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Regular Article

Biodistribution of the recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in rats☆

Eva Herzog [a,*], Stephen Harris [b], Claire Henson [b], Andrew McEwen [b], Sabrina Schenk [a], Marc W. Nolte [a], Ingo Pragst [a], Gerhard Dickneite [a], Stefan Schulte [a], Sabine Zollner [a]

[a] CSL Behring GmbH, Preclinical Research and Development, 35041 Marburg, Germany
[b] Quotient Bioresearch Metabolic Chemistry, Rushden, UK

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ABSTRACT

Introduction: The recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) is undergoing clinical trials for prophylaxis and on-demand treatment of haemophilia B patients. The aim of this study was to investigate the pharmacokinetics, whole-body and knee joint distribution of rIX-FP following intravenous administration to rats, compared with a marketed, non-fused rFIX and recombinant human albumin.
Material and Methods: [^{3}H]-rIX-FP, [^{3}H]-rFIX or [^{3}H]-albumin were administered to rats followed by quantitative whole-body autoradiography over 24 or 240 hours, and the tissue distribution as well as elimination of radioactivity were measured.
Results: Elimination of all radioactivity derived from the three proteins was shown to occur primarily via the urine. The tissue distribution of [^{3}H]-rIX-FP and [^{3}H]-rFIX (but not of [^{3}H]-albumin) was comparable, both penetrating predominantly into bone, and well-perfused tissues, suggesting that the rIX moiety determines the distribution pattern of rIX-FP, while the albumin moity is responsible for the prolonged plasma and tissue retention. Detailed knee-joint analysis indicated rapid presence of [^{3}H]-rIX-FP and [^{3}H]-rFIX in synovial and mineralised bone tissue, mostly localised to the zone of calcified cartilage. Longest retention times were observed in the bone marrow and the endosteum of long bones. Intriguingly, [^{3}H]-rIX-FP- and [^{3}H]-albumin-derived radioactive signals were detectable up to 240 hours, while [^{3}H]-rFIX-derived radioactivity rapidly declined after 1 hour post-dosing correlating to the extended plasma half-life of [^{3}H]-rIX-FP.
Conclusion: The prolonged plasma and tissue retention of rIX-FP achieved by albumin fusion may allow a reduction in dosing frequency leading to increased therapeutic compliance and convenience.
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Introduction

Factor IX (FIX) is the zymogen of a serine protease that circulates in plasma at an average concentration of 5 μg/ml [1]. Activated FIX (FIXa) is an integral part of the tenase enzyme complex activating factor X, ultimately leading to blood coagulation [2,3]. FIX-replacement therapy, using recombinant (rFIX) or plasma-derived (pdFIX) FIX is a safe and efficacious treatment approach to reduce bleeding rates among haemophilia B patients, while limiting haemarthrosis and arthropathy (potential causes of disability) [4]. Unfortunately, the relatively short terminal half-life of FIX of approximately 18–34 hours, necessitates frequent infusions every 2 or 3 days to maintain FIX clotting activity above 1% and to prevent spontaneous bleeding [5,6].

To improve therapeutic compliance and convenience, a novel recombinant fusion protein linking coagulation factor IX with albumin via a cleavable peptide linker (rIX-FP) has been designed to prolong the circulation time of FIX significantly; thus, improving its pharmacokinetic profile while maintaining its efficacy [7–9]. Understanding the tissue distribution of this novel coagulation factor construct is important to ensure that it reaches those areas necessary for maximum haemostatic efficacy. However, there is limited information available on the tissue distribution of FIX (rFIX/pdFIX) following intravenous administration and how fusion with albumin, as in the case of rIX-FP, may affect it. Therefore, this study aimed to investigate the pharmacokinetics and whole-body distribution of rIX-FP following intravenous administration of radiolabeled rIX-FP to rats, compared with a marketed, non-fused rFIX and recombinant human albumin, with particular focus on the bone–joint distribution profile.

Abbreviations: FIX, factor IX; FIXa, activated FIX; rFIX, recombinant FIX; pdFIX, plasma-derived FIX; rIX-FP, recombinant fusion protein linking factor IX with albumin; QWBA, quantitative whole-body autoradiography; LSC, liquid scintillation counting; LMW, low-molecular-weight; ATIII, antithrombin III; FcRn, neonatal Fc-receptor.

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* Corresponding author at: CSL Behring GmbH, Emil-von-Behring-Str. 76, 35041 Marburg, Germany. Tel.: +49 6421 39 6106; fax: +49 6421 39 4663.
E-mail address: eva.herzog@cslbehring.com (E. Herzog).

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Material and Methods

Radiolabelling and Characterisation of rIX-FP and rFIX

rIX-FP (CSL Behring GmbH, Germany), rFIX (BeneFIX®, Pfizer Pharma GmbH, Berlin, Germany) or albumin (Recombumin®, Novozymes Biopharma, Bagsvaerd, Denmark) were radiolabeled with N-succinimidyl-[2,3-^{3}H]-propionate (Quotient Bioresearch, Cardiff, UK), using the method described by Müller [10].

Treatments

Male Sprague-Dawley rats (Charles River, UK) received a slow bolus radioactive dose of 11−16 MBq/kg (320−420 μCi/kg) [^{3}H]-rIX-FP, [^{3}H]-rFIX, or [^{3}H]-albumin. All animals received care in compliance with the European Convention on Animal Care and the study was approved by the local Ethics Committee.

Plasma Analysis

Following intravenous dosing, blood samples (~5−10 ml) were collected into individual heparinised containers using cardiac puncture under anaesthesia (isofluorane) at the following post-dose time points (one animal/time point): 0.25, 1, 3, 8, 24, 72, 120, and 240 hours for [^{3}H]-rIX-FP- and [^{3}H]-albumin-treated animals, and based on previous pharmacokinetic data, 0.25, 1, 3, and 24 hours for [^{3}H]-rFIX-treated animals. The whole-blood samples were centrifuged and the resultant plasma harvested.

