Accepted Manuscript

Title: In vitro and preclinical assessment of an intranasal spray formulation of parathyroid hormone PTH 1-34 for the treatment of osteoporosis

Authors: Allan J. Williams, Faron Jordan, Gareth King, Andrew L. Lewis, Lisbeth Illum, Tahir Masud, Alan C. Perkins, Richard G. Pearson

PII: S0378-5173(17)30999-7
DOI: https://doi.org/10.1016/j.ijpharm.2017.10.029
Reference: IJP 17085

To appear in: *International Journal of Pharmaceutics*

Received date: 23-4-2017
Revised date: 11-10-2017
Accepted date: 12-10-2017

Please cite this article as: Williams, Allan J., Jordan, Faron, King, Gareth, Lewis, Andrew L., Illum, Lisbeth, Masud, Tahir, Perkins, Alan C., Pearson, Richard G., In vitro and preclinical assessment of an intranasal spray formulation of parathyroid hormone PTH 1-34 for the treatment of osteoporosis.International Journal of Pharmaceutics https://doi.org/10.1016/j.ijpharm.2017.10.029

In vitro and preclinical assessment of an intranasal spray formulation of parathyroid hormone PTH 1-34 for the treatment of osteoporosis

Allan J Williams[1], Faron Jordan[2], Gareth King[2], Andrew L. Lewis [2], Lisbeth Illum [3] Tahir Masud[4], Alan C Perkins[5], and Richard G Pearson*****[1]

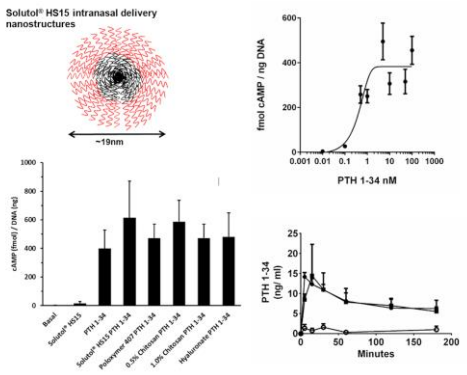
[1] School of Medicine, Division of Rheumatology, Orthopaedics and Dermatology, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK; [2]Critical Pharmaceuticals Limited, BioCity Nottingham, Pennyfoot Street, Nottingham, NG1 1GF, UK; [3] IDentity, 19 Cavendish Crescent North, The Park, Nottingham [4] Nottingham University Hospitals NHS Trust, Queen's Medical Centre, Nottingham, NG7 2UH, UK; [5] Radiological Sciences, School of Medicine, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK

***Corresponding author**

Email addresses:

Dr Richard G Pearson: richard.pearson@nottingham.ac.uk

Graphical abstract



Highlights

- Nasal spray formulations of PTH 1-34 containing absorption enhancers, either Solutol® HS15, poloxamer 407, chitosan or sodium hyaluronate, did not affect the PTH 1-34 bioactivity when tested within an *in vitro* assay.
- An intranasal formulation of PTH 1-34 containing Solutol® HS15 had comparable pharmacokinetics to a subcutaneous PTH 1-34 injection in the preclinical rat model.
- A potential clinical application of PTH 1-34 spray formulation for the treatment of osteoporosis has been established.

Abstract

Osteoporosis treatment with PTH 1-34 injections significantly reduces the incidence of bone fracture. Potential further reductions in fracture rate should be observed through nasal spray delivery to address the poor compliance associated with patient dislike of repeated PTH 1-34 subcutaneous injections. *In vitro* human osteoblast-like Saos-2 cell intracellular cAMP levels were used to define PTH 1-34 nasal spray formulation bioactivity. The chemically

synthesised PTH 1-34 had an EC_{50} of 0.76nM. Absorption enhancers polyethylene glycol (15)-hydroxystearate (Solutol® HS15), poloxamer 407, chitosan or sodium hyaluronate did not diminish the bioactivity of PTH 1-34 within an *in vitro* cell culture model ($p>0.05$). We also demonstrated the effectiveness of the transmucosal absorption enhancer Solutol® HS15 in a nasal spray formulation using a preclinical pharmacokinetic model. In Sprague-Dawley rats without the absorption enhancer the uptake of PTH 1-34 into the blood via intranasal delivery produced a Cmax of 2.1±0.5 ng/ml compared to 13.7±1.6 ng/ml with Solutol® HS15 enhancer ($p=0.016$) and a Cmax14.8±8 ng/ml in subcutaneous injections. Together these data illustrate that the nasal spray formulation bioactivity *in vitro* is not affected by the nasal spray absorption enhancers investigated, and the Solutol® HS15 nasal spray formulation had an equivalent pharmacokinetic profile to subcutaneous injection in the rat model. The Solutol® HS15 formulation therefore demonstrated potential as a PTH 1-34 nasal spray formulation for the treatment of osteoporosis.

Keywords

Osteoporosis, teriparatide, formulation, nasal spray, osteoblast, drug delivery

1. Introduction

Osteoporosis is a disease characterised by a reduction in bone density with corresponding changes in bone microarchitecture, leading to increased fracture risk particularly of the hip (Genant et al., 2013), vertebrae (Michalska et al., 2012) and distal radius (Genant et al., 2010). In the UK, annually there are more than 50,000 osteoporotic hip fractures which often lead to more serious health consequences or mortality. 10% of these patients fail to survive for one month and 30% do not survive beyond one year (Abrahamsen et al., 2009; Clark et al., 2012). This human cost is mirrored by a health economic implication in the UK of £2 billion per annum. When global statistics are considered the numbers escalate. For example,

in China there are approximately >700,000 osteoporotic hip fractures annually (Kanis et al., 2012).

Bisphosphonates, which reduce osteoclast-mediated bone resorption, are the principle drug class used to treat osteoporosis. Anabolic osteoporosis drugs induce new bone production through an increase in osteoblast number (Jilka et al., 2009, Jilka et al., 1999). These anabolic drugs are based upon parathyroid hormone (PTH 1-84, PTH 1-34, teriparatide, Forsteo/Forteo or Teribone™) (Baron and Hesse, 2012; Neer et al., 2001). Teriparatide refers to recombinant, as opposed to chemically synthesised, PTH 1-34. Whilst a sustained high serum concentration of PTH suppresses bone formation (Horwitz et al., 2005), intermittent self-administered injections stimulate bone formation (Neer et al., 2001). Hence, the regimen of administration is essential for obtaining the therapeutic benefit of PTH. The therapeutic window of PTH 1-34 for the treatment of osteoporosis is currently limited to a 24 month period. Forsteo (US PTH 1-34, Eli Lilly) and Forteo (EU PTH 1-34, Eli Lilly) are currently available as a daily 20 µg subcutaneous (SC) injection. Teribone™ (Japan PTH 1-34, Ashai Kasei) is prescribed as a once weekly 56.5 µg SC injection (Nakamura et al., 2012). The flank is the recommended SC injection site.

Patient compliance to self-injectable PTH 1-84 and PTH 1-34 can be poor (Black et al., 2013; Yu et al., 2012), and some studies have shown that only 50% of patients continue administering the drug for 6 months after commencing treatment (Briot et al., 2009; Foster et al., 2011). The reasons for poor compliance to therapy are numerous, but include a dislike of using needles. 10% of the world's population have a needle phobia (Jenkins, 2014). Self-injecting can be associated with needle-stick injuries, bleeding, pain at the injection site, bruising and inflammation. Improved compliance to osteoporotic medicines is associated with lower fracture risk therefore providing a significant patient benefit (Weycker et al., 2007). Non-injectable delivery of PTH 1-34 for the treatment of osteoporosis is therefore an important unmet clinical need.

Orally delivered protein and polypeptide-based drugs is an attractive option although it is normally inefficacious, due to enzymatic degradation, acid-induced hydrolysis and low absorption in the gastrointestinal tract (Goldberg and Gomez-Orellana, 2003). However, some promising new technologies have been developed. These include Emisphere's Eligen® based on co-administration with 5-CNAC and Unigene's oral peptide delivery system Peptelligence® based on the use of enteric coated tablets. Reported clinical results are encouraging but are inferior to SC injection of PTH with neither of these technologies having progressed to market (Haemmerle et al., 2012; Henriksen et al., 2013; Sturmer et al., 2013).

Intranasal delivery offers great potential but is a challenging route for proteins and peptides, largely because of unfavourable bioavailability and often poorly tolerated intranasal membrane absorption (permeation) enhancers (Brayden et al., 2012; Illum, 2012; Illum et al., 2012; Lems and den Heijer, 2013). Both new delivery devices and intranasal mucosal permeation enhancers are being developed to improve nasal delivery of these large molecules (Djupesland, 2013; Fortuna et al., 2014; Pires et al., 2009). Together these advances aim to surmount the physiochemical and biological barriers previously preventing successful intranasal spray delivery of biological drugs. Our formulation development strategy focuses upon polyethylene glycol (15)-hydroxystearate (Solutol® HS15) for intranasal absorption-enhancement. The PTH 1-34 intranasal spray formulation is being developed to achieve the required pharmacokinetic profile, mimicking the pulsatile subcutaneous injections with an initial fast absorption profile, which has been shown to promote new bone formation.

