

Cross-Reactivity Studies of Gutta-Percha, Gutta-Balata, and Natural Rubber Latex (*Hevea brasiliensis*)

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Gutta-percha and gutta-balata are derived from the *Paliquium gutta* and *Mimusops globsa* trees, respectively, that are in the same botanical family as the rubber tree *Hevea brasiliensis*. For this reason the potential for immunological cross-reactivity between the gutta-percha and gutta-balata used in endodontics and natural rubber latex (NRL) has been the subject of some controversy, because these products may be used in latex-allergic individuals. The objective of this study was to investigate the potential cross-reactivity between gutta-percha, gutta-balata, and NRL. Physiological extracts of seven commercially available gutta-percha products, raw gutta-percha, raw gutta-balata, and synthetic *trans*-polyisoprene were each analyzed for cross-reactivity with NRL in a competitive radioallergosorbent test inhibition assay. No detectable cross-reactivity was observed with any of the raw or clinically used gutta-percha products. In contrast the raw gutta-balata released proteins that were cross-reactive with *Hevea* latex. We conclude that the absence of gutta-percha proteins that can react with *Hevea* latex-specific IgE antibody supports the minimal potential for commercially available gutta-percha to induce allergic symptoms in individuals sensitized to NRL. Because gutta-balata is sometimes added to commercial gutta-percha products caution should be exercised if products containing gutta-balata are used in endodontic care of latex-allergic individuals.

Reports of immediate-type hypersensitivity to natural rubber latex (NRL), produced by the rubber tree *Hevea brasiliensis*, have increased dramatically since the first case of latex hypersensitivity was reported in 1979 (1). This has been partly attributed to the increased use of rubber medical products after the Center for Disease Control and Prevention's recommendation for universal infection control precautions in 1986. Today an estimated 5% to

17% of adult health care workers and up to 67% of children with spina bifida have become sensitized to NRL (2, 3).

Because avoidance of latex allergen exposure is the only practical method of managing latex-allergic individuals, medical and dental environments have been particularly scrutinized with regard to the removal of *Hevea* latex-containing products. In the dental office powdered latex gloves, latex rubber dams, and other dipped NRL-containing products have been obvious targets for replacement with synthetic alternatives.

Another concern is the potential for cross-reactivity between gutta-percha and NRL. Gutta-percha is a naturally occurring *trans*-polyisoprene polymer that is extracted in Southeast Asia from the leaves of the *Palaquium* (gutta-percha) tree. Gutta-balata is a naturally occurring *trans*-polyisoprene polymer that is extracted from the bark of the *Mimusops globsa* tree. The *trans* form of the polyisoprene produces a crystalline rigidity that contrasts with the elasticity of the *cis*-polyisoprene derived from the latex of the *Hevea brasiliensis* tree. The potential for cross-reactivity between gutta-percha, gutta-balata, and NRL is due to the fact that these products are derived from trees in the same botanical family.

Several studies have suggested that gutta-percha may release proteins that induce reactions in latex-allergic individuals. In 1986 Gazelius et al. (4) described a latex-allergic female nurse who experienced pulsating pain accompanied by tachycardia, dyspnea, and fever 2 h after obturation of her mandibular left second molar. Although these symptoms were uncharacteristic of those routinely experienced by patients with latex allergy, they were still attributed to the gutta-percha obturation because the pain persisted until the gutta-percha was removed 3 months later. A more recent report by Boxer et al. (5) described a latex-allergic dental hygienist who experienced immediate lip and gingival swelling and diffuse urticaria after the insertion of gutta-percha points into her maxillary molar by a general dentist. The case was referred to an endodontist due to persistent symptoms. Radiographic examination revealed overextended obturation of the palatal root canal. The endodontist retreated the tooth and placed silver points within the confines of the root canal. Immediately after removal of the gutta-percha, relief of the oral discomfort was noted, and the urticaria resolved several hours thereafter. These studies, however, have not proven cross-reactivity between *Hevea* latex and gutta-percha. Boxer's group was unsuccessful in extracting cross-reactive proteins from commercially available gutta-percha. Moreover Knowles et al. (6), and Kleier and Shibilski (7), presented case reports in which gutta-

TABLE 1. Description of test materials

Product Description	Type	Source
Raw gutta-percha	White raw*	Endoden Corp. (Monrovia, CA)
Raw gutta-balata	Natural raw*	Endoden Corp. (Monrovia, CA)
Gutta-percha	Cones	Union Broach (York, PA)
Gutta-percha	Cones	Hygenic Corp. (Mahwah, NJ)
Gutta-percha	Cones	Densply/Caulk Corp. (Milford, DE)
Gutta-percha	Pellets	Obtura Corp. (Fenton, MO)
Gutta-percha	Cones	Kerr Corp. (Orange, CA)
Gutta-percha	Cones	Henry Schein (Melville, NY)
Gutta-percha	Cartridges	Ultrafill Corp. (Mahwah, NJ)
Synthetic gutta-percha	Pellets/synthetic <i>trans</i> -polyisoprene*	Endoden Corp. (Monrovia, CA)
<i>Hevea brasiliensis</i> (positive control)	Powdered NRL examination glove*	Allegiance Corp. (Chicago, IL)
Vinyl (negative control)	Powdered vinyl examination glove*	Allegiance Corp. (Chicago, IL)

* These materials contain no filler, plasticizers, or radiopaque material.

percha was successfully used to obturate teeth in patients with a documented IgE antibody-mediated allergy to NRL. They observed no associated allergic symptoms. Thus these two cases dispute the presence of any *Hevea* latex cross-reactive protein in gutta-percha.

