Taylor & Francis
Taylor & Francis Group

Expert Opinion on Drug Delivery

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iedd20

In vivo dermal delivery of bleomycin with electronic pneumatic injection: drug visualization and quantification with mass spectrometry

Liora Bik, Martijn van Doorn, Anders C. N. Hansen, Christian Janfelt, Uffe H. Olesen, Merete Haedersdal, Catharina M. Lerche & Kristoffer Hendel

To cite this article: Liora Bik, Martijn van Doorn, Anders C. N. Hansen, Christian Janfelt, Uffe H. Olesen, Merete Haedersdal, Catharina M. Lerche & Kristoffer Hendel (2022) In vivo dermal delivery of bleomycin with electronic pneumatic injection: drug visualization and quantification with mass spectrometry, Expert Opinion on Drug Delivery, 19:2, 213-219, DOI: 10.1080/17425247.2022.2035719

To link to this article: https://doi.org/10.1080/17425247.2022.2035719

Published online: 08 Feb 2022.

EXPERT OPINION ON DRUG DELIVERY
2022, VOL. 19, NO. 2, 213–219
https://doi.org/10.1080/17425247.2022.2035719

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

OPEN ACCESS Check for updates

In vivo dermal delivery of bleomycin with electronic pneumatic injection: drug visualization and quantification with mass spectrometry

Liora Bik [a,b*], Martijn van Doorn [a], Anders C. N. Hansen [c], Christian Janfelt [c], Uffe H. Olesen [b], Merete Haedersdal [b], Catharina M. Lerche [b,c] and Kristoffer Hendel [b]

[a]Department of Dermatology, Erasmus Mc University Medical Center Rotterdam, Rotterdam, The Netherlands; [b]Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark; [c]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

Background: Intralesional bleomycin (BLM) administration by needle injection is effective for keloids and warts but has significant drawbacks, including treatment-related pain and operator-depended success rates. Electronic pneumatic injection (EPI) is a promising, less painful, needle-free method that potentially enables precise and controlled dermal drug delivery. Here, we aimed to explore the cutaneous pharmacokinetics, biodistribution patterns, and tolerability of BLM administered by EPI *in vivo*.

Research Design and Methods: In a pig model, EPI with BLM or saline (SAL) were evaluated after 1, 48 and 216 hours. Mass spectrometry quantification and imaging were used to assess BLM concentrations and biodistribution patterns in skin biopsies. Tolerability was assessed by scoring local skin reactions (LSR) and measuring transepidermal water loss (TEWL).

Results: Directly after BLM injection a peak concentration of 109.2 μg/cm^3 (43.9–175.2) was measured in skin biopsies. After 9 days BLM was undetectable. EPI resulted in a focal BLM biodistribution in the mid-dermal delivery zone resembling a triangular shape. Mild LSRs were resolved spontaneously and TEWL was unaffected.

Conclusions: BLM administered by EPI resulted in quantifiable and focal mid-dermal distribution of BLM. The high skin bioavailability holds a great potential for clinical effects and warrants further evaluation in future human studies.

ARTICLE HISTORY
Received 22 November 2021
Accepted 25 January 2022

KEYWORDS
Bleomycin; drug delivery; LC-MS; MALDI; needle-free injection; skin

1. Introduction

Bleomycin (BLM) is an antineoplastic agent used in dermatology as off-label drug for the treatment of (recalcitrant) common warts, keloids, hypertrophic scars, and non-melanoma skin cancer[1], [2]. These skin lesions are readily accessible for intralesional treatment and require lower dosages of BLM compared to intravenous treatment[1]. BLM's mechanism of action originates from its ability to bind and break DNA strands via generation of free radicals by oxidation of iron molecules, ultimately leading to cell cycle arrest and apoptosis [3–5]. The large molar mass and highly hydrophilic properties of BLM (1415 Da, LogP −7.5) precludes passive dermal uptake after topical administration. Therefore, several mechanical and energy-based administration techniques have been developed to enhance the dermal drug delivery of hydrophilic macromolecules [6–9].

The most commonly used mechanical drug delivery technique for intralesional bleomycin treatment is the conventional needle-syringe injections [1]. However, needle injections are associated with important drawbacks such as treatment-related pain and highly operator-depended success rates. Energy-based devices offer great potential to deliver drugs into skin in a less painful, more precise and controlled manner, without the use of needles. Electronic pneumatic injection (EPI) devices can facilitate drug delivery by generating a high-velocity jet stream exceeding 100 m/s that penetrates the epidermis and disperses liquid drugs in its target tissue [10,11]. Factors that influence jet penetration dimensions in the skin include the velocity profile, fluid properties like viscosity, and mechanical skin properties such as Young's modulus (skin elasticity)[12]. Advanced air-powered EPI devices allow for the pre-defined selection of pressure and injection volumes, offering tailored treatments with potentially better dermal bioavailability. In addition, EPI can be used with liquid formulations developed for conventional injections with hypodermis needles allowing new applications to be easily deployed such as dermal delivery of anesthetics, vaccines and chemotherapeutics[11]. Moreover, EPI facilitates a sterile and safe treatment by using clean plastic disposable nozzles.

