Introduction

Biopharmaceuticals, such as vaccines, hormones, and therapeutic antibodies, are the fastest growing drugs in the pharmaceutical industry. However, most of these drugs should be administered by injection, which is one of the least favorable routes of taking medicines. Though injection is the ideal drug delivery method in terms of dose adjustment, bioavailability, and the rapid onset of efficacy, most people are unwilling to receive injections because of injection-related pain. Pain perception is increased in children compared with adults. The psychological stress imposed by repeated injections in chronically ill children is enormous and may result in needle phobia and reduced medical adherence. To overcome these disadvantages of injections, many approaches have been investigated so far. The first one is developing other administration routes, such as oral, topical, and inhalation. Inhalable insulin, Afrezza, approved in 2014 by the US FDA, is a good example of this approach. However, the market acceptance of Afrezza should be monitored carefully from the patients’ and physicians’ points of view. The other one is reducing administration frequency and at the same time mitigating pain associated with the frequent use of needles. Sustained-release formulation using micro-encapsulation or sustained-elimination technology using PEGylation or additional glycosylation are some of these technologies. Neulasta (PEGylated filgrastim), Pegasys (PEGylated interferon alpha), Nesp (Darbepoetin alpha, two glycosylation sites added), and Eutropin Plus (Somatropin microparticle encapsulated by hyaluronic acid) are the most successful examples. Another approach is developing convenient drug-device combination products, such as pen injectors containing cartridges and auto-injectors containing prefilled syringes. This approach has been widely used for SC injection products that enable self-administration, such as human growth hormone, insulin analogues, follitropin alfa, interferons, and monoclonal antibodies to treat rheumatoid arthritis. Since injection pain is closely related to the composition of the formulation and injection procedure, drug-device combination products should be developed by considering every aspect of injection pain.

This paper will cover the method of improving patient compliance with biopharmaceutical drugs by reducing injection-
associated pain in terms of formulation, device, and injection procedure.

**Formulation**

Proteins are very susceptible to degradations, such as aggregation, fragmentation, deamidation, oxidation, and isomerization. Therefore, formulation development is focused on stabilizing the active pharmaceutical agent, API, in an acceptable storage condition and period. In many cases, however, good stability of proteins does not occur with low injection pain. There are several formulation factors that affect injection-related pain.

1. **pH**

   pH is the most important factor regarding protein stability and injection pain. Physiological pH 7.4 is definitely the most preferred pH for injections. However, the pH of the formulation should be determined to minimize pH-dependent degradation, such as isomerization at an acidic pH or disulfide scrambling at a basic pH, as well as reduce aggregation and deamidation, which can occur at both acidic and basic pH. Particularly, the asparagine deamidation reaction is greatly affected by pH (2), and Asp-Pro bonds are well known to be easily cleaved at a low pH (3).

   In general, the freeze-dried formulation of proteins shows good stability at around a neutral pH. However, the optimum pH of aqueous formulations is slightly acidic in many cases. For example, the liquid formulation of human growth hormone is stable at around pH 6.0, whereas its freeze-dried formulation is stable at around a neutral pH. Interferons and Etanercept also use a slightly acidic pH in liquid formulation to increase shelf life.

2. **Buffer**

   The role of buffer in pharmaceutical formulation is maintaining the pH of the solution during its shelf life. Commonly used buffers for recombinant protein formulations are summarized in Table 1 along with the pH ranges at which they are effective. The typical buffers used to control the pH in protein formulations are usually used in the concentration range of 5–100 mM. Since those buffer species already exist in our body, they do not seem to cause any injection pain. However, several studies have reported that the citrate buffer causes injection pain.

   Double-blind, placebo-controlled, randomized, cross-over studies were conducted to identify the pain-causing component in epoetin alfa preparation (4). It was found out that the local pain experienced after SC administration of epoetin alfa preparation is mainly caused by the citrate component of the buffered solution.

   Similar results were obtained from the direct comparison study with commercially available human growth hormone products (5). In this study, perception of pain was evaluated by the volunteers immediately after injection and 2 min after injection of three formulations into the thigh, which differed with respect to pH and buffers (histidine, citrate, and saline). Significantly more participants (38/54) found that the citrate buffer caused more pain than the histidine buffer immediately after injection ($P=0.002$). Histidine buffer did not cause more pain than saline buffer ($P=0.996$). After 2 min, there was no difference between the histidine and the citrate buffers ($P=1.00$), nor between the histidine and saline buffers ($P=1.00$).

   Buffer concentration also affects injection site pain. As pH of the solution diverges from physiological pH, the impact of buffer concentration or buffer strength becomes more important. Effect of buffer concentration and pH on injection pain was studied with the formulations, made isotonic with NaCl, and ranged in pH from 6 to 7 with phosphate buffer concentrations of 5 to 50 mM (7). The local tolerance after injection was assessed as injection pain on a visual analogue scale, pain duration, and local tolerance (redness, paleness, and oedema). The discomfort at the injection site was lowest with 10 mM phosphate, pH 7. Injection of buffer at pH 6 (50 mM phosphate) caused significantly more pain than using 10 mM phosphate, whereas the pain at pH 6 using 10 mM phosphate did not differ significantly from that experienced by injection of the solution at pH 7 using either 10 or 50 mM phosphate. Therefore, the buffer strength should be kept as low as possible to avoid pain upon injection.