The purpose of this study was to use an established immunological method called the radioallergosorbent test (RAST) inhibition analysis to examine whether raw gutta-percha, raw gutta-balata, or seven clinically used gutta-percha products actually release proteins that cross-react with *Hevea* latex.

MATERIALS AND METHODS

Extraction of Test Materials

The raw gutta-percha (partially processed, deresinated, and polymerized), commercially available gutta-percha, gutta-balata, NRL, and vinyl products used in this study are described in Table 1.

Before extraction the raw gutta-percha, raw gutta-balata, and synthetic *trans*-polyisoprene samples that contained no filler, plasticizer, or radiopaque material additives were cut into 5 mm slivers. Commercially available gutta-percha cones containing ~20% gutta-percha, according to Friedman et al. (8), were cut into 5 mm lengths. Powdered examination latex gloves and vinyl gloves (Allegiance Corporation, Chicago, IL) were extracted as positive and negative controls, respectively. All these preparations were extracted using a ratio of 1 ml of extraction buffer (phosphate-buffered saline containing 1% bovine serum albumin and 0.05% Tween 20 (Sigma Chemical Company, St. Louis, MO) per gram of material) for 16 to 18 h at 23°C with constant agitation. The only exceptions were the Obtura (Obtura Corporation, Fenton, MO) and Ultrafill gutta-percha (Ultrafill Corporation, Mahwah, NJ) that were heated with their appropriate devices and injected as a fine thread into duplicate tubes containing 60°C extraction buffer (1 ml/g). These were extracted at 60°C for 16 h to maximize the removal of any protein. After extraction each test material was removed, the tube was centrifuged ($1,000 \times g$), and the supernatant was analyzed immediately.

RAST Inhibition Analysis

The gutta-percha and gutta-balata extracts were analyzed for cross-reactive allergen by competitive RAST inhibition analysis

(9). Dilutions of nonammoniated latex reference (0.1 ml, E8-Latex, FDA-CBER, Bethesda, MD), 100,000 AU/ml, in which 1 U = 38.9 ng tested at 10,000 to 0.17 units/ml), or test extracts were pipetted in duplicate 12 × 75 mm plastic test tubes (Sarstedt, Newton, NC) containing 0.1 ml of human IgE anti-latex serum pool. After 4-h incubation at 23°C, 0.3 ml of nonammoniated latex allergen-solid phase was added, and the mixture was rotated for 12 to 14 h at 23°C. After a buffer wash, ^{125} I-anti-human IgE Fc was added and the tubes rotated 12 to 14 h at 23°C. Following a final wash bound radioactivity was detected in a Cobra multiwell gamma counter (Packard Instruments, Downers Grove, IL). Allergen results were interpolated from an E8 latex (FDA-CBER, Bethesda, MD) reference curve and reported in nanograms of allergen per gram (ng/g) of test material.

The IgE anti-latex source was a pool of serum from 180 latex-allergic adults that has been shown to contain IgE antibody specific for the latex allergen Hev b 1-7 (data not shown).

RESULTS

No *Hevea* latex cross-reactive allergenic protein was detected in the extracts prepared from the vinyl glove (negative control). The powdered latex examination gloves (positive control) contained levels of protein (30,808 ng/g) that were cross-reactive with *Hevea* latex allergen-specific human IgE antibody.

No *Hevea* latex cross-reactive allergenic protein was detected in the extracts prepared from raw or commercially processed gutta-percha or from the synthetic *trans*-polyisoprene (Table 2). Levels below 40 ng/g represent a minimal risk to *Hevea* latex IgE-sensitized patients.

The raw gutta-balata extracts, however, contained levels of protein (237 ng/g) that were cross-reactive with *Hevea* latex allergen-specific human IgE antibody.

