and a good tolerogen (see *Results*), properties not shared by the corresponding 4- and 6-methylated regioisomers which are good sensitizing agents only [7]. Based on these results we have speculated that the induction of immune tolerance in the mouse may be mediated by thiol-linked protein conjugates of the hapten [7].

Although genetic homogeneity considerations make the mouse a useful model for contact sensitivity studies, it does not provide as good an opportunity to study tolerance because the hypersensitivity generally is lost within 2–3 weeks after sensitization due to the induction of negative regulating antibodies (anti-idiotypic antibodies) [8,9]. Therefore, in order to characterize more fully the immunologic properties of 5-Me-PDC, we have extended our studies to the guinea pig which remains the standard experimental species for the study of contact allergy.

MATERIALS AND METHODS

Animals Female outbred Hartley strain guinea pigs were obtained from the Animal Care Unit, University of California, San Francisco. They were fed on a pelleted diet and supplied with water ad libitum.

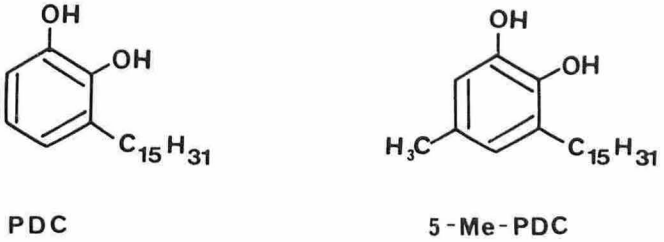

Figure 1. 3-n-Pentadecylcatechol (*PDC*) a component from poison ivy urushiol and 5-methyl-3-n-pentadecylcatechol (*5-Me-PDC*).

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Abbreviations:
DNFB: 2,4-dinitrofluorobenzene
5-Me-PDC: 5-methyl-3-n-pentadecylcatechol
PCD: 3-n-pentadecylcatechol

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Table I. Challenge Tests with Urushiol on Urushiol-Sensitized Guinea Pigs

Amount[a] in μg (nmol)	Reading (h)	Skin Reaction[b] 3	2	1	0.5	0	Sensitive/Total	Average Response[c]
12.5 (40)	24	0	3	3	1	0	7/7	1.36
	48	0	4	3	0	0	7/7	1.57
	72	0	4	3	0	0	7/7	1.57
	96	0	2	4	1	0	7/7	1.21
6.25 (20)	24	0	0	5	2	0	7/7	0.86
	48	0	1	5	1	0	7/7	1.07
	72	0	3	3	1	0	7/7	1.35
	96	0	2	4	1	0	7/7	1.21
3.1 (10)	24	0	0	4	3	0	7/7	0.78
	48	0	0	3	3	1	6/7	0.64
	72	0	0	0	5	2	5/7	0.34

[a]An acetone:olive oil (4:1) solution (25 μl) containing the indicated amount was applied on a 2-cm² area of the clipped and shaved flank.

[b]Intensity of reaction to urushiol was evaluated 14 days after sensitization attempt according to the following scale: 0 = no reaction, 0.5 = discrete erythema, 1 = confluent erythema, 2 = intense erythema and swelling, 3 = severe erythema going well beyond the testing area.

[c]The values of this column are mathematical averages of the assigned scale, numbers attributed to skin reactions. The summation of skin reaction ratings is divided by the total number of animals in the group. These numerical values are not precise but do reflect the trend in the severity of the skin reactions observed.

Chemicals The synthesis of 5-Me-PDC will be described elsewhere.* Poison ivy urushiol was kindly provided by Dr. H. Baer (NIH, Bethesda, Maryland) and 2,4-dinitrofluorobenzene (DNFB) was purchased from the Aldrich Chemical Company.

Sensitization and Challenge Test Procedures Guinea pigs were sensitized to urushiol or DNFB epicutaneously. In brief, 1 mg (3.0 μmol) of urushiol in 100 μl of acetone:olive oil (4:1) was applied to a 4-cm² area of the clipped and shaved flank of the guinea pig. Following the same protocol, 0.5 mg (2.7 μmol) of DNFB in 100 μl of acetone:olive oil (4:1) was used. After a period of 15 days for urushiol and 5 days for DNFB the animals were challenged on a 2-cm² flank area with nonirritating concentrations of urushiol [6.25 μg (20 nmol) in 25 μl] or DNFB [50 μg (270 nmol) in 25 μl] in acetone or acetone:olive oil (4:1), respectively. Skin reactions were read 24, 48, 72, and 96 h after application of the challenge dose and were rated according to the following scale: 0 = no reaction, 0.5 = discrete erythema, 1 = confluent erythema, 2 = erythema and swelling (induration), 3 = severe erythema and swelling which extends well beyond the testing area, 4 = ulceration or necrosis.