Dermal drug delivery of BLM with EPI in living skin has not been investigated previously. Therefore, in this study we aimed to explore EPI-delivery of BLM by evaluating BLM skin

CONTACT Liora Bik * l.bik@erasmusmc.nl Department of Dermatology, Erasmus Mc, Doctor, Molewaterplein 403015 GD, Rotterdam, The Netherlands

concentration over time, biodistribution patterns and local tolerability in *in vivo* pig skin to support future studies in human subjects.

2. Materials and methods

2.1. Study design

BLM was administered by EPI in *in vivo* pig skin (Figure 1) and followed-up after 1 hour, 48 hours, and 216 hours. An overview of sample sizes and evaluation methods is available in Table 1. Cutaneous pharmacokinetics were quantified by liquid chromatography–mass spectrometry (LC-MS). Biodistribution patterns were visualized by matrix-assisted laser desorption ionization imaging (MALDI-MSI). Local tolerability was clinically evaluated by grading local skin reactions (LSR) and quantifying transepidermal water loss (TEWL) as a measure for skin integrity.

2.2. Animals

The use of four female pigs (62–75 kg) was approved by the Danish Animal Inspectorate (license 2017–15-0201-01204). The study was conducted according to the Federation of European Laboratory Animal Science Associations guidelines and followed the Animal Research: Reporting In Vivo Experiments (ARRIVE 2.0) guidelines. Two weeks before the start of the study, the pigs were placed in a solitary confinement where they could acclimate. The general anesthesia procedure on study days 1, 2 (48 h) and 9 (216 h) was performed with intramuscular benzodiazepine combined with inhalation of isoflurane (2%). Anesthesia was maintained by isoflurane and intravenous bolus of propofol [13]. Test sites of 2 × 2 cm were evenly distributed along the spine and demarcated with a black marker on each animal after carefully hair removal using an electric razor (Figure 1 -A). After finalization of the study, the animals were euthanized while under general anesthesia with i.v. pentobarbital.

2.3. Formulation of bleomycin

BLM (batch 7K062C; Baxter; Deerfield; IL; USA) was diluted in natrium chloride 0.9% (SAL) to a concentration of 15,000 IU/ml [13,14].

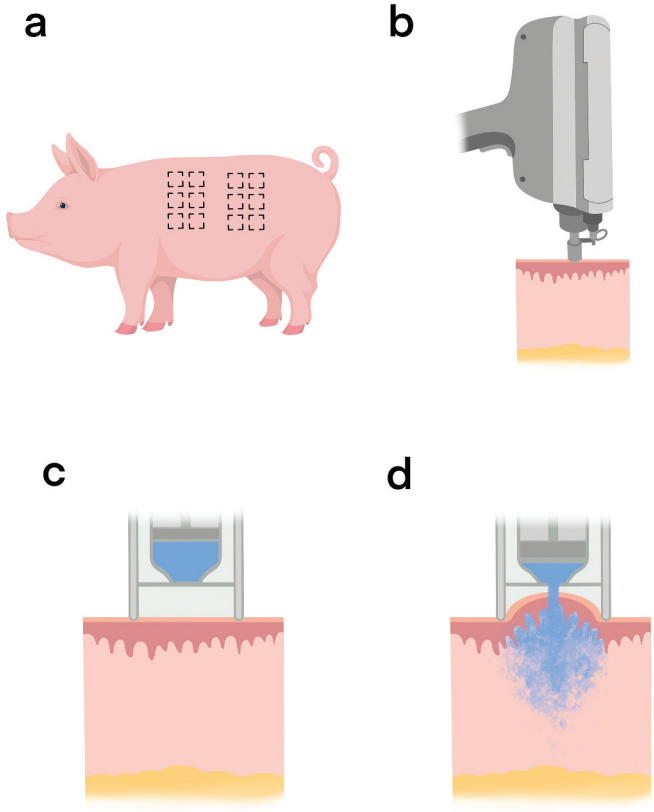

Figure 1. Study design. Schematic illustration of the *in vivo* animal study showing the investigated intervention of intradermal electronic pneumatic injection with bleomycin. A) An *in vivo* pig model with along the spine demarcated black test sites of 2 × 2 cm. B) Before injection, the jet injector was placed perpendicularly on the skin surface. C) Cross-section illustration of the jet injector tip on skin with the selected injection volume of bleomycin (blue) loaded in the reservoir. D) During the injection phase, the high-velocity jet stream of bleomycin penetrates the epidermis followed by vertical and lateral dispersion into the mid-deep intradermal delivery zone, forming a visible skin papule.

