CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.1433584

Available online at: http://www.iajps.com Research Article

STANDARDIZED IN VITRO ANALYSIS OF THE DEGRADABILITY OF HYALURONIC ACID FILLERS BY HYALURONIDASE

[1]Dr. Fatima Amin, [2]Dr. Umaira Maqsood, [3]Dr. Quratul Ain

[1]WMO, RHC Eminabad, Gujranwala.
[2]WMO, RHC Kalaswala, Pasrur, Sialkot.
[3]WMO, Children Hospital, Faisalabad.

Abstract:

Hyaluronidase is fundamentally a "Hyaluronic Acid" (HA) digesting enzyme which particularly recognized as the adjuvant regarding infiltration anesthesia. There is another "off-label" or hidden utilization of hyaluronidase which considered as gold standard specifically for the HA filler-associated impediment management. Yet, according to the current situation, there are just a few types of research which may analyze the HA fillers degradability of difference by hyaluronidase.

This research intended to analyze the HA-filler interactions and hyaluronidase in the manners of time-dependent utilizing a proper and latest standardization in vitro method.

(BEL; Merz) Belotero Balance Lidocaine, Comparable HA-fillers, (EMV; Galderma) Emervel Classic and (JUV; Allergan) Juvederm Ultra 3, were hatched with the fluorescent dye and HYAL; Hylase "Dessau" Riemser) bovine hyaluronidase or control (NaCI) with specific monitoring by time-lapse video-microscopy. HA-fillers degradation was analyzed as a decline in fluorescence intensity of HA-filler plus control vs HA-filler plus hyaluronidase, enumerated by image analysis with the assistance of a computer.

Hyaluronidase represented an important degradation of HA-fillers EMV and BEL. It was possible to measure degradation at 7h (EMV) and 5h (BEL), significance was originally grasped at 13 h (EMV) and 14 h (BEL). There was no JUV effect observes in hyaluronidase.

In the comparative analysis of HA-fillers and hyaluronidase through vitro method, the time-lapse microscopy qualifies systematically.

Keywords:*Vitro Analysis, Hyaluronic Acid Fillers, Degradability, Hyaluronidase*

Corresponding author:
Dr. Fatima Amin,
WMO, RHC Eminabad,
Gujranwala.

QR code

Please cite this article in press Fatima Amin et al., ***Standardized In Vitro Analysis Of The Degradability Of Hyaluronic Acid Fillers By Hyaluronidase,*** *Indo Am. J. P. Sci, 2018; 05(09).*

1.0 INTRODUCTION:

In actual, (HA) hyaluronic acid is principally a (GAG) non-sulfated glycosaminoglycan and it is an important component of (ECM) extracellular matrix of skin. In the development of skin aging, a decline in the HA of skin content is deliberated the most important feature. Currently, the reversible "HA-based dermal fillers" injection is commonly observed as the standard for augmentation of tissue and re-contouring facial or deep skin hydration. Probable difficulties in the treatment of fillers are from overcorrections of unaesthetic, Tyndall effects. Accordingly, there is another effect of lower eyelid edema, which also follows tear-trough growth, infections, to granulomas up to tissue necrosis and in some rare cases blindness through vascular occlusions (Alaverdyan, 2004).

In the handling of filler treatment complications, there is the availability of some particular antidotes is a common reason for the preference utilization of HA-based fillers as compared to injectable fillers (like CHA, calcium hydroxylapatite). By the timely hyaluronidase infiltration it may destroy HA-fillers and may prevent from highly adverse vascular complications as the instant hyaluronidase availability is observed a need for those physicians who inject HA. Besides the possibility of hyaluronidase, it is discussed controversially whether the HA-fillers may be mortified by hyaluronidase effective. A change in or even confrontation to "degradability" may further be associated to HA-filler concentration, its unified properties, and the cross-linking degrees (Alsoufi, 2011).

