ACP Gel: A New Hyaluronic Acid–Based Injectable for Facial Rejuvenation. Preclinical Data in a Rabbit Model

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Background: Facial aging results from reduced biosynthetic activity of dermal fibroblasts and decreased deposition of extracellular matrix components, such as hyaluronic acid, a glycosaminoglycan responsible for skin hydration and turgidity. Exogenous hyaluronic acid injections provide a safe though short-term solution for facial rejuvenation. Using a rabbit model, the authors investigated residence time and tolerability of ACP gel, a new hyaluronic acid cross-linked derivative, compared with high-molecular-weight native hyaluronic acid currently used for facial rejuvenation (Ial System).

Methods: ACP gel 1% and 2%, Ial System, and saline were intradermally injected into the backs of 12 New Zealand rabbits: six animals were used to follow volume maintenance and redness up to 10 days and the other six animals were euthanized at days 2, 6, 8, 10, 14, and 21 (one animal per time point) to histologically assess biocompatibility.

Results: ACP gel 2% had the longest residence time, showing a significantly better volume maintenance than the other samples, especially in the initial study period (71 percent of original volume versus 23 percent and 21 percent of ACP gel 1% and Ial System, respectively, at day 2). Macroscopically, no adverse events were observed in the treated animals. Histologic examination confirmed the absence of adverse events, persistent inflammation, tissue degeneration, or necrosis. ACP gel macrophage-mediated phagocytosis was more persistent with respect to the Ial System, consistent with its longer residence time.

Conclusion: ACP gel 2% is a promising dermal biorevitalizer, characterized by a high safety profile and prolonged residence time in relation to native high-molecular-weight hyaluronic acid. (*Plast. Reconstr. Surg.* 118: 341, 2006.)

Aging of the face is both an aesthetic and a clinical problem: reduced dermal vascularization and decreased biosynthesis of important extracellular matrix components by fibroblasts result in loss of skin turgidity and elasticity.[1] In particular, the amount of hyaluronic acid, a glycosaminoglycan with an enormous ability to bind water and maintain tissue hydration, is deeply impaired in mature skin.[2,3] Hyaluronic acid has fundamental structural properties, mainly attributable to its capability to coordinate extracellular matrix components, and important biological roles in the young healthy skin: it favors fibroblast proliferation and migration[4,5]; acts as a free radical scavenger[6]; and, because of optimal tissue hydration, improves nutrient exchange between vessels and dermis. The decrease of hyaluronic acid activities in the aging skin contributes to dermal thinning and folding.

Hyaluronic acid is conserved among species and is totally biocompatible,[7] thus hyaluronic acid exogenous injections are safe and can temporarily improve the signs of facial aging. Hyaluronic acid injection into mature dermis can indeed restore skin tone, fullness, and elasticity, but native hyaluronic acid has the limit of being rapidly degraded and it resides for just a few days in the dermis.[8] For this reason, chemically stabilized forms of hyaluronic acid are being proposed as advanced biorevitalizing agents with prolonged effects.

The aim of this study was to compare the intradermal residence time and histologic behavior of two different concentrations of ACP, a new cross-linked hyaluronic acid derivative, with

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Ial System, native high-molecular-weight hyaluronic acid currently used for facial rejuvenation.[9] For this purpose, a rabbit model allowing direct comparison of the different gels was used.

MATERIALS AND METHODS

Hydrogels

Steam sterilized ACP gel at the concentration of 1% and 2% in saline solution was supplied by Fidia Advanced Biopolymers S.r.l. Ial System was provided by Fidia farmaceutici S.p.a. One milliliter of gel was used for each treatment.

Study Design

Each of the 12 animals used in the study received four intradermal injections: ACP gel 1%, ACP gel 2%, Ial System, and saline solution as a control. The animals were divided into two groups of six animals each (group A and group B). The volume of the treated areas and redness were observed in group A for a 10-day period, after which the rabbits were euthanized. For histologic evaluation, animals of group B were euthanized at time points 2, 6, 8, 10, 14, and 21 days (one animal per time point).

Animals and Treatments

Twelve male New Zealand rabbits (Harlan, Italy), aged 3 months and weighing 170 to 230 g, were used in this study. Under general anesthesia [intravenous injection of 0.25 ml/kg Zoletil/Rompun/saline solution, 1.0:0.5:3.5 (v/v/v)], animals were shaved in the dorsal region, which was disinfected before gel injection. The four treatment sites were identified and marked (Fig. 1, *above*). The samples to test were intradermally injected through a 20-gauge half needle (Fig. 1, *below*).