The aim of this work was to develop a PTH 1-34 Solutol® HS15 based intranasal spray formulation with appropriate bioactivity, pharmacokinetics and bioavailability for the treatment of osteoporosis in patients. We present data on the duration of the intracellular 3'-5'-cyclic adenosine monophosphate (cAMP) cascade evoked by the addition of PTH 1-34 in different formulations to Saos-2 human osteoblast-like cell cultures. In addition we present the half maximal effective concentration to validate the *in vitro* human osteoblast culture

system. We have compared the bioactivity within an *in vitro* model, of a range of formulations and present the pharmacokinetic profiles of the Solutol® HS15 formulation delivered intranasally in a pre-clinical rat model.

2. Materials and Methods

2.1 Materials

PTH 1-34 was chemically synthesised by Polypeptide Inc. (Torrance, CA, USA). Chitosan (~150 kDa) and sodium hyaluronate (620-1200 kDa) were both obtained from Novomatrix (USA). Solutol® HS15 (345 Da) and Poloxamer 407 (9.8-14.6 kDa) were provided by BASF (UK). All other reagents were purchased from Sigma, UK, unless stated.

2.2 Tissue culture

Saos-2 human osteoblast-like cells are a well-characterised *in vitro* model, previously shown to express PTHR1 receptors and elicit a cAMP response when treated with parathyroid hormone (Rodan et al., 1987). Human osteoblast-like Saos-2 cells were purchased from the European Collection of Cell Cultures (Salisbury, UK). Cells were cultured at 37°C, 5% CO_2 in 0.2 µm sterile filtered McCoy's 5A, 10% foetal calf serum (FCS), 5mM β-glycerophosphate, 50 µg/ml L-ascorbic acid and media changed every 2-3 days. Experiments were performed within a 5-passage window. Cells were seeded at 2×10^4 cm^{-2} in tissue culture plates (Corning, UK) and cultured at a sub-confluent density. Mineralisation was confirmed using alizarin red staining at days 7 and 15. Briefly, cells were washed using PBS pH7.4, fixed in 70% ethanol, rehydrated in water and stained using 0.5% alizarin red solution. Digital images were captured using a Nikon Eclipse TS100 inverted microscope (Nikon Instruments, UK).

2.3 In vitro bioactivity of PTH 1-34 formulations

Saos-2 cells were exposed to; (1) Phosphate buffered saline (PBS) pH 7.4 0.5% BSA (basal), (2) 7.5% w/v Solutol® HS15 in acetate buffer (pH4) (representing vehicle), (3) 1 mg/ml PTH 1-34 in acetate buffer (pH 4) (no enhancer), or 1 mg/ml PTH 1-34 drug

formulations with adsorption enhancer either (4) 7.5% w/v Solutol® HS15, (5) 0.5% poloxamer 407, (6) 0.5% chitosan, (7) 1% chitosan (8) 0.5% hyaluronate. In brief, the Solutol formulation was prepared by rendering Solutol HS15 molten through raising the temperature to 60 ^{0}C. Molten Solutol® HS15 was then dissolved in pH4 0.1 M acetate buffer also at 60 ^{0}C. The resulting 7.5 % w/v Solutol HS15 was allowed to cool and used to prepare the 1 mg/ml PTH 1-34 Solutol® HS15 formulation. Similarly, the remaining 1 mg/ml PTH 1-34 formulations were prepared using 0.5% Poloxamer, 0.5% and 1% Chitosan and 0.5% Hyaluronate solutions. Before exposure to the Soas-2 cell cultures the intranasal delivery formulations underwent a 2 stage 1 in 25,000 dilution; 1/125 in PBS/ 0.5% BSA followed by 1/200 in McCoy's 5A/ 0.5% BSA culture medium (Table 1). This placed formulations with PTH (1-34) at a suitable concentration (~10 nM) for measuring the cAMP response of Soas-2 cells. This dilution is also representative of the dilution of the drug which occurs between the administration of a nasal spray formulation and exposure to the osteoblast in bone within a preclinical or clinical scenario. This is based upon serum concentrations and Cmax from human teriparatide pharmacokinetic studies (Satterwhite et al., 2010; Sturmer et al., 2013). Bioactivity was determined by measuring the secondary messenger cAMP produced as a result of PTH 1-34 binding to the PTH receptor. The Saos-2 cells were FCS-restricted for 16h prior to simulation. Following 30 minutes incubation with 25µM rollipram in culture medium, the Saos-2 cells were stimulated with the PTH 1-34 formulations or controls (1-8) and incubated for a further 60 minutes at 37°C, 5% CO_2, washed three times in PBS pH7.4 and lysed according to manufacturer's instructions. Samples were stored at -20°C until cAMP was quantified using ELISA (R&D Systems, Abingdon, UK). Cell numbers were normalised using PicoGreen® DNA quantification (Molecular Probes®, Invitrogen, Paisley, UK).

2.4 Pre-clinical pharmacokinetic studies

Male Sprague-Dawley rats, 250g-350g, were housed separately in the Biomedical Support Unit facilities at the University of Nottingham for a minimum of 7 days for acclimatisation

prior to the start of the experiment. Environmental temperature and relative humidity were maintained at 19±2 °C and 55±10 % respectively. Drinking water and a commercial balanced diet was supplied ad libitum.

The intranasal formulation of 1 mg/ml PTH 1-34, 7.5% w/v Solutol® HS15, 5% w/v mannitol in acetate buffer (pH4) was administered to rats (100 µg/kg). A second group of rats received an intranasal formulation of 1mg/ml PTH 1-34, 5% w/v mannitol in acetate buffer without 7.5% w/v Solutol® HS15 absorption enhancer. A third group of rats received a formulation a 1 mg/ml PTH 1-34 dosed subcutaneously to enable the calculation of relative bioavailability (80 µg/kg). Rats were sedated in groups of four with 2% v/v isoflurane vapour which was effective for the duration of the intranasal dosing procedure. 25-30 □l of the intranasal formulation was placed approximately 5mm into one nostril using a Gilson® pipette. Non-sedated rats received subcutaneous doses. All animal work was performed under UK Home Office Licence No. 40/3360.

The nasal spray PTH 1-34 bioavailability was calculated relative to subcutaneous injection in the rat model. A longitudinal series of blood samples was collected in heparinized tubes from the tail vein for 3h post administration. The total amount of blood removed from each animal was below 7% during the whole of the experiment. Plasma was prepared by centrifugation of whole blood at 13,000*g* for 10min at room temperature. The plasma was immediately frozen at -20°C prior to analysis for PTH 1-34 on an Acquity UPLC with Quattro premier XE mass spectrometer (Waters, Elstree, UK) (Chambers et al., 2013).

2.5 Statistical analysis

Data were subjected to statistical analysis using Prism 6.05 (GraphPad Inc) and Excel 14.0 (Microsoft). Normality of data was assessed using Kolmogorov-Smirnov and distribution plots. ANOVA was accompanied by the Dunnett's post-hoc test. Throughout the manuscript $p<0.05$ was considered to be statistically significant. Pharmacokinetic data were processed using WinNonLin 5.3 (Pharsight, Certara, USA).

3. Results

The fundamental phenotypic characteristic of Soas-2 cells to deposit bone mineral on exposure to β-glycerophosphate and ascorbic acid was confirmed by observing a positive Alizarin red stain (Figure 1A). Soas-2 cells proliferated, after an initial lag period of four days post seeding, cell numbers made a rapid linear increase over the subsequent 7 days from 1.6×10^5 cm^{-2} to 1.4×10^6 cm^{-2} (Figure 1B). A longitudinal study of the production of intracellular cAMP by Soas-2 cells following a 10nM dose of PTH 1-34 identified at what time-point the cytosolic concentration was at maximum (Figure 2). The study covered the first 72 hours post exposure. Baseline intracellular cAMP levels were consistently negligible (3.3±0.4 fmol cAMP/ ng DNA). There was a rapid increase in cAMP to approximately 33-fold from baseline 1 minute after dosing the PTH 1-34, which increased to 99-fold by 10 minutes. The peak of 406±23 fmol cAMP/ng DNA was observed 60 min post PTH 1-34 dose. During the following 3 hours post-maximal cAMP levels decreased rapidly to 107±14 fmol cAMP/ng DNA. At 24h the intracellular cAMP concentration was 9.7±0.8 fmol cAMP/ng DNA, returning to baseline for the remainder of the experimental period. Therefore intracellular cAMP was assayed 1h post treatment in subsequent formulation experiments.

