J. Pharm. Pharmacol. 1996, 48: 1012–1015
Received November 16, 1995
Accepted February 27, 1996

Local Tolerance of Subcutaneous Injections

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Abstract

Human insulin-like growth factor I (hIGF-I) has several possible clinical applications. Because subcutaneous administration of the drug can cause pain, local tolerance to injection of different formulations with or without hIGF-I has been investigated in man using isotonic saline solution as reference.

The formulations, made isotonic with NaCl, ranged in pH from 6 to 7 with phosphate buffer concentrations of 5 to 50 mM. 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 on injection of the solution at pH 7 using either 10 or 50 mM phosphate. hIGF-I itself did not seem to cause pain.

We concluded that for subcutaneous injections at non-physiological pH, the buffer strength should be kept as low as possible to avoid pain upon injection. We also hypothesize that when a non-physiological pH must be used for stability reasons, a lower buffer strength enables more rapid normalization of the pH at the injection site.

Human insulin-like growth factor (hIGF-I) has insulin-like and growth-promoting effects and there are possibilities for future clinical use of recombinant hIGF-I in a wide variety of applications. hIGF-I is most stable at pH 6 (Fransson et al 1994) and a parenteral formulation at this non-physiological pH was developed for subcutaneous administration. The severe pain experienced by several patients in early clinical trials on injection with hIGF-I has been investigated in this study. In numerous studies with other drugs (Ipp et al 1990; Zindel 1989; Gazzaniga et al 1993) subjects have reported pain upon subcutaneous injection. Factors that might cause pain include the injection volume and the speed of injection (Frenken et al 1994), osmolality (Doenicke et al 1992), the pH of the formulation (Barnet & Kapp 1992), injection site (Ipp et al 1990), the size and quality of the injection needle (Coley et al 1987), the presence of irritating substances (Frenken et al 1993) and the temperature of the solution (Ross & Soltes 1995).

The purpose of buffers in pharmaceutical formulations is to maintain a stable pH, usually that at which the drug is most stable. The ability of a buffer to maintain a pH value is dependent on the pK_a of the buffer, on the pH and on the concentration (Flynn 1980). We hypothesized that the injection pain could be reduced if a formulation with a lower buffer capacity was used for hIGF-I.

Several clinical trials on analgesics have shown that pain scales can be a reliable and objective method of assessing the analgesic properties of drugs (Baños et al 1989). It is generally accepted that visual analogue scales are more sensitive and accurate than other measures, thus being generally advised in the evaluation of the intensity of pain (Langley & Sheppard 1985).

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The aim of this study was to evaluate how pH, buffer concentration and presence of hIGF-I affect local tolerance to subcutaneous injection of the solution.

Materials and Methods

Study design

The investigation was designed as a double-blind, randomized, 10 × 10 Graeco-Latin square with the three factors: subject (1–10), site of injection (1–10) and injection order number (1–10). Eight formulations and two identical reference solutions containing 150 mM NaCl were compared and were balanced in respect of the site and of the injection number in the sequence of ten injections given to each subject. All injections were to be in 4 × 4 cm regions on the lower left and right arms, which are relatively sensitive to pain.

A published 10 × 10 Graeco-Latin square (Peng 1967) was taken as a basis (Table 1). The ten rows of this square correspond to subjects, the ten columns correspond to injection sites, the ten Latin letters A–J correspond to formulations, and the ten numerical indices correspond to the injection number in the sequence of injections given to each subject. The assignments of subjects to rows, of injection sites to columns, and of letters A–J to formulations, were performed randomly. The resulting assignments were then transferred to personal maps specifying the site for each injection in each subject.

Formulations

Although phosphate is not ideal at pH 6, because of its low buffer capacity at this pH, sodium phosphate was chosen as buffer, because citrate buffer causes pain (Frenken et al 1993). The compositions of the eight formulations and the reference solution are listed in Table 2. The formulations were prepared by mixing disodium phosphate, monosodium phosphate and

Table 1. The Graeco-Latin square experimental design. The columns correspond to the injection sites, the rows correspond to the subjects and the indices correspond to the injection number.