Each animal was individually caged and housed in standard controlled conditions. The rabbits were observed for macroscopic adverse events, such as extended edema and redness, during the days after injection. In particular, redness induced by the treatments was evaluated for 10 days in group A using a scoring scheme from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, and 3 = marked).

Volume Calculation

On group A, the extent of cutaneous swelling was measured daily up to day 10: the mound volume was mathematically calculated using the formula $(2/3\ \pi) \times r1 \times r2 \times r3$, describing the volume of a hemiellipse, where r1, r2, and r3 represent the width, length, and height of the swollen area.[10] The volume reduction at the different time points was described as mean percentage ± SD with respect to the volume at time 0 (time of injection). Statistical differences among volumes in different treatments were evaluated by analysis of variance for repeated measures with balanced design; comparisons between treatments were made with Bonferroni t test. Significant differences were achieved at $p < 0.05$.

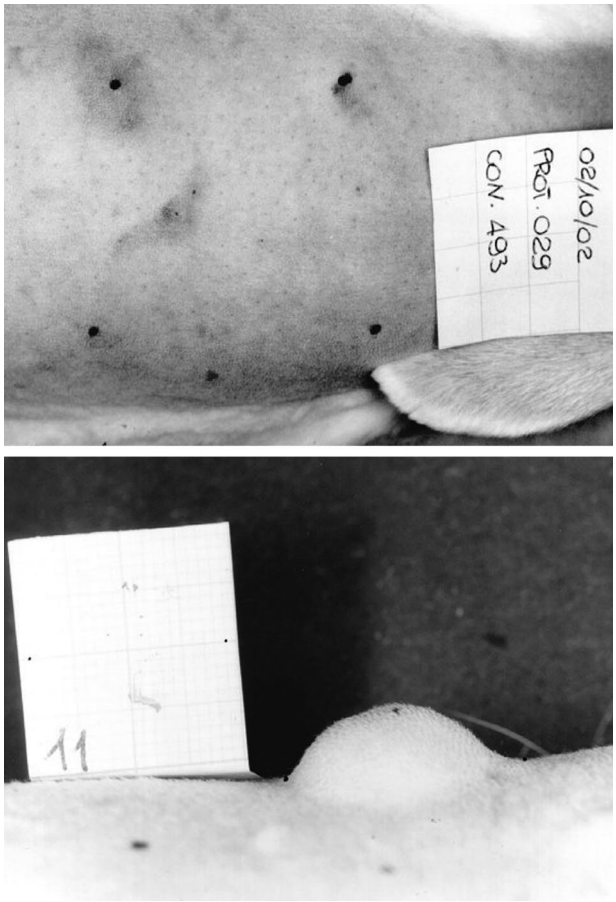

Fig. 1. (*Above*) Injection sites: ACP 2% and 1% samples, Ial System, and saline solution were injected into four sites of each rabbit back. (*Below*) Dermal mound formed after sample injection. The tissue corresponding to the injection site was drawn up applying a 25-mmHg vacuum, and samples were intradermally injected using a 20-gauge half needle.

Histologic Analysis

The animals in group B were euthanized at 2, 6, 8, 10, 14, and 21 days after intradermal injections. The samples were excised from the treatment site, fixed in 4% formalin, and embedded in paraffin. Sections were stained with hematoxylin-

eosin and processed for routine microscopy. Histomorphologic analysis investigated the tissue reaction by naked eye evaluation of the presence of macrophages, giant cell reaction, granulomatosis, fibrosis, degenerative events, or tissue necrosis. These parameters were evaluated as a function of time using scores from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, and 3 = pronounced).

RESULTS

Hydrogel Injection

Macroscopic Adverse Event Evaluation

Injection of all gel samples and saline was accomplished without complications. None of the animals showed pain or severe adverse events, including infections, ulcerations, or areas of erythema, during the whole period of the study. In particular, edema exceeding the injection area was not observed in any of the treated animals. In group A, in which redness was specifically followed for 10 days, cutaneous redness was induced by ACP gel 1% and ACP gel 2% treatments: at 1 day, the redness was mild to moderate, with mean scores 1.5 ± 0.2 and 2.2 ± 0.4, respectively. Redness rapidly reduced with time; it was slight 3 days after injection (scores < 1) and completely disappeared by day 7 (Fig. 2).