For purposes of comparison only, a numerical "average response" value was calculated for each set of readings by summing the individual ratings and dividing the sum by the total number of animals in the experimental group.

Induction of Tolerance Guinea pigs were tolerized by epicutaneous application of 5-Me-PDC (1 mg, 3 μmol) dissolved in 100 μl acetone:olive oil (4:1) on a 4-cm² area of the clipped and shaved flank 4 weeks and 2 weeks before sensitization was attempted with urushiol, as described above. A different skin area was used for each application.

RESULTS

Assessment of Primary Irritating Threshold Dose Prior to initiating the study, we treated a group of naive animals with varying amounts of urushiol (50 μg/2 cm² to 3.1 μg/2 cm²) to determine the primary irritating threshold dose and the appropriate dose range to assess induction of hypersensitivity. Skin readings at 24, 48, and 72 h after urushiol painting established that 1 of 7 animals showed a mild skin irritation (0.5 rating) after exposure to 12.5 μg/2 cm² urushiol. Doses higher than 12.5 μg/2 cm² were toxic. No skin reaction could be observed with 6.25 μg or 3.1 μg urushiol. Therefore, although the 12.5-μg dose was included in some of our studies as an upper limit, we have used the nonirritating 6.25- and 3.1-μg doses only in the assessment of hypersensitivity induction. Primary irritation is also reflected in Table III (negative controls or group 3).

Sensitization to Urushiol A positive control group (group 1) consisting of 7 guinea pigs was sensitized to poison ivy urushiol by epicutaneous application according to the standard procedure described above. The results of this study are summarized in Table I and Fig 2. All animals in this positive control group were shown to be sensitized although 2 animals did not display a detectable reaction at the lowest (3.1 μg) challenge dose. Furthermore, the reactions reflect a true delayed type hypersensitization. The maximum intensity of the skin reactions was reached 48–72 h after the epicutaneous challenge, a response characteristic of allergenic long-chain catechols [7]. All animals challenged with the 6.25-μg dose displayed confluent erythema and infiltration. Based on these results, the 6.25-μg dose was considered to be an adequate challenge dose for these studies.

Induction of Immune Tolerance to Urushiol by Epicutaneous Pretreatment with 5-Me-PDC Group 2 animals were pretreated epicutaneously with 5-Me-PDC on day 1 and again, on a different skin area, on day 15. Two pretreatments were required to demonstrate a significant difference between the sensitized group and the 5-Me-PDC pretreated group in response to a challenging dose of urushiol. When 1 epicutaneous pretreatment was given, only 2 of 6 animals (data not shown) became tolerant to urushiol. After the second 5-Me-PDC application a rest of 15 days was allowed, then (day 30) the animals received a sensitizing dose of urushiol following the same protocol as described for group 1 animals. The animals finally were challenged with urushiol on day 44, 2 weeks after application of the sensitizing dose.

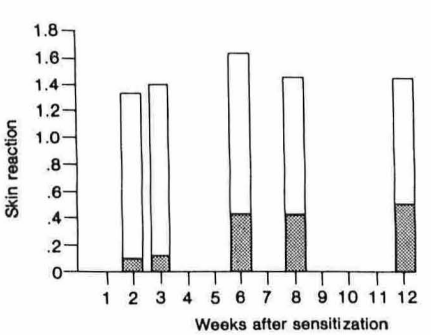

Figure 2. Specific induction of immune tolerance to urushiol by epicutaneous pretreatment with 5-Me-PDC. □, Group 1, sensitized to urushiol (positive control). ■, Group 2, tolerized with 5-Me-PDC.