The half maximal effective concentration (EC_{50}) of the chemically synthesised PTH 1-34 was determined by stimulating osteoblast-like Saos-2 cells with 0.01nM - 100nM of PTH 1-34 and the cell lysates assayed for cAMP. Inter-well variations were normalised using DNA and values are presented as mean ± standard error of the mean (SEM) (Figure 3). An EC_{50} of 0.76nM was calculated for PTH 1-34 in the Saos-2 cell culture system.

The data demonstrated that none of the absorption enhancers (Solutol® HS15, poloxamer 407, chitosan or sodium hyaluronate), which aim to promote the entry of PTH 1-34 into the blood from the nasal cavity, altered the bioactivity of PTH 1-34 within the cell culture system ($p > 0.05$) (Figure 4). Bioactivity was assessed by quantifying the intracellular secondary messenger cAMP within osteoblast-like Saos-2 cells, which express the PTHR1 receptors on their cell surface. Soas-2 cells exposed to the negative control PBS pH 7.4 0.5% BSA

quantified basal cAMP values (1.6±0.4 fmol cAMP/ ng DNA). 7.5% Solutol® HS15 (16±6.5 fmol cAMP/ng DNA) did not differ significantly from cAMP basal ($p>0.05$). PTH 1-34 formulations containing 0.5% hyaluronate (482±104 fmol cAMP/ng DNA), 0.5% poloxamer 407 (460±70 fmol cAMP/ng DNA), 1% chitosan (405±77 fmol cAMP/ng DNA) or 0.5% chitosan (588±89 fmol cAMP/ng DNA) or 7.5% Solutol® HS15 (614±135 fmol cAMP/ng DNA) did not differ significantly from stimulation with PTH 1-34 without nasal absorption enhancers (369±72 fmol cAMP/ng DNA) ($p>0.05$). We therefore continued to the preclinical phase with our nasal spray formulation containing Solutol® HS15 as our *in vitro* study did not identify that it was inferior compared to formulations containing either poloxamer 407, chitosan or sodium hyaluronate. Indeed none of these absorption enhancers at the concentrations tested had an effect on the bioactivity of PTH 1-34 ($p>0.05$).

The pharmacokinetic profile after the administration of PTH 1-34 is defined for the intranasal delivery of the formulation with and without Solutol® HS15, plus a subcutaneous PTH 1-34 injection without Solutol® HS15 (Figure 5, Table 2). Intranasally administered PTH 1-34 without Solutol® HS15 was poorly absorbed as identified by the low AUC value (open circles, AUC=140±66.5 ng/ml/min) compared to the subcutaneously administered PTH 1-34 (squares, AUC=1432±409.7 ng/ml/min) and the intranasally administered PTH 1-34 enhanced with Solutol® HS15 was absorbed well (closed circles, AUC=1393±226.8 ng/ml/min). The bioavailability of intranasally delivered PTH 1-34 enhanced with Solutol® HS15 in rats was calculated relative to the subcutaneous dose and found to be 78%. There were no significant differences between the pharmacokinetic parameters of the SC injection and those of intranasally delivered PTH 1-34 enhanced with Solutol® HS15 as shown in Table 2.

The PTH 1-34 half-life for Solutol® HS15 enhanced intranasal delivery did not differ from that observed for subcutaneous injection. The peak serum concentration (Cmax) of PTH 1-34 was equivalent in subcutaneous and intranasal enhanced administrations, both being significantly greater than non-enhanced intranasal delivery of PTH 1-34 ($p=0.016$). The

pharmacokinetic data obtained in rats provides evidence that the Solutol® HS15 formulation of PTH 1-34 have potential for intranasal delivery to humans.

4. Discussion

In this study the bioactivity of intranasal formulations of PTH 1-34 was demonstrated *in vitro*, using the human osteoblast-like cell line Saos-2. The data demonstrated that the intracellular cAMP secondary messenger response in Saos-2 cells to PTH 1-34 was not significantly different between PTH 1-34 alone or the PTH 1-34 within formulations containing either Solutol® HS15, hyaluronate, poloxamer 407 or chitosan. The osteoblastic phenotype of Saos-2 cells was exemplified by the production of bone mineral under controlled cell culture conditions, as demonstrated in Figure 1A. Soas-2 cells are a well-established cell line suited to investigating the response of osteoblast-like cells to PTH and its analogues. The cells proliferated rapidly under the culture conditions, as shown in Figure 1B. The doubling time during the exponential growth phase was 31h, similar to values previously published of 32 and 37 hours (McQuillan et al., 1995; Rodan et al., 1987). Fundamentally, this cell line is considered suitable as an *in vitro* bioassay for PTH 1-34 formulation development due to the expression of ~100,000 PTHR1 receptors per cell (Alokail and Peddie, 2007).

A seminal research publication by the late Gideon Rodan's group regarding the characterisation of Saos-2 cells, established the EC_{50} of PTH 1-34 to be 2.8 nM using an adenylate cyclase secondary messenger assay. This assay identified the incorporation of tritiated adenine into cAMP, whereas in the present studies a cAMP ELISA based assay was used (Rodan et al., 1987). More recently Murrills et al demonstrated an EC_{50} of 0.62 nM in Saos-2 cells (Murrills et al., 2004). Our results identified an EC_{50} value of 0.76 nM for chemically synthesised PTH 1-34. Indeed, it has been reported that the sensitivity of Soas-2 cells to PTH 1-34 increases until confluent monolayers are reached, this being in part attributed to less basal cAMP being produced. Basal cAMP values are subtracted prior to representation of cAMP intracellular concentration post stimulation. Variation in EC_{50} values between laboratories is likely to be due to differences in Saos-2 proliferation and differentiation states, variations in tissue culture conditions or the PTH 1-34 preparation. Together, the data in our *in vitro* studies suggest that the chemically synthesised PTH 1-34

in the various intranasal spray formulations did not have a detrimental effect on the cAMP signalling pathway in human osteoblast-like Saos-2 cells, indicating that PTH bioactivity was not altered (Chauvin et al., 2002; Vilardaga et al., 2002).

Several alternatives to intranasal delivery of PTH are being investigated for the purpose of improving patient adherence to the administration of biological drugs (Cai et al., 2014). Successful oral delivery requires the PTH 1-34 formulation to resist proteolysis induced by the harsh acidic gastric microenvironment. New oral delivery approaches such as PTH 1-34 loaded thiolated chitosan nanoparticles and/or pegylated chitosan nanoparticles as well as PTH 1-34 Solutol HS 15/ labrasol emulsions have shown promise in animal models as an alternative administration to injection (Narayanan et al., 2013, 2014) but as yet have not been translated to the clinic. However, it should be born in mind when interpreting these results that the gastric pathway in animal models may differ significantly from that of humans.

The intranasal route has proven an established route for delivery of a number of drugs to the general circulation and is an expanding field of research (Illum, 2012; Illum et al., 2012; Riese et al., 2014). A key determinant of the efficacy of a nasal spray formulation of PTH 1-34 is the pharmacokinetics. To investigate this the rat preclinical model was used and it showed that serum levels of PTH 1-34 were significantly greater when Solutol® HS15 was included in the nasal delivery formulation. We have previously demonstrated that Solutol® HS15 (CriticalSorb™) is an essential formulation component for the enhancement of the systemic delivery of human growth hormone via the intranasal route (Illum et al., 2012). In those studies, the bioavailability in conscious rats of an insulin Solutol® HS15 formulation delivered nasally was found to be 100 % relative to a subcutaneous injection for the first two hours. The bioavailability of insulin in from the nasal delivery formulation without Solutol® HS15 was near zero (Lewis et al., 2013). Interestingly, the bioavailability of hGH in combination with 10% and 7.5% Solutol® HS15 after nasal administration in rats were found to be 49.9 % and 34.3 % respectively (Illum et al., 2012). Solutol® HS15, a mixture of mono-

and diesters of 12-hydroxystearate (macrogol 15-hydroxystearate) functions as a non-ionic surfactant with a hydrophilic-lipophilic balance (HLB) value of 14-16. Due to the amphiphilic nature of its components Solutol® HS15 forms micelles (CMC ~ 0.005% w/v) in solution with a radius of ~6.5 nm. The size of the micelles is not affected by interaction with peptides/proteins and importantly when co-dissolved with Solutol® HS15, the peptides/proteins retain their tertiary structure (Shubber et al., 2015). This indicates that there is no direct interaction between the micellar structures and the drug. Solutol® HS15 has been found to work mainly by enhancing the transcellular transport of the drug across the mucosal membrane and is non-toxic to the mucosal tissue (Brayden et al., 2012). The Solutol® HS15 formulation is compliant with the European Medicines Agency guidelines on pharmaceutical quality of inhalation and nasal products (EMEA/CHMP/QWP/49313/2005). The rational regarding pursuing a formulation containing Solutol® HS15 was supported by data previously published investigating the effect of repeated dosing of Solutol® HS15 on the nasal mucosa (Illum, 2012). However this was only a pilot study in a rat model over a 5 day period. Further data, perhaps in a larger animal model, would be beneficial in assessing if there is any irritation to the nasal mucosa by the absorption enhancer Solutol® HS15 when dosed repeatedly over an extended period.