Residence Time

Maintenance of Gel Volume

After injection of the treatments, a regular mound formed, which was measured using a caliper in group A. Comparison among the hyaluronic acid–based gels tested showed a statistically significant difference ($p < 0.05$, analysis of variance for repeated measures), and ACP gel 2% showed the best volume maintenance (Fig. 3). From time point 0 to 4 days, the percentage of volume reduction was significantly lower in ACP gel 2% treatment when compared with ACP gel 1% and Ial System ($p < 0.05$). It is noteworthy that, at day 2, the volume of ACP gel 2% was 71 percent with respect to time 0, a considerable result if compared with ACP gel 1% and Ial System at the same time point, which corresponded to 23 percent and 21 percent of the original volume, respectively ($p < 0.05$, Bonferroni test).

Tolerability

Histologic Analysis

Excision of samples at the time the animals were euthanized showed that the hydrogels remained localized in the injection site. Histology revealed an initial phlogosis in all the treatments except for saline, which regressed for ACP gel 2% in 8 days (Fig. 4). Macrophage reaction was detected both in Ial System and ACP gel samples, but with different intensity. The difference in macrophage reaction entity in Ial System and ACP gel 2% is shown in Figure 5, which clearly shows the more relevant ongoing phagocytosis in ACP gel 2% at day 6. Neither ACP gel preparations nor Ial System induced any giant cell reaction, fibrosis, granulomatosis, degenerative phenomena, or necrosis.

DISCUSSION

Biorevitalizing injectable agents are a convenient approach to facial rejuvenation: through a

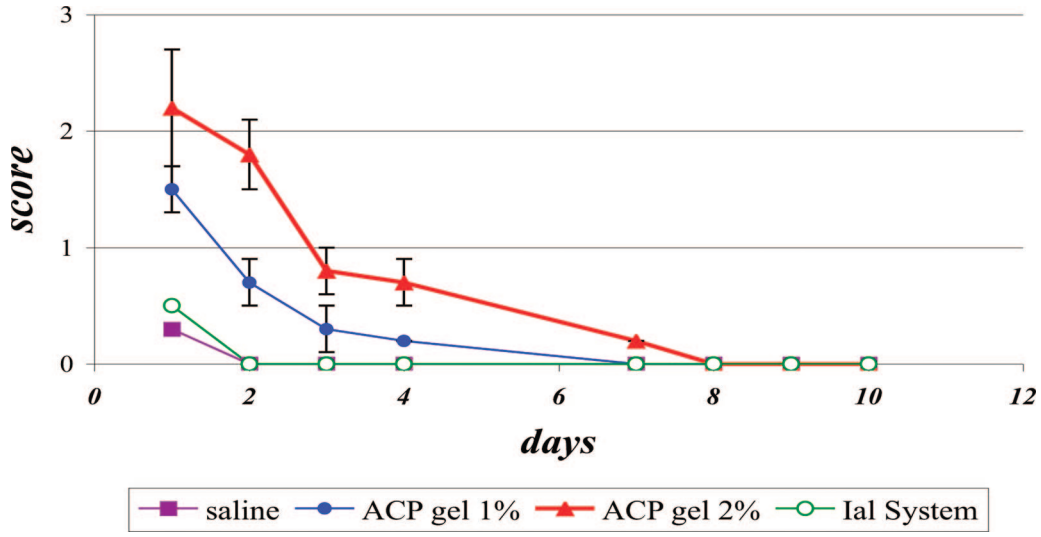

Fig. 2. Skin redness as a function of time. Redness induced by sample injection was evaluated using scores from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, and 3 = marked).

