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THERAPEUTICS

Glutathione-PEGylated liposomal methylprednisolone in comparison to free methylprednisolone: slow release characteristics and prolonged lymphocyte depression in a first-in-human study

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AIMS

Intravenous high-dose free methylprednisolone (MP) hemisuccinate is the primary treatment for an acute relapse in relapsing–remitting multiple sclerosis. However, it is inconvenient and its side effects are undesirable. Both dose and dosing frequency can be reduced by incorporating free MP in glutathione-PEGylated liposomes, creating a slow-release formulation with reduced toxicity and prolonged peripheral efficacy. This first-in-human study was designed to assess the safety, pharmacokinetics and pharmacodynamics of glutathione-PEGylated liposomes containing MP (2B3–201).

METHODS

The first part was a double-blind, three-way cross over study in 18 healthy male subjects, receiving ascending doses of 2B3–201, active comparator (free MP) or placebo. Part 2 of the study was an open-label infusion of 2B3–201 (different doses), exploring pretreatment with antihistamines and different infusion schedules in another 18 healthy male subjects, and a cross-over study in six healthy female subjects. MP plasma concentrations, lymphocyte counts, adrenocorticotropic hormone, osteocalcin and fasting glucose were determined. Safety and tolerability profiles were assessed based on adverse events, safety measurements and central nervous system tests.

RESULTS

The most frequent recorded AE related to 2B3–201 was an infusion related reaction (89%). 2B3–201 was shown to have a plasma half-life between 24 and 37 h and caused a prolonged decrease in the lymphocyte count, adrenocorticotropic hormone and osteocalcin, and a rise in fasting glucose.

CONCLUSION

2B3–201 is considered safe, with no clinically relevant changes in central nervous system safety parameters and no serious adverse events. In addition, 2B3–201 shows a long plasma half-life and prolonged immunosuppressive effects.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Intravenous high-dose methylprednisolone (MP) is the primary treatment for an acute relapse in relapsing–remitting multiple sclerosis. Although effective, this treatment is inconvenient and its side effects are undesirable.
- Dose and dosing frequency can be reduced by incorporating MP in glutathione-PEGylated liposome, creating a central nervous system-targeted formulation, 2B3–201, with reduced toxicity and prolonged efficacy.

WHAT THIS STUDY ADDS

- 2B3–201 at doses up to 450 mg was considered safe.
- 2B3–201 has a slow-release like pharmacokinetic profile of MP, with a plasma half-life between 24 and 37 h.
- 2B3–201 caused a prolonged decrease in the lymphocyte count, adrenocorticotropic hormone and osteocalcin, and a mild rise in fasting glucose.

Introduction

Multiple sclerosis (MS) is one of the most prevalent neuroinflammatory diseases and the leading cause of chronic disability in young adults. In MS, central nervous system (CNS) infiltration of leucocytes leads to overt inflammation and demyelination and results in neuronal dysfunction [1] High-dose **methylprednisolone (MP) hemisuccinate**, given 500–1000 mg daily for 3–5 consecutive days, is the primary treatment for an acute relapse in relapsing–remitting (RR) MS [2]. However, it is often given intravenously and causes undesirable short-term and long-term side effects include insomnia, depression and agitation [3, 4].

Both dose and dosing frequency of glucocorticoids may significantly be reduced by incorporating steroids in (PEGylated) liposomes, which is expected to result in reduced systemic toxicity while maintaining peripheral efficacy [5]. The additional conjugation of glutathione (GSH) to target active GSH transporters on the blood–brain barrier, has been shown to facilitate the delivery of the liposome-encapsulated drug into the brain [6, 7].

2B3–201 is MP hemisuccinate encapsulated in GSH-PEGylated liposomes that has been developed with the aim to enhance the sustained delivery of MP into the brain, thereby potentially augmenting CNS activity. Preclinical studies in animal models showed that 2B3–201 at therapeutic levels in animal models had fewer behavioural side effects (unpublished) and a superior efficacy compared to MP hemisuccinate [8–10]. Also, plasma circulation of 2B3–201-derived MP was significantly increased by encapsulation in GSH-PEGylated liposomes [11]. Based on these preclinical data, we expected a longer half-life and fewer side effects of 2B3–201 in human subjects when compared to MP.

In this first-in-human study we aimed to assess the safety, pharmacokinetic and pharmacodynamic profile of 2B3–201 in healthy male and female subjects. Plasma concentrations of lymphocytes, osteocalcin, adrenocorticotropic hormone (ACTH) and fasting glucose were used as pharmacodynamic endpoints, as intravenous administration of prednisolone causes rapid inhibition of the hypothalamic–pituitary–adrenal axis [12], glucose homeostasis disturbances, and depletion of osteocalcin [13, 14] and lymphocytes [15]. CNS effects were measured with the NeuroCart [16].