DISCUSSION

The procedures used in the manufacturing of commercial-grade gutta-percha differ widely from those used in the production of *Hevea* latex. Raw gutta-percha *trans*-polyisoprene is isolated from mature *Paliquium gutta* tree leaves that have been ground to a fine pulp (Lentz, personal communication). The pulp is mixed with water, heated to 75°C to release the gutta-percha threads, and then

TABLE 2. Results of the RAST inhibition assay analysis for cross-reactive allergen

Study Code	Product Description	Type	Source	Cross-reactive Latex Allergen* (ng/g)
A	Raw gutta-percha	White raw	Endoden Corp.	<40†
B	Raw balata	Natural raw	Endoden Corp.	237 (284, 187)
C	Gutta-percha‡	Cones	Union Broach	<40
D	Gutta-percha‡	Cones	Hygenic Corp.	<40
E	Gutta-percha‡	Cones	Densply/Caulk Corp.	<40
F	Gutta-percha‡	Pellets	Obtura Corp. —23°C	<40
G	Gutta-percha‡	Pellets	Obtura Corp.—200°/60°C	<40
H	Gutta-percha‡	Cones	Kerr Corp.	<40
I	Gutta-percha‡	Cones	Henry Schein	<40
J	Gutta-percha‡	Cartridges	Ultrafill Corp.—23°C	<40
K	Gutta-percha‡	Cartridges	Ultrafill Corp.—200°/60°C	<40
L	Gutta-percha‡	Pellets/synthetic <i>trans</i> -polyisoprene	Endoden Corp.	<40
M	<i>Hevea brasiliensis</i> (positive control)	Powdered latex examination glove	Allegiance Corp.	30,808
N	Vinyl (negative control)	Powdered vinyl examination glove	Allegiance Corp.	<40

* *Hevea brasiliensis* cross-reactive allergen in nanogram/gram as assessed in the RAST inhibition assay. Allergen measurements are the mean of duplicate extractions each analyzed in duplicate.

† Levels <40 ng/g represent a minimal risk to *Hevea* latex IgE-sensitized patients.

‡ Gutta-balata content as a percentage of total mass ranged from 0.25% to 0.36% in commercial gutta-percha products (10).

cooled to 45°C. The gutta-percha flocculates, producing a “yellow gutta” that contains 60% polyisoprene and 40% contaminants (resin, protein, dirt, and water). Conversion of the yellow gutta to the final ultrapure white gutta that is used commercially is accomplished by the Obach technique (Lentz, personal communication). This method involves mixing of yellow gutta with cold industrial gasoline at below 0°C temperatures.

The gasoline treatment not only flocculates the gutta-percha *trans*-polyisoprene, but it dissolves the resins and is believed to denature any residual gutta-percha proteins. After removal of the cold gasoline, deresinated gutta threads are redissolved in warm gasoline at 75°C, and the dirt particulate is allowed to precipitate. The residual greenish-yellow solution is bleached with activated clay, filtered to remove any remaining particulate, and then steam-distilled to remove the gasoline. The final ultrapure white gutta-percha has a gasoline scent before it is modified with fillers (sometimes including gutta-balata) into its final commercial product formulation.

Ten tons of gutta leaves yields 0.13 tons of white raw gutta-percha that is composed of 92% polyisoprene, 1.5% resin, and 6.5% water (8, 10). Importantly, however, the harsh organic solvent purification results in the denaturation of any plant protein that resides in the unprocessed yellow gutta-percha. The white gutta-percha is further diluted with fillers, radiopaque material, and plasticizers such that the final gutta-percha cones used in endodontic procedures contain ~20% gutta-percha, 56% zinc oxide filler, 11% radiopacifier (barium sulfate), and 3% plasticizers (waxes or resins) (8). Occasionally when gutta-percha is in short supply, the manufacturers add some gutta-balata or synthetic *trans*-polyisoprene to the gutta-percha cones. The synthetic *trans*-polyisoprene would not be expected to contain any *Hevea* latex cross-reactive proteins. It is, however, a problem when gutta-balata is added, because the manufacturers do not disclose its presence or percentage and our study has shown that raw gutta-balata releases proteins that cross-react with *Hevea* latex. Use of a gutta-balata-containing product could potentially place a highly latex allergic patient at risk for an allergic reaction even when proper instrumentation and obturation techniques are used to confine the material within the root canal system. It may also be a particular problem for the dentist who treats an asymptomatic individual who is in the early

stages of their sensitization and therefore provides a negative history of latex allergy despite having detectable latex-specific IgE antibody in their skin and/or blood (11).

According to Mr. Nicholas Lentz, Vice-President of Endodont, Inc., one of the direct manufacturers of gutta-percha in the United States, the percentage of gutta-balata in relation to the total mass of other components (gutta-percha and additives) in the commercially available cones is reportedly very low (<1% of total mass) (Lentz, personal communication).

The practical risk of using gutta-percha products with gutta-balata additive may therefore be considered minor. This would seem to be in agreement with the clinical findings of Knowles et al. (6) and Kleier and Shibilski (7), who used commercially available gutta-percha to obturate canals in highly sensitized NRL-allergic patients without precipitating any allergic reactions.

In conclusion the absence of extractable protein from raw and commercial sources of gutta-percha that is cross-reactive with *Hevea* latex indicates that their use in individuals who are sensitized (IgE antibody positive) to NRL represents a minimal risk for the induction of allergic symptoms. Manufacturers of gutta-percha products should be encouraged to eliminate the addition of any gutta-balata in their formulation, because raw gutta-balata release protein that can cross-react with *Hevea* latex and that may place a NRL-allergic individual at risk for allergic reactions.

The opinions or assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy, Department of Defense, or the United States Government.

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