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Vaccine delivery by precipitation (VDBP) induces tolerance to poison ivy.

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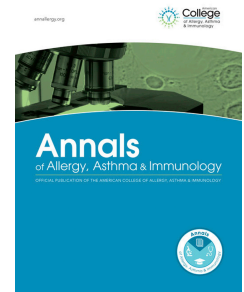
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1 TITLE PAGE

2 Letter

3
4 Title: Vaccine delivery by precipitation (VDBP) induces tolerance to poison ivy.

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6
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13 Conflicts of interest: The authors hold US and EU patents for the technology reported in
14 this manuscript.15
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Vaccine delivery by precipitation (VDBP) induces tolerance to poison ivy

Key words:

Immunotherapy; Poison Ivy; Urushiol.

Abbreviations used:

GC/MS = Gas chromatography / mass spectrometry

IM = Intramuscular injection

PI = Poison ivy

Pi1 to *Pi7* = Vaccine formulations *Pi1* to *Pi7*

Reduct-fold = factor by which patch test sensitivity is reduced typically 4-16 weeks
following treatment

SQ = Subcutaneous injection

Tx = Treatment

VDBP = Vaccine Delivery by Precipitation

Previous poison ivy vaccines, comprising urushiol in sterile vegetable oils injected SQ, were withdrawn in 1994 for failure to demonstrate statistical efficacy (personal communication J.Slater). We'd found them anecdotally safe and effective in some patients. For highly allergic patients for whom avoidance was not practical we offered immunotherapy with home-made vaccines in accordance with Declaration of Helsinki guidelines for ethical use of "unproven interventions"¹. Informed consent was obtained from all patients. For simplicity and because it's self-sterilizing we extracted urushiol and prepared vaccines in ethanol. We injected it IM because concentrated ethanol is a tissue irritant and small volumes injected IM would be rapidly diluted by tissue fluid. We measured urushiol concentration by GC/MS. We quantitated patch test sensitivity by the method of Marks² with 10-fold increasing concentrations of urushiol and used the number of micrograms of urushiol to produce Marks's "grade 3" reaction as our measure of sensitivity. The most sensitive two of our first four patients achieved durable, measurable tolerance³, challenging us to figure out how we were achieving more effective tolerance to poison ivy than ever reported in previously sensitized patients and how to increase the % responding.

Previous poison ivy vaccines in vegetable oils became repositories from which urushiol diffused by the molecule. Vaccines in ethanol injected IM precipitate solid urushiol particles within the injected muscle as the ethanol is diluted by tissue water and the urushiol becomes insoluble. The more rapid the dilution, the larger the number and smaller the size of the particles. Xiang et al reviewed uptake of vaccines as a function

of particle size. They reported efficient naive dendritic cell uptake of 0.5 to 5 μ particles by macropinocytosis⁴.

0.1 ml of a 25 mg/ml solution of urushiol in ethanol contains 2.5 μ g urushiol. If precipitated into 2 μ diameter particles it will deposit 0.6 billion such particles around the injection site. VDBP deposits hundreds of millions of antigen particles sized for efficient uptake by naive dendritic cells which in muscle appear to be of lineages and in a cytokine milieu favorable for tolerance induction.

Table 1 shows outcomes for our first 35 courses of treatment. *Pi1* and *Pi2* were crude 7 day ethanol extract of fresh leaves containing 1.12 and 1.92mg urushiol/ml. *Pi3* was a 50 mg/ml concentrate purified by the method of ElSohly⁵. *Pi4* was 75% by volume *Pi2* mixed with 25% *Pi3*. *Pi5* (50mg/ml) and *Pi6* (110mg/ml) were vacuum-concentrated unpurified crude extracts. *Pi7* was 50% *Pi6* and 50% *Pi2*.

The most sensitive two of our first four cases responded to cumulative urushiol doses of 0.78 to 0.84 mg. As injected ethanol volume is a limiting factor for both safety (increasing volume increases both discomfort and risk of tissue injury as larger volumes will be diluted more slowly) and efficacy (slower dilution of larger injected volumes will yield smaller numbers of larger particles and may miss the 0.5-5 μ target range for efficient dendritic cell uptake) we next studied *Pi3*, a concentrated formulation of urushiol purified by the method of ElSohly⁴. Patient 1 (the first author) didn't respond to

0.84 mg of crude extract vaccine *Pi1*. He had a 15-fold reduction in sensitivity to 77.2 mg in purified concentrate *Pi3* but with eosinophilia and dermographism lasting 3 months. Patient 11 had a 67-fold sensitivity reduction and 9-12 month clinical response to 3.61 mg of *Pi2* but did not respond to 6.6 mg of *Pi3*. We concluded that something with adjuvant activity in crude extracts is lost in purification, and that mixtures of crude ethanol extracts with concentrates to increase the dose of urushiol per unit volume of ethanol might be an effective way to increase dose without unacceptable increases in injected ethanol volume. We did this in vaccine *Pi4* with which patient 11 had a 10-fold sensitivity reduction and a clinical response still present at 36 months and patient 1 had a 1000-fold sensitivity reduction still present at 28 months.