Other researchers have employed nasal absorption enhancer systems to improve the nasal delivery of PTH. Zelos Therapeutics showed that N-dodecyl-□-D-maltoside, an enhancer with a similar absorption enhancing mechanism to that of Solutol® HS15, was able to improve the nasal absorption of a cyclic PTH 1-31 analogue to 35-40% in non-human primates (Krause D et al., 2009). Matsumoto et al performed a clinical nasal dosing study of PTH 1-34 with 90 osteoporotic subjects, mean age 66.5 years, and showed nasal administration of 1000 µg PTH 1-34 (a 50 fold dose increase over the 20µg/daily sub cutaneous injection) increased lumbar bone mineral density by 2.4% in 3 months, but concluded greater bioavailability was needed (Matsumoto et al., 2006).

Clinical applications of nasal spray formulations of PTH 1-34 could be used in combination with other bone regeneration techniques including implanted biomaterial (Reznikov et al., 2016). Combining bone fracture surgery with post-operative PTH 1-34 nasal spray prescription could improve outcome in scenarios such as Charcot arthroplasty (Pearson et al., 2011; Tamai et al., 2013) and in difficult to treat ankle union (Campbell et al., 2015). Furthermore there is potential to combine intranasal PTH 1-34 with experimental therapeutics such as inhibitors of osteoclast-mediated bone resorption, to develop dual action drug combinations for the treatment of osteoporosis.

5. Conclusion

Overall our data demonstrate the development of a novel intranasal spray formulation of PTH 1-34 that generates comparable bioactivity to subcutaneous injections in a preclinical model. This work exemplifies the potential of Solutol® HS15 (CriticalSorb™) enhanced PTH 1-34 intranasal spray formulations as an attractive alternative to subcutaneous injections. The ease of use of intranasal sprays aims to increase adherence to PTH 1-34 therapy; increasing efficacy, patient benefit and reducing healthcare costs. Therefore the pharmacokinetics of the Solutol® HS15 formulation must now be addressed within a Phase I clinical trial to demonstrate the translation from a small animal model to man.

Conflicts of interest: Critical Pharmaceuticals: Patent no. WO 2010/029374A1. All other authors have no conflict of interest.

Acknowledgements

We would like to thank Dr Phil Clarke, Preclinical Oncology, University of Nottingham, UK; Kirk Jeffery (Critical Pharmaceuticals Ltd, Nottingham, UK) and Brian Trussell for expert technical assistance and access to specific laboratory facilities. This study was supported by research funding from Engineering and Physical Sciences Research Council (Grant EP/K502364/1) and Technology Strategy Board (Grant 101161).

References

Abrahamsen, B., van Staa, T., Ariely, R., Olson, M., Cooper, C., 2009. Excess mortality following hip fracture: a systematic epidemiological review. Osteoporosis Int. 20, 1633-1650.
Alokail, M.S., Peddie, M.J., 2007. Characterisation of ligand binding to the parathyroid hormone/parathyroid hormone-related peptide receptor in MCF7 breast cancer cells and SaOS-2 osteosarcoma cells. Cell biochemistry and function 25, 139-147.
Baron, R., Hesse, E., 2012. Update on bone anabolics in osteoporosis treatment: rationale, current status, and perspectives. J Clin Endocrinol Metab 97, 311-325.
Black, D.M., Bilezikian, J.P., Greenspan, S.L., Wuster, C., Munoz-Torres, M., Bone, H.G., Rosen, C.J., Andersen, H.S., Hanley, D.A., 2013. Improved adherence with PTH(1-84) in an extension trial for 24 months results in enhanced BMD gains in the treatment of postmenopausal women with osteoporosis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 24, 1503-1511.
Brayden, D.J., Bzik, V.A., Lewis, A.L., Illum, L., 2012. CriticalSorb promotes permeation of flux markers across isolated rat intestinal mucosae and Caco-2 monolayers. Pharmaceutical research 29, 2543-2554.
Briot, K., Ravaud, P., Dargent-Molina, P., Zylberman, M., Liu-Leage, S., Roux, C., 2009. Persistence with teriparatide in postmenopausal osteoporosis; impact of a patient education and follow-up program: the French experience. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 20, 625-630.
Cai, Y., Xu, M., Yuan, M., Liu, Z., Yuan, W., 2014. Developments in human growth hormone preparations: sustained-release, prolonged half-life, novel injection devices, and alternative delivery routes. International journal of nanomedicine 9, 3527-3538.
Campbell, E.J., Campbell, G.M., Hanley, D.A., 2015. The effect of parathyroid hormone and teriparatide on fracture healing. Expert Opin Biol Ther 15, 119-129.
Chambers, E., Lame, M., Bhattacharya, D., Fountain, K., 2013. Dealing with Complicated Peptides in Bioanalysis: LC/MS/MS of Teriparatide, AAPSBiotech.
Chauvin, S., Bencsik, M., Bambino, T., Nissenson, R.A., 2002. Parathyroid hormone receptor recycling: role of receptor dephosphorylation and beta-arrestin. Molecular endocrinology 16, 2720-2732.
Clark, E.M., Gould, V.C., Morrison, L., Masud, T., Tobias, J., 2012. Determinants of fracture risk in a UK-population-based cohort of older women: a cross-sectional analysis of the Cohort for Skeletal Health in Bristol and Avon (COSHIBA). Age and ageing 41, 46-52.
Djupesland, P.G., 2013. Nasal drug delivery devices: characteristics and performance in a clinical perspective-a review. Drug delivery and translational research 3, 42-62.
Fortuna, A., Alves, G., Serralheiro, A., Sousa, J., Falcao, A., 2014. Intranasal delivery of systemic-acting drugs: Small-molecules and biomacromolecules. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 88, 8-27.
Foster, S.A., Foley, K.A., Meadows, E.S., Johnston, J.A., Wang, S.S., Pohl, G.M., Long, S.R., 2011. Adherence and persistence with teriparatide among patients with commercial, Medicare, and Medicaid insurance. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 22, 551-557.
Genant, H.K., Engelke, K., Hanley, D.A., Brown, J.P., Omizo, M., Bone, H.G., Kivitz, A.J., Fuerst, T., Wang, H., Austin, M., Libanati, C., 2010. Denosumab improves density and strength parameters as measured by QCT of the radius in postmenopausal women with low bone mineral density. Bone 47, 131-139.
Genant, H.K., Libanati, C., Engelke, K., Zanchetta, J.R., Hoiseth, A., Yuen, C.K., Stonkus, S., Bolognese, M.A., Franek, E., Fuerst, T., Radcliffe, H.S., McClung, M.R., 2013. Improvements in hip trabecular, subcortical, and cortical density and mass in postmenopausal women with osteoporosis treated with denosumab. Bone 56, 482-488.
Goldberg, M., Gomez-Orellana, I., 2003. Challenges for the oral delivery of macromolecules. Nature reviews. Drug discovery 2, 289-295.
Haemmerle, S.P., Mindeholm, L., Launonen, A., Kiese, B., Loeffler, R., Harfst, E., Azria, M., Arnold, M., John, M.R., 2012. The single dose pharmacokinetic profile of a novel oral human parathyroid hormone formulation in healthy postmenopausal women. Bone 50, 965-973.
Henriksen, K., Andersen, J.R., Riis, B.J., Mehta, N., Tavakkol, R., Alexandersen, P., Byrjalsen, I., Valter, I., Nedergaard, B.S., Teglbjaerg, C.S., Stern, W., Sturmer, A., Mitta, S., Nino, A.J., Fitzpatrick, L.A., Christiansen, C., Karsdal, M.A., 2013. Evaluation of the efficacy, safety and pharmacokinetic profile of oral recombinant human parathyroid hormone rhPTH(1-31)NH2 in postmenopausal women with osteoporosis. Bone 53, 160-166.
Horwitz, M.J., Tedesco, M.B., Sereika, S.M., Syed, M.A., Garcia-Ocana, A., Bisello, A., Hollis, B.W., Rosen, C.J., Wysolmerski, J.J., Dann, P., Gundberg, C., Stewart, A.F., 2005. Continuous PTH and PTHrP infusion causes suppression of bone formation and discordant effects on 1,25(OH)2 vitamin D. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 20, 1792-1803.
Illum, L., 2012. Nasal drug delivery - recent developments and future prospects. Journal of controlled release : official journal of the Controlled Release Society 161, 254-263.
Illum, L., Jordan, F., Lewis, A.L., 2012. CriticalSorb: a novel efficient nasal delivery system for human growth hormone based on Solutol HS15. Journal of controlled release : official journal of the Controlled Release Society 162, 194-200.