2.4. Electronic pneumatic injection

EPI with BLM or SAL was performed using an electronically controlled pneumatic jet injector device (EnerJet 2.0; PerfAction Technologies Ltd.; Rehovot; Israel) with a pressure of 6 bar (device range: 2–6 bar) and injection volume of 100 µL (device range: 50–130 µL) [13]. The device uses compressed air as pressure source, produces jet stream

Table 1. Overview study design

Drug delivery			Mass spectrometry quantitation and imaging							Tolerability						
Interventions			*LC-MS (h) n = 6–8*				*MALDI-MSI (h) n = 1*			*LSR n = 8*			*TEWL n = 6*			
Description	BLM	SAL	0	1	48	216	0	1	48	1	48	216	B	1	48	216
EPI + BLM	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EPI + SAL	-	+	-	+	-	-	-	-	-	+	+	+	-	+	+	+
			Total LC-MS *n = 37*				Total MALDI-MS*n = 3*			Total LSR *n = 48*			Total TEWL *n = 42*			

EPI: Electronic Pneumatic Injection
BLM: Bleomycin
SAL: Saline
B: Baseline measurement of untreated skin
LC-MS: Liquid chromatography–mass spectrometry
MALDI-MSI: Matrix-assisted laser desorption/ionization-mass spectrometry imaging
LSR: Local skin reaction
TEWL: Transepidermal waterloss

velocities of up to 150 m/s and has a nozzle orifice diameter of 200 µm[15]. Single injections with EPI were provided per square centimeter in the marked test sites and contained 1500 IU of bleomycin per 100 µL. A clear skin papule was accepted as the end-point for intradermal drug delivery [15].

2.5. Tolerability assessment

Skin tolerability was assessed by comparing clinical local skin reactions (LSR) at baseline, 1 hour, 2 days, and 9 days after EPI. Erythema and edema were scored independently by two evaluators (LB and KH) using the OECD test scale (Draize scale; 0–4) as described in previous studies (n = 48) [15–17]. Transepidermal water loss (TEWL; g/h/m [2]) was measured using a DermaLab skin analysis device with TEWL probe (Cortex Technology; Hadsund; Denmark), representing skin barrier integrity (n = 42).

2.6. Sample preparation

The excised punch biopsies of 8 mm at the injection site were snap frozen and stored at −80°C. For LC-MS, full-skin biopsies were sliced in multiple parts, mixed with 1 mL phosphate-buffered saline and disrupted with a tissue lyser (TissueLyser II, Retsch, Haan Germany) [13,18,19]. The fluid was extracted after centrifugation for LC-MS analyses. For MALDI-MSI analyses, the full-skin biopsies were sectioned with a cryomicrotome and fixated on a microscope slide.

2.7. LC-MS quantification

A total of 37 punch biopsies were taken from the test areas for LC-MS quantification and prepared as previously described [13]. A Thermo TSQ Vantage triple-quadrupole mass spectrometer was used for LC-MS analyses in combination with a Thermo Accela high-performance liquid chromatography system (Thermo Fisher Scientific, Waltham, MA, USA). The system was operated with a limit of quantification (LOQ) of 14.49 ng/ml. Eight-point calibration curves were created with a 0.5 mg/ml BLM stock solution (Sigma-Aldrich Corp., St. Louis, MO, USA) and repeated pre- and post of sample list testing. BLM B2 and BLM A2 were quantified in MRM mode using the transitions *m/z* 713.5 →530.0 and *m/z* 708.0 → 493.5, respectively.

2.8. MALDI-MS imaging

Punch biopsies (8-mm) were taken from the test site for MALDI-MS imaging at 0 hours (n = 1), 1 hour (n = 1) and 48 hours (n = 1), and prepared as in a previous study [13]. AP-SMALDI5 and AP-SMALDI10 MALDI-MSI ion sources (TransMIT GmbH, Giessen, Germany) mounted on a Thermo Q-Exactive Orbitrap mass spectrometer were used to perform MALDI-MS imaging at a mass resolution of 140,000 at *m/z* 200 and a scan range of *m/z* 225–1750. The analyzed biomolecules included BLM and skin lipids to clearly distinguish the BLM spatial biodistribution pattern from the surrounding skin. BLM B2 was detected at *m/z* 1425.5188 and a skin lipid molecule at m/z 782.5670. Images were generated using MSiReader version 1.02[20].

