Topical disposition of two strengths of a I-125-rhEGF jelly in rat skin wounds

Article *in* Biopharmaceutics & Drug Disposition · July 2004

DOI: 10.1002/bdd.393 · Source: PubMed

12 authors, including:

Jorge Duconge
University of Puerto Rico, Medical Sciences Campus
126 PUBLICATIONS **700** CITATIONS
SEE PROFILE

Ana Aguilera Barreto
Center for Genetic Engineering and Biotechnology
21 PUBLICATIONS **46** CITATIONS
SEE PROFILE

Maria Becquer
University of Havana
19 PUBLICATIONS **84** CITATIONS
SEE PROFILE

Daniel Alvarez
Medipan s.a
6 PUBLICATIONS **289** CITATIONS
SEE PROFILE

BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **25**: 193–201 (2004)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/bdd.393

Topical Disposition of Two Strengths of a ^{125}I-rhEGF Jelly in Rat Skin Wounds

J. Duconge[a,*], P.A. Prats[b], C. Valenzuela[b], A. Aguilera[b], I. Rojas[b], M.A. Becquer[a], D. Alvarez[a], L. Estrada[c], S. Alfonso-Ortíz[a,†], E. Hardy-Rando[b], O. García-Pulpeiro[d] and E. Fernández-Sánchez[a]

[a] *Laboratory of Biopharmaceutics, Department of Pharmacology, Institute of Pharmacy and Foods, University of Havana, Ave 23 and 222, La Coronela, Havana 36, CP 13600, Cuba*

[b] *Center for Genetic Engineering and Biotechnology, Havana, Ave 31 e/ 158 y 190. P.O. Box 6162, Havana 10600, Cuba*

[c] *Department of Pharmacy, 'C.J. Finlay' University Hospital, Havana 10600, Cuba*

[d] *Pharmaceutical Laboratories 'Roberto Escudero', Research and Development Section, Havana, Cuba*

ABSTRACT: Growth factors have proved to be an effective therapeutic strategy. However, some controversies have arisen concerning their efficacy in topical wound treatments. Stabilization of epidermal growth factors at the wound site and long-lasting receptor occupancy are important factors for wound repair. This study evaluated the cumulative profiles of two jellies containing 10 or 20 μg of ^{125}I-rhEGF per gram of jelly, in a rat full-thickness skin lesion model. The prolonged time-courses at the wound sites for both strengths compared with saline solutions previously evaluated using a similar skin lesion model are reported. It seems that these two topical formulations that provide more sustained amounts of ^{125}I-rhEGF over the period of sampling, would probably achieve the required wound healing response in terms of cell proliferation, collagen deposition and protein synthesis. Further studies need to be developed in order to elucidate whether such an *in vivo* disposition pattern is consistent with an earlier and stronger promotion of wound healing events. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: ^{125}I-rhEGF; jelly; topical disposition; strength; non-linearity

Introduction

The promotion of dermal and epidermal regeneration is a crucial aim in the treatment of acute and chronic wounds. Growth factors have proved to be an effective therapeutic strategy. However, controversy exists over the efficacy of many topical wound treatments, particularly growth factors, and questions have arisen as to the real value of these agents.

For instance, exogenous epidermal growth factor (EGF) accelerates wound healing, but treatment effects are often modest. It is the opinion of some researchers that failures are due to the existence of local factors such as proteases in the wound fluid that make local protein administration ineffective [1, 2, 3]. It is clear that new delivery systems and therapeutic strategies need to be developed to improve dermal and epidermal regeneration.

Several findings indicate that stabilizing EGF at the wound site and achieving long lasting receptor occupancies are important factors in permitting the expression of its healing effects and suggest that topical formulations stabilizing EGF would be suitable dosage forms for the acceleration of wound repair.