Methods

Design

Initially a randomized, double-blind, placebo- and active comparator- controlled three-way crossover study with three cohorts of six healthy males each was performed. Subsequently the study was extended while applying a parallel open label design with four cohorts, each containing six healthy subjects.

In cohorts 1, 2 and 3, a single dose of 150 mg, 300 mg and 450 mg 2B3–201 respectively was tested and compared to free MP and placebo (Table 1). The time interval between the occasions in the cross-over parts was 1 week. Cohorts 4, 5 and 6 had a single dose of 300 mg (cohort 5) and 450 mg (cohorts 4 and 6) 2B3–201 tested while applying altered infusion schedules and pre-treatment with clemastine. Cohort 7 included females and compared 450 mg 2B3–201 to 1000 mg of free MP in a double-blind crossover design.

An interim analysis was conducted after completion of cohorts 1, 2 and 3, at which point, safety, pharmacokinetics and pharmacodynamics results were evaluated and a decision to continue to the next cohort was made.

The study was approved by the Medical Ethics Committee of the BEBO Foundation (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

Subjects

Forty-six healthy subjects were recruited via the CHDR database and advertisements. All subjects gave written informed consent and were subsequently medically screened before entry into the study. Healthy subjects were not allowed to smoke >10 cigarettes per day and had to refrain from smoking during the study days. In the 48 h prior to the study days they were asked not to drink alcohol and to avoid xanthine- containing drinks. The use of medication was not allowed during the study period (except occasional use of paracetamol, up to 1 g per day). Healthy subjects with a positive Mantoux test and or recent (<1 month prior to screening) or current significant infection, were not enrolled.

Table 1

Summary of study characteristics

Cohort	1	2	3	4	5	6*	7
2B3-201 dose	150 mg	300 mg	450 mg	450 mg	300 mg	450 mg	450 mg
Population	Healthy males	Healthy males	Healthy males	Healthy males	Healthy males	Healthy males	Healthy females
Design	Randomized	Randomized	Randomized				Randomized
	Crossover	Crossover	Crossover				Crossover
	Placebo-controlled	Placebo-controlled	Placebo-controlled				
	Active comparator: methylprednisolone	Active comparator: methylprednisolone**	Active comparator: methylprednisolone				Active comparator: methylprednisolone
	Double blind	Double blind	Double blind	Open-label	Open-label	Open-label	Double blind
Number of subjects	6	6	6	6	6	6	6

*Cohort 6 had an infusion that was twice as long, data from this cohort has not been used in our pharmacokinetic and pharmacodynamic analyses.
**Dose of methylprednisolone was an intravenous infusion of 300 (cohort 2 only) or 1000 mg.

Treatments

Seven study cohorts with a total of 46 subjects received an infusion with 150 mg, 300 mg or 450 mg 2B3–201, 300 mg or 1000 mg free MP, or placebo. An overview of all cohorts can be found in Table 1. Subjects in cohorts 1–3 had 3 study periods, during which they either received 2B3–201, free MP or placebo (5% dextrose). Cohorts 4 and 6 received open label infusions of 450 mg 2B3–201, and cohort 4 also assessed the pretreatment effect of 2 mg clemastine on adverse events. Subjects in cohort 5 received 300 mg 2B3–201 and were also pretreated with clemastine. In cohort 6, a longer infusion duration was assessed. In cohort 7, healthy female subjects received 450 mg 2B3–201 while being pretreated with clemastine, and 1000 mg free MP in a double blind two-way cross-over fashion.

Safety

Adverse events, electrocardiogram (ECG), lymphocyte count, fasting glucose, blood pressure and heart rate measurements were collected throughout the study. Twelve-lead ECG recordings were made using Electrocardiograph Marquette 800/5500 or Dash 3000. Blood pressure and heart rate were assessed using a Nihon-Kohden BSM-1101 K monitor or a Colin Pressmate BP 8800 or a Dash 4000. All ECG, blood pressure and heart rate measurements were performed after subjects had been resting in a supine position for at least 5 min.

Pharmacokinetics

Whole blood samples were taken for assay of the active component MP and the encapsulated prodrug MP hemisuccinate. Blood samples were taken 0.25 h predose and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 26, 48 and 72 h postdose for all cohorts, and up to 288 h for cohorts 4–7. The blood was drawn in 2 mL NaF/K-oxalate tubes, directly placed on ice and then centrifuged (2000 *g*, 10 min, at 2–8°C), transferred to 2 mL Sarsted tubes and stored at –80°C within 30 min after sampling. The concentrations of MP and MP hemisuccinate in human sodium fluoride / potassium oxalate plasma were determined using a validated liquid chromatography with tandem mass spectrometry (LC–MS/MS) assays by Analytical Biochemical Lab (Assen, the Netherlands). The lower limit of quantitation (LLOQ) was 1 ng ml^{-1} for MP. Concentrations for MP were calculated by interpolation from a calibration curve while applying a range of 1–1000 ng ml^{-1}.