LC-MS and MALDI-MSI were validated prior to the study to guarantee accuracy and reliability of the bleomycin analysis methods.

2.9. Statistics

Descriptive data of LC-MS quantification and TEWL measurements were presented as medians and interquartile range (Q1-Q3) and separately tested by Mann-Whitney test. LSR data were normally distributed and therefore presented as mean and SD and tested by independent samples T-test. An alpha level of ≤0.05 was considered statistically significant. SPSS version 25 (IBM Corporation; Armonk; NY; USA) was used for statistical analyses.

3. Results

3.1. Cutaneous concentrations of BLM measured by LC-MS

A total of 37 samples were analyzed with LC-MS. Skin biopsies taken directly after EPI + BLM resulted in a peak BLM

Table 2. LC-MS quantification and MALDI-MSI of bleomycin delivery by electronic pneumatic injection in skin

		Quantified (LC-MS)			Imaged (MALDI-MSI)
Intervention	Time	µg/cm[3]	IQR	% of maximum concentration*	Spatial distribution
EPI + BLM	0 h	109.2	(43.9– 175.2)	100.0%	Superficial dermis
	1 h	63.5	(22.3–79.3)	58.2%	Mid-deep dermis
	48 h	2.5	(1.4–3.6)	2.3%	Not detected
	216 h	0.0	(0.0–0.7)	0.0%	–
EPI + SAL	1 h	0.0	(0.0–0.0)	0.0%	–

*Percentage of maximum concentration calculated with the highest measured bleomycin concentration (EPI + BLM at 0 h).
EPI: Electronic Pneumatic Injection
BLM: Bleomycin
SAL: Saline
LC-MS: Liquid chromatography–mass spectrometry
MALDI-MSI: Matrix-assisted laser desorption/ionization–mass spectrometry imaging
IQR: Interquartile range (Q1–Q3)
IU: International units
Not imaged

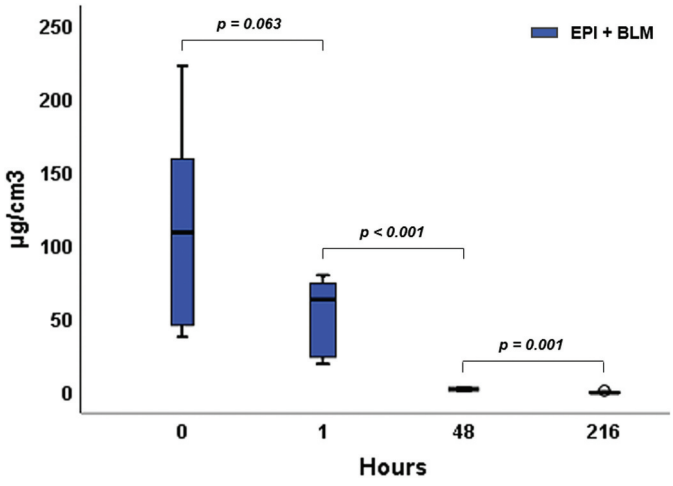

Figure 2. Box plots of bleomycin quantification by liquid chromatography-mass spectrometry. Bleomycin concentration per cubic centimeter of skin visualized as box plots with quartile boxes and min-max whiskers. EPI successfully delivered high concentrations of bleomycin in the skin directly after injection, 0 hours, and was completely cleared at 216 hours.

concentration of 109.2 µg/cm [3] (43.9–175.2; Table 2; Figure 2). After 1 hour, the BLM concentration was 63.5 µg/cm [3] (22.3–79.3). Over the following days, BLM concentrations significantly decreased to almost undetectable: 2.5 µg/cm [3] (1.4–3.6; $p < 0.001$) after 2 days and complete clearance from all skin samples after 9 days. Control interventions (EPI + SAL) contained no BLM that was detectable by LC-MS quantification at 1 hour (Table 2).

3.2. Biodistribution pattern visualized by MALDI-MS imaging

A total of three samples were analyzed for intradermal delivery of BLM by EPI with MALDI-MS imaging (Table 2; Figure 3 A-B). The biodistribution patterns were similar between 0 hours and 1 hour. Directly after injection, BLM was distributed in a triangular pattern, which was primarily located at the entry point of injection and spread out laterally in the papillary dermis. A slightly wider and homogenous biodistribution pattern was observed after 1 h, specifically from papillary to reticular dermis. Two days following injection, BLM was not visible in any of the skin samples (Figure 3 A, right panel).