If an essential adjuvant was lost in purification we thought vacuum concentration without purification might be a simple way to retain it, in vaccines *Pi5* and *Pi6*. As only 2 of 5 patients treated with these two vaccines had the 10-fold or greater sensitivity reduction that correlates with durable clinical response we decided to mix our most potent concentrate (*Pi6*) with an equal volume of fresh crude extract as vaccine *Pi7*.

Our observational series did not have pre-selected endpoints. The parameters we identified as most relevant are “reduct-fold,” the factor by which patch test sensitivity was reduced by treatment, and “Clinical response” (Yes or No as reported by the patient). When available we also tracked the intervals at which tolerance was still present (by tolerance to patch test &/or reported natural exposure) and lost. Cases #16,

18 and 30 and patient 24b with a blank space in the “reduct-fold” column reported convincing clinical tolerance to natural exposure but did not return for post-treatment patch tests.

Eighteen of 20 courses in 18 patients treated with vaccines containing at least 50% by volume of unmodified crude ethanol extract “spiked” with either purified or unpurified concentrated urushiol (to increase urushiol dose) produced convincing clinical &/or patch test tolerance at urushiol doses from 3.36 to 22.4 mg.

We found a 39,000-fold variation in pre-Tx patch test reactivity in patients reporting similar symptoms. “Reduct-fold” ≥ 10 had an excellent correlation with clinical response. Within this dose range for combination vaccines, pre-treatment sensitivity did not.

We found unpurified urushiol concentrates less stable than purified concentrates. Going forward we plan to apply for formal clinical trials with a combination vaccine $\sim Pi4$. We don’t believe it practical to attempt to identify the adjuvant in crude ethanol extracts without an animal model with a tight dose-response curve, which to our knowledge doesn’t exist.

Treatment with ~ 0.5 , ~ 2 and ~ 20 mg urushiol at 2-6 week intervals was safe and effective for most patients. Some needed short term prednisone &/or topical

106 triamcinolone for injection site reactions, more often by patients requesting treatment in
107 less steps to reduce travel. This did not appear to affect outcomes.

108
109 The standard for disclosure in scientific reporting is increasingly becoming that of sufficient
110 detail to enable a reader to completely reproduce the reported observations. However, we
111 encourage readers of this article NOT to try to reproduce our work without a way to measure the
112 urushiol content of their vaccines. The reason is a greater than 10-fold variation in the urushiol
113 content of similarly prepared crude ethanol extracts, coupled with incomplete knowledge of the
114 factors that affect that yield. Without knowing content it's impossible to extrapolate our
115 experience for either safety or efficacy.