Considering the probable filler injection risks, the established degradability of HA-filler specifically by hyaluronidase may be considered as "safety-measure" and probable modest edge regarding other dermal fillers manufacturers. According to this specific background, this research is based on the systematic analysis of the degradability of HA-fillers in a time-dependent manner by bovine hyaluronidase with the use of vitro approach through standardized video-microscopy (Brody, 2005).

2.0 MATERIALS AND METHODS:

Particularly for this study we used three market available HA-fillers such as, first one (BEL) "Belotero Balance Lidocaine" BDDE, HA 22.5 milligram/millilitre, with lidocaine; provided by a German Company "Merz Pharmaceutical Frankfurt" and manufacture in Geneva Switzerland by ANTEIS SA Company, second (EMV) Emervel Classic; cross-linked, sizing BDDE, biphasic, HA 20.0 milligram/millilitre, lidocaine; Galderma; produced by Swedish "Q-Med AB" Company and finally (JUV) Juvederm Ulta 3; crosslinked "Hyalcross" BDDE HA 24.0 milligram/millilitre, lidocaine; manufactured by the United States of America "Pharm-Allergan" CA (Buhren et al., 2018).

Initial, 50 µl of every filler was blended with green fluorescent "10 µl" cell linker dye (Sigma PKH67) in 10 u/ml 10 units of (HYAL; Hylase "Dessau") bovine testicular hyaluronidase, it also signifies the German standard usage of hyaluronidase, specifically it is equal to NaCI volume and placed in BD Bioscience 24 well plates. Secondly, the fluorescent gel was positioned in 350 µl of "serum-free keratinocyte medium" while the wells were positioned in the workstation of time-lapse video microscopy (Carl Zeiss Microscopy at the temperature of 37C with 5% CO_2). With the dependency of the HA-filler integrity, the fluorescent dye endured enclosed in gel, which gives the high fluorescent intensity output, or the dye diluted in the surrounding medium, with decline fluorescence intensity output. The intensity of fluorescence was recorded over twenty hours (FITC channel, 5 X magnifications). All images were taken by CCD chip (Zeiss) and the workstation used was controlled by software named Axiovision 4.7 (Zeiss) (Buhren et al., 2018).

HA-fillers degradation was analyzed as an alteration in HA-filler HYAL vs. HA-filler plus control fluorescence (CTR; NaCI), $n \leq 6$ according to one condition, measured by BioVoxxel Fiji ImageJ, a computer-based analysis of images. The below-mentioned figure explored the schematic illustration of the study.