3.3. Tolerability

Clinical evaluation of the treated test sites showed that EPI + BLM resulted in mild LSR, primarily erythema and edema at 1 hour, that were close to completely resolved at day 9 (Figure 4). Compared to control intervention (EPI + SAL), BLM treatment resulted in significantly higher LSRs at 1 hour, 2 and 9-days ($p \leq 0.025$). Skin integrity after EPI interventions, measured by TEWL, was not compromised for any time points when compared to baseline ($p > 0.05$).

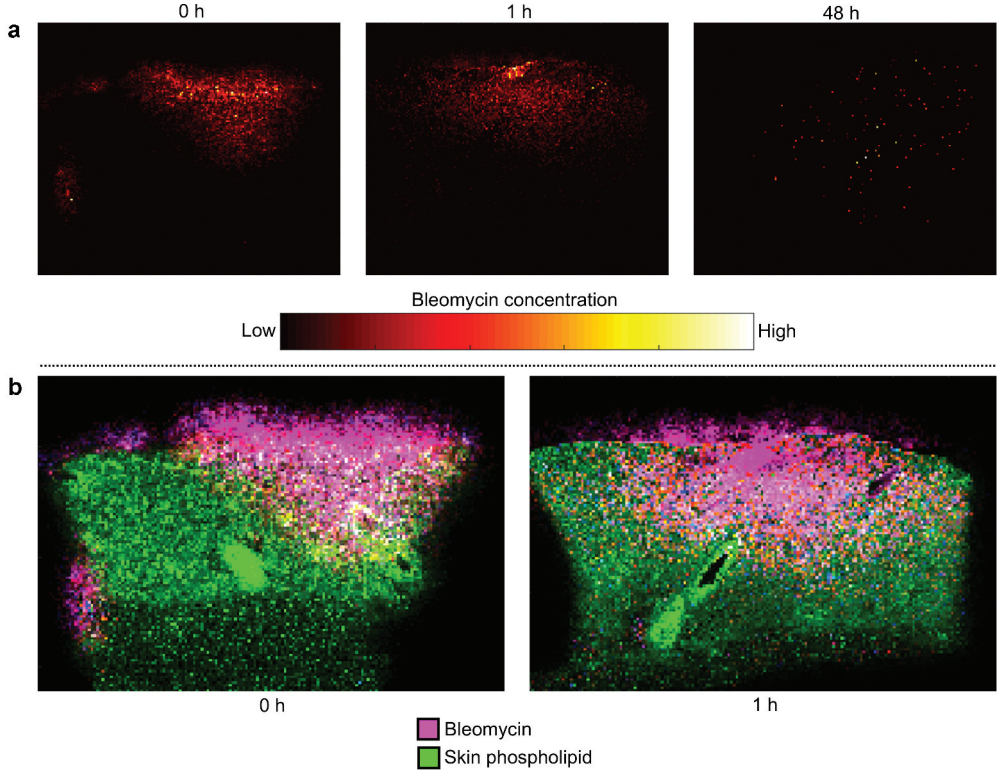

Figure 3. Skin samples showing bleomycin B2 and a skin phospholipid molecule visualized by mass spectrometry MALDI-MS imaging. A. Bleomycin biodistribution patterns after 0 hours, 1 hour and 48 hours after administration by electronic pneumatic injection. White/yellow indicates the strongest bleomycin signal, whereas black represents the lack of bleomycin detection. The colors illustrate the intensity of bleomycin signal per image. No direct comparison of bleomycin concentration can be made between images. B. Focal distribution of bleomycin (pink) in the mid-deep dermal delivery zone administered by electronic pneumatic injection, surrounded by untreated skin (phospholipid molecule; green).