The following pharmacokinetic variables were calculated: area under the plasma concentration–time curve (AUC) from time 0 to the time of the last quantifiable concentration ($AUC_{0\text{-}t}$) and from time 0 extrapolated to infinity ($AUC_{0\text{-}inf}$); maximal observed plasma drug concentration (C_{max}); time to maximum observed plasma drug concentration (t_{max}); half-life ($t_{½}$), volume of distribution (Vd); and clearance. For the noncompartmental analysis only MP concentrations up to 74 h were used.

Pharmacodynamics

Lymphocyte count. Time points for measurement of lymphocytes were 2 h predose (cohorts 1–3 only), 15 min predose (cohorts 4–7) and 1, 2, 4, 8, 12, 24, 48 and 72 h postdose for all cohorts, and up to 288 h postdose for cohorts 4–7. The 2 mL EDTA-sample was directly, without preprocessing, sent to a hospital haematology and chemistry laboratory for analysis. The normal range for lymphocyte count was 1.00–3.50 × 10^9 l^{-1}.

Osteocalcin. Serum osteocalcin was measured several times per occasion: predose on day 0, 8, 24, 48 and 72 h postdose for all cohorts, and up to 288 h postdose for cohorts 4–7. Intact osteocalcin was measured in serum with ELISA [13], the normal range used was 0.4–4.0 nmol l^{-1}.

ACTH. ACTH was measured 12 times per occasion. Samples were taken 0.25 h predose and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 26, 48 and 72 h postdose for all cohorts, and up to 288 h postdose for cohorts 4–7. The ACTH samples (2 ml in an EDTA-tube) were put on ice immediately and centrifuged within 10 min. Normal range was <75 ng l^{-1}.

Fasting glucose. As a pharmacodynamics and safety marker, measurement of fasting glucose levels was performed. Samples were taken predose, 2, 6, 12, 24 and 72 h postdose for all cohorts, and up to 288 h for cohorts 4–7. 2 ml was collected in a NaF tube; the used normal range was 3.1–6.4 mmol l^{-1}.

Complement and IgE. To confirm if the observed infusion related reactions in cohort 1 were complement mediated and not allergic reactions, we measured, for cohorts 2–7, complement factors SC5b-9, C3a, C4d and Bb (4 mL blood EDTA tube) and IgE (2 mL blood, EDTA tube). These samples were taken predose (depending on cohort at –20, –9 or –7 min) and 5, 30 and 120 min after start of the infusion.

CNS tests. CNS tests performed with the NeuroCart included: pharmaco-EEG [17–19], maze learning [20], visual verbal learning test, Stroop test [21], adaptive tracking [22], VAS Bond and Lader [23] and VAS Bowdle [24] and saccadic and smooth pursuit eye movements [25].

Statistics

To compare the pharmacodynamics and pharmacokinetics between treatments the mean and standard deviation were calculated per time point by treatment. Cohorts with the same treatment are combined into one treatment group. For MP, values below LLOQ are set to 0 ng ml^{-1} before dosing and set to half of LLOQ (0.5 ng ml^{-1}) after dosing. For ACTH, all values below LLOQ were set to half of LLOQ (2.5 ng l^{-1}).

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [26], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18.

Results

Demographics

A total of 46 subjects participated, of whom 41 completed the study. Five subjects retracted consent during the study, and four of them were replaced. The subjects who participated in this study were all healthy young adults; subject characteristics are listed in Table 2.

Safety

No clinically relevant changes were observed in ECG, physical examination or vital signs (temperature, heart rate, systolic and diastolic blood pressure). Safety laboratory assessments for blood haematology, chemistry and urinalysis also showed no clinically meaningful abnormalities with the exception of a decrease in lymphocytes, which will be discussed in more detail in the pharmacodynamics section.

The most frequently reported adverse events related to 2B3–201 were infusion related reactions, defined as any sign or symptom experienced by the subject within 4 h after the start of the infusion [27, 28]. Symptoms related to infusion that occurred within 4 h after start of the infusion, such as chest discomfort, urticaria, angioedema and back pain, were clustered [29]. Infusion reaction related symptoms were reported by 41 of the 46 healthy subjects (89%). Other frequently reported adverse events were somnolence (15%), gastroesophageal reflux disease (8%), back pain (not assessed as an infusion related reaction) (8%), fatigue (8%) and dizziness (8%).

