

SCIENTIFIC DISCUSSION

1. Introduction

Homocystinuria is a serious life-long disease and is associated with a high morbidity and mortality. Treatment aims to reduce homocysteine accumulation and to restore the transmethylation capacity by normalising the concentrations of S-adenosylmethionine (SAH) and S-adenosylhomocysteine (SAM).

Homocystinuria is an inherited disorder of the metabolism of the amino acid methionine leading to accumulation of homocysteine in the blood and urine. This is due to a dysfunction in one of the metabolic pathways responsible for transsulfuration and remethylation of homocysteine. It is considered the second most common inborn error of amino acid metabolism after phenylketonuria. The major clinical manifestations include mental retardation, dislocation of the optic lens (ectopia lentis), skeletal abnormalities and a tendency to thromboembolic episodes. The estimated incidence of homocystinuria due to Cystathionine beta-synthase (CBS) deficiency is 1 in 335 000 births worldwide, with marked regional variations.

The aim of the treatment of homocystinuria is to normalize homocysteine levels by several ways in varying combinations such as: boosting residual enzyme activity with vitamin B6, vitamin B12 and folates, reducing load on the metabolic pathway affected with low-methionine diet (CBS deficiency), supplementation with the deficient products downstream of the enzyme abnormality (cysteine for CBS deficiency and methionine for impaired remethylation), using the alternative pathway to eliminate the toxic substrate i.e. pharmacologic treatment with betaine which remethylates homocysteine to methionine.

Betaine hydrochloride is a drug product available over-the-counter used to acidify the gastric juice in patients with maldigestion due to achlorhydria. Betaine dihydrogen citrate is available as a drug product in Germany (Flacar®, Fa. Schwabe) and recommended for fatty liver and other liver diseases in amounts of 2-4 grams per day.

Betaine anhydrous (Cystadane) is given to patients with homocystinuria in Europe on a compassionate use basis. Betaine anhydrous has been obtained from chemical companies to treat patients with homocystinuria for many years.

These forms of betaine dissolve easily and completely at physiologic pH in the gastrointestinal tract. Due to its molecular structure and rapid absorption kinetics it is generally accepted that the bioavailability of oral betaine is high, although this has never been formally evaluated. The bioavailability of all betaine salts should theoretically be similar although this has also not been formally tested.

Currently there is no treatment to correct the basic genetic causes of homocystinuria. Consequently, therapy is directed at correcting the biochemical abnormalities of these disorders.

Betaine anhydrous was granted orphan medicinal product status for treatment of the orphan condition homocystinuria in July 2001.

Betaine (trimethyl glycine) acts by remethylation of homocysteine to methionine, and thereby reduces levels of homocysteine. Betaine is found naturally in many foods, including sugar beets, however levels found are insufficient to achieve reduction in homocysteine levels in patients suffering from the condition. Patients are usually treated with a dose of 3g twice a day of betaine.

Cystadane (betaine) is formulated as an oral powder allowing for weight-adapted dosage in the important patient group of children.

The Applicant has submitted a complete mixed application (containing own data from the company and bibliographic data) through the Centralised procedure.

Cystadane is indicated for the treatment of homocystinuria, involving deficiencies or defects in:

- Cystathionine beta-synthase (CBS),
- 5, 10-methylenetetrahydrofolate reductase (MTHFR),
- Cobalamin cofactor metabolism (cbl).

2. Quality aspects

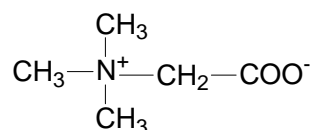
Introduction

Betaine is a well known, naturally occurring drug substance that can be isolated from sugar beets. The drug product is an oral powder consisting of 1 g of betaine (betaine free base) without any excipients.

It is packaged in 300 ml High-Density polyethylene (HDPE) bottle with polypropylene (PP) child resistant caps and three polystyrene spoons as measuring devices for the administration of the product. It is proposed as an orphan drug for the treatment of homocystinuria.

