Single-Arm Study for the Characterization of Human Tissue Response to Injectable Poly-L-Lactic Acid

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BACKGROUND Injectable poly-L-lactic acid (PLLA) is a synthetic polymer indicated for the correction of facial wrinkles and folds. Animal studies have shown that implantation of PLLA stimulates collagen synthesis; human studies have been limited.

OBJECTIVE To investigate human tissue response to injectable PLLA.

METHODS AND MATERIALS In this exploratory single-arm, open-label study, 14 healthy subjects were administered injectable PLLA; punch biopsies at 3, 6, and 12 months were analyzed for qualitative and quantitative changes from baseline in collagen types I and III and assessed for inflammatory responses.

RESULTS Quantitative and qualitative increases were observed for collagen types I and III at 3 and 6 months and were statistically significant for collagen type I at 3 and 6 months. Post hoc analyses at 12 months showed nominal collagen increases but were hindered by technical difficulties. The degree of inflammatory response was similar to baseline at 3, 6, and 12 months; all subjects were found to have no or mild inflammation after baseline. Adverse events were mild and among those reported previously.

CONCLUSION Results of this study in humans found statistically significant stimulation of collagen type I with no or mild inflammatory response after administration of injectable PLLA.

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Over the past decade, there has been increasing awareness that volume loss is an important factor in the manifestation of facial aging.[1,2] As a result, the use of nonsurgical procedures has increased, and various products with different mechanisms of action are available.[2,3] Injectable poly-L-lactic acid (PLLA; Sculptra Aesthetic, Sculptra; Valeant Aesthetics, a division of Valeant Pharmaceuticals, North America LLC, Bridgewater, NJ) is a biodegradable, biocompatible device made of a synthetic polymer derived from the alpha hydroxy acid family and is supplied as microparticles along with carboxymethylcellulose and mannitol.[4–6] It is indicated for the correction of wrinkles and nasolabial fold contour deficiencies in immunocompetent individuals and for the restoration and/or correction of the signs of facial lipoatrophy in individuals with human immunodeficiency virus.[5,6] It does not require allergy testing because of its nonanimal origin.[7] Although polymers of lactic acid have been used for many years in a variety of medical devices, investigations into the mechanism by which PLLA elicits greater volume after injection have been limited to animal studies and case reports. These studies and reports have documented the gradual formation of new collagen after administration of

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PLLA.[7,8] There are at least 28 different types of collagens;[9] type I collagen accounts for approximately 80% and type III collagen for approximately 10% of the total collagen in the adult human dermis.[10] Type III collagen is the primary component of early granulation tissue and is abundant in embryonic tissue.[11] It also plays an important role in fibrillogenesis.[9,12] Together, types I and III collagen form the broad extracellular fibers that confer the tensile strength of the human dermis.[9] The purpose of the present study was to characterize the human tissue response to injectable PLLA by examining new formation of types I and III collagen and to evaluate inflammatory responses.

Methods

Study Design

This exploratory, phase 2, single-arm, open-label study (U.S. National Institutes of Health, ClinicalTrials.gov Identifier: NCT00869687) conducted in Canada was approved by an institutional review board and complied with the recommendations of the Helsinki 18th World Health Congress, as well as local guidelines and regulations. Healthy subjects age 35 to 55 who signed informed consent statements were eligible for inclusion. Subjects were excluded if they were pregnant, nursing, or not taking measures to avoid pregnancy; had a history of allergic or anaphylactic reactions, including hypersensitivity to local anesthetics, latex, silicon, or any of the product's constituents, keloids, bleeding disorders, or cancer other than basal cell carcinoma within 5 years; or had a history of alcohol or drug abuse. Individuals were also excluded if they had an active inflammatory process or infection (acneform outbreak, cutaneous eruptions, viral eruptions, cancerous or precancerous lesions, or any other active skin disease); scars in the area to be treated; or had received injections of fillers, laser resurfacing, or intense pulsed light therapy in the preauricular, ear, or neck areas or botulinum toxin type A in the auricular area. Individuals with clinically important disease within 3 months of the study (as judged by the investigator, such as significant laboratory abnormalities, myocardial infarction, stroke, connective tissue diseases, hepatitis, asthma, or rheumatoid arthritis), laboratory parameters outside the reference range, any serious skin disease, human immunodeficiency virus, or diabetes mellitus were also excluded. Immunosuppressive medications or systemic steroids within 6 months of treatment, antiplatelet medications within 7 days before biopsies, and prescription facial wrinkle therapies (retinoic acid derivatives, prescription-strength alpha-hydroxy and beta-hydroxy acids, and idebenone 1%) were not permitted.