*Correspondence to: Laboratory of Biopharmaceutics, Department of Pharmacology, Institute of Pharmacy and Foods, University of Havana, Ave. 23 and 222, La Coronela, Havana 36, CP 13600, Cuba. E-mail: jduconge@yahoo.com.mx

†Current address: Center of Immunoassay, SUM 1, Ave 25 and 134, Playa, Havana, Cuba.

Received 29 January 2003
Revised 20 October 2003

Our research group has been working with a silver sulfadiazine-based rhEGF cream, and has obtained contradictory results. It was found that silver itself retarded the wound healing process besides having deleterious effects on EGF, mediated through the generation of silver free radicals, although others have found the opposite [4].

Evidence indicates that mitogenic stimulation by EGF requires at least an 8–12 h exposure to make the majority of the cells divide [5]. Even exposures for as short as 2 h have produced a significant increase in healing rates, and increasing the time of exposure further increases the rate of wound healing [6].

Simple and competitive dosage forms are adequate for treating skin lesions successfully. Our previous report [7] found a rapid disappearance of ^{125}I-rhEGF solutions from full-thickness skin wounds in rats and hypothesized that other dosage forms such as a jelly or a cream could prolong EGF permanence at the lesion site without incurring the expense of more complicated strategies.

This paper reports prolonged courses of two strengths of a ^{125}I-rhEGF jelly at wound sites compared with saline solutions previously evaluated in a similar skin lesion model.

Materials and Methods

Growth factor

Highly purified recombinant human EGF was produced at the Center for Genetic Engineering and Biotechnology (CIGB, Havana, Cuba). Purified $NaI^{125} \cdot EGF$ (0.1 mg/ml) radiolabelled by the chloramine-T method (Amersham, Arlington Heights, IL, USA) was used (radiochemical purity 93%; efficiency 72% and specific activity 200 μCi/μg).

Formulation

The ^{125}I-rhEGF topical jelly formulation was developed by the Center for Genetic Engineering and Biotechnology, Havana, Cuba [8] containing ^{125}I-rhEGF (0.001%, 10 μg/g, lower strength, or, 0.002%, 20 μg/g, higher strength), carbopol 940 (0.50%), propylenglycol (10%), NaOH (0.20%), EDTA-Na_2 (0.010%), methyl-paraben (0.18%), propyl-paraben (0.02%) and sterile water. All these aqueous phase excipients were obtained from Sigma (St Louis, MO, USA) and Merck (Darmstadt, Germany).

^{125}I-rhEGF cumulative profiles in skin lesions

All animal procedures were carried out under the approval of the Ethics Committee for Laboratory Animal Use at CIGB. All the invasive procedures were conducted under sodium pentobarbital anaesthesia (40 mg/kg body weight). Adult male Sprague-Dawley rats (247–265 g, 7–9 wk old) were intraperitoneally anaesthetized and four 1×1 cm full-thickness skin lesions were made on the upper and lower back. Lesions were allowed to evolve untreated for 2 h. Then, 400 mg of jelly containing either 10 or 20 μg of ^{125}I-rhEGF per gram of jelly was delivered to each lesion site. Each lesion was considered as an experimental unit ($n = 12$). Three rats per experimental group were killed and 12 lesions were sampled at 2, 4, 6, 12, 24, 48 and 72 h. Skin specimens were excised, flushed and then washed twice with ice-cold phosphate buffered saline (pH 7.4), and weighed before measurement of radioactivity in a 1272 gamma counter (LKB, Sweden). The resultant tissue-associated radioactivity was expressed as the percentage of the administered dose per gram of tissue from the wound site (%D/g). A ^{125}I-rhEGF-radioactivity standard (100% radioactivity) was included for correction of radioactivity measurements.

Serial plasma samples were obtained by puncture of the retrorbital plexus. Individual urine samples were taken by means of sterile urine collectors, at the same experimental time points. Blood was placed in heparinized tubes and centrifuged at 6000 rpm, for 1 min. Finally, 300 μl aliquots were pipetted into LP3 tubes for determining TCA-precipitable plasma-associated radioactivity. One hundred microliters of plasma was added to the same volume of 30% TCA (v/v), vortexed and incubated for 30 min on ice, centrifuged for 5 min at 6000 rpm, and the sediment fraction was counted using a 1272 gamma counter (LKB, Sweden). The total urine volume was registered and 300 μl aliquots were

transferred to LP3 tubes to determine the excreted radioactivity.

