Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Pharmaceutical Sciences

Verifying the Safety of Microbial Contamination in Self-Injectors of Glucagon-like peptide-1 Receptor Agonists

Atsushi Ishimura[1*], Atsushi Inose[2]

[1]Division of Practical Pharmaceutical Sciences, Nihon Pharmaceutical University, 10281 Komuro, Ina-machi, Kitaadachi-gun, Saitama 362-0806, Japan
[2]Division of Life Science Pharmaceutics, Nihon Pharmaceutical University, 10281 Komuro, Ina-machi, Kitaadachi-gun, Saitama 362-0806, Japan

DOI: 10.36347/sajp.2024.v13i03.001 | **Received:** 03.02.2024 | **Accepted:** 08.03.2024 | **Published:** 11.03.2024

***Corresponding author:** Atsushi Ishimura
Division of Practical Pharmaceutical Sciences, Nihon Pharmaceutical University, 10281 Komuro, Ina-machi, Kitaadachi-gun, Saitama 362-0806, Japan

Abstract **Original Research Article**

Glucagon-like peptide-1 receptor agonists (GLP-1RAs) enhance insulin secretion in patients with type 2 diabetes mellitus in a glucose concentration-dependent manner. Many GLP-1RA formulations are administered in divided doses using a needle attached to the patient. Prior to use, the formulation is store in a cool place owing to stability. However, it is store at room temperature after use owing to problems with the injector. Therefore, the split-dose GLP-1RAs formulations contain phenolic antiseptics (phenol and cresol). Contamination of the formulation with microorganisms must be considered because patient self-injection is not performed under aseptic conditions. Therefore, this study verified the safety against microbial contamination during use based on Escherichia coli survival in split-dose GLP-1RA formulations. Unused liraglutide, exenatide, and lixisenatide were contaminated with E. coli stored at 25°C (room temperature) and cultured over time. E. coli gradually decreased immediately after suspension and E. coli did not survive after 60-90 min. The preservative of the split-dose GLP-1RAs formulation exhibited sufficient sterilizing power at 25°C, and it was inferred that room temperature is preferable for storage after use considering the formulation storage conditions, including injector failure and drug denaturation.

Keywords: Glucagon-like peptide-1 receptor agonists, phenol-based disinfectants, room temperature.

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is an incretin, a class of gastrointestinal hormone that enhances insulin secretion in a glucose concentration-dependent manner. Drugs developed for this purpose are GLP-1 receptor agonists (GLP-1RAs), which provide good glycemic control and weight loss with a low risk of hypoglycemia in patients with type 2 diabetes (Htike ZZ *et al.*, 2017). Major international guidelines recommend the use of GLP-1RA for the treatment of diabetes (Kamata M *et al.*, 2017). In Japan, six components can be prescribed in a variety of formulations (injections or tablets) and doses (daily or weekly). Peptide drugs containing GLP-1RAs are unsuitable for oral administration because their large molecular weight makes them less permeable in the gastrointestinal tract and they are degraded by enzymes in the stomach. The recent launch of oral dosage and storage methods are complicated and most formulations remain injectable. Additionally, many GLP-1RAs formulations were administered at divided doses by patients with a needle attached. The formulation is store in a cool place prior to use, owing to stability. However, it is store at room temperature after use owing to problems with the injector. The Japanese Pharmacopoeia's formulation regulations for injectable drugs stipulate that "an appropriate preservative in an amount sufficient to prevent the growth of microorganisms may be added to those to be administered in divided doses" so that the required sterility for injectable drugs is maintained throughout the period of use, even if microorganisms are introduced into injectable drugs to be used in divided doses. Therefore, GLP-1RAs preparations administered in divided doses contain a phenolic antiseptic (phenol or cresol) as a preservative that exerts bactericidal effects by disrupting the cell membranes of microorganisms and altering their enzymatic proteins. However, microorganism contamination of the formulation must be considered because patient self-injection is not performed under aseptic conditions. Therefore, this study verified the

safety of microbial contamination based on Escherichia coli survival in a split-dose GLP-1RA formulation.

METHODS

One of the colonies was touched with a platinum ear and suspended in 1 mL of PBS. Subsequently, 10 µL of the suspension was suspended in 1 mL each of unused liraglutide, exenatide, and lixisenatide. The absence of contamination was confirmed by pure culture of the Japanese Pharmacopoeia strain MG1655 in fixed medium. Samples were stored at 25°C for 30, 60, 90, 120, and 180 minutes, and 50 µL of each specimen was transferred to Soybean Casein Digest Canten medium, with three copies of each incubated at 35°C for 24 hours to check for survival. Positive results were observed for bacterial growth of the bacteria (community formation). The average number of colonies on the three pieces of medium were calculated if there were positive results. Controls were cultured under the same conditions to confirm that the bacteria were viable before contact with liraglutide, exenatide, and lixisenatide.