Eligibility was established during a screening period of up to 4 weeks. Administration of injectable PLLA in the postauricular area was conducted at three treatment sessions spaced 4 weeks apart (baseline, weeks 4 and 8). At baseline and before the first injection, a 3.5-mm punch biopsy was taken immediately adjacent to the injection area, and subsequent 3.5-mm punch biopsies were taken from different sites within the injection area 3, 6, and 12 months after the first injection session. The biopsies were microscopically evaluated for collagen and inflammation using hematoxylin- and eosin–stained sections. Collagen was also evaluated histochemically using Picro Sirius Red stain.

Treatment

Injectable PLLA was reconstituted using 5 mL of sterile water for injection at least 2 hours before use. One vial of reconstituted PLLA was used per subject, and the remaining amount in the vial was discarded. Before injection, the area was treated with topical anesthetic according to the investigator's judgment. Three 0.05-mL deep dermis depot injections were made per session to individual sites in a designated 1.5-cm postauricular circular area (behind the postauricular crease at the midlobe level) for a total of 0.15 mL per treatment session (eight injections along the circumference of the circular area and one at the center to encompass the entire site).

Study Evaluations

The primary efficacy variable was quantitative change in the intensity level of type I collagen from baseline to 6 months after administration of injectable PLLA. Secondary efficacy variables were quantitative changes in the intensity level of type I collagen from baseline to 3 and 12 months; quantitative changes in the intensity level of type III collagen from baseline to 3, 6, and 12 months; qualitative assessment of the changes from baseline in types I and III collagen at 3, 6, and 12 months; and qualitative histologic evaluation of inflammation.

Histologic Methods

Biopsy specimens were divided longitudinally (one sample for histology and one for histochemical staining), snap frozen, and later embedded in paraffin at a centralized laboratory (Bostwick Scientific, Glen Allen, VA) for sectioning and staining. Briefly, tissue sections were deparaffinized, hydrated, and stained using Weigert's iron hematoxylin before staining with Picro Sirius Red.[13] Quantitative assessment of collagen types I and III was determined using digital microscopy with the intensity of collagen fiber staining given as a percentage, with a range of 0 to 100.[14] A dermatopathologist unblinded to subject identification at baseline visit but blinded to postbaseline visits qualitatively assessed collagen types I and III. Digitized images for qualitative assessment were rated on a 7-point scale (−3 = severe decrease, −2 = moderate decrease, −1 = small decrease, 0 = no change, 1 = small increase, 2 = moderate increase, 3 = severe increase). A blinded dermatopathologist qualitatively evaluated inflammatory response on a 4-point scale based on number of lymphocytes around superficial dermal vessels (0 = absent, no lymphocytes; 1 = mild, 1–25 lymphocytes; 2 = moderate, 26–60 lymphocytes; 3 = severe, >60 lymphocytes) after histologic examination of tissue samples stained with hematoxylin and eosin.

Statistical Analysis

Descriptive statistics were used to summarize demographic and baseline characteristics and the intensity (%) of collagen types I and III. *p*-values from one-sample *t*-tests were calculated to test for significance of changes from baseline. The analysis performed on the observed cases was the primary analysis. As supportive analyses, the descriptive statistics and *p*-values from one-sample *t*-tests were calculated based on the data with a last observation carried forward (LOCF) imputation using a repeated-measurement analysis applied to the efficacy variables. All statistical tests were two-sided, with a significance level of .05, and evaluated using a 95% confidence level. With a sample size of 10 evaluable subjects, the study had 80% power to detect an effect size of 1.0 in change from baseline in type I collagen intensity level, using a one-sample *t*-test at a two-sided significance level of .05.