Kinetic analysis

Analysis of the drug disposition pooled data was performed by a non-compartmental analysis (NCA) with a combined linear/log linear trapezoidal rule approach. A time zero value was added for extrapolation purposes. The linear trapezoidal rule was used up to the peak level, after which the logarithmic trapezoidal rule was applied. Lambda z is a first-order rate constant associated with the terminal (log linear) segment of the curve. It was estimated by linear regression of the included terminal data points. Goodness of fit for the terminal elimination phase, adjusted for the number of points used, was also provided. The largest adjusted regression was selected in order to estimate lambda z, with one caveat: if the adjustment did not improve, but was within 0.0001 of the largest value, the regression with the larger number of points was used.

Parameters extrapolated to infinity, such as *AUC* (i.e. area under the disposition curve) and *AUMC* (i.e. area under the first moment of the disposition curve), were computed based on the last predicted level, where the predicted value was based on the linear regression performed to estimate the terminal lambda first-order rate constant. Computing these parameters based on the last observed level was discouraged in order to avoid large estimation errors. The time to peak value was determined as the time of the maximum observed level (i.e. maximum %D/g) considering the entire curve; and the peak level was that corresponding to the above mentioned time to peak value. For all these purposes WinNonlin professional software (Version 2.1, Pharsight Inc., 1997, NC, USA) was used.

In the case of flat, modified-release formulation, the plateau time was a more suitable parameter than the time to peak. The plateau time was defined as the time span of one dosing cycle, during which the drug levels deviated from the peak level, by less than a clinically specified percentage. The time during which ^{125}I-rhEGF levels were at least half the peak level (50%) was considered by Meier *et al.* [9] to correlate to the width of the efficacy range, and corresponded to the half-value duration (HVD).

For assessing the extent of persistency of the modified-release formulation, simple solutions, i.e. ^{125}I-rhEGF saline solutions, and modified-release jelly formulations, with two ^{125}I-rhEGF strengths (10 and 20 μg/g), were chosen under the null hypothesis that the peak level would not differ by more than 20%. Based on their half-value duration ratio (R_{HVD}), the following ratings were taken into consideration: $R_{HVD} < 1.5$ is no retardation; $1.5 \leqslant R_{HVD} < 3$, is weak to medium retardation; $R_{HVD} \geqslant 3$ is strong retardation.

The ^{125}I-rhEGF *in situ* availability ($F_{in\ situ}$) from the jelly formulation at the lesion site was determined by the ratio of the *AUC* for each strength to the *AUC* of the saline solution, using the corresponding dose correction factor. For these purposes the previously reported experimental data for saline solutions containing 200 and 400 ng of ^{125}I-rhEGF were considered [7].

Statistical analysis

Measures of central tendency and dispersion (mean, median, minimum and maximum values, standard deviation and inter-quartile range) were calculated in each case. The assumptions of normality (Shapiro-Wilks test) and homogeneity of variances (Levene test) among the groups were verified at each time. Differences between the groups at each time-point were assessed by the independent-samples *t*-test or the non-parametric Mann-Whitney *U*-test, depending on the occurrence of the previously verified assumptions. Also, time variations in every group were analysed by the Dunn test. In all cases, the analysis was made using the software SPSS for Windows, version 10.0, and a p value < 0.008 was considered statistically significant, using the Bonferrony adjustment.