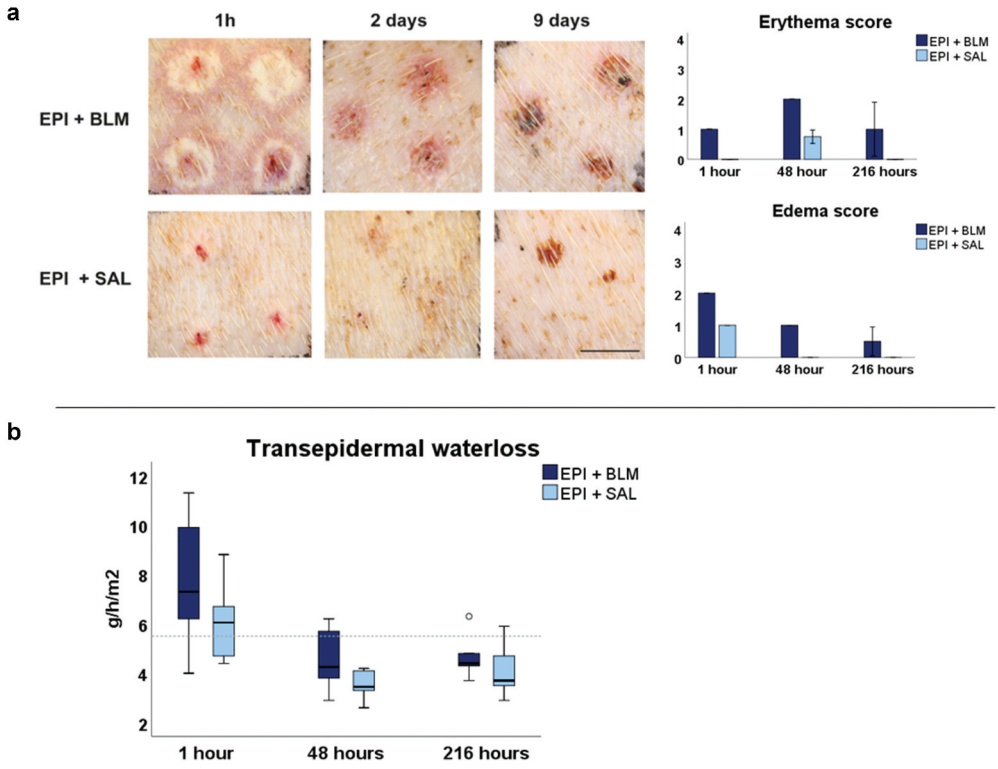

Figure 4. Tolerability assessment of electronic pneumatic injection. Clinical photography's and bar graphs with error bars of erythema and edema scores demonstrate mild local skin reactions at 1 hour after bleomycin (BLM) delivery by EPI, which were close to completely resolved at day 9. EPI with normal saline (SAL) served as control. B. Boxplot presenting the median and interquartile ranges (and min/max whiskers) of transepidermal waterloss (TEWL), representing skin integrity, after EPI with BLM or SAL. The horizontal line marks TEWL baseline measurement of untreated skin. No significantly difference was found in TEWL compared to baseline for both interventions at all time-points.

4. Discussion

In this *in vivo* pig study, BLM delivery to the skin using electronic pneumatic injection (EPI) was investigated for the first time. We found that EPI successfully delivered BLM in the dermis with the highest BLM concentration of 109.2 µg/cm [3] (43.9–175.2) using a pressure setting of 6 bar and 100 µL within the first hour of injection. As expected, the peak concentration measured by mass spectrometry occurred directly after injection and slowly decreased to an undetectable level after 9 days. Interestingly, in our previous *in vivo* pig skin study, we observed a 2-fold higher dermal BLM concentration of 210.9 µg/cm [3] (210.9–357.7) after needle injections with the same volume and BLM concentration [13]. This could partly be explained by an undelivered dosage of up to 20% of the injection volume remaining on skin surface [21]. The velocity of the liquid jet stream is approximately fifty times faster than the volumetric rate of fracture of porcine skin, causing backflow of the jet during injection visible as residual fluid on skin surface [22]. However, despite this small loss of injection fluid, EPI was still able to deliver a high dosage of BLM in a fast and standardized manner. To determine the required dosage for a therapeutic effect, we evaluated the intradermal dosages of BLM when administered with EPI compared to the intravenous route of administration which is commonly used in dermato-oncology. At eight minutes after intravenous administration with the therapeutic dosage of BLM (15,000 IU m^{-2}), an intratumoral concentration of ~0.1 µg/g of BLM was measured leading to significant clinical effects in head and neck cancer [23]. In this study, EPI delivery of BLM (15,000 IU/ml; injection volume of 100 µL) resulted in a high intradermal concentration of 109.2 µg/cm [3], corresponding to ~100 µg/g, directly after injection. By directly targeting the skin, EPI drug delivery with a relatively low dosage leading to a high dermal concentration could potentially result in a better clinical response, while at the same time it could reduce the risk of systemic adverse events.

Mass spectrometry imaging showed EPI to successfully generate a lateral and homogeneous distribution in the mid-deep dermis. Similar biodistribution patterns were observed in *ex vivo* pig skin studies immediately after EPI with aqueous solutions [15,24]. Moreover, clear detection of BLM with MALDI-MSI suggests that BLM molecules were unaffected by the high shear stresses caused by the high-velocity jet stream and small nozzle diameter of EPI [11]. In this study, a pressure level of 6 bar was required to generate an immediate skin papule: EPI's clinical endpoint for intradermal drug delivery [21]. The optimal device settings, however, will vary depending on the tissue consistency, clinical indication and treated anatomical location [15,21,24]. Tissue targets that are not easily penetrated and infiltrated by needle injection, such as keloid scars, are therefore well-suited for EPI's high-velocity jet injection [25].