Results

This study was conducted between March 2009 and May 2010. Forty-four subjects were screened and 17 qualified. Three of these were not treated because it was expected that only 14 subjects would be needed to meet the necessary sample size of 10 evaluable subjects by the end of the study. All of the 14 treated subjects completed the treatment, and only one discontinued before the end of the study, because of relocation away from the investigational site area. All subjects were white; 64% were male and 36% female. Mean age was 43 (Table 1).

Quantitatively, the mean level of type I collagen intensity increased significantly, from 21.2% at baseline to 35.3% at 3 months ($p = .02$) and to 33.7% at 6 months ($p = .03$) (Table 2, Figure 1). The LOCF repeated-measurement supportive statistical analyses supported the findings at 6 months. Mean levels of type III collagen intensity increased from 2.8% at baseline to 5.7% at 3 months and 5.7% at 6 months; statistical significance was not reached ($p > .05$) (Table 2, Figure 1).

Qualitative analysis by a dermatopathologist indicated that there was a mild, moderate, or severe increase in type I collagen from baseline for

approximately 7%, 29%, and 29% (64% total) of subjects, respectively, at 3 months and 36%, 29%, and 14% (79% total) at 6 months. There was a mild, moderate, or severe increase from baseline in type III collagen in approximately 36%, 36%, and 7% (79% total) of subjects, respectively, at 3 months and 29%, 43%, and 0% (72% total) at 6 months. Figure 2 shows micrographs of the Picro Sirius Red collagen staining in samples from 2 subjects at baseline and 3 and 6 months after injection.

TABLE 1. Demographic Characteristics

Characteristic	*Value (*N = *14)*
Age, years	
Mean ± SD	43 ± 3.6
Median	42
Range	38–50
Sex, *n* (%)	
Male	9 (64.3)
Female	5 (35.7)
Race, n (%)	14 (100.0)
Caucasian	
Ethnicity, *n* (%)	
Hispanic	5 (35.7)
Not Hispanic	9 (64.3)
Weight, kg	
Mean ± SD	91.1 (22.4)
Median	92.9
Range	58.0–136.2
Height, cm	
Mean ± SD	171 (14.1)
Median	174
Range	151–201

SD, standard deviation.

Histologic assessment of inflammation at baseline indicated that 12 subjects had mild inflammation, one had moderate inflammation, and one had no inflammation; at 3 months, eight subjects were assessed to have mild inflammation, and six had no inflammation. At 6 months, no subjects had moderate or severe inflammation, 10 had mild inflammation, and four had no inflammation. Figure 3 shows histologic sections of samples taken at 6 months that contain injectable PLLA crystals.

Post hoc analyses using Picro Sirius Red staining of specimens collected at 12 months were performed in a separate batch, 6 months after the original batch was analyzed. The originally analyzed batch was composed of samples taken at baseline and 3

TABLE 2. Collagen Intensity Level (%) at Baseline and After Treatment With Injectable Poly-L-Lactic Acid

	Type I Collagen		*Type III Collagen*	
	*Value (*N = *14)*	*Change from Baseline*	*Value (*N = *14)*	*Change from Baseline*
Baseline				
Mean ± SD	21.2 ± 15.4		2.8 ± 5.7	
Median	14.2		0.1	
Range	5.7–45.2		0.0–18.4	
Month 3				
Mean ± SD	35.3 ± 10.7	14.1 ± 19.1	5.7 ± 5.5	3.0 ± 9.0
Median	35.9	22.0	5.6	4.7
Range	9.0–52.8	−14.1–38.0	0.1–19.1	−17.5–18.7
95% CI		3.0–25.1		−2.2–8.1
p-value*		.02		.24
Month 6				
Mean ± SD	33.7 ± 8.2	12.5 ± 18.6	5.7 ± 4.6	2.9 ± 8.0
Median	33.6	15.0	4.6	4.1
Range	15.2–52.5	−17.2–46.8	0.1–12.1	−17.6–12.0
95% CI		1.7–23.2		−1.8–7.5
p-value*		.03		.20

CI, confidence interval; SD, standard deviation.
*Based on 1-sample *t*-tests.