Pretreatment with 2 mg clemastine (cohorts 4, 5 and 7) at 20 min before infusion did not result in fewer infusion related reactions: all subjects in these cohorts showed symptoms of an infusion related reaction (see Table 3). Not all infusion related reactions resulted in (temporary) halt of the infusion or lowering of the infusion speed.

All adverse events were mild in severity, short lasting and self-limiting. One adverse event related to 1000 mg free MP was classified as moderate: a male subject (cohort 1) developed an acute tonsillitis with fever 3 days after the infusion. He was subsequently treated with pheneticillin and recovered fully.

Table 2

Subject characteristics

Cohort	1	2	3	4	5	6	7
2B3–201 dose	150 mg	300 mg	450 mg	450 mg	300 mg	450 mg	450 mg
Population	Healthy males	Healthy males	Healthy males	Healthy males	Healthy males	Healthy males	Healthy females
Age (years), mean (range)	25 (20–30)	24.9 (19–36)	25.3 (20–45)	21.3 (19–25)	25.5 (20–35)	19.8 (18–23)	23 (20–30)
Weight (kg), mean (range)	77.6 (66–95)	71.3 (66–77)	75.5 (68–88)	81.1 (68.9–116.6)	71.0 (59.8–85.6)	72.1 (65.9–77.8)	66.8 (54.4–81.2)
Height (cm), mean (range)	183.9 (177–191)	179.9 (159–189)	183.4 (165–194)	184.9 (178.4–199.6)	177.3 (165.0–188.2)	184.2 (179.2–191.5)	171.5 (162.7–179.0)
BMI (kg m^{-2}), mean (range)	23.0 (20–28)	22.1 (20–26)	22.6 (18–26)	23.5 (21.5–29.3)	22.7 (18.4–25.2)	21.2 (20.2–23.1)	22.8 (19.1–26.9)
Number of subjects	6	6	6 (1 dropout)	6	6	5 (1 dropout)	6 (2 dropouts)

BMI, body mass index

Table 3

Infusion related reactions (IRRs) per cohort

Cohort	1	2	3	4	5	6	7
2B3–201 dose	150 mg	300 mg	450 mg	450 mg	300 mg	450 mg	450 mg
No. of IRRs	4	5	5	6	6	6	6
Infusion (temporary) stopped due to IRR symptoms	2	3	5	2	4	6	3

Complement and IgE measurements showed that 2B3–201 caused a parallel rise of C3a and Bb and no increase in C4d and IgE levels were observed (Figure 1).

Pharmacokinetics

A concentration–time graph for MP plasma concentration at different dose levels of 2B3–201 derived MP and free MP is shown in Figure 2. Pharmacokinetic parameters per cohort are listed in Table 4. Plasma concentrations of 2B3–201 derived MP were measured up to 7 days (300 and 450 mg), for 300 mg and 1000 mg free MP concentrations were measurable until 2 days after infusion.

2B3–201 derived MP had a maximum plasma concentration of 545 mg ml^{-1} (450 mg 2B3–201), which contrasts with the maximum plasma concentration of 7290 ng ml^{-1} for free MP (1000 mg). The T_{max} was 5.9 h for 2B3–201 derived MP while free MP had a T_{max} of 2.16–4.2 h. Plasma half-life for 2B3–201 derived MP was between 24 and 37 h. Free MP had a half-life of 2.2–4 hr. A *t*-test showed a significant difference in AUC and C_{max} (*P* values of respectively 0.003 and 0.006) between males and females and in weight (*P* value = 0.03), but not in BMI. Observed differences were tested for correlation with weight and BMI with a Spearman correlation. Correlation was found for weight, with values of –0.335 (weight and C_{max}, *P* value = 0.03) and –0.39 (weight and AUC, *P* value = 0.01), but not for body mass index (BMI), with values of 0.11 (BMI and C_{max}) and 0.052 (BMI and AUC). Concentrations and pharmacokinetic parameters for MP hemisuccinate are not reported (data on file).