Jenkins, K., 2014. II. Needle phobia: a psychological perspective. British journal of anaesthesia 113, 4-6.
Kanis, J.A., Oden, A., McCloskey, E.V., Johansson, H., Wahl, D.A., Cooper, C., Quali, I.O.F.W.G.E., 2012. A systematic review of hip fracture incidence and probability of fracture worldwide. Osteoporosis Int. 23, 2239-2256.
Krause D, Eddy P, Merutka G, MacDonald B, 2009. Intranasal (IN), Pharmacokinetic (PK) and Bioavailability of ZT-034 a Parathyroid Hormone (PTH) Analog. JBMR 24, S352.
Lems, W.F., den Heijer, M., 2013. Established and forthcoming drugs for the treatment of osteoporosis. The Netherlands journal of medicine 71, 188-193.
Lewis, A.L., Jordan, F., Illum, L., 2013. CriticalSorb (TM): enabling systemic delivery of macromolecules via the nasal route. Drug delivery and translational research 3, 26-32.
Matsumoto, T., Shiraki, M., Hagino, H., Iinuma, H., Nakamura, T., 2006. Daily nasal spray of hPTH(1-34) for 3 months increases bone mass in osteoporotic subjects: a pilot study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 17, 1532-1538.
McQuillan, D.J., Richardson, M.D., Bateman, J.F., 1995. Matrix deposition by a calcifying human osteogenic sarcoma cell line (SAOS-2). Bone 16, 415-426.
Michalska, D., Luchavova, M., Zikan, V., Raska, I., Jr., Kubena, A.A., Stepan, J.J., 2012. Effects of morning vs. evening teriparatide injection on bone mineral density and bone turnover markers in postmenopausal osteoporosis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 23, 2885-2891.
Murrills, R.J., Matteo, J.J., Samuel, R.L., Andrews, J.L., Bhat, B.M., Coleburn, V.E., Kharode, Y.P., Bex, F.J., 2004. In vitro and in vivo activities of C-terminally truncated PTH peptides reveal a disconnect between cAMP signaling and functional activity. Bone 35, 1263-1272.
Nakamura, T., Sugimoto, T., Nakano, T., Kishimoto, H., Ito, M., Fukunaga, M., Hagino, H., Sone, T., Yoshikawa, H., Nishizawa, Y., Fujita, T., Shiraki, M., 2012. Randomized Teriparatide [human parathyroid hormone (PTH) 1-34] Once-Weekly Efficacy Research (TOWER) trial for examining the reduction in new vertebral fractures in subjects with primary osteoporosis and high fracture risk. J Clin Endocrinol Metab 97, 3097-3106.
Narayanan, D., Anitha, A., Jayakumar, R., Chennazhi, K.P., 2013. In vitro and in vivo evaluation of osteoporosis therapeutic peptide PTH 1-34 loaded pegylated chitosan nanoparticles. Molecular pharmaceutics 10, 4159-4167.
Narayanan, D., Anitha, A., Jayakumar, R., Chennazhi, K.P., 2014. PTH 1-34 loaded thiolated chitosan nanoparticles for osteoporosis: oral bioavailability and anabolic effect on primary osteoblast cells. Journal of biomedical nanotechnology 10, 166-178.
Neer, R.M., Arnaud, C.D., Zanchetta, J.R., Prince, R., Gaich, G.A., Reginster, J.Y., Hodsman, A.B., Eriksen, E.F., Ish-Shalom, S., Genant, H.K., Wang, O., Mitlak, B.H., 2001. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 344, 1434-1441.
Pearson, R.G., Shu, K.S.S., Lowe, J., Game, F.L., Jeffcoate, W.J., Scammell, B.E., 2011. Histological features of acute Charcot neuroarthropathy in diabetes. Diabetologia 54, S11-S11.
Pires, A., Fortuna, A., Alves, G., Falcao, A., 2009. Intranasal drug delivery: how, why and what for? Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques 12, 288-311.
Reznikov, N., Steele, J.A.M., Fratzl, P., Stevens, M.M., 2016. A materials science vision of extracellular matrix mineralization. Nat. Rev. Mater. 1, 14.
Riese, P., Sakthivel, P., Trittel, S., Guzman, C.A., 2014. Intranasal formulations: promising strategy to deliver vaccines. Expert opinion on drug delivery, 1-16.
Rodan, S.B., Imai, Y., Thiede, M.A., Wesolowski, G., Thompson, D., Bar-Shavit, Z., Shull, S., Mann, K., Rodan, G.A., 1987. Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. Cancer research 47, 4961-4966.
Satterwhite, J., Heathman, M., Miller, P.D., Marin, F., Glass, E.V., Dobnig, H., 2010. Pharmacokinetics of Teriparatide (rhPTH 1-34) and Calcium Pharmacodynamics in Postmenopausal Women with Osteoporosis. Calcif. Tissue Int. 87, 485-492.
Shubber, S., Vllasaliu, D., Rauch, C., Jordan, F., Illum, L., Stolnik, S., 2015. Mechanism of Mucosal Permeability Enhancement of CriticalSorb® (Solutol® HS15) Investigated In Vitro in Cell Cultures. Pharmaceutical research 32, 516-527.
Sturmer, A., Mehta, N., Giacchi, J., Cagatay, T., Tavakkol, R., Mitta, S., Fitzpatrick, L., Wald, J., Trang, J., Stern, W., 2013. Pharmacokinetics of Oral Recombinant Human Parathyroid Hormone rhPTH(1-31)NH2 in Postmenopausal Women with Osteoporosis. Clinical Pharmacokinetics 52, 995-1004.
Tamai, K., Takamatsu, K., Kazuki, K., 2013. Successful treatment of nonunion with teriparatide after failed ankle arthrodesis for Charcot arthropathy. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 24, 2729-2732.
Vilardaga, J.P., Krasel, C., Chauvin, S., Bambino, T., Lohse, M.J., Nissenson, R.A., 2002. Internalization determinants of the parathyroid hormone receptor differentially regulate beta-arrestin/receptor association. The Journal of biological chemistry 277, 8121-8129.
Weycker, D., Macarios, D., Edelsberg, J., Oster, G., 2007. Compliance with osteoporosis drug therapy and risk of fracture. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 18, 271-277.

Yu, S., Burge, R.T., Foster, S.A., Gelwicks, S., Meadows, E.S., 2012. The impact of teriparatide adherence and persistence on fracture outcomes. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 23, 1103-1113.

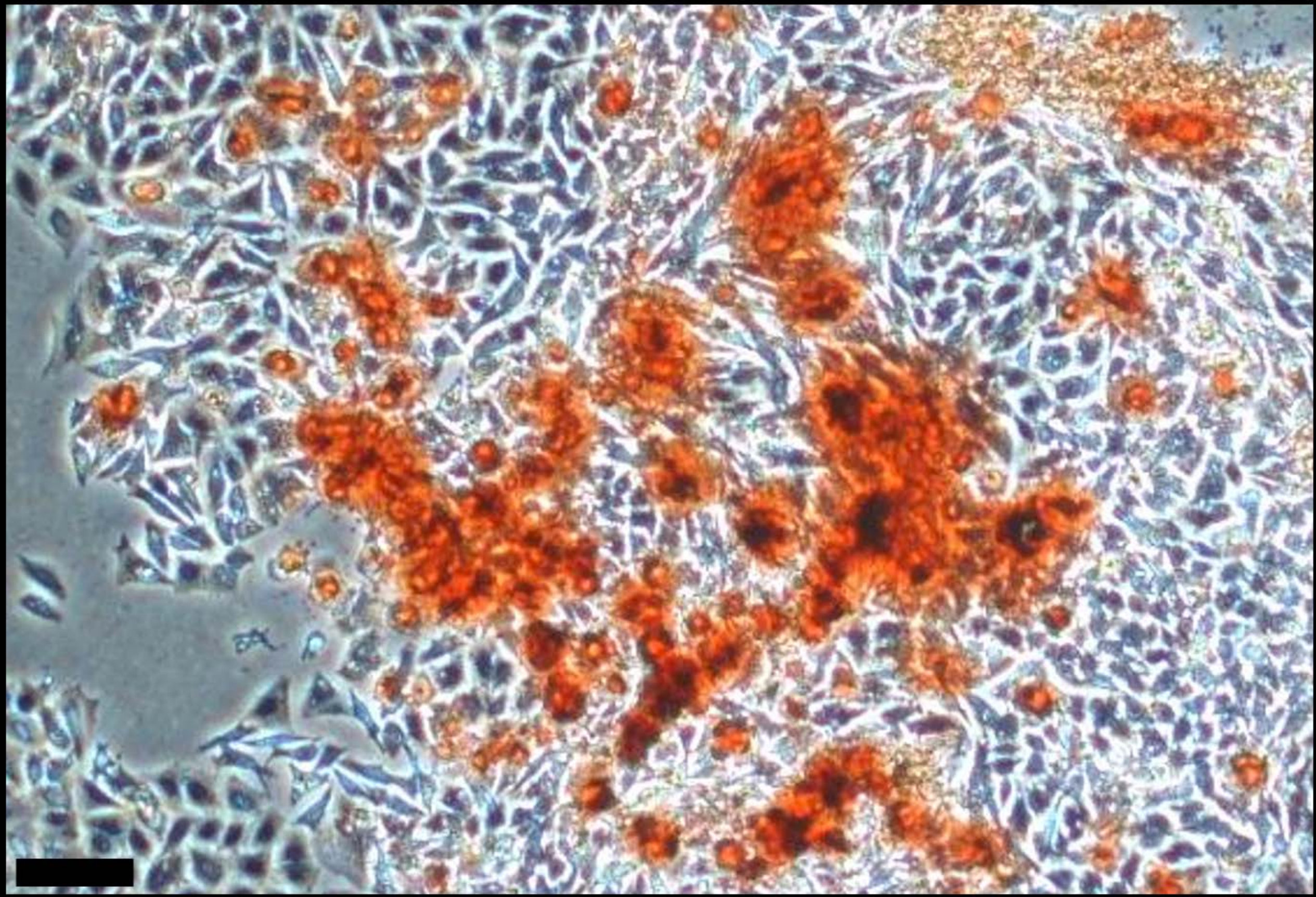
Figure 1. **Human osteoblast-like Saos-2 cell mineralisation and proliferation characteristics**. **A** Photomicrograph of adherent osteoblast-like cells depicting a mineralization phenotype. Alizarin red produces a red complex when exposed to calcium mineral deposits. Image taken after Soas-2 cells were cultured for 7 days within a mineralisation promoting culture media containing the active ingredient 5mM β-glycerophosphate. This demonstrated that the cell line at the passage numbers we used had maintained an osteoblastic phenotype; Bar = 100µm. **B** Soas-2 cell population growth. There is a lag phase in the proliferation of Soas-2 cells following adhesion to the tissue culture plastic (1-4 days). When cultured in the standard medium McCoy's 5A with 10% foetal calf serum (FCS) (solid circles) cells proliferate rapidly between days 4-11. Soas-2 cultured in mineralization medium (solid squares) containing 5 mM β-glycerophosphate did not proliferate (1-11 days) (n=2, mean ± SEM, $p<0.05$).