Row (subject no.)	Column (site of injection)									
	8	5	7	3	4	10	6	9	1	2
6	A_1	E_8	B_9	H_7	C_{10}	J_4	I_6	D_5	G_2	F_3
8	I_7	B_2	F_8	C_9	H_1	D_{10}	J_5	E_6	A_3	G_4
10	J_6	I_1	C_3	G_8	D_9	H_2	E_{10}	F_7	B_4	A_5
3	F_{10}	J_7	I_2	D_4	A_8	E_9	H_3	G_1	C_5	B_6
7	H_4	G_{10}	J_1	I_3	E_5	B_8	F_9	A_2	D_6	C_7
9	G_9	H_5	A_{10}	J_2	I_4	F_6	C_8	B_3	E_7	D_1
5	D_8	A_9	H_6	B_{10}	J_3	I_5	G_7	C_4	F_1	E_2
4	B_5	C_6	D_7	E_1	F_2	G_3	A_4	H_8	I_9	J_{10}
2	C_2	D_3	E_4	F_5	G_6	A_7	B_1	I_{10}	J_8	H_9
1	E_3	F_4	G_5	A_6	B_7	C_1	D_2	J_9	H_{10}	I_8

sodium chloride in water for injection to give isotonic solutions of different pH and buffer concentration. hIGF-I was added to some of the solutions by ultrafiltration.

Study population

Ten healthy adult male volunteers, aged 18–40 and sensitive to injection pain, were recruited to the study. All potential participants were injected with 0·2 mL of a vehicle known to inflict pain on injection (pH 6, 50 mM phosphate) over 20 s at a pre-study medical examination. At 30 s after injection each subject assessed the injection pain on a 100 mm horizontal visual analogue scale (0 mm = no pain, 100 mm = severe pain). Subjects that marked > 20 mm on this scale were eligible for inclusion in the study.

Injection procedure

After randomization, all subjects received a total of ten injections (0·2 mL injection^{-1}). The ten injections, representing the eight formulations and the two sodium chloride references, were injected subcutaneously using a 0·5 mL 28 G Microfine syringe (Beckton and Dickinson, USA) with a fixed cannula. The solutions were stored at 2–8°C until the day of use, when they were equilibrated to room temperature before injection. All injections were given subcutaneously over 20 s by one person.

For each subject each of the ten injections was made every 15 min in a specified order and site on the lower left and right arms. The subjects filled in assessment sheets after each injection. In order to estimate the local tolerance to injection, the variables evaluated were: injection pain 30 s after each injection, assessed on a 100 mm visual analogue scale (*0 mm = no pain, 100 mm = severe pain*); duration of injection pain, measured with a stop-watch given to each subject; local tolerance in terms of redness, paleness and oedema, assessed by one of the staff on a yes or no scale; and, for some subjects, description of the injection pain in words (e.g. burning, itching).

An erroneous exchange of injection 2 between subjects 7 and 8 occurred during the study. Subject 7 did not receive formulation I and received formulation B twice, whereas subject 8 received formulation I twice and did not receive formulation B. All other subjects received all the formulations once. This imbalance was taken into account in the statistical handling of the data (see below).

Table 2. The pH and concentrations of sodium phosphate buffer, sodium chloride and hIGF-I in the formulations.

Formulation	pH	Phosphate (mM)	Sodium chloride (mM)	hIGF-I (mg mL^{-1})
A	6·0	50	112	0
B	6·0	10	145	0
C	7·0	50	112	0
D	7·0	10	145	0
E	6·0	5	150	0
F	6·0	50	112	5
G	6·0	10	145	5
H	7·0	50	112	5
I*	8	0	150	0
J*	8	0	150	0

*Reference solutions

Blood glucose

On treatment with hIGF-I there is a minor risk of hypoglycaemia. At the doses used in this study, this was considered to be very unlikely because the injections were given to non-fasting subjects. In order to minimize the possibilities of hypoglycaemia, non-fasting glucose was measured before the first injection and after the last, and food was supplied during the study day.