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Table II. Skin Reactions to Urushiol Sensitized Guinea Pigs Pretreated with 5-Me-PDC (Group 2)[a]

Amount[a] in μg (nmol)	Reading (h)	Skin Reaction 3	2	1	0.5	0	Sensitive/Total	Average Response
12.5 (40)	24	0	0	0	1	7	1/8	<0.1
	48	0	0	1	1	6	2/8	0.2
	72	0	0	1	1	6	2/8	0.2
	96	0	0	1	3	4	4/8	0.4
6.25 (20)	24	0	0	0	0	8	0/8	0
	48	0	0	0	2	6	2/8	0.12
	72	0	0	0	2	6	2/8	0.12
	96	0	0	0	2	6	2/8	0.12

[a]See footnote to Table I for details.

Skin reactions were read 24, 48, 72, and 96 h after application of the challenging doses of urushiol. The results (Table II, Fig 2) clearly demonstrate that epicutaneous application of 5-Me-PDC induces immune tolerance to urushiol in the guinea pig. Thus with the nonirritating 6.25-μg challenge dose, only 2 animals gave a weakly positive reaction while the remaining 6 animals showed no skin reaction even after 72 h. The maximum "average response" in this group was only 0.12 which should be compared with the 1.35 value observed with the positive control group at 72 h. The fact that 2 of the 8 animals showed a moderate degree of sensitization may be ascribed to the genetic heterogeneity that exists in an outbred strain of a given species. It is also noteworthy that no spontaneous sensitization to 5-Me-PDC occurred on 5-Me-PDC-treated skin sites, although we did not perform challenge tests with the tolerogen.

Skin Reaction of Urushiol in Naive Guinea Pigs: Negative Control In order to provide an unambiguous comparison, a group of naive animals (group 3) was challenged on the same time and dosage schedule with urushiol as the urushiol-sensitized (group 1) and 5-Me-PDC-pretreated/urushiol treated (group 2) animals. The results (Table III, Fig 2) confirm our preliminary observation that a 12.5-μg challenge dose of urushiol is about the threshold irritating dose since 1 of the 8 animals in this group displayed a mild skin irritation. It will be noted that no evidence of irritation was observed with a 6.25-μg dose which was used as the challenge dose in our sensitization and tolerance studies.

Specificity of Tolerance Induced with 5-Me-PDC To determine whether the immune tolerance induced with 5-Me-PDC was specific to urushiol, we proceeded to sensitize the urushiol-tolerant guinea pigs (group 2) with a second potent contact sensitizer, DNFB. Guinea pigs which had been tolerized to urushiol with 5-Me-PDC 50 days earlier were treated with the sensitizing dose (0.5 mg) of DNFB and 5 days later were tested with the challenge dose (50 μg) of DNFB. Skin reactions were read 24 h after the application of the challenge dose. The results are summarized in Fig 2.

All of the 5-Me-PDC-pretreated animals (group 2), even though they still were nonresponsive to urushiol, could be sensitized to DNFB and displayed an average skin response of 1.7 which means that most of DNFB-challenged sites showed erythema and induration. Urushiol-tolerant guinea pigs and urushiol-hyposensitized guinea pigs responded equally well to DNFB. As a further check, a group of 3 naive animals were sensitized to and subsequently challenged with DNFB. The skin responses of this positive control group (data not shown) were essentially identical to those of the experimental group of urushiol-tolerant animals.

Persistence of Tolerance to Urushiol Following the initial challenge, group 2 animals were tested further at 2-week intervals with 6.25 μg of urushiol to assess the duration of the immune tolerance induced by 5-Me-PDC. Group 1 animals served as the control group. The maximum skin reactions (usually observed 72 h after application of the challenge dose) are recorded in Table IV and Fig 3. The urushiol-sensitized animals continued to give strong reactions to challenge doses of urushiol even after 12 weeks. It is possible that the exhaustive testing may have heightened the responsiveness of these animals. The tolerized animals, however, did not show a significant hypersensitivity reaction until week 6. Even after 8 weeks, 3 of the animals in this group of 8 still did not display a reaction.