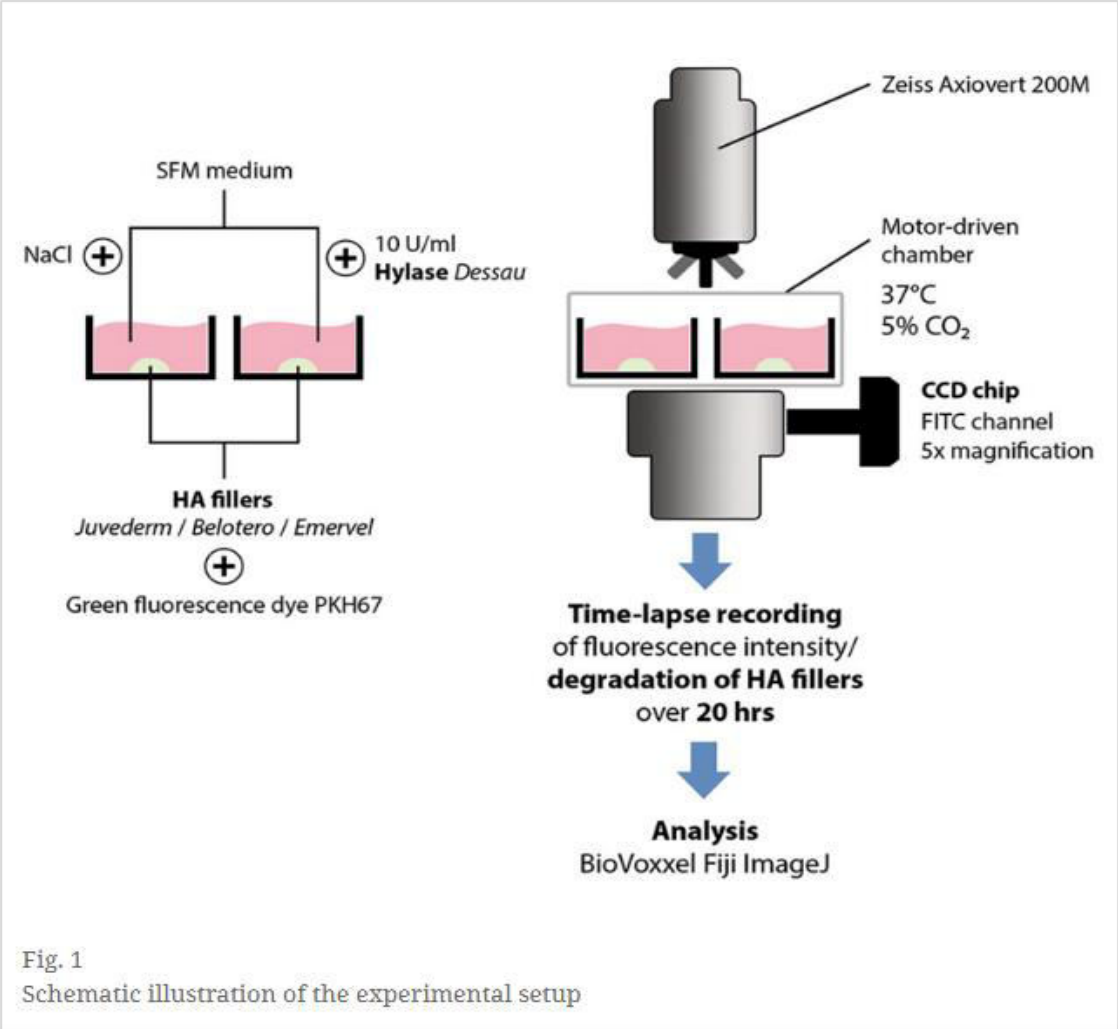

Fig. 1
Schematic illustration of the experimental setup

(Source: Buhren et al., 2018)

The intensities of raw fluorescence were calculated every 10 min and there is 6 uninterrupted calculation were averaged to gain a modern value for every one full hour. For easier interpretation and visualization, outputs were represented on a multiplicative calibration that is intensities of average fluorescence for every one full hour which further divided by intensities of corresponding fluorescence at the time point 0 hours. On every point, intensities of normal fluorescence between dual groups were also associated with a nonparametric usage of "Wilcoxon Rank-Sum Test". The two condition's difference was also calculated by p values lesser than 0.05 and declared as statistically significant (Cavallini et al., 2013).

3.0 RESULTS:

All possible conditions ("BEL+CTR, BEL + HYAL, EMV + CTR, EMV + HYAL, JUV + CTR and JUV + HYAL) represented the intensities of comparable fluorescence at 0 h. Furthermore, specifically for each condition, we recorded a minor to the medium fluctuation of complete over the experimental course. Important curves separation for CTR vs HYAL treated fillers and later a filler degradation was experienced only for EMV and BEL. There is also a degradation of HYAL by BEL (n=5) was experienced at the fifth hour and grasped significance (p=0.03) initializing at the fourteenth hour. There is also a degradation of HYAL by EMV (n=6) which was experienced at the seventh hour and reached

significance (p=0.04) initiating at the thirteenth hour. In overall observance, the strongest degradation was experienced for BEL and no HYAL significant degradation separated curved was experienced, specifically for JUV (n=4) (Buhren et al., 2018).

4.0 DISCUSSION:

Some studies have methodically analyzed for the HA-fillers' degradation by hyaluronidase. Some researchers used a specific test which was based on "colorimetric determination" of N-acetyl-D-Glucosamine discharged from eleven multiple bovine hyaluronidase HA-fillers. Another study showed that in vitro method by another assessment of the products of degradation of three multiple HA-fillers using size exclusion chromatography with ovine hyaluronidase (Buhren et al., 2018).