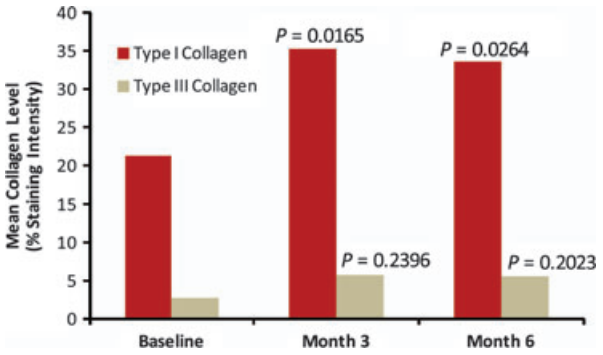

Figure 1. Collagen levels at baseline and after treatment with injectable poly-L-lactic acid (PLLA). *p*-values reflect statistical significance of changes from baseline after injectable PLLA treatment.

and 6 months. The post hoc analyzed batch included samples taken at baseline and 3, 6, and 12 months, but the staining intensity levels of collagen types I and III were much lower in the post hoc batch than in the original batch, with a mean baseline type I collagen intensity of 4.4%, compared with 21.2% in the original analysis and a mean baseline type III collagen intensity of 0.4%, compared with 4.4% in the original analysis. Qualitative analysis by a dermatopathologist indicated that there was a mild, moderate, or severe increase in type I collagen from baseline in approximately 7%, 21%, and 0% (29% total) of subjects, respectively, at 3 months; 7%, 0%, and 0% (7% total) at 6 months; and 23%, 8%, and 0% (31% total) at 12 months. There was a mild, moderate, or severe increase in type III collagen in approximately 14%, 14%, and 0% (29% total) of subjects, respectively, at 3 months; 14%, 21%, and 0% (36% total) at 6 months; and 23%, 23%, and 0% (46% total) at 12 months. At 12 months, a dermatopathologist found that no subjects had moderate or severe inflammation.

With regard to adverse events, five subjects experienced papules, with one papule developing into a nodule. By the end of the study, the papules had resolved spontaneously in all but two subjects. Other adverse events were among those reported in the product package insert, such as upper respiratory tract infection, postprocedural edema, and oropharyngeal pain. Nodules (≥ 0.5 cm in diameter) and papules (<0.5 cm in diameter) were defined as palpable, solid lesions, usually found in the dermal or subcutaneous tissue above, level with, or below the skin surface.

Discussion

This exploratory, phase 2, single-arm, open-label study investigated the human tissue response to injectable PLLA by examining new collagen formation and inflammatory reaction in 14 healthy volunteers. The mean level of type I collagen increased statistically significantly from baseline to 6 months. During the same time, the level of type III collagen increase did not reach statistical significance. These increases in types I and III collagen over 6 months were also observed in more than 70% of subjects as assessed by qualitative analysis of specimens. Histologic assessment of inflammation indicated that at 3, 6, and 12 months, no subjects had moderate or severe inflammation.

Kulkarni and colleagues[15] examined the histologic tissue response to PLLA in guinea pigs. After subcutaneous implantation of PLLA powder, they observed a very mild inflammatory response with evidence of a foreign body reaction in the first week, marked fibroblastic activity and proliferation by 2 weeks, and gradual ingrowth of tissue fibers by 4 weeks, with no further indication of inflammatory reaction.[15] In 1993, Gogolewski and colleagues[8] examined the tissue response to subcutaneous PLLA implantation in mice for 6 months. Proliferating fibroblasts and mature vascularized fibrous tissue were typical of the tissue response, with degradation of PLLA accompanied by increased collagen deposition with no acute inflammation.[8]

These preclinical studies suggest that, as PLLA was degraded, an evolving connective tissue response resulted in the gradual filling, with new collagen fibers, of the space that the implant originally