The concentration of glucose was measured with a Reflolux photometer (Boehringer Mannheim, Germany). Blood samples were taken from the finger tips from all subjects on the study day. The glucose levels were measured before the injections, and 0·5 and 3 h after the last injection.

Statistics and data analysis

The primary variable in the statistical analysis consisted of the visual analogue scale assessments of pain at each injection. Comparison of the eight formulations and two references was based on a graphical version of Tukey's *T*-procedure adapted to a 10 × 10 Graeco-Latin square design. Comparison between the effects of the formulation on the visual analogue scale responses could then be made in terms of so-called LSMEANS which, briefly, estimated what the means would have been if the design had been balanced. A slightly conservative variant of Tukey's *T*-procedure was used, namely to replace the

standard error of the difference between any two LSMEANS by the maximum 7·237 of all such standard errors. The 5% upper quartile of the studentized range distribution with parameter k = 9 (formulations) and 64 degrees of freedom is equal to 4·54 (Hochberg & Tamhane 1987). The 'uncertainty' intervals based on Tukey's *T*-procedure to be used in the graphical display, each have endpoints of the form LSMEAN ± 4·54 × 7·237/8, i.e. LSMEAN ± 11·6. The formal comparisons of the formulation effects using Tukey's *T*-procedure were considered to be approximate in view of the visual analogue scale used. The residual plot indicated, as expected, that the variability in the visual analogue scale scores was relatively small at low levels, although the statistical model used actually assumes constant variability. This was compensated somewhat by the use of a slightly conservative variant of Tukey's *T* procedure, with somewhat too large 'uncertainty' intervals.

Results and Discussion

As shown in Fig. 1 the different formulations caused different amounts of injection pain. The LSMEANS of the visual analogue scale scores and the end-points of the corresponding 'uncertainty' intervals are shown in the figure. Non-overlapping intervals indicate significant (at the 5% level) differences between the effects of the formulations. Even though there were quite large inter-individual differences, as shown by Table 3, pH 6, 50 mM phosphate formulations clearly caused more injection pain than pH 6, 10 mM phosphate formulations. It was shown that injection of hIGF-I, (pH 6, 50 mM phosphate), caused injection pain in at least 90% of the subjects in the study (> 10 mm on visual analogue scale), whereas only 30% of the subjects in the study marked more than 10 mm on the visual analogue scale on injection with a formulation of hIGF-I of the same pH but with only 10 mm phosphate. Further reduction in buffer concentration to 5 mm phosphate did not reduce pain further. Because formulations with or without hIGF-I caused similar amounts of injection pain, it is concluded that hIGF-I itself did not cause injection pain.

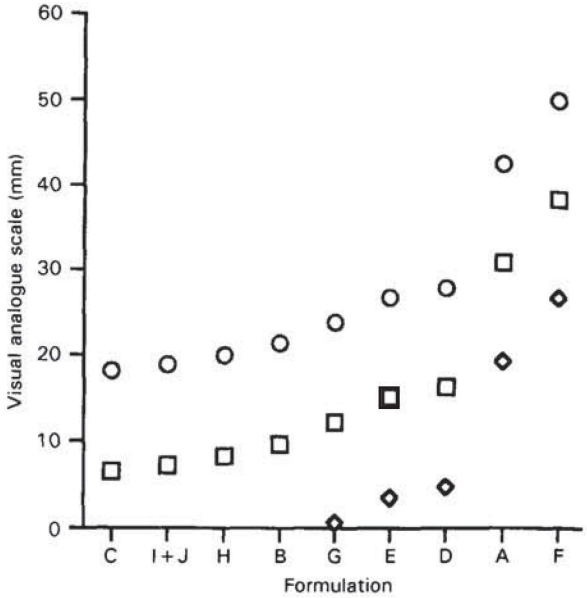

FIG. 1. Assessment of injection pain by visual analogue scale (□). Results from statistical evaluation of 'uncertainty intervals', upper (○) and lower limit (◇) for simultaneous comparisons of formulations at an approximate significance level of 5% based on Tukey's *T*-method. The formulations are described in Table 2.