According to the comparison with our study, both referred studies described the toughest resistance in JUV HA-filler with highly cross-linking of 24 mg/ml regarding degradation. As the equal product of EMV, the biphasic 20 mg/ml HA-filler of Restylane was associated as highly subtle. Some other researchers used an approach of photography to compare visually the human recombinant hyaluronidase comparison with four multiple HF-fillers. Accordingly, authors of that report again explained that EMV Restylane was degraded through hyaluronidase, in the most subtle way in the shape of dose-dependent. Parallel to our outcomes BEL reserved it shape most followed by JUV (Tan, Malhotra and Ali, 2016).

Recently, in a vivo human research seven multiple HA-fillers (also containing BEL, EMV, and JUV) were accordingly injected into the deep skin of fifteen respondents, trailed by ovine hyaluronidase 20 to 40 units secondary injections. Resulted in the HA-fillers degradation was specifically observed by palpation for fourteen days following. Summarizing the example, the observation of all HA-fillers preserved with 20 and 40 U hyaluronidase, represented an important decline in volume. According to our research BEL (Belotero) was discovered the quickest to dissolve (Kim, Chong and Mok, 2014).

Different cited researches show an important heterogeneity with HA-filler analysis regard, hyaluronidases and highly noted investigational setups and approaches to assessment. As contrasting all other arrangements our research is featured by a greater level of standardization, computer-based quantification, independent and according to follow the HA-fillers interactions with hyaluronidase over a full course of time due to specific time-lapse video documentation. We also noted that there was a mild to medium fluctuation in baseline or intensities of CTR-fluorescence regarding every specific filler over the experimental course. Accordingly, this fluctuation is probably caused by an alteration in the filler form; which is also represented by outcomes. Only an efficient filler degradation found and there is a specific dilution of the dye in the mediocre may give output, in particular, HYAL vs CTR separation curves resulted by treated fillers of both, these were also analyzed for EMV and BEL. To understand the outputs we designed all values for NaCI treated control against HYAL treated fillers (Kim, Chong and Mok, 2014).

Furthermore, outputs confirmed by other researches which analyzed some other parts of the hyaluronidase and HA-fillers interactions. Specifically mentioned Kim et al. (2011) rabbit ear model to show the hyaluronidase properly cures skin necrosis if promptly rejected in four hours after vascular obstruction of an artery while utilizing EMV. In recent years, some research also uses the same method of rabbit ear to demonstrate that hyaluronidase subcutaneous injection is highly effective as compared to intra-arterial injection. Furthermore, the same researchers prove that there is degradation in EMV by hyaluronidase within one hour and also stated that hyaluronidase successfully degraded EMV in the vivo murine model (Kim, Chong and Mok, 2014).

With the specification of hyaluronidase interaction and according to the outputs of JUV are considered more inconsistent. Other researchers strongly recommend that there is high resistance to degradation in contradiction of ovine or bovine hyaluronidase. They also showed that ovine or bovine or recombinant hyaluronidase of human successfully degrades JUV. These important but controversial outputs may be relevant to differences in bovine, ovine and recombinant human applied hyaluronidases with some proposed doses and incubation setups durations. These studies denote that HA contents, as well as the techniques of cross-linking, have a high effect on the resistance versus hyaluronidase. The highest monophasic JUV cross-linking degree may control the access by HA substrate enzyme, while the EMV biphasic nature

and highly exclusive particles offer and generate a high surface attack (Tan, Malhotra and Ali, 2016).

Our study showed that HA highest content fillers (JUV, 24 milligrams/milliliter) were highly resilient to specific degradation comparing to BEL 22.5 milligram/milliliter; EMV 20 milligram/milliliter lower concentrations. Therefore, we also demonstrated that the BEL monophasic was comparably degradation sensitive as biphasic EMV. Accordingly, this study also demonstrated that videomicroscopy (time-lapse) showed an effective and exclusive approach to HA-fillers degradability assessment by hyaluronidase in the manner of time dependency. According to the study, we may not analyze the different doses effect of kinds of hyaluronidase (Buhren et al., 2018).