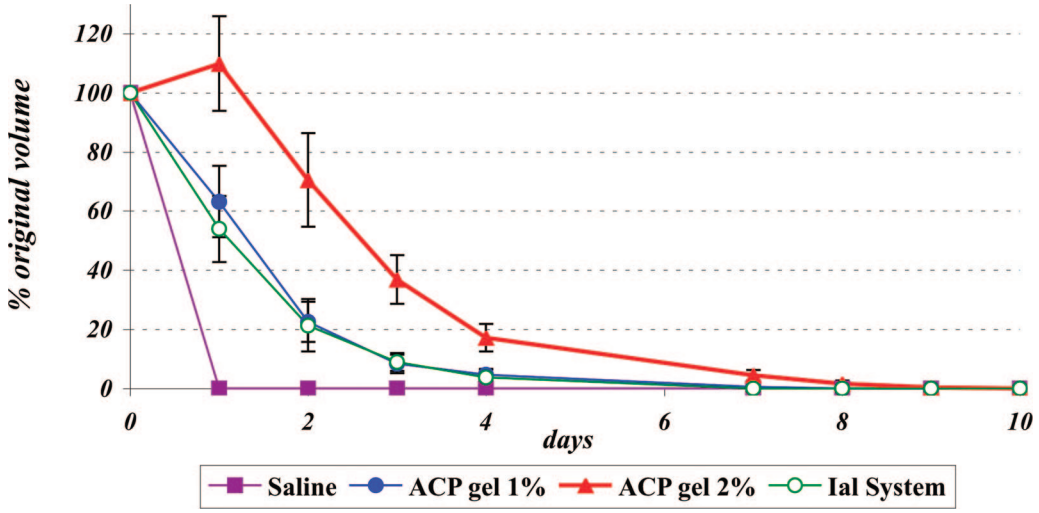

Fig. 3. Percentage of volume maintenance for all treatments as a function of time.

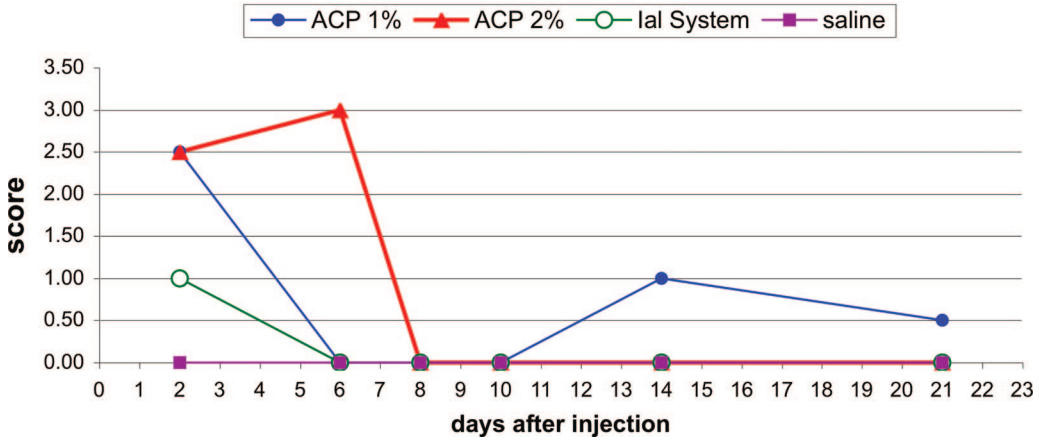

Fig. 4. Phlogosis as a function of time, according to histologic evaluation. A scoring scheme was used for naked eye analysis, ranging from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, and 3 = pronounced).

minimally invasive, painless, and non–time-consuming treatment, biocompatible and absorbable substances are injected in the mature dermis. These substances act by stimulating the normal dermal physiology, which is deeply impaired with aging and environmental factors, such as ultraviolet light, smoke, and pollution. Among biorevitalizers (including vitamins, coenzymes, minerals, and amino acids), hyaluronic acid has captured the most interest because of its extremely high safety profile and biological functions. Hyaluronic acid is indeed a highly conserved, nonimmunogenic, naturally occurring glycosaminoglycan, highly represented in the extracellular matrix of young and healthy skin. Hyaluronic acid has important structural roles in the dermis, where it coordinates collagen, proteoglycans, and fibronectin to form macromolecular structures of the extracellular matrix,[11,12] and it also has hydrating properties, because of its ability to bind high volumes of water. Most importantly, hyaluronic acid directly acts on fibroblast proliferation and migration[13] and stimulates collagen deposition.[14] When hyaluronic acid dermal content decreases with age, causing loss of turgidity, loss of tissue dehydration, and formation of facial folds,[2,3] hyaluronic acid exogenous application can temporarily restore a more physiologic and healthy dermal state.

Intradermal high-molecular-weight hyaluronic acid injection proved to be effective in recovery of skin turgidity and elasticity[9] and it is widely used as a nonsurgical antiaging cosmetic treatment. Natural hyaluronic acid is rapidly resorbed in the dermis.[8] To prolong the hydrating and stimulatory effects, hyaluronic acid can be