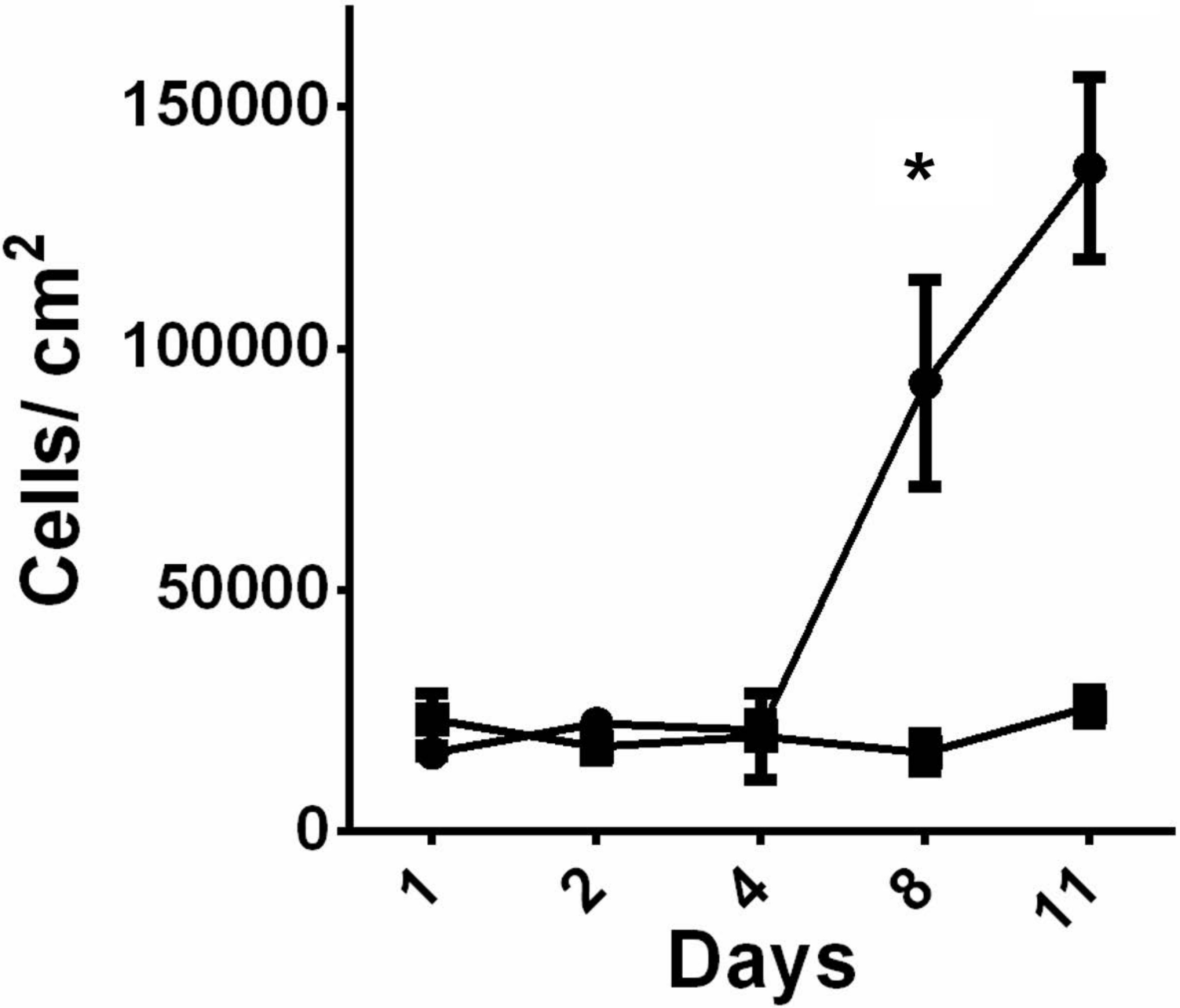
Figure 2. **Time course of the production of the intracellular secondary messenger cAMP by Soas-2 cells post PTH 1-34 stimulation.** Bioassay sensitivity when quantifying differences in intracellular cAMP concentration caused by receptor binding of PTH (1-34) is dependent on the time point assayed post stimulation. Mineralising Saos-2 cells were FCS-restricted for 16h prior to 30min incubation with 25µM rollipram in McCoy 5A. Cells were either stimulated with the 10 mM PTH 1-34 (squares) or PBS pH 7.4 (circles) in 0.5% BSA for 72 hours at 37°C, 5% CO_2. At each time point cells were washed three times in PBS pH7.4 and the cell lysate was quantified using ELISA (R&D Systems, Abingdon, UK). (n=3, mean ±SEM).

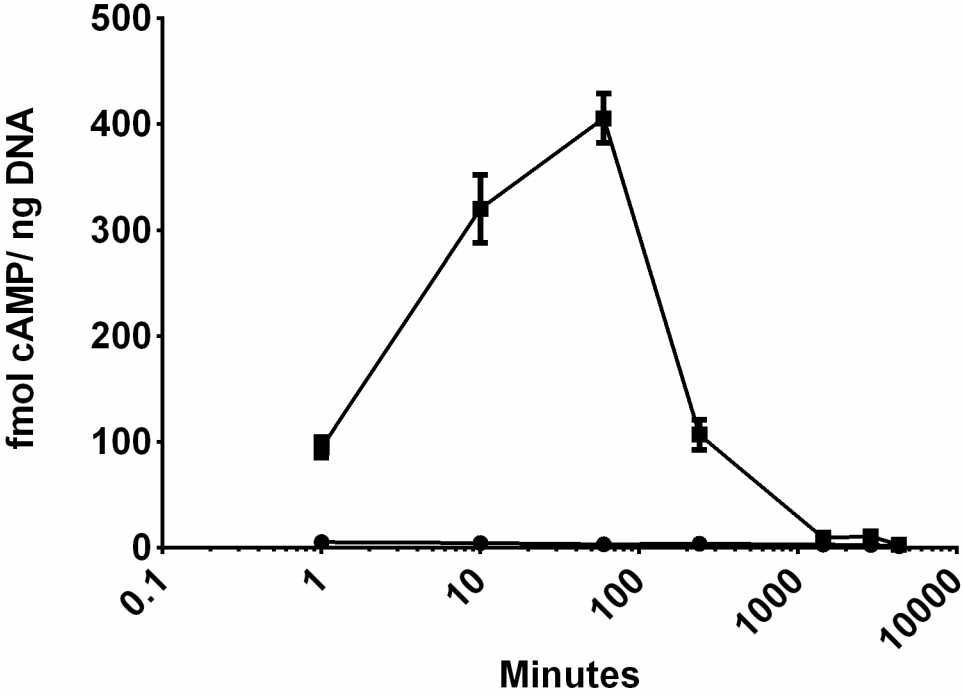
Figure 3. **Half maximal effective concentration (EC_{50}) of PTH 1-34 stimulation on Soas-2 cell cAMP intracellular messenger production**. Mineralising human osteoblast-like Saos-2 cells were FCS-restricted for 16h prior to 30min incubation with 25µM rollipram in McCoy 5A. Cells were then stimulated with a range of PTH 1-34 concentrations (0.01nM – 100nM) for 1 hour at 37°C, 5% CO_2. At each time point cells were washed three times in PBS pH7.4 and the cell lysate was quantified using ELISA (R&D Systems, Abingdon, UK). Inter-well variation was minimised by presenting cAMP data normalised to DNA. An EC50 of 0.76nM was calculated for PTH 1-34 in the Saos-2 cell culture system. (n=3, mean ±SEM).