Similarly, hyaluronidase high doses may serve to JUV degrade as it is proved from some past studies also. Higher hyaluronidase doses will similarly give output in swift degradation of HA-fillers in an initial hour and it may frequently assess in the vivo clinical situation. As taking the hyaluronidase account it will not only HA-filler degrader but also EMC in surrounding HA may reasonably concern about the injection of hyaluronidase high doses which may give output in decline of physiological HA as in the treated atmosphere. Yet, the turnover of a non-stabilized and half-life, in skin physiological HA is only regarded for twenty-four hours which imply the equilibrium and always recognized in a few hours (Buhren et al., 2018).

5.0 CONCLUSION:

As concluding note, with the concern of molecular mechanisms, our outputs further suggest HA content and cross-linking technique is the main factor which regulates the hyaluronidase sensitivity. Futuristic researches may extend our study to analyses of dose-response and a wider range of multiple hyaluronidases and HA-fillers.

REFERENCES:

1. Alaverdyan, M. (2004). Hyaluronidase and hyaluronic acid in certain infectious and immunological phenomena. Bulletin of Experimental Biology and Medicine, 53(1), pp.75-78.
2. Alsoufi, A. (2011). Hyaluronic acid fillers. Prime, 1(5), pp.62-64.
3. Brody, H. (2005). Use of Hyaluronidase in the Treatment of Granulomatous Hyaluronic Acid Reactions or Unwanted Hyaluronic Acid Misplacement. Dermatologic Surgery, 31(8), pp.893-897.
4. Buhren, B., Schrumpf, H., Bölke, E., Kammers, K. and Gerber, P. (2018). Standardized in vitro analysis of the degradability of hyaluronic acid fillers by hyaluronidase. European Journal of Medical Research, 23(1).
5. Burten, J. (2004). THE HYALURONIC ACID-HYALURONIDASE SYSTEM. Journal of the American Medical Association, 128(5), p.365.
6. Cavallini, M., Gazzola, R., Metalla, M. and Vaienti, L. (2013). The Role of Hyaluronidase in the Treatment of Complications From Hyaluronic Acid Dermal Fillers. Aesthetic Surgery Journal, 33(8), pp.1167-1174.
7. FALCONE, S., DOERFLER, A. and BERG, R. (2007). Novel Synthetic Dermal Fillers Based on Sodium Carboxymethylcellulose: Comparison with Crosslinked Hyaluronic Acid-Based Dermal Fillers. Dermatologic Surgery, 33, pp.S136-S143.
8. Jones, D., Tezel, A. and Borrell, M. (2010). In Vitro Resistance to Degradation of Hyaluronic Acid Dermal Fillers by Ovine Testicular Hyaluronidase. Dermatologic Surgery, 36(Sup 1), pp.804-809.
9. Kim, J., Chong, Y. and Mok, H. (2014). Shell-Crosslinked Hyaluronic Acid Nanogels for Live Monitoring of Hyaluronidase Activity In Vivo. Macromolecular Bioscience, 14(6), pp.881-888.
10. Monheit, G. and Prather, C. (2007). Hyaluronic acid fillers for the male patient. Dermatologic Therapy, 20(6), pp.394-406.
11. NOJIMA, T., ISHIMOTO, M., SASAKI, M. and MATSUMURA, G. (2010). BIOCHEMICAL STUDIES ON HYALURONIC ACID AND HYALURONIDASE. III. ACTIVATION OF SKIN HYALURONIDASE BY THE ACTION OF BLOOD CORPUSCLES. The Journal of Biochemistry, 39(1), pp.77-79.
12. Tan, P., Malhotra, R. and Ali, S. (2016). Hyaluronic acid fillers and hyaluronidase use in regional eye blocks. Anaesthesia, 71(8), pp.988-989.