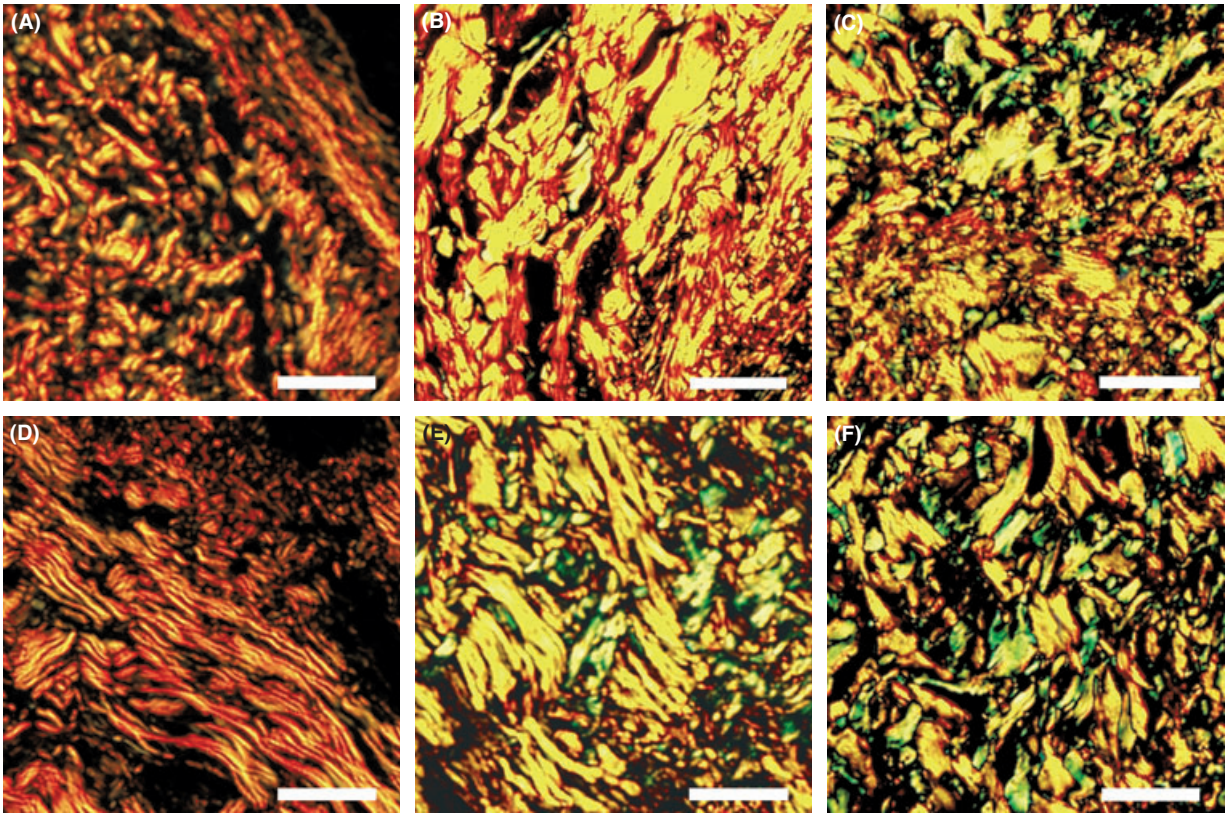

Figure 2. Picro Sirius Red collagen staining. Samples from two subjects (A–C and D–F) taken at baseline and after treatment with injectable poly-L-lactic acid (PLLA). Larger yellow fibers are consistent with collagen type I; smaller green fibers are consistent with collagen type III. (A) Baseline: average intensities for collagen types I and III were 9.1 and 0.1%, respectively. (B) 3 months after treatment with injectable PLLA: average intensities for collagen types I and III were 47.1 and 1.2%, respectively. (C) 6 months after treatment with injectable PLLA: average intensities for collagen types I and III were 36.6 and 12.12%, respectively. (D) Baseline: average intensities for collagen types I and III were 8.9 and 0.0%, respectively. (E) 3 months after treatment with injectable PLLA: average intensities for collagen types I and III were 36.4 and 10.0%, respectively. (F) 6 months after treatment with injectable PLLA: average intensities for collagen types I and III were 32.0 and 9.8%, respectively. The scale bar represents 50 μm.