Active Substance

Betaine anhydrous is not described in the European Pharmacopoeia and scientific data have been submitted in the form of The Active Substance Master File (ASMF). The active substance is in the anhydrous form and the structural formula is provided below:



Betaine anhydrous exists either as white crystals or a crystalline powder with a weak characteristic odour. It is freely soluble in water. It has melting point range of 301-305°C and a bulk density of 0.71g/l. The pH of a 1% aqueous solution was found to be 5.0 - 8.0. It is hygroscopic.

Manufacture

Description of Manufacturing Process and Process Controls

Betaine technical grade is extracted from sugar beet molasses as a result of a purification process.

Betaine occurs with an average abundance of 0.2 % - 0.3 % in the common variety of sugar beets (*Beta vulgaris* L) and is a natural key-intermediate of the amino acid and methyl-metabolism in all vertebrates.

Efforts of the sugar industry to increase sugar yields have led to the development of chromatographic processes for large scale desugarization of molasses. Ion exclusion is used to separate sugar and non-sugar fractions of molasses.

The manufacturing process can be summarised as follow:

1: Pre-process

Raw material – dilution – filtration – twice [ion exclusion chromatography – concentration by evaporation of the Betaine fraction] – separation by centrifugation – drying to Betaine technical grade.

2: Betaine process

Starting material - dissolution with distilled water - activated carbon treatment – filtration, microfiltration - crystallisation by evaporation – separation by centrifugation – vacuum drying – sieving – packaging – weighing.

Sections related to control of materials, control of critical steps and intermediates, process validation and manufacturing process development are suitably detailed in the closed part of the ASMF.

The structure has been satisfactorily elucidated by elemental analysis and spectral analyses i.e. X-ray powder diffraction, UV, IR, and ¹H- NMR spectroscopic analysis. The solid state is characterised by refractive index, thermal analysis, thermogravic analysis, and microscopic appearance.

Potential impurities arising from the manufacturing process are amino acids and sugars. They are quantitatively removed during the purification process. Their presence is controlled by adequate analytical methods.

Appropriate data and discussion regarding potential degradation products have been included.

Residual solvents testing was not deemed necessary since only water was used during the manufacturing process of betaine.

- Specification

Since betaine anhydrous is not described in any pharmacopoeia, an internal monograph has been developed by the ASMF holder as well as by the applicant, Orphan Europe. Both specifications are detailed. They differ slightly but are both acceptable.

Appropriate specification for betaine includes parameters such as appearance, identification, pH, loss on drying, assay, related substances, particle size, bulk volume, microbiological quality. Analytical methods are in accordance with Ph.Eur.

Impurity limits have been adequately justified by batch analysis, stability studies, toxicological and clinical studies.

Analytical methods to control the active substance such as test on foreign matter, assay and related sugar impurities (HPLC) and other amino acids (AAA), identification (IR), limit tests for chloride, sulphate, iron and heavy metals, pH, loss on drying, sulphated ash and total viable aerobic count have been suitably described and validated by both the applicant and the ASMF holder in accordance with ICH guidelines.

Data are provided for 3 batches of active betaine anhydrous tested by the ASMF holder and 3 batches tested by the applicant. Results are in line with the respective specification and confirm the consistency and uniformity of the process.

Stability

Stability Summary and Conclusions

Stability studies were carried out by the ASMF holder on 3 batches kept in the commercial container at room temperature (not ICH conditions, but temperature and humidity were continuously monitored). Criteria tested included: appearance, odour, loss on drying, assay, pH, transmittance, related substances, total viable aerobic count and yeasts and moulds.

Results were provided up to 6 years, the active substance remained very stable except few out-of-spec results found after 3 years for loss on drying. The ASMF holder has committed to conduct post-approval stability studies under ICH conditions.