An hIGF-I preparation of pH 7 is not feasible because of the lower stability of hIGF-I. This pH caused less injection pain than pH 6, however. At pH 6, a lower buffer concentration resulted in less discomfort, possibly because a higher buffer concentration results in a slower change in solution pH at the injection site. The frequency and intensity of occurring redness, paleness and oedema at the injection site also decreased on reducing the buffer concentration or increasing the pH.

A non-physiological pH changes the chemical equilibrium between physiological buffers in subcutis and cutis tissue. This change causes transport of potassium ions which depolarize the nerve endings, causing pain (Guyton 1991). The magnitude

Table 3. Summary of the assessments of pain on a visual analogue scale (mm) for each formulation versus subject number. The formulations are described in Table 1.

Subject number	Formulation								
	A	B	C	D	E	F	G	H	I+J
1	29·0	16·0	0·0	0·0	14·0	13·0	2·0	11·0	0·0
2	2·0	11·0	0·0	7·0	14·0	57·0	21·0	0·0	0·0
3	1·0	1·0	0·0	10·0	3·0	0·0	5·0	7·0	1·0
4	45·0	28·0	19·0	30·0	33·0	33·0	17·0	15·0	20·5
5	56·0	0·0	15·0	7·0	38·0	78·0	60·0	0·0	8·5
6	26·0	7·0	2·0	6·0	23·0	21·0	8·0	9·0	2·0
7	18,0	10·5	5·0	2·0	2·0	13·0	0·0	3·0	0·0
8	64·0	–*	0·0	28·0	17·0	75·0	2·0	2·0	10·3
9	20·0	0·0	12·0	0·0	3·0	41·0	7·0	27·0	29·5
10	47·0	0·0	12·0	73·0	4·0	53·0	0·0	9·0	1·5
Mean	30·9	9·7	6·5	16·3	15·1	38·4	12·2	8·3	7·2

*Subject number 8 did not receive formulation B.

and duration of the depolarization would be dependent on the pH and buffer concentration. In other words, the induced depolarization of the nerve endings is more quickly normalized at lower buffer concentrations.

Pain is difficult to evaluate, partly because of its subjective character and the complex feelings that pain evokes. The subjective sensation of pain differs greatly within individuals, as is obvious from the large inter-individual differences in visual analogue scale scores in Table 3.

The Graeco-Latin square design used in this study was chosen in order to obtain as much information as possible from a limited number of subjects. This design requires complete balance in order to achieve convincing results on completion of the study. In this study, however, imbalance occurred as stated at the end of the section on injection procedure. It was, therefore, necessary to modify the statistics somewhat on calculation of the results.

There is a significant difference in pain, measured on a visual analogue scale, caused by injections at pH 6 and pH 7 when the buffer concentration is high (50 mM). The pain experienced at pH 6 can, however, be reduced substantially by reducing the buffer concentration. It is the buffer capacity of the solution (i.e. resistance to pH changes) that determines how stable the pH value will be when the solution is injected into the tissue. The results from this study show that because the hIGF-I formulation of pH 6 in 10 mM phosphate results in less pain at the injection site it is a more suitable hIGF-I formulation of than that of pH 6 in 50 mM phosphate. As the formulations with hIGF-I and the corresponding vehicles cause a similar degree of injection pain, it is shown that the pain is not caused by the hIGF-I itself.

Acknowledgements

We thank Associate Professor Bertil Karlmark and Dr Märta Ryde for their expertise, Dr Ebba Florin-Robertsson for valuable comments during the preparation of the manuscript, and Dr Olivier Gilbaud for the statistical analysis.

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