Pharmacodynamics

Lymphocytes (Figure 3a). The effects of 2B3–201 derived MP, free MP and placebo on lymphocytes are shown in Figure 3a. Administration of 2B3–201 and free MP resulted in a maximal decrease in lymphocyte count 6–12 h after dosing. The decrease in lymphocyte count,

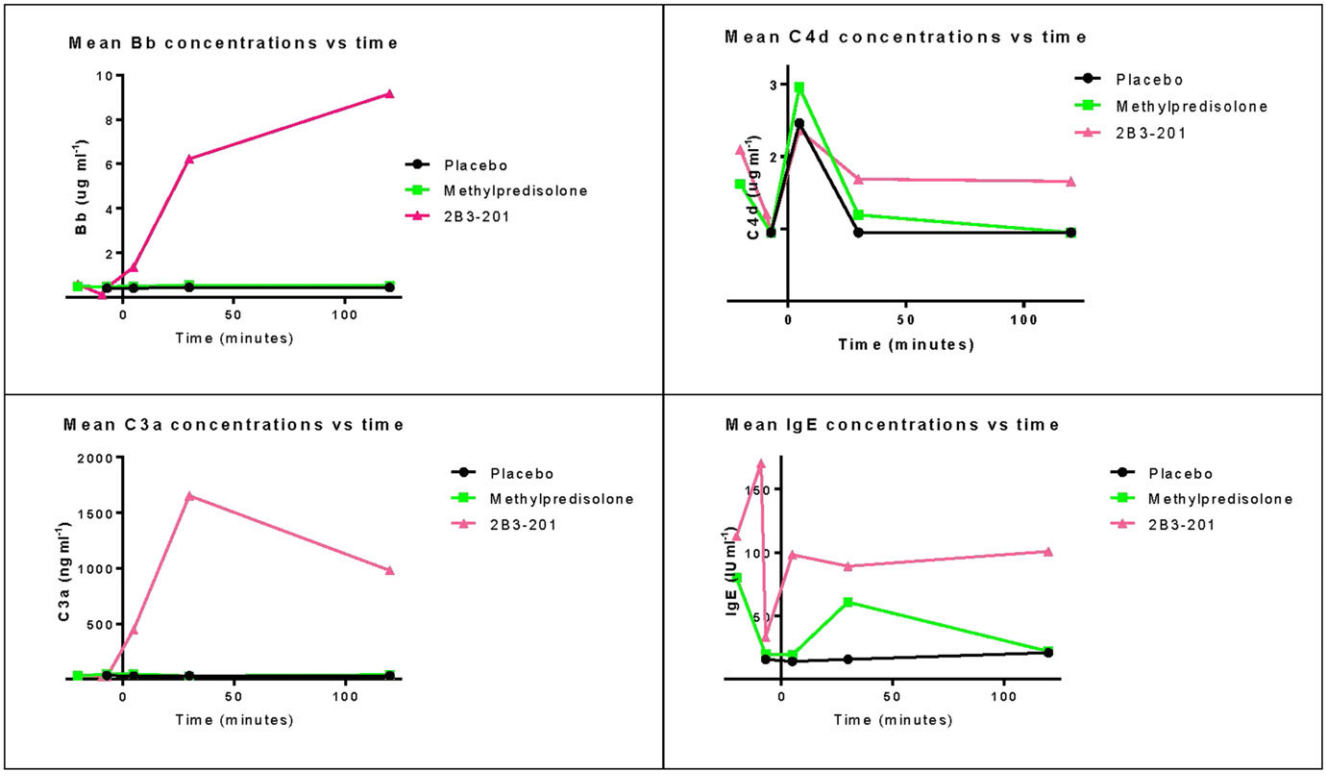

Figure 1

Mean values of Bb, C4d, C3a and IgE concentrations for 2B3–201, methylprednisolone and placebo

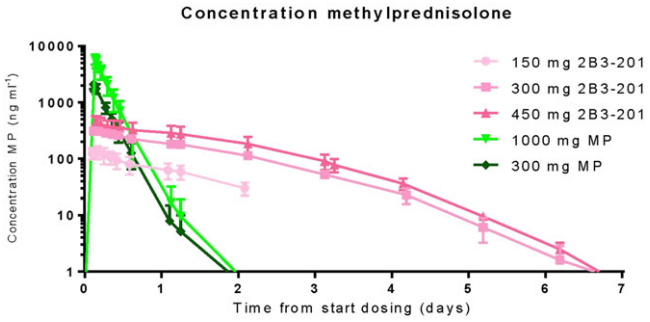

Figure 2

Serum methylprednisolone (MP) concentrations for 150, 300 and 450 mg 2B3–201, and 300 and 1000 mg MP. The concentrations of 150 mg 2B3–201 have only been measured for 50 h

persisted for 2 days after dosing after 150 mg 2B3–201 administration, for 3 days after dosing with 300 and 450 mg 2B3–201. Infusion of 300 mg and 1000 mg free MP resulted in a maximal decrease for 24 h. Seven days after dosing, lymphocyte values for all active groups had returned to baseline.