All interventions were well-tolerated with only mild LSRs that were close to completely resolved at the end of the study

and skin integrity quantified by transepidermal water loss was not compromised. EPI with BLM led to more prominent acute LSR compared to EPI with no active substances. BLM provokes an inflammatory process by inducing cell cycle arrest and apoptosis, visible as transient acute LSR that could be related to temporary mild pain sensation [5,26]. EPI alone, however, also induced slight LSRs, probably as a result of the high-powered jet-tissue interaction causing skin structure disruption, visible on confocal microscopy imaging as dermal vacuoles and sup-epidermal clefting [15]. In comparison, we previously showed that needle injection with SAL showed no LSRs for up to 9 days [13].

The strengths of this study include the use of an *in vivo* pig skin model to mimic vital human skin, evaluation of bleomycin concentration over time and biodistribution patterns with mass spectrometry and tolerability assessment. Limitations of this study include the low number of mass spectrometry imaging samples. In addition, systemic adverse event monitoring was not performed. In a human study, blood samples drawn 40 minutes after an intravenous bolus of 30.000 IU BLM resulting in a blood concentration of 1.42 mg/ml [27]. Blood samples from living pigs 1 hour after intradermal needle injections of ≥225.000 IU of BLM resulted in a comparable concentration of 1.48 mg/ml [13]. In clinical practice, however, intralesional dosages usually do not exceed 6.000 IU of BLM per treatment, making the risk of systemic adverse events through percutaneous systemic uptake highly unlikely [1]. Lastly, a reference intervention group such as needle injection with bleomycin was lacking that precluded direct comparison of EPI to other drug delivery techniques.

5. Conclusions

In conclusion, EPI with BLM results in a quantifiable and focal mid-dermal spread of BLM in *in vivo* pig skin. Clinical evaluation of LSR and TEWL indicates that the intervention was well-tolerated. In addition, EPI enables needle-free drug delivery in a fast and standardized manner, while providing tailored treatments by selection of tunable settings such as pressure and injection volume. The high bioavailability, tolerability and ease-to-use administration technique indicate that BLM delivered by EPI holds great potential for clinical effects as dermatological treatment and warrants further investigation in future human studies.

Acknowledgments

The authors like to thank Diana Høeg for her technical assistance during this study. In addition, support from the Carlsberg Foundation, Independent Research Fund Denmark | Technology and Production Sciences (grant no. 9041-00203B)) and the Lundbeck Foundation (R307-2018-3318) is gratefully acknowledged.

Author contributions

Conception and design: L Bik, U Olesen, M Haedersdal, C Lerche, K Hendel. Analysis and interpretation of data, drafting of the paper or revising it critically for intellectual content, final approval of the version to be published, agree to be accountable for all aspects of the work: All authors.

Declaration of interest

The authors have disclosed that the EnerJet device was provided by PerfAction as part of a research collaboration. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Abbreviations

EPI: Electronic pneumatic injection
BLM: Bleomycin
SAL: Saline

Funding

This work was supported by the Candys Foundation under Grant [17-236].

ORCID

Liora Bik http://orcid.org/0000-0002-4624-5383
Martijn van Doorn http://orcid.org/0000-0003-1672-7899
Anders C. N. Hansen http://orcid.org/0000-0002-5576-0088
Christian Janfelt http://orcid.org/0000-0002-4626-3426
Uffe H. Olesen http://orcid.org/0000-0002-4578-684X
Merete Haedersdal http://orcid.org/0000-0003-1250-2035
Catharina M. Lerche http://orcid.org/0000-0003-3653-6424
Kristoffer Hendel http://orcid.org/0000-0003-1845-1224

References

1. Bik L, Sangers T, Greveling K, et al. Efficacy and tolerability of intralesional bleomycin in dermatology: a systematic review. J Am Acad Dermatol. 2020;83(3):888–903.
2. Saitta P, Krishnamurthy K, Brown LH. Bleomycin in dermatology: a review of intralesional applications. Dermatol Surg. 2008;34 (10):1299–1313.
3. Povirk LF, Hogan M, Dattagupta N. Binding of bleomycin to DNA: intercalation of the bithiazole rings. Biochemistry. 1979;18 (1):96–101.
4. Burger RM, Peisach J, Horwitz SB. Activated bleomycin. A transient complex of drug, iron, and oxygen that degrades DNA. J Biol Chem. 1981;256(22):11636–11644.
5. Tounekti O, Pron G, Belehradek J Jr., et al. Bleomycin, an apoptosis-mimetic drug that induces two types of cell death depending on the number of molecules internalized. Cancer Res. 1993;53(22):5462–5469.
6. Wenande E, Anderson RR, Haedersdal M. Fundamentals of fractional laser-assisted drug delivery: an in-depth guide to experimental methodology and data interpretation. Adv Drug Deliv Rev. 2020;153:169–184.
7. Lee W-R, Shen S-C, Fang C-L, et al. Topical delivery of methotrexate via skin pretreated with physical enhancement techniques: low-fluence erbium:YAG laser and electroporation. Lasers Surg Med. 2008;40(7):468–476.
8. Mikolajewska P, Donnelly RF, Garland MJ, et al. Microneedle pre-treatment of human skin improves 5-Aminolevulininc Acid (ALA)- and 5-Aminolevulinic Acid Methyl Ester (MAL)-Induced