DISCUSSION

These studies have provided additional evidence that the 5-methyl analog of PDC is capable of inducing tolerance to urushiol in experimental animals. In view of the structural similarity of this tolerogen to immunogenic substances including PDC, 4-Me-PDC, 6-Me-PDC, and urushiol itself, the influence of the 5-position in this system in directing the immune response is remarkable. One interpretation of these results is based on our chemical model studies with various ring-methylated analogs of PDC. The key

Table III. Skin Reactions to Urushiol on Untreated Guinea Pigs (Negative Controls—Primary Toxicity Assessment) (Group 3)

Amount[a] in μg (nmol)	Reading (h)	Skin Reaction 3	2	1	0.5	0	Positive/Total	Average Response
25 (80)	24	0	0	7	1	0	8/8	0.94
	48	0	0	6	2	0	8/8	0.87
	72	0	0	4	2	2	6/8	0.62
12.5 (40)	24	0	0	0	0	8	0/8	0
	48	0	0	0	1	7	1/8	0.1
	72	0	0	0	1	7	1/8	0.1
6.25 (20)	24	0	0	0	0	8	0/8	0
	48	0	0	0	0	8	0/8	0
	72	0	0	0	0	8	0/8	0
3.1 (10)	24	0	0	0	0	8	0/8	0
	48	0	0	0	0	8	0/8	0

[a]See footnote to Table I for details.

Table IV. Persistence of Immune Tolerance to Urushiol Induced with 5-Me-PDC

Weeks after Sensitization Attempt	Group 1		Group 2	
	A	B	A	B
2	7/7	1.06	2/8	0.12
3	7/7	1.14	2/8	0.12
6	6/7	1.50	4/8	0.44
8	6/7	1.39	4/8	0.44
12	6/7	1.43	5/8	0.50

A = Number of animals sensitive to urushiol out of a total number of animals in the group. B = Average of skin reaction (see footnote to Table I for definition).

finding of that study is that the electrophilic quinones derived from the parent haptens react in a regiospecific manner with model amino (at C-5) and thiol (at C-6) nucleophiles [6]. It may be reasonable to assume that the selectivity observed in these model reactions also obtains in the critical protein conjugation reactions which convert the hapten to the complete antigen. Such an interpretation is consistent with the proposal that linkage of the hapten to the carrier protein via a sulfhydryl bond might lead to the selective induction of suppressor cells whereas linkage via an amino nucleophile might lead to the selective induction of effector cells. Subsequent studies with DNFB and 2,4-dinitrocyanatobenzene have led to similar conclusions [10]. The highly electrophilic DNFB is a potent sensitizer and forms amino-linked protein conjugates whereas the less reactive cyanato analog is a tolerogen and forms thiol-linked protein conjugates. The reactivity of the quinone derived from 5-Me-PDC with sulfhydral reagents also points to the possibility that such an intermediate should react readily with glutathione. In a separate study we have demonstrated the chemical feasibility of this pathway by isolation and chemical characterization of the 6-S-N-acetylcysteinyl adduct of 5-Me-PDC.* The mechanisms by which small organic compounds induce delayed contact hypersensitization must involve

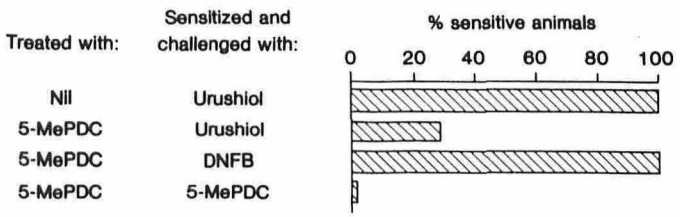

Figure 3. Persistence of tolerance to urushiol induced by epicutaneous pretreatment with 5-Me-PDC.

*D.J. Liberato, J.-L. Stampf, V. Byers, and N. Castagnoli, Jr., manuscript in preparation.

complex pathways and a variety of regulatory factors [9]. The induction of tolerance and hyposensitization to urushiol in guinea pigs by i.v. injection of PDC-associated autologous red blood cells 2 weeks prior to attempted contact sensitization [11] or i.v. administration of O,O-*bis*acetyl-PDC [12] have been reported. The present contribution establishes that a ring-methylated derivative of PDC can induce tolerance by epicutaneous application. The duration of the induced tolerance by the i.v. administration of the *bis*acetyl derivative and by the epicutaneous administration of the 5-Me-PDC (6–8 weeks) is comparable. The possibility of developing an analog of PDC with therapeutic potential based on further structural modification of the catechol ring is currently under consideration.

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