Stability studies were performed by the applicant on 3 production-scale batches kept in a smaller version of the commercial container under ICH conditions (up to 24 months at 25 °C / 60% RH and 6 months at 40 °C / 75% RH). Criteria tested included: description, identification, appearance of constituted solution, pH, loss on drying, saccharide & amino acid impurities, assay, and microbial limits. All results remained within the specification after long-term and accelerated storage except 2 results (loss on drying test) adequately justified. No saccharide or amino acid impurities were detected, and microbial results were within set specifications.

Results showed that forced degradation studies (oxidation, acid and alkali hydrolysis, elevated temperature and light) did not adversely affect the active substance.

Based on the presented data, the proposed retest period of 2 years by the applicant is considered satisfactory. However, the ASMF holder has committed to conduct post-approval stability studies under ICH conditions.

Medicinal Product

Pharmaceutical Development

Betaine in the anhydrous form is a white crystalline powder. Dissolution studies were omitted since it is freely soluble in water.

It exists as anhydrous, monohydrate and hydrochloride forms. The applicant has justified its choice of the anhydrous form; the hydrochloride was discounted on organoleptic reasoning, and the monohydrate was not chosen due to poor flow properties of the compound.

The applicant has discussed in detail the implications of formation of the monohydrate form, and the effect of humidity and high temperature on the product. Humidity conditions above 50% were found to have a negative impact on the powder with moisture absorbance and deliquescence observed. Consequently filling conditions are maintained below 40% humidity.

The applicant has provided justification for a finished product consisting solely of the active, on the grounds that the drug substance has ideal flow characteristics, is freely soluble in water, has a low angle of repose and the quantity to be consumed by the patient (up to 20 g daily) and this is considered satisfactory.

Adventitious Agents

Potential contaminants originating from the starting material used for synthesis of the drug substance have been convincingly ruled out.

Manufacture of the Product

The manufacturing process has been adequately detailed and consists solely of filling the bottle with the active substance, outer labelling and packaging, QC control and EU release (Orphan Europe, France). An overage has been added for the filling and is adequately justified.

Parameters such as temperature and relative humidity control were identified as critical parameters and controlled during the filling process.

A process validation protocol describing the operations, equipment used and tests to be performed have been provided, as well as validation results of three consecutive batches. All three lots complied with the acceptance criteria.

Product Specification

Appropriate finished product specification at release and end of shelf-life have been provided. It includes parameters such as identification (IR, HPLC), appearance of the constituted solution, odour

testing, loss on drying, Impurity (GCP), assay of betaine (HPLC), uniformity of mass of delivered doses from multidose containers (Ph. Eur. 2.9.27) and microbiological quality (skip testing). Analytical methods have been described and validated apart from the methods described in Ph.Eur.

Batch analyses are provided for 3 pilot batches of finished product. All results appear to meet the finished product specification. Assay was always above 98%, loss on drying was below 1.0% and no amino acid or saccharide impurities were detected.

The applicant has referred to the section on impurities as per section 3.2.S.3.2 impurities and as the finished product consists only of the active, this is considered satisfactory. Limits and specifications are adequately justified.

Cystadane is packed in a 300cc round, opaque, white, HDPE bottle with a white Polypropylene child resistant closure. Three polystyrene measuring spoons are enclosed in the carton for each bottle. Information on the measuring devices can be found in section 3.2.R regional information and the applicant commits to perform post-approval additional testing and to provide results for the “uniformity of mass of delivered doses”.

Sources and specifications of the bottle and cap are specified and supported by certificates of analysis from the finished product manufacturer.

Primary packaging materials such as HDPE and PP are in line with the Ph.Eur. Requirements.