occupied. Although the form of the PLLA implants in these earlier animal studies differed from the injectable PLLA formulation currently approved, they were later supported by histologic observations of case report biopsies taken from subjects treated with injectable PLLA.[7] Biopsies taken from 8 to 24 months after injection demonstrated progressive degradation of injectable PLLA, with associated gradual ingrowth of collagen type I, indicating that the long-term mechanism of action was not physical filling with injectable PLLA but was the production of fibrous tissue that maintained volume after the implant was resorbed.[7] The results of the present exploratory study are consistent with these previous findings and with a mechanism of volume increase wherein dermal collagen formation is stimulated without an appreciable accompanying inflammatory response. Although quantitative increases in type I procollagen have been reported after injection of nonanimal stabilized (cross-linked) hyaluronic acid, increased collagen represents only a partial component of the volume restoration properties of this type of filler in addition to the physical occupation space.[16] Thus, the mechanism of PLLA collagen stimulation is in contrast to other volume replacement products that achieve immediate volume restoration through space-occupying properties

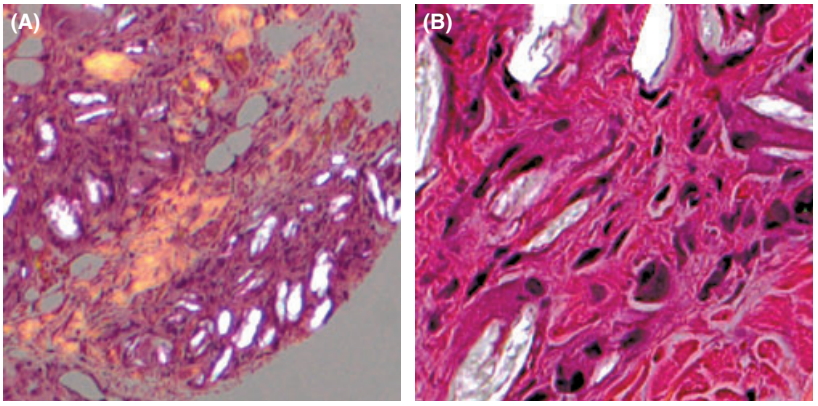

Figure 3. Histologic examination of a tissue sample taken 6 months after injectable poly-L-lactic acid (PLLA) treatment. Specimen is stained with hematoxylin and eosin and viewed using polarized light microscopy. PLLA crystals appear as bright white and can be seen as engulfed by multinucleated histiocytes. (A) Polarized collagen appears as bright orange (×10). (B) Nonpolarized collagen appears as red in the lower right corner (×40).

of the material that is gradually lost as the material is metabolized or resorbed, with only a small component of volume restoration due to collagen formation.

The qualitative assessments of the changes in collagen types I and III were limited in that blinding was not possible because it was necessary to pair reference baseline samples with samples taken from the same subjects at later visits. The batches of Picro Sirius Red staining limited the quantitative and qualitative assessments in changes in collagen types I and III. In the post hoc analysis, mean type I collagen intensity increased from 4.4% at baseline to 4.8% at 3 months, 4.9% at 6 months, and 6.3% at 12 months; mean changes from baseline did not reach statistical significance. Similarly, mean type III collagen intensity increased from 0.4% at baseline to 0.5% at 3 months, 0.5% at 6 months, and 0.5% at 12 months but did not reach statistical significance at any time. The staining intensity levels of collagen types I and III were much higher in the original analysis batch (samples taken at baseline and 3 and 6 months) than in the post hoc analysis batch (samples taken at baseline and 3, 6, and 12 months). The lower staining intensity of the post hoc batch could have been because of multiple factors, including degradation of the tissue samples and differences in the lot or batch of the stain or the apparatus or other methods used. Because the tissue samples were 2 μm thick, it is also possible that the collagen layer was surpassed when the tissue sample was cut from the paraffin block. The lower staining intensity of the post hoc batch may be why statistical significance was not reached for the quantitative assessment of type I collagen change from baseline for this batch and why qualitative collagen increases were not observed in as many subjects in the post hoc batch as in the original batch. The nodules observed in this study may have been due to lack of massage, the adherent periauricular skin, or possibly injection into the deep dermis in this area of thin skin.

Conclusions

In this exploratory, phase 2, open-label study of 14 healthy volunteers, the results of previous studies were confirmed, and a statistically significant increase in collagen type I was observed after treatment with injectable PLLA without an appreciable accompanying inflammatory response. Changes from baseline for collagen type III were not statistically significant.

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