ACTH (Figure 3b). ACTH concentrations were below the lower limit of quantification for almost all subjects 3 h after administration of active study medication. The decrease of ACTH was sustained for 3 days (150 mg) and 4 days (300 and 450 mg) in the 2B3–201 dosing groups, whereas for free MP ACTH plasma levels were no longer decreased after the first day after dosing, demonstrated a slight compensatory increase on days 2 and 3, and had returned to baseline values from day 4 onwards.

Osteocalcin (Figure 3c). All the active treatment groups showed a decrease in osteocalcin concentrations in the first 24 h after dosing. In the 1000 mg MP dosing group, osteocalcin concentrations started to rise again after 24 h. For the 2B3–201 dosing groups, the decrease in osteocalcin concentrations persisted for at least 4 days.

Fasting glucose (Figure 3d). An increase in fasting glucose was visible for all active treatment groups. Peak concentrations were measured 12 h after dosing for free MP cohorts, and 15 h after dosing for 2B3–201 cohorts. Fasting glucose concentrations were below 6 mmol l^{-1} (the upper limit of subjects in fasting condition) after 2 days for cohorts with free MP and 150 mg 2B3–201. For subjects who received 300 mg and 450 mg 2B3–201, fasting blood glucose had returned to levels below 6 mmol l^{-1} after 4 days.

CNS tests. No relevant changes in the effects on CNS between 2B3–201 and free MP could be observed.

Discussion

This first-in-human study with 2B3–201, a formulation of MP-encapsulated GSH-PEG liposomes, showed prolonged MP concentrations in serum, and as a consequence a sustained decrease in the levels of lymphocytes, osteocalcin and ACTH and increased fasting glucose over a longer period of time.

Based on pharmacokinetic properties, 2B3–201 acts like a slow release product. The estimated terminal half-life of 2B3–201 derived MP is ten times longer than free MP. Also, the C_{max} is lower for 2B3–201 (360–545 ng ml^{-1}) than for free MP (5120–7290 ng ml^{-1}). Based on graphical inspection, it is likely that the pharmacokinetics of 2B3–201 derived MP is characterized by first order kinetics. The observed pharmacokinetic profile of free MP corresponded with literature [30, 31] and information in the Summary of Product Characteristics.

Pharmacokinetics of 450 mg 2B3–201 in women were different from 450 mg 2B3–201 in men: the C_{max} and AUC were higher, and the half-life was longer (Table 4). This can be explained by relative lower weight of women resulting in a

Table 4

Pharmacokinetic parameters of methylprednisolone, calculated from 0–72 h

Dose (mg)	Cohort	Compound	Cmax (ng ml^{-1}) (SD)	Tmax (h) (SD)	AUC_0_inf (ng h l^{-1}) (SD)	Ka (h^{-1}) (SD)	T ½ (h) (SD)
150	**1 (*n* = 6)**	2B3–201	138 (37.1)	4.56 (1.3)	4370 (1000)	0.029 (0.0045)	24.4 (3.9)
300	**2 (*n* = 6)**	2B3–201	375 (96.6)	4.48 (1.4)	13 400 (3220)	0.028 (0.0032)	25 (2.5)
300	**5 (*n* = 6)**	2B3–201	282 (35)	4.19 (0.9)	14 800 (4060)	0.025 (0.0086)	31 (9.5)
450	**3 (*n* = 6)**	2B3–201	501 (144)	4.85 (0.75)	17 900 (2690)	0.024 (0.0029)	29.2 (3.5)
450	**4 (*n* = 6)**	2B3–201	360 (57.5)	5.12 (1.4)	17 400 (4020)	0.024 (0.0034)	28.9 (4.3)
450	**7 (*n* = 6)**	2B3–201	545 (99.7)	5.90 (2.9)	31 800 (15500)	0.0250 (0.010)	37.0 (29)
300	**2 (*n* = 6)**	Free MP	2140 (371)	2.67 (0.20)	11 900 (2490)	0.258 (0.039)	2.74 (0.44)
1000	**1 (*n* = 6)**	Free MP	5930 (580)	2.16 (0.37)	27 800 (7370)	0.286 (0.041)	2.47 (0.38)
1000	**3 (*n* = 6)**	Free MP	5120(1120)	3.95 (0.029)	28 800 (10800)	0.286 (0.032)	2.45 (0.30)
1000	**7 (*n* = 6)**	Free MP	7290 (1740)	4.20 (0.26)	42 900 (13400)	0.208 (0.082)	3.81 (1.6)