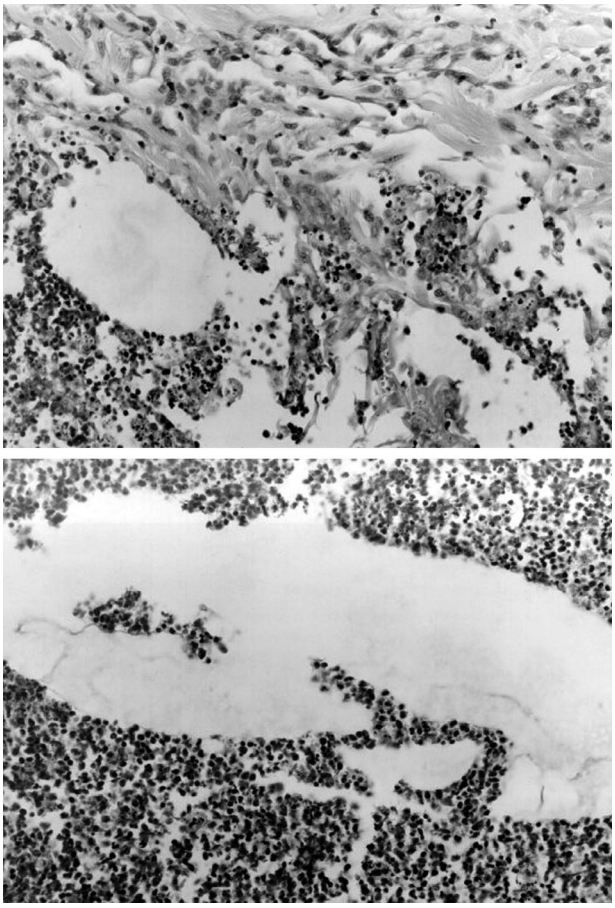

Fig. 5. (*Above*) Macrophage-mediated phagocytosis in Ial System treatment on day 6 postinjection (hematoxylin and eosin staining). (*Below*) Intense macrophage infiltration next to the semitransparent ACP 2% gel on day 6 postinjection (hematoxylin and eosin staining).

chemically stabilized by modifications affecting its degradation rate.

We used a rabbit model, allowing a direct intraanimal comparison of dermally injected hyaluronic acid and a new hydrogel based on ACP, a hyaluronic acid derivative. We compared tolerability and residence time for 21 days, a longer time period than the 1-week subcutaneous degradation time of ACP observed in a previous model (Fidia Advanced Biopolymers ACP toxicologic files biodegradation/toxicity study in rats, unpublished data) and longer than hyaluronic acid residence time reported in the literature.[8]

Our results demonstrate that ACP is a promising advanced biorevitalizing gel: it maintains the optimal safety profile of natural hyaluronic acid, but it is characterized by prolonged residence time. Both of these features are attributable to a unique chemical structure: cross-linked hyaluronic acid without the insertion of different molecules. Cross-linking is a method of increasing hyaluronic acid residence time and viscosity, and cross-links of a different nature can be found in stabilized hyaluronic acid fillers. In most stabilized hyaluronic acid fillers, cross-linking is obtained through molecules creating bridges between hyaluronic acid chains; therefore, there are new chemical species, different from hyaluronic acid and without the same safety profile, which are released during the product degradation in the dermis. ACP cross-linking, instead, is obtained by esterification of the carboxyl and hydroxyl groups of the same or of different hyaluronic acid molecules, resulting in intra–hyaluronic acid and inter–hyaluronic acid bonds, without insertion of any other molecule.[15]

ACP at a concentration of 2% in saline guaranteed a longer residence time than Ial System, the high-molecular-weight hyaluronic acid currently used for facial rejuvenation ($p < 0.005$ up to 4 days after injection). The lower degradation rate is a fundamental aspect for ACP gel biorevitalizing action: the effects on dermal rejuvenation will be more persistent, because of the prolonged bioavailability of hyaluronic acid released during the process of ACP gel degradation in the treated site.

Macrophage phagocytosis for ACP gel was more relevant with respect to Ial System: ACP attraction of macrophages on the injection site represents an important feature. Macrophages are indeed known to release growth factors, such as interleukin-1 and tumor necrosis factor, positively affecting fibroblast proliferation and extracellular matrix component deposition.[16]

The performance observed for ACP gel 2% was not associated with any irritation event, permanent redness, or persistent macroscopically evident edema. No severe adverse events, persistent inflammation, or degenerative/necrotic events occurred in the dermal district of the rabbit. These data confirm the optimal tolerability of ACP gel, already observed in humans in abdominopelvic surgery.[17–20]

CONCLUSIONS

ACP cross-linked hyaluronic acid at a concentration of 2% in saline has a dermal residence time that is more prolonged than that of Ial System, high-molecular-weight hyaluronic acid, and it is characterized by an extremely high tolerability and biocompatibility profile. ACP gel 2% may be considered a promising biorevitalizing agent for facial rejuvenation, which guarantees to the

treated area a prolonged bioavailability of hyaluronic acid.

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