Excretion Balance

After dosing, animals selected for the final autoradiography time point (240 hours for [^{3}H]-rIX-FP or [^{3}H]-albumin, and 24 hours for [^{3}H]-rFIX) were returned to glass metabolism cages for separate urine (at 0−8 hours, 8−24 hours and 24-hour intervals thereafter) and faeces (at 24-hour intervals) collection. At the end of the observation period, the metabolism cages were rinsed with water followed by methanol, and the washings collected for quantitative radiochemical analysis.

Quantitative Whole-body Autoradiography (QWBA)

QWBA analysis was performed based on the technique of Ullberg [11] at time points up to 24 ([^{3}H]-rFIX) or 240 hours ([^{3}H]-rIX-FP, [^{3}H]-albumin). Sagittal sections of the body were assessed at up to five different levels: Level 1, exorbital lachrymal gland; Level 2, intra-orbital lachrymal gland; Level 3, harderian gland/adrenal gland; Level 4, thyroid gland; and Level 5, brain and spinal cord. A calibration curve was created via SeeScan using data from the ^{3}H-blood standards from which tissue concentrations of radioactivity were determined (nCi/g). Percentage total dose/tissue was also determined. Additionally, one hind limb from each animal was used for separate sectioning and a more detailed analysis of the knee joint.

Quantitative Radiochemical and Chromatographic Analysis

Liquid scintillation counting (LSC) was used to determine the radioactivity level (disintegrations/minute) of the dose formulation, plasma, urine, faeces, and cage wash using a Packard 2300TR liquid scintillation analyser (PerkinElmer, Waltham, MA, USA) with automatic external standard quench correction and Ultima Gold XR scintillation fluid (PerkinElmer). Proportions of radioactivity in purified ^{3}H-labeled protein solutions, plasma and urine samples were determined by high-performance liquid chromatography (HPLC; Agilent 1100 HPLC system with ultraviolet detection at 280 nm) using a Phenomenex BIOSEP SEC 2000 size exclusion column (300 × 7.8 mm; mobile phase 100 mM sodium phosphate or 0.1 M phosphate buffer for protein solutions and body fluids, respectively). FIX antigen levels were also measured to confirm the presence of rIX-FP in tissues.

Results

Characterisation of [^{3}H]-rIX-FP and [^{3}H]-rFIX Preparations

Radiochemical purity of the [^{3}H]-rIX-FP, [^{3}H]-rFIX, and [^{3}H]-albumin formulations was > 90% as determined by HPLC analysis and shown in Fig. S1a i, ii, and iii, respectively. Assessment of the biological activity confirmed that 100% of rIX-FP specific activity was maintained following [^{3}H]-labelling based on measurements of FIX activity (Fig. S1b).

Analysis of Circulating Radioactivity and Pharmacokinetics

Plasma analysis confirmed the mean dose levels of ~400 μCi/kg in each treatment group: [^{3}H]-rIX-FP, 409 μCi/kg; [^{3}H]-rFIX, 425 μCi/kg; and [^{3}H]-albumin, 382 μCi/kg. For direct comparisons of FIX products, radioactive dose level differences were considered when interpreting the plasma and tissue concentration data. Plasma radioactivity levels and representative radiochromatograms of [^{3}H]-rIX-FP, [^{3}H]-rFIX, and [^{3}H]-albumin are shown in Table S1 and Fig. S2 (a-c), respectively. Levels of intact protein recovered in plasma are shown in Table 1 and Fig. 1a.

[^{3}H]-rIX-FP

Unchanged [^{3}H]-rIX-FP (peak at ~7 minutes) was the major component in plasma, accounting for ~100% of all radioactivity up to 8 hours post-dosing (see Table 1). At 24 hours, this declined to 73.0%, with the remaining radioactivity composed of two peaks that co-chromatographed with albumin (at ~8.5 minutes) and a low-molecular-weight (LMW) component (at 13.4 minutes), and represented 16.5% and 3.7% of the total radioactivity, respectively (Table S1 and Fig. S2a). [^{3}H]-rIX-FP levels continued to decline with time, with < 1.0% at 120 hours and almost undetectable at 240 hours (Table 1).

Table 1
Recovery* of radioactivity in rat plasma, urine, and faeces following a single intravenous administration of [^{3}H]-rIX-FP, [^{3}H]-rFIX, or [^{3}H]-albumin.

Sample	Time (hours)	[^{3}H]-rIX-FP	[^{3}H]-rFIX	[^{3}H]-albumin
Plasma	0.25	100	91.7	100
	1	100	86.3	100
	3	100	68.6	98.3
	8	100	–	96.3
	24	73.0	44.3	97.4
	72	60.7	–	79.3
	120	0.97	–	73.6
	240	0.29	–	BLQ
Urine	0–8	13.5	11.7	10.7
	8–24	26.4	39.3†	36.7
	24–48	16.9	–	28.7
	48–240	16.1	–	37.2
	Subtotal	72.9	51.0	113.3
Faeces	0–24	0.9	8.9	3.7
	24–48	0.8	–	1.9
	48–240	2.6	–	6.9
	Subtotal	4.3	8.9	12.5
Cage wash	**Subtotal**	3.7	2.9	0.7
Total		80.8	62.8	126.5

*Expressed as % of overall radioactivity administered. †Sample collection for rFIX was 0−6 hours. BLQ, below limit of quantification. rFIX, recombinant factor IX; rIX-FP, recombinant fusion protein linking coagulation factor IX with albumin; –, no measurement for that time point.

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