SD, standard deviation

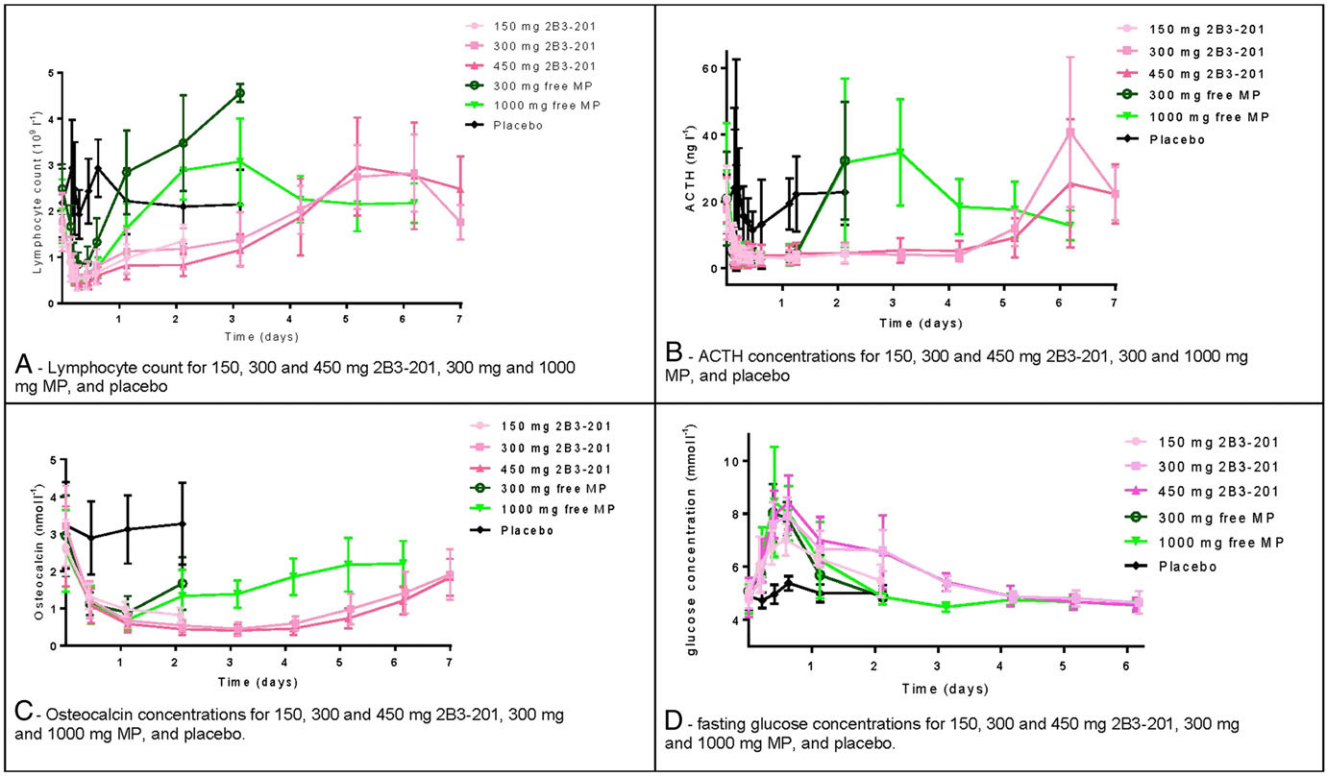

Figure 3

Pharmacodynamic measurements: graphs of lymphocyte count (A), adrenocorticotropic hormone concentrations (B), Osteocalcin concentrations (C) and fasting glucose concentrations (D) for different 2B3–201 doses, 300 and 1000 mg free methylprednisolone, and placebo

higher concentration of MP in serum, and a longer residence time of 2B3–201 compared to men, as the clearance is comparable (men: 0.09–0.11 l h^{-1}, women: 0.09 l h^{-1}).

A limitation of the cross-over part of the study was the time interval between the cohorts. In cohort 3, dosing of 450 mg of 2B3–201 resulted for two subjects in low concentrations of MP study in predose samples at the start of subsequent occasions. However, we believe that this did not influence the major outcome as pharmacodynamic parameters lymphocytes, ACTH and fasting glucose were back to baseline in <7 days after the infusion. Also, the other four subjects in cohort 3 did not have measurable predose pharmacokinetic results.

As a consequence of prolonged plasma concentrations of 2B3–201 derived MP, a pronounced decrease in lymphocytes was observed for all dose levels for 3 days, an effect that lasted markedly longer than in the free MP groups (1 day). Similar prolonged pharmacodynamic effects were observed for the decreases in concentrations of osteocalcin and ACTH, as well as a rise in fasting blood glucose. All these effects were present over a longer period after dosing 2B3–201 in comparison to free MP. Even though we observed prolonged effects of 2B3–201, we could not observe significant differences in effects on CNS functioning between 2B3–201 and free MP.