RESULTS

E. coli survival gradually decreased immediately after suspension in liraglutide, exenatide, and lixisenatide, and E. coli died after 60-90 min (Table 1).

Table 1: *E. coli* survival in GLP-1RA formulations

Number of E. coli colonies on control medium: 1392						
	Immediately following	30 min later	60 min later	90 min later	120 min later	180 min later
Liraglutide	147	1	negative	negative	negative	negative
Exenatide	117	negative	1	negative	negative	negative
Lixisenatide	1	negative	1	negative	negative	negative

DISCUSSION

E. coli survival gradually decreased immediately after suspension in liraglutide, exenatide, and lixisenatide, and E. coli died after 60-90 min at 25°C. This suggests that the phenol system in the GLP-1RA formulation has an appropriate bactericidal effect. Seven GLP-1RA formulations comprising six components can prescribe in Japan. Of these, four were split-dose formulations. Split-dose formulations contain a phenolic antiseptic (phenol or cresol) as a preservative, although the formulation and dosage of the preservative vary depending on the pharmaceutical company. Three of the four split-dose formulations were used in this study. This was because the same pharmaceutical company manufactured two of the four formulations, and the concentration of the preservative was the same; hence, three formulations were used.

GLP-1RA formulations are pre-filled formulations (hereafter referred to as "pen formulations") because they are based on self-injection. Peptide preparations such as insulin and GLP-1RAs are stored differently before and after use since they are in a cool place and after use at room temperature, owing to the stability of the preparations. There are two reasons for storage at room temperature. (1) Injection of cold formulations is painful at the injection site, so it is necessary to bring the temperature of the formulation back to room temperature; however, condensation from this temperature difference may cause injector failure. (2) Peptide formulations do not affect the potency of the drug if stored at room temperature for approximately one month. This study was conducted under 25°C conditions, considering near room temperature conditions after use. Cresol is a powerful preservative that requires twice the normal dosage when stored at 4°C owing to its reduced bactericidal power (Kishida M *et al.*, 1991). The basic conditions for the use of preservatives are drug concentration, temperature of action, and duration of action, each of which is closely related to the other and the disinfectant effect cannot be fully demonstrated if any of these conditions are lacking (Shinya Y, 1986). This is because the bactericidal action of a disinfectant is a chemical reaction between the drug and the bacteria; if the working temperature is not high, the reaction rate between the disinfectant and the bacteria, the speed of penetration into the bacteria, and the speed of diffusion will also be higher, suggesting that temperature works synergistically with bactericidal action.

This study inferred that the sterilizing power of the preservative for the split-dose GLP-1RAs formulation was sufficient at 25°C. It was inferred that room temperature was preferable for storage after use considering formulation storage conditions such as injector failure and drug denaturation. However, Bacillus cereus is widely present in nature, is heat resistant and may not be sterilized, even at temperatures that may cause drug denaturation. The stability of a drug product must be guaranteed as the first priority if microorganisms cannot be sterilized; therefore, it is important to prevent them from contaminating the drug product. It was considered necessary for healthcare professionals to emphasize the importance of basic cleanliness procedures to patients, such as hand washing before self-injection in a clean environment and wiping the rubber plug and injection site with alcohol cotton.

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

- Htike, Z. Z., Zaccardi, F., Papamargaritis, D., Webb, D. R., Khunti, K., & Davies, M. J. (2017). Efficacy

and safety of glucagon-like peptide-1 receptor agonists in type 2 diabetes: a systematic review and mixed-treatment comparison analysis. *Diabetes, Obesity and Metabolism*, *19*(4), 524-536.

- Kamata, M., Kubota, A., Shikishima, T., Inoue, A., Kaneshige, S., Nomiyama, T., Ogata, K., Yanase, T. & Kamimura, H. (2017). Evaluation of the safety and efficacy of change to Dulaglutide (a once-weekly GLP-1 receptor agonist). *Japanese Journal of Pharmaceutical and Diabetes*, 6, 193-200.
- Kishida, M., Terazawa, C., Ohta, H., Choshi, T., Horimoto, S., Hayashida, S., Nagao, Y., Ishibashi, F. & Takashina, S. (1991). Study on operative procedure and preservative condition and bacterial contamination of insulin vial. *Medicine and Drug Journal*, 27, 548-554.
- Shinya, Y. (1986). Cautions in using disinfectants. *Medicine and Drug Journal*, 22, 2001-2006.