3. **Excipient**

   Various excipients have been used to improve the stability of biopharmaceutical formulation. Commonly used excipients can be grouped into surfactants such as polysorbate and poloxamer; sugars such as sucrose and trehalose; polyols such as mannitol and sorbitol; amino acids such as glycine, methionine, and arginine; or salt. While these stabilizers do not seem to cause any
injection pain, preservatives contained in multiple-dose prepara-
tions have been known to affect patient compliance in terms of
injection pain and odor.

A recent review summarized two studies regarding local dis-
comfort and pain at the injection site in association with preser-
vatives. In a study to compare benzyl alcohol (0.9% and 1.5%)
and m-cresol (0.25%), greater discomfort was consistently report-
ed for 0.25% m-cresol than for the either of the benzyl alcohol
solutions by both the patients and the nurses. In another study to
compare phenol and benzyl alcohol, 19 out of 30 healthy volun-
teers reported significantly less pain with 0.45% phenol than 1.5%
benzyl alcohol.

Benzyl alcohol and phenol are good preservatives; they are also
known to have local anesthetic function and result in low injec-
tion pain. On the contrary, m-cresol gives stronger odor and
more pain than phenol and benzyl alcohol.

4. Osmolality

Control of osmolality of the formulation is important to ensure
that the product delivered to the patient is isotonic (285–295
mOsm/kg). As the osmolality goes higher or lower from the iso-
tonic range, injection pain can increase.

For example, recombinant human papillomavirus vaccine,
Gardasil, is notorious for severe injection pain, which is mainly
due to hyper-osmolality. The concentration of sodium chloride
in Gardasil is more than twice (1.91%) the isotonic concentration
(0.9%). However, it is supposed to be unstable in isotonic condi-
tions.

5. Delivering volume

When high injection pain is expected due to pH or osmolality
of the solution, reducing delivering volume by increasing concen-
tration of protein is another option on the assumption that the
drug itself does not cause pain.

Device

1. Needle

Venipuncture requires the use of needles typically as large as
22–21 gauge inserted to depths of 25–38 mm to withdraw mil-
illiters of blood. In contrast, vaccines usually require injection of
less than 1 ml of fluid; therefore, 25- to 22-gauge needles with
a length of 16–38 mm are adequate. For the staked-needle type
of prefilled syringes, 27-gauge is a standard needle in 1-mL-
long prefilled syringes. However, 29-gauge needle with thin wall,
which has the same inner diameter as 27-gauge is becoming
popular recently. Insulin delivery, which involves even smaller
volumes and is typically carried out by patients in diverse every-
day settings, benefits from still smaller needles, usually of 31–29
gauge inserted to a depth of 6–13 mm.

To mitigate pain from hypodermic injections, the effect of nee-
dle geometry on pain has been investigated. Needle gauge has
been shown to significantly affect the frequency of pain during
needle insertion into the skin of human subjects. For example,
insertion of a 27-or 28-gauge needle had an approximately 50%chance of being reported as painful, which was significantly
greater than that for insertion of a 31-gauge needle, which had a
39% chance of causing pain.

Recently, fine needles such as 32- and 33-gauge needles 5 mm
in length have been introduced. However, fine needles are not
suitable for all applications. For example, rapid delivery of large
volumes and administration of formulations with large particu-
lates require larger needles. In addition, fine needles are accom-
panied by an increase in gliding force of the plunger due to with
narrow inner diameter of the needle. Thus, there is a trade-off
between pain and other delivery considerations when smaller
needles are used. The correct balance must be obtained for each
application.

2. Injection device

Drug-device combination products such as pen injector and
autoinjector are very popular these days. Examples include in-
sulin analogues, human growth hormone, follitropin alfa, and
parathyroid hormone for pen injectors, and TNF-alpha blockers,
pegylated interferon, and darbepoetin alpha for autoinjectors.

Basically, devices have been designed to improve compliance of
patient by supplying convenient and safe injection procedures.
They also play a role in reducing injection pain by introducing
fine needles for pen injectors. In addition, patients can feel more
less anxiety by hiding needles throughout the whole injection
process.

Injection Procedure

To avoid unnecessary pain due to inappropriate usage of the
drug, various factors associated with the injection procedure
should be standardized. When it comes to the self-administration
drug, education for the patient is very important. Since most bio-
pharmaceutical drugs are stored at 2–8°C, they should be taken out of the refrigerator and kept at room temperature for about 30 minutes before injection. If the temperature of the solution is low, it can cause injection pain.

One of the factors associated with increased patient discomfort was the injection technique used by clinicians. Further strategies to minimize pain during the injection procedure are to have a good technique, to give clients appropriate information, to be a calm and confident nurse, to use a drawing-up needle, to use the smallest diameter needle, to flick the skin or tap the injection site before swabbing, to stretch the skin, to enter the skin quickly, to distract the patient, and to inject the medication slowly.

Conclusion

Formulation development of biopharmaceuticals should be focused on minimizing injection pain as well as increasing stability of proteins. In cases where acidic or basic pH of drug solution is inevitable, concentration or strength of buffer should be minimized as long as pH is maintained through the shelf life. Citrate buffer is not recommended because it is known to cause injection site pain. If the formulation is designed for multiple doses, care should be taken to select a preservative system that can affect injection pain. Using fine needles can definitely reduce the injection pain. However, it also increases gliding force of the plunger, which can make injection time longer or requires more power to inject. Therefore, the correct balance must be obtained for each application. Finally, injection procedures should be well standardized and patients educated, particularly in the case of self-administration.

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