Treatment with 2B3–201 led to the occurrence of mild infusion related reactions in 89% of all subjects. Increased levels of complement concentrations were found in all subjects after receiving 2B3–201 300 mg and 450 mg, although not all subjects reported symptoms related to an infusion reaction (Table 3). From this study, we can conclude that complement is activated due to administration of 2B3–201, resulting in a rise in C3a (Figure 1). With a simultaneous rise of complement factor Bb (specific for the alternative pathway, Figure 1), and a lack of rise in C4d (Figure 1) concentration (specific for classical pathway), we can conclude that 2B3–201 activated the alternative complement activation pathway. IgE concentrations (Figure 1) were not increased, indicating no anaphylactic reaction was initiated. These results correspond well with what is known as *complement activation related pseudo allergy* (CARPA). The relationship between liposomal drug delivery and CARPA is well known, and the observed symptoms in this study match those previously described by others [32–34].

There were a couple of adjustments described that may decrease the development of a CARPA reaction. First adjustment is to start the infusion with a low infusion rate [32]. We lowered the infusion rate during cohort 1. Also, lowering the infusion speed when symptoms occur, and re-challenging subjects has also reported to be effective [29]. The same could be observed in our study: the infusions of only three subjects were eventually permanently halted as a result of an infusion related reaction. All other subjects received the complete infusion.

Another study reported that a low concentration of liposomes in the infusion fluid also led to fewer infusion related reactions [35], which was implemented in cohort 1. In

cohorts 2–7 the concentration of the liposomes was, however, still relatively high, as compared to the adjusted concentration in cohort 1 without the observed infusion related reactions. The effect of pretreatment with clemastine has been discussed in literature [29, 35], and although this had not been effective in all studies, it was decided to administer 2 mg clemastine 20 min before start of the infusion in cohorts 4, 5 and 7. In our study design, subjects received 2B3–201 once, so a reported decrease of infusion related reaction with multiple dosing [35] of the same compound was not addressed.

Our current actions did not lead to a decrease in the number of reported infusion related reactions, although we did observe a reduced need to change the infusion speed because of infusion related reactions. Reducing the concentration of the liposomes at the start of the infusion may offer a solution in the future.

Methylprednisolone is first choice in medication for acute relapses in MS [36, 37]. In certain European countries, usually 3 consecutive days of infusions are given [38]. Based on study results in 2015 which revealed that use of oral administration of MP was noninferior to intravenous MP [39], the National Institute for Health and Care Excellence (UK) adapted their guideline accordingly [40]. Nevertheless, use of intravenous MP remains part of clinical practice especially for patients who suffer from severe relapses and those who do not respond to oral treatment. With these practices in mind, use of 2B3–201 as a 1-day intravenous treatment may be a good alternative.

Moreover, to reduce the burden of 3–5 days of hospital visits, and healthcare costs related to the days of admissions in other countries, a single infusion of 2B3–201 could be beneficial for patients and reduce side effects caused by high doses of MP. In the current study, 2B3–201 derived MP was measurable and active for 7 days after infusion, resulting in a sustained decrease of lymphocyte count, ACTH and osteocalcin, and an increase in fasting glucose. Now, studies with single administrations in patients with RRMS and a relapse measuring clinical improvement and comparing single administrations of 2B3–201 to 3 day treatments with regular MP are warranted to demonstrate this further.

Even though the infusion related reactions were all mild and self-limiting, these reactions caused by 2B3–201 in the current setting were frequent and intense. MP treatment in MS reduces symptoms of the MS relapse on the short term, but for most patients it does not influence the disease progression in the long term [37]. It is important that the side effect profile is acceptable for the patient. The observed infusion related reactions, if not resolved, may therefore limit the future widespread use of 2B3–201 as a standard therapy for the treatment of relapses in patients with RRMS.

Competing Interests

K.M.S.K., R.G.J.A.Z., E.S.K. and G.J.G. declare no conflict of interest. The study reported in this manuscript was sponsored by 2-BBB medicines BV, Leiden, the Netherlands. W.G. and I.S. were former employees of 2-BBB medicines BV, P.J.G. is the CEO of 2-BBB medicines BV.

Contributors

K.M.S.K. acquired and interpreted the data, drafted the paper and co-designed the study. R.G.J.A.Z. acquired and interpreted the data, reviewed the paper and co-designed the study. I.S. interpreted the data and reviewed the paper. W.G. interpreted the data, reviewed the paper and co-designed the study. P.J.G. interpreted the data, critically reviewed the paper and designed the study. E.S.K. performed statistical analyses and co-designed the study. G.J.G. acquired and interpreted the data, critically reviewed the paper and designed the study.

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