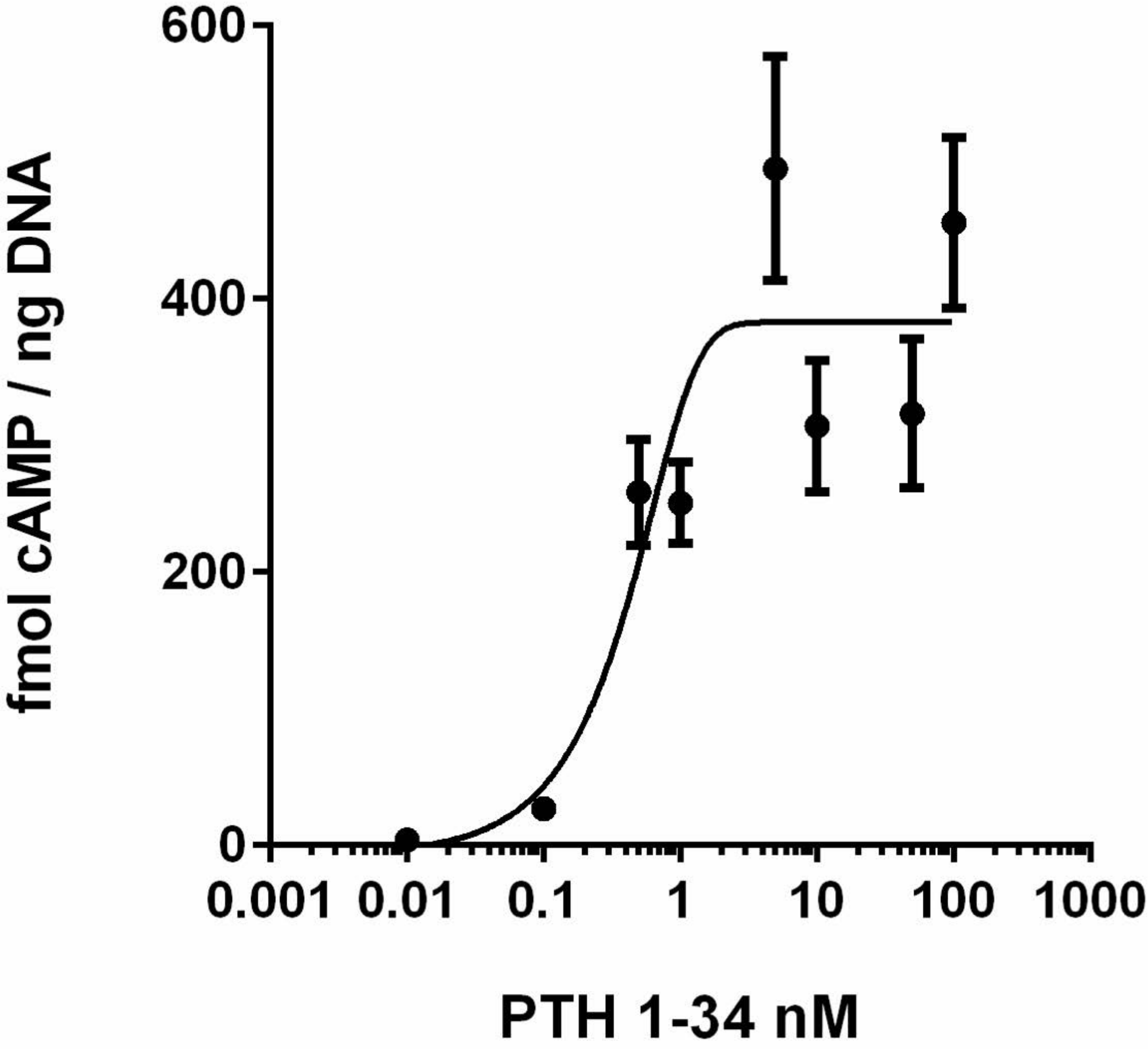
Figure 4. **Solutol® HS15 PTH 1-34 bioactivity in mineralising human osteoblast-like Saos-2 cells**. Basal - PBS pH 7.4 0.05% BSA. 7.5% Solutol® HS15 sodium acetate (pH 4) vehicle, 7.5% Solutol® HS15 + PTH 1-34 PTH, 0.5% Hyaluronate + PTH 1-34, 0.5% Poloxamer 407 + PTH 1-34, 1% chitosan + PTH 1-34, 0.5% chitosan + PTH 1-34. Cells were stimulated with dilutions of the above (1/25,000) of formulations. In each case the percentage composition of formulation components are prior to dilution, PTH 1-34 was 1 mg/ml in the formulations prior to dilution equating to an exposure of ~10nM per well. Intracellular cAMP was normalised to genomic DNA quantity per well. (n=3, mean ±SEM).

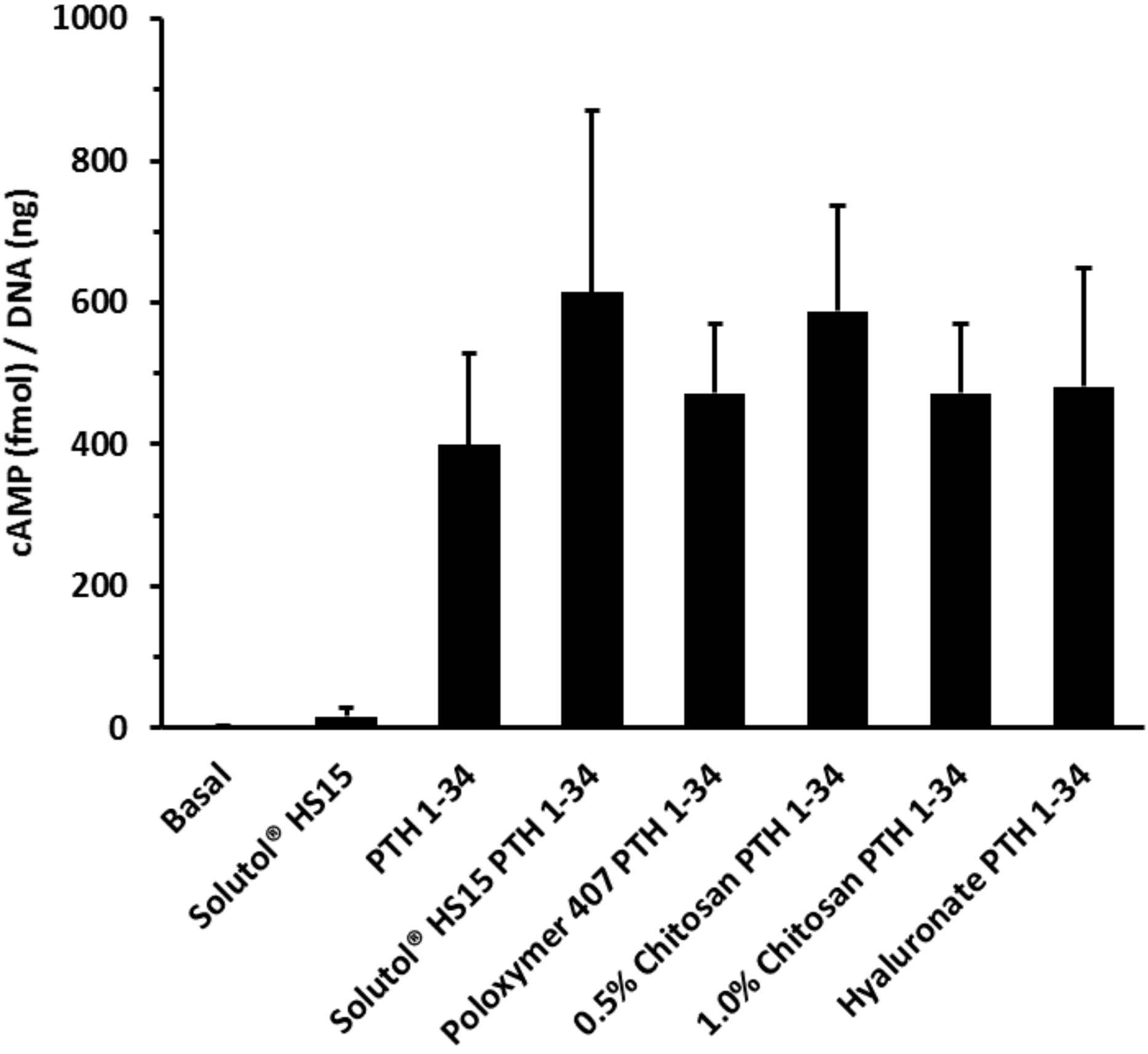
Figure 5. **Pharmacokinetic profile of Solutol® HS15 intranasal administration of PTH 1-34 in rats**. PTH 1-34 concentration in rat plasma following administration via subcutaneous and intranasal routes. Intranasally administered 100 µg/kg 1 mg/ml PTH 1-34 in 5% w/v mannitol within acetate buffer (pH4) not containing Solutol® HS15 was poorly absorbed (○).Intranasal delivery of 100 µg/kg of 1 mg/ml PTH 1-34 in 5% w/v mannitol within acetate buffer (pH4) with 7.5% Solutol® HS15 (●).Subcutaneous injection of 80 µg/kg 1 mg/ml PTH 1-34 without Solutol® HS15 was used to calculate bioavailability (■). Bioavailability of 78% for the intranasal administration of PTH 1-34 in Solutol® HS15 containing formulation compared to subcutaneous administration. Calculation was dose corrected (n=4, mean ± SD).