PpIX production for topical Photodynamic Therapy Without Increase in Pain or Erythema. Pharm Res. 2010;27 (10):2213–2220.
9. Christensen RL, Omland SH, Persson DP, et al. Topical delivery of nivolumab, a therapeutic antibody, by fractional laser and pneumatic injection. Lasers Surg Med. 2021;53(1):154–161.
10. Barolet D, Benohanian A. Current trends in needle-free jet injection: an update. Clin Cosmet Investig Dermatol. 2018;11:231–238.
11. Mitragotri S. Current status and future prospects of needle-free liquid jet injectors. Nat Rev Drug Discov. 2006;5(7):543–548.
12. Mohizin A, Kim JK. Current engineering and clinical aspects of needle-free injectors: a review. J Mech Sci Technol. 2018;32 (12):5737–5747.
13. Hendel K, Hansen ACN, Bik L, et al. Bleomycin administered by laser-assisted drug delivery or intradermal needle-injection results in distinct biodistribution patterns in skin: in vivo investigations with mass spectrometry imaging. Drug Deliv. 2021;28(1):1141–1149.
14. Hendel KK, Bagger C, Olesen UH, et al. Fractional laser-assisted topical delivery of bleomycin quantified by LC-MS and visualized by MALDI mass spectrometry imaging. Drug Deliv. 2019;26(1):244–251.
15. Bik L, van Doorn Mba, Biskup E, et al. Electronic pneumatic injection-assisted dermal drug delivery visualized by ex vivo confocal microscopy. Lasers Surg Med. 2021;53(1):141–147.
16. Wenande E, Tam J, Bhayana B, et al. Laser-assisted delivery of synergistic combination chemotherapy in in vivo skin. J Control Release. 2018;275:242–253.
17. Olesen UH, Clergeaud G, Hendel KK, et al. Enhanced and sustained cutaneous delivery of vismodegib by ablative fractional laser and microemulsion formulation. J Invest Dermatol. 2020;140 (10):2051–2059.
18. Oecd. Test No. 404: Acute Dermal Irritation/Corrosion2015.
19. Wenande E, Olesen UH, Nielsen MMB, et al. Fractional laser-assisted topical delivery leads to enhanced, accelerated and deeper cutaneous 5-fluorouracil uptake. Expert Opin Drug Deliv. 2017;14 (3):307–317.
20. Bokhart MT, Nazari M, Garrard KP, et al. MSiReader v1.0: evolving open-source mass spectrometry imaging software for targeted and untargeted analyses. J Am Soc Mass Spectrom. 2018;29 (1):8–16.
21. Bik L, van Doorn MBA, and Boeijink N, et al. Clinical endpoints of needle-free jet injector treatment: an in depth understanding of immediate skin responses. Lasers Surg Med. 2022 Jan 23. Epub ahead of print. .
22. Baxter J, Mitragotri S. Jet-induced skin puncture and its impact on needle-free jet injections: experimental studies and a predictive model. J Control Release. 2005;106(3):361–373.
23. Kosjek T, Krajnc A, Gornik T, et al. Identification and quantification of bleomycin in serum and tumor tissue by liquid chromatography coupled to high resolution mass spectrometry. Talanta. 2016;160:164–171.
24. Erlendsson AM, Haedersdal M, Rossi AM. Needle-Free injection assisted drug delivery-histological characterization of cutaneous deposition. Lasers Surg Med. 2020;52(1):33–37.
25. Levenberg A, Vinshtok Y, Artzi O. Potentials for implementing pressure-controlled jet injection in management of keloids with intralesional 5FU and corticosteroids. J Cosmet Dermatol. 2020;19 (8):1966–1972.
26. Yamamoto T. Bleomycin and the skin. Br J Dermatol. 2006;155 (5):869–875.
27. Groselj A, Krzan M, Kosjek T, et al. Bleomycin pharmacokinetics of bolus bleomycin dose in elderly cancer patients treated with electrochemotherapy. Cancer Chemother Pharmacol. 2016;77 (5):939–947.