Stability of the Product

Three pilot batches of the finished product stored in the commercial packaging have been placed on stability under ICH conditions (up to 24 months at $25 \pm 2^\circ\text{C}$ / $60 \pm 5\% \text{RH}$ and 6 months at $40 \pm 2^\circ\text{C}$ / $75 \pm 5\% \text{RH}$). Results are in accordance with the specification, and in particular assay remains within the range 95 – 105%, and neither saccharide nor amino acid impurities were detected at any time point for for both storage conditions. Based on the available data, a shelf life of 2 years with the precaution “do not store above 25°C ” and “keep the bottle tightly closed in order to protect against moisture” can be granted. The applicant commits to perform stability studies under ICH conditions and in accordance with revised specification.

In-use stability studies reflecting the conditions of use of the product were performed. The finished product was stored at ambient temperature and under high humidity conditions (75% RH) since the active substance is hygroscopic. The bottle was opened daily and a sample was withdrawn. Analysis of the remaining powder was performed until 31 days in order to cover the time required when dosages under 6 g/per day are used for children under 10 years of age. Results were all within the proposed specification.

Additional results of stability testing performed on two batches of the finished product, in aqueous solution and in simulated gastric fluid were presented. The quantity of powder (6 g) dissolved was equivalent to a dose of the finished product as per section 4.2 of the SPC. Satisfactory assay results were provided for product solutions over 24 h in water and 2 h in simulated gastric fluid.

The applicant has committed to continue testing of the 3 batches to the end of the shelf life (2 years) and to place 1 batch per year under stability. Any deviation would be reported.

Discussion on chemical, pharmaceutical and biological aspects

Generally, satisfactory documentation has been provided and the minor objections raised in the Day 120 List of Questions and D 180 List of Outstanding Issues were solved during the evaluation procedure. The active substance betaine is well-characterised and the retained specification is acceptable.

Results showed that betaine is stable and stability data support the proposed re-test period.

Regarding the finished product, the manufacturing process is a simple filling process and has been adequately described and controlled. It should ensure a consistent quality for the product. Appropriate specification has been selected for this oral powder.

Stability studies under ICH conditions have demonstrated the good stability of the finished product. Stability data support the proposed shelf-life (before opening and in-use) and storage conditions as defined in the Summary of Product Characteristics.

At the time of the CHMP opinion, there were some outstanding minor quality issues with no impact on the benefit/risk. The applicant undertook to provide with the necessary information as follow-up measures within an agreed timeframe and to submit variations if required following the evaluation of this additional information.

3. Non-clinical aspects

Introduction

The pharmacology and the toxicology sections are based on the applicant's own data and bibliographic data. The applicant performed one primary pharmacodynamic study and four toxicology studies (one acute toxicity testing and three tests for genotoxicity). All other data was of bibliographic origin.

The search for preclinical bibliographic data was performed in two steps.

The first step was conducted in 1995 by the literature search service of the Minnesota Technology's Project Outreach. In this attempt articles on animal studies with betaine from 1967 to 1995 were acquired from the databases MEDLINE, Toxlit and PUBMED. The search was performed for CAS registry "107-43-7" or for "betaine" or "trimethylglycine". 71 articles were considered useful for the "non-clinical development program" at this time point.

The second step was conducted in 2005 with a literature search in several database such as MEDLINE, TOXLINE and BIOSIS for the period 1995-2005. The key words combined terms like "cystadane, betaine, betaine-anhydrous, trimethylglycine, N-trimethylglycine, methanaminium, in vitro, animal-study, animal-model, pharmacology, pharmacokinetics, toxicology or toxicity". About 150 articles were identified and 58 were considered useful.

The applicant focused on 45 relevant references.