Results

Figure 1 depicts the ^{125}I-rhEGF local disposition profiles, after topical application of a jelly formulation containing two different strengths (i.e. 10 and 20 μg/g) in a full-thickness skin lesion model in rats. Table 1 shows the ^{125}I-rhEGF-

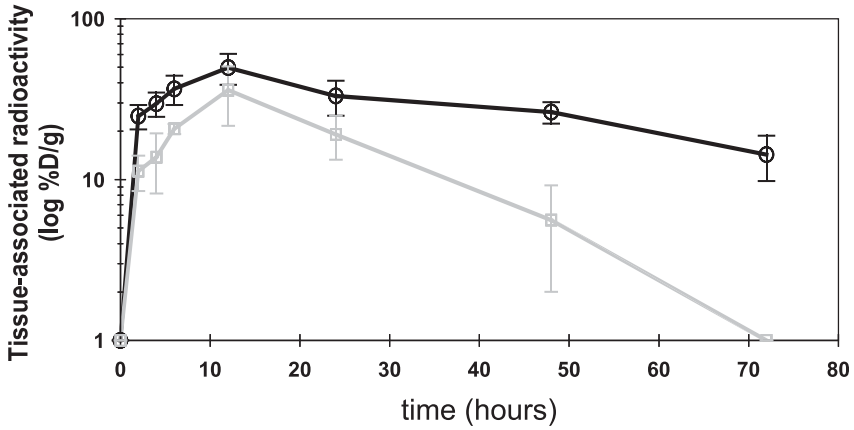

Figure 1. Disposition profiles after application of a jelly formulation containing each dosage strength under study, i.e. 10 (□) and 20 µg ^{125}I-rhEGF (○) per gram of jelly, onto full-thickness skin wounds in rats. Values represent the mean ± SD of 12 replicates per sampling time

Table 1. Cumulative profiles of ^{125}I-rhEGF-associated radioactivity at the lesion sites expressed as percentage of the dose applied per gram of tissue (%D/g), after topical administration of both strengths (mean ± SD)

Dosage strength (µg/g)	Time (h)						
	2	4	6	12	24	48	72
10	11.3(± 2.8)[a]	13.8(± 5.6)[a]	20.7(± 1.5)[a]	36.1(± 14.5)[a]	19.1(± 5.8)[a]	5.6(± 3.6)[a]	ND
20	24.8(± 4.3)[b]	29.7(± 5.1)[b]	36.7(± 7.6)[b]	49.7(± 10.8)[a]	33.1(± 8.1)[b]	26.3(± 4.0)[b]	14.3(± 4.5)

[a,b]Different superscript means significant differences ($p < 0.008$).
ND, no drug was detected at 144 h.

associated radioactivity per gram of tissue (%D/g) at each time point. Statistically significant differences were detected between the two strengths at all times evaluated, except for 12 h when the differences were not evident despite figures suggesting otherwise.

Interestingly, both jellies showed a rather similar kinetic pattern as judged by the shape of the curves depicting their respective accumulation behaviour at the wound site. There was a remarkable difference, however, in the extent of disposition as seen from their individual *AUC*. Even though the time to peak was the same for both formulations (12 h), the tissue-associated radioactivity peaked to 49.7%D/g for the higher strength, while the peak value was only 36.1% D/g for the lower one, which showed a sharper decline after 24 h, reaching a value of zero at 72 h, while its counterpart was still radioactively detectable.

Certainly, the local application of both jellies elicited typical deposition responses for the experimental animal model used, with less than 25%D/g being detected during the first 2 h. Table 2 shows average kinetic parameters characterizing each formulation studied. Accordingly, the relatively slow elimination rate from the wound site is a common feature for both strengths, although the 20 µg/g jelly showed a more protracted course as indicated by a larger MRT, HVD and *in situ* availability ($F_{in\ situ}$), a fact reinforced by the diffusion-limited release pattern of the jellies. Moreover, we must be aware of the capacity-limited (i.e. saturation) non-linear kinetic at the lesion site, which was evident when comparing the disposition parameters of both jellies. An interesting plateau was observed between 24 and 48 h for the higher dosage strength (see Figure 1), which is probably associated with the non-linear pattern.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.