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Table 1		CLINICAL EXPERIENCE WITH IM POISON IV TUBERCULOID VACCINES IN ETHANOL 2009-2017																
case	pt #	pt	Tx	Pre-Tx	Inj	Tx	Ratio Tx to	Post-Tx	time	reduct-	Clinical	Response	Lost	Adverse				
			complete	grade-3 µg	Tx dose mg	vol ml	# steps	pre-sens	Gr3	grade-3 µg	post-Tx	fold	Vaccine	response	maintained	protection by	Effect	
1	1a	70y M	06/24/09	0.5085	0.84	0.86	6	1652	1.13	1m	2	Pi 1	N	n/a	n/a			
2	2	31y M	08/06/09	0.0226	0.78	0.96	7	34425	2.26	9m>3wk	100	Pi 1	Y	9m	14 mo			
3	3	57y F	09/08/09	0.113	0.84	0.96	7	7442	0.113	2m	1	Pi 1	N	n/a	n/a			
4	4	14y F	12/26/09	0.005085	0.84	0.52	8	165388	0.113	3m=3wk	22	Pi 1	Y	3m+	??			
5	8a	66y F	07/26/10	0.00384	1.43	0.96	7	371875	19.2	8m>1m	5000	Pi 2	Y	40 m+	63 mo			
6	10	12y M	09/21/10	0.0576	1.43	0.99	8	24809	0.0576	1m	1	Pi 2	N	n/a	n/a			
7	11a	65y F	07/15/11	0.0576	3.61	1.71	10	62674	3.84	2m	67	Pi 2	Y	9m	12 mo			
8	1b	73y M	04/23/12	0.5	77.20	2.49	14	154400	7.5	1m=3m	15	Pi 3	No exposure	>3, < 16 mos	16 mo		1	
9	11b	66y F	12/27/12	0.05	6.60	0.71	7	132100	0.05	3m	1	Pi 3	N	n/a	n/a			
10	11c	67y F	10/10/13	0.05	2.59	0.44	2	51720	0.5	3 wks	10	Pi 4	Y	36m+			2	
11	1c	74y M	11/14/13	0.5	15.93	1.23	8	31860	500	6m=14mo	1000	Pi 4	No exposure	28m+			3	
12	19	55y F	04/08/14	0.015	7.56	0.44	5	504000	1	6 wk	67	Pi 5	Y	6m+				
13	14	57yM	07/31/14	22.5	17.56	0.54	6	780	22.5	0d	1	Pi 5	Y					
NOTE: Tested same day completed treatment as patient moving out of area																		
14	20	53y M	08/14/14	0.225	17.56	0.64	6	78044	0.5	4m	2	Pi 5	Y					
NOTE: Above patient taking adalimumab for psoriasis from Dec 2013. ?? if improved tolerance to natural PI exposure 2/ IT, that drug or both.																		
15	21	65y F	08/21/14	0.015	17.56	0.64	6	1170667	0.5	4m	33	Pi 5	Y	16m+ clin	16 mo patch			
16	23	23y F	06/29/15	5	17.37	1.24	7	3474	Didn't return for post-Tx testing		NT	Pi 4	Y: Farmer, reported full tolerance to same exposure.					
17	24a	25y M	06/22/15	0.035	17.37	1.24	7	496286	5	2m	143	Pi 4	Y	3m+	4m partial			
18	24b		09/30/15	Tree trimmer c/partial loss /tol @ 4 mo, full restore after 11.2 mg booster									Pi 4	Y	14m+			
19	28	29y M	08/06/15	5	18.2	1.3	2	3640	Didn't return for post-Tx testing		NT	Pi 4	Y: Tree-trimmer reported full tolerance.				4	
20	26	16y M	10/05/15	0.0225	17.37	1.24	7	772000	0.015	1m	1	Pi 4	No exposure					
21	31a	65y M	10/21/15	150	17.92	0.32	2	119	1500		10	Pi7	Y (VG not E)	3m+				
22	31b		03/24/16	VG but not 100% initial protection, 100% for 9m+ after 16.8 mg booster									Pi7	Y	9m+			
23	8b	72y F	02/04/16	0.5	8.68	0.36	6	17360	1000	3m	2000	Pi7	Y	6m+			5	
24	37a	47y F	02/08/16	0.5	18.7	0.17	2	37400	0.33	3m	1	Pi6	Unsure (dog)					
25	37b	47y F	05/09/16	0.35	17.9	0.32	1	51143	5	6 wk	14	Pi7	Y	11m+				
26	35	46y F	03/31/16	0.023	17.9	0.32	1	778261	25	3m	1087	Pi7	Y	6m+			6	
27	36	15y M	03/31/16	0.05	17.9	0.32	1	358000	1.5	3m	30	Pi7	Y	6m+				
28	38	44y F	05/09/16	0.225	17.9	0.32	2	79556	2.25	7m	10	Pi7	Y	6m+				
29	42	53y M	07/25/16	0.15	22.4	0.4	1	149333	3.5	6m	23	Pi7	Y	6m+			7	
30	43	24y M	08/04/16	0.05	21.3	0.38	2	426000	22.5	3m	450	Pi7	Y	7m+				
31	39	47y F	07/21/16	0.0035	3.36	0.06	1	960000	0.225	6m	64	Pi7	Y	7m+			8	
32	46	39y M	08/29/16	0.005	22.4	0.4	1	4480000	Didn't return for post-Tx testing		NT	Pi7	Y					
33	47	59y F	09/22/16	0.011	5.04	0.18	2	458182	7.7	3m	700	Pi7	Y	7m+				
34	48	71y F	10/12/16	0.035	0.56	0.1	1	16000	Pt chose to stop after 1st dose		NT	Pi7	(N)				9	
35	49	64y F	02/06/17	0.5	5.04	0.18	2	10080	0.5	6 wk	1	Pi7	?				10	

var x 39K

Adverse effects:

- 1 Dermographism lasting ~3 mos with eosinophilia (to 1200 /mm3) after high dose (total 77 mg) urushiol. Eos 300 / mm3 @ 4 mos.
- 2 Slowly resolving (weeks) tender local "lumps" to 3 cm on abbreviated (rapid build-up) dosing schedule. Did not give prednisone.
- 3 Eosinophilia (to 800 /mm3) w/out dermatographism 2 days after cumulative 16 mg, 100 / mm3 @ 7 mos.
- 4 Painful local swelling required 4 days' prednisone 40 mg/d for each of 2 doses.
- 5 Painful local induration to 6 cm by 6d @ step 4 of 5 dose sched., resp to 4 days pred 40 mg/d then QOD taper 20, 10, d/c Good clin & patch test resp s/ 5th dose.
- 6 Dermographism c/ Eos up from baseline 210 to 440 / mm3
- 7 Transient tender lumps @ 1 step inj sites, local PI @ 1 of 4 sites, all QNS/want Tx. By 12d mild PI partially o/lapping sites /recent gr 6&7 patch test reactions. Eos 300 (base 200).
- 8 Tender swelling lasting 2d @ ea of 1st pr of 1.68 mg inj's (planned 2-step schedule). Stopped Tx 2/ good clinical control c/ response confirmed by post-Tx patch.
- 9 Vesicular reaction at 1st dose shot sites, triamcinolone QNS to control, needed 7 day pred taper. Did not achieve protection but decided not to continue Tx.
- 10 Mild local / 1st set shots, uncomfortable local lumps + o/lying PI dermatitis lasting 3 wks despite topical triamcinolone / 2nd dose; 3rd dose deferred.