Pharmacology

Betaine (trimethylglycin, CAS: 107-43-7, Mw: 117.15) is a natural occurring quaternary amine closely related to the amino acid glycine. Its primary function in mammals is within the metabolic cycle of methionine. Catabolism of methionine occurs by transfer of the adenosyl group from ATP to the sulphur group of methionine forming S-adenosylmethionin. Subsequently S-adenosylhomocystein is formed by transfer of the methyl group to an appropriate acceptor such as phosphatidyl ethanolamine. S-adenosylhomocysteine is then hydrolysed to homocysteine and adenosine. Methionine can be regenerated (1) by transfer of the methyl group of methyltetrahydrofolate to homocysteine by homocysteine methyltransferase (MS) (resulting in methionine and tetrahydrofolate) or (2) by transfer of the methyl group of betaine by the betainehomocysteinmethyltransferase (BHMT) (resulting in methionine and dimethylglycine). These reactions constitute the well known activated methyl cycle. In addition homocysteine is an intermediate in the synthesis of cysteine (3). Therefore homocysteine and serine are condensed to cystathionine by the cystathionine synthetase (CBS). Cystathionine is deaminated and cleaved by cystathioninase to cysteine and α -ketobutyrate.

The catabolic pathway of methionine and the role of homocysteine are well established and can be found in several textbooks of biochemistry (Biochemistry; Streyer 1988, third edition). The role of betaine is well defined too. The rationale for developing betaine as treatment for homocysteinuria is reasonable.

Primary pharmacodynamics

Several pivotal primary pharmacodynamics studies were provided which showed that the BHMT-catalysed reaction is inducible in rats, mice and chicks. A second set of experiments in MS-inactivated bats showed that betaine is able to prevent neurological impairment and that betaine is able to stimulate the BHMT dependent pathway under these conditions. A third set of experiments in ethanol fed rats showed that betaine is able to prevent 5-methyltetrahydrofolate accumulation indicating an activation of the BHMT pathway. In summary pivotal studies were presented which demonstrated that the BHMT pathway can be activated by betaine in several mammalian species.

Secondary pharmacodynamics

Some secondary pharmacodynamics studies demonstrated antioxidative, anticonvulsant, osmolytic function and anti-apoptotic effects of betaine in several models.

In the rat, betaine was demonstrated to protect the liver against ethanol-induced injury and to prevent vitamin A depletion. The antioxidative effect of betaine was related to its ability to increase hepatic SAM (S-adenosylhomocysteine) levels and to prevent fatty liver in ethanol-fed rats.

In rats and mice, betaine (intraperitoneal administration) blocked homocysteine-induced convulsions and antagonised strychnine-, electroshock-, and PTZ- (pentilentetrazole) induced seizures. Following local applications, it also blocked neuronal excitation induced by glutamate and by homocysteine in the rat. Since PTZ is known to block chloride channels and reduce GABAergic inhibition, an interaction is consistent with the physiological effect of betaine as an osmolytic protectant.

The protective function of betaine as an organic osmolyte was demonstrated in the rat.

The increase of superoxide dismutase and catalase activities and the decrease of glutathione peroxidase activity induced by subcutaneous administration of carbon tetrachloride was normalised in the kidney of rats pretreated with betaine. Pre-incubation of rat culture hepatocytes with betaine largely prevented bile acid induced apoptosis. In vivo betaine supplementation in water significantly ameliorated hepatocyte apoptosis following bile duct ligation.

Safety pharmacology programme

No specific studies regarding safety pharmacology (except for CNS parameters) and pharmacodynamic interaction were presented. Non-clinical and human data was submitted regarding the influence of betaine on renal system, CNS and CVS. With respect to the safety pharmacology the data was incomplete.

The applicant had not provided any relevant recent clinical data concerning potential effects on the CVS in particular on the electrophysiology. The influence of betaine on the electrophysiology of cardiac action was not discussed. Since homocysteinuria itself increases the risk for cardiovascular diseases it can be assumed that treated patients are closely monitored by their physicians and potential electrophysiological disturbances would have been reported in the past. Also, taking into account that betaine is a natural constituent of the human nutrition the CHMP found the lack of non-clinical data acceptable.

Pharmacodynamic drug interactions

No specific studies have been presented.

Pharmacokinetics

Betaine and its metabolite DMG (Dimethylglycine) were determined by isocratic HPLC procedure with u.v. detection. The detection limits were specified to be 0.005 mM/l for betaine and 0.002 mM/l for DMG.