Cells/ cm2
150000
100000
50000
0
*
*
1
2
4
8
11
Days


500
400
300
200
100
0
fmol cAMP/ ng DNA
0.1
1
10
100
1000
10000
Minutes


600
400
200
0
fmol cAMP / ng DNA
0.001
0.01
0.1
1
10
100
1000
PTH 1-34 nM


1000
800
600
400
200
0
cAMP (fmol) / DNA (ng)
Basal
Solutol® HS15
PTH 1-34
Solutol® HS15 PTH 1-34
Poloxymer 407 PTH 1-34
0.5% Chitosan PTH 1-34
1.0% Chitosan PTH 1-34
Hyaluronate PTH 1-34

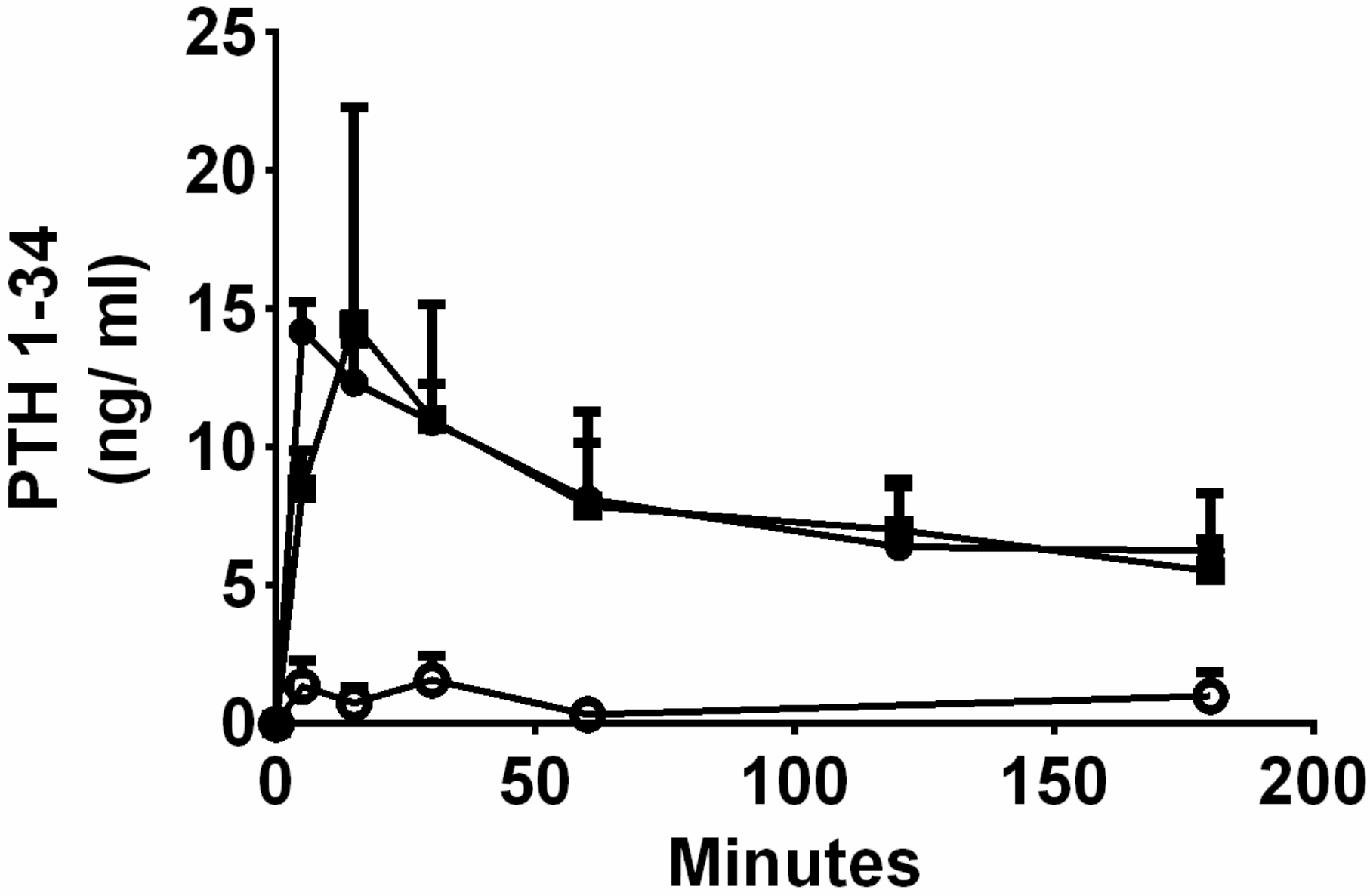

PTH 1-34
(ng/ ml)
25
20
15
10
5
0
0
50
100
150
200
Minutes

Table 1. **Formulation and Soas-2 osteoblast-like cells exposure concentration of drug and excipients**. Three controls were included; (1) 0.5% Bovine serum albumin (BSA), (2) 7.5% Solutol™ HS15 which was the concentration in the Solutol™ HS15 containing formulation and (3) 1 mg/ml PTH (1-34) alone. Formulations containing 1 mg/ml PTH (1-34) and absorption enhancers, 7.5% Solutol HS15, 0.5% Poloxamer, and 0.5% and 1% Chitosan, were also prepared (4-8). Dilution of these controls and formulations were made with standard medium McCoy's 5A with 10% foetal calf serum (FCS) in all cases. The concentration of PTH (1-34) and excipients exposed to the Soas-2 cells is listed.

Table 2. **Pharmacokinetic parameters of PTH (1-34) administered sub-cutaneously, intranasally with Solutol® HS15 enhancement and intranasally with no enhancer in rats**. The peak serum concentration (Cmax) of PTH 1-34, the time at which peak serum concentration of PTH (1-34) was observed (Tmax), the area under the curve (AUC) and PTH (1-34) half-life were determined. No statistical difference between subcutaneous injection and intranasal administrations using Solutol® HS15 were observed for all pharmacokinetic parameters ($p>0.05$).

Table 1

	Formulation	Concentration of drug and excipient exposed to Soas-2 cells
1	0.5% BSA	$2x10^{-5}$ BSA
2	7.5% Solutol HS15	$3x10^{-4}$ Solutol HS15
3	1 mg /ml PTH 1-34	10 nM PTH
4	7.5% Solutol HS15 + 1 mg/ml PTH 1-34	$3x10^{-4}$ % Solutol HS15 + 10 nM PTH
5	0.5% Poloxamer 407 + 1 mg/ml PTH 1-34	$2x10^{-5}$ % Poloxamer 407 + 10 nM PTH
6	0.5% Chitosan + 1 mg/ml PTH 1-34	$2x10^{-5}$ % Chitosan + 10 nM PTH
7	1 % Chitosan + 1 mg/ml PTH 1-34	$4x10^{-5}$ % Chitosan + 10 nM PTH
8	0.5% Hyaluronate + 1 mg/ml PTH 1-34	$2x10^{-5}$ % Hyaluronate + 10 nM PTH 1-34

Table 2

Pharmacokinetic Parameters	Sub-cutaneous PTH 1-34	Intranasal PTH 1-34 + Solutol® HS15	Intranasal PTH 1-34 – no enhancer
Cmax (ng/ml)	14.8 ± 7.5	13.7 ± 1.6	2.1 ± 0.5
Tmax (min)	16.3 ± 10.3	10.0 ± 5.8	13.3 ± 14.4
AUC0-3 (min*ng/ml)	1432 ± 409.7	1393 ± 226.8	140 ± 66.5
Half Life (min)	189.5 ± 110.0	157.0 ± 68.1	#
F_{rel}0-3 (%)	-	78.0	7.8

(n=4, mean ± SD) # not determined