No specific pharmacokinetic animal studies have been performed. With respect to the long-standing use of betaine in humans the applicant presented human data.

No data was presented concerning tissue distribution, protein binding, placental transfer, secretion into the milk and pharmacokinetic drug interactions. Since no animal data was presented no interspecies comparisons were possible.

Safety margins are currently neither available nor considered necessary.

Toxicology

Single dose toxicity

The single dose toxicity studies were consistent with a relatively low toxicity of betaine. The main symptoms could be characterised as known symptoms of the CNS and gastrointestinal bleeding.

Published data: the acute toxicity of betaine is low. In the mouse, LD₅₀ values of 10,800 mg/kg and 830 mg/kg by the subcutaneous and intravenous route respectively were reported.

Applicant's data: In the one single-dose study conducted in rats at dose levels up to 20,000 mg/kg, the combined (male and female) LD₅₀ was 11,179 mg/kg. The major antemortem sign of toxicity was CNS depression. Necropsy of decedants revealed areas of body surface staining, altered stomach, small intestine and caecum contents and darkened glandular gastric mucosa and brain. Red fluid was observed in the small intestine of 2 females and in the small intestine and brain of one male at 20,000 mg/kg. Necropsy of survivors was unremarkable.

The signs of CNS depression were consistent with the anticonvulsant effects discussed in the secondary pharmacodynamics section. The delayed deaths (1/10 at 10,000 and 12,500 mg/kg) appeared to be the result of gastro-intestinal irritation and bleeding, not present in survivors and in the non-lethal doses of 5000 mg/kg.

Repeat dose toxicity (with toxicokinetics)

Studies were mainly reported from the published literature but one study was conducted with Cystadane by the applicant.

Published data: The studies conducted at BIBRA involved a dose range finding study to evaluate reversibility. The highest dose used allowed the observation of some biological effects which disappeared during the recovery period. In the definitive study betaine was added to the diet in rats at 1%, 2% and 5% (17.5 g/day) for 14 or 28 or 90 days. There was no clear evidence of toxicity but at higher betaine intakes several serum clinical chemistry parameters were altered including increased ALKP and LDH levels, decreased ALT activity, increased gamma GT and bilirubin in females and decreased albumin and protein levels in males. The Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of the red blood cells were reduced and hepatocytes developed fatty droplets in a dose related manner. Females were more affected than males. These effects, except those on the reduced MCV and MCH, were reversed during the recovery period.

In the Brandeis University studies conducted in rats at dietary dose levels of 0.5%, 0.75%, 1 and 5% (25 g/day) for 28 days or 5% for 90 days, hepatic lipid droplets were investigated in more detail. Liver lipid was reduced by betaine. There were no significant adverse effects of clinical significance, but the

MCV was again reduced at 5% betaine in the 28 day study but all other parameters were completely reversible.

The influence of betaine on the liver and a possible imbalance in the folate-mediated metabolism was discussed. The observed effects of betaine on haematological parameters were attributed mainly to nutritional differences between the BIBRA and the Brandeis study part. The rat chow used in the Brandeis study supplied much more nutrients, vitamins and minerals than the chow used in the BIBRA part. It is reasonable that haematological effects may occur in juvenile animals which were ill-nourished especially in individuals undergoing pronounced growth. With respect to the influence of betaine on the liver it was shown that betaine enhances lipoprotein secretion and possibly apoprotein synthesis. The observed droplets likely represent VLDL particles (VLDL synthesis may be impaired due to a limited energy/protein supply in the BIBRA part), since no changes were apparent in the TGSR in the Brandeis part of the study. Again, differences in the composition of the chow may contribute to the observed influence on the liver. The data was considered satisfactory. With respect to the extensive clinical experience no evidence of adverse effects on the liver has been identified.

A 4-week study in male rats to investigate the effects of dietary betaine supplementation (0.5% w/v, approximately 1.3 g/kg) on methionine metabolism in control and ethanol-fed animals revealed that betaine had no effects on body weight gain but prevented weight loss induced by ethanol and protected against alcoholic fatty liver (Barak *et al.*, 1993).

Applicant's data: (This study has been also reported in the Pharmacodynamics Section). Liquid dietary administration of betaine at 0%, 0.5% (1.3 g/kg/day) or 1% (2.6 g/kg/day) w/v of betaine for 29 days revealed no significant treatment related effects on food consumption, body weight gain or in histopathological examination.

The repeated-dose toxicity studies do not comply with the Note for Guidance on repeated Dose Toxicity (CPMP/SWP/1042/99). However, in view of the extensive clinical experience, this was considered acceptable.

Table 2

Study ID	Species/Sex/ Number/Group	Dose/Route	Duration	NOEL/ NOAEL (mg/kg/day)	Major findings
Applicant's study. ID not given	Rat/10 sex/group	Oral, dietary Dose range 1.3 - 2.6g/kg/day	29 days	>2.6g/kg/day	None in parameters evaluated
BIBRA studies	Rat/10 sex/group	Oral, dietary Dose range 1, 2 and 5% (17.5g/day)	14 days	Not established	Clinical chemistry and red blood cell parameter changes
	Rat/40 sex/group		90 days		
	Rat/20F/group		28 days		
Brandeis studies	Rat/8 sex/group	Oral, dietary 0.5, 0.75, 1 and 5% (25g/day)	28 days	1%	Reversible decrease in MCV at 5% in 28 day study

Genotoxicity

As expected, betaine tested negative in the genotoxicity testing. Betaine was tested for mutagenicity in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 with or without metabolic activation at concentration levels up to 5000µg /plate. This study was negative. The clastogenic potential was assessed in vitro using human lymphocytes with or without metabolic activation at concentration levels up to 10,000µg/mL for 3 hours (activated cells) or 24 hours (non activated cells). This study was negative. The clastogenic potential was assessed in vivo in a mouse micronucleus test at dose levels up to 2000mg/kg p.o. This study was negative.

In the bacterial mutagenicity assay, the relevant guideline states that the standard set of bacterial strains should include strains that will detect point mutations at A-T (adenine-thymine) sites such as *S. typhimurium* TA102 or *E.coli* WP2 uvrA. The package presented did not have these strains. However, in view of the nature of the product, the package submitted was considered acceptable.

Table 3

Type of test/study ID/GLP	Test system	Concentration range/ Metabolising system	Results
Gene mutations in bacteria M/AMES/17027	<i>Salmonella</i> strains TA1535, TA1537, TA1538, TA98 TA100	+/- S9 8-5000µg/plate	Negative
Chromosomal aberrations in vitro M/HL/17028	Human lymphocytes	+/- S9 1000-10,000µg/mL	Negative
Chromosomal aberrations in vivo M/MMN/17029	Mouse, micronuclei in bone marrow	+/- S9 500-2000mg/kg	Negative

Carcinogenicity

Long-term studies

There are no reports in the literature of carcinogenicity caused by treatment with betaine at doses up to 30 g daily over 6 months or 8-20 g/day for 1 to 3 years, although in view of the short duration of treatment, these findings cannot be taken as strong evidence of lack of carcinogenic potential.

As betaine is a naturally constitute of mammalian cells occurring in quite high amounts and taking into account its already long term use in clinical practice long term carcinogenicity studies were considered not necessary by the CHMP.

No data was provided concerning short or medium-term studies.

Reproduction Toxicity

No relevant studies have been conducted. The applicant stated that in view of the nature of the product and the extensive clinical experience, it was not relevant to conduct animal studies.

The applicant stated that little is known about the interactions between choline and folate and homocysteine metabolism during pregnancy despite the fact that pregnancy places considerable stress on maternal folate and choline stores and that choline is a critical nutrient for the foetus. Furthermore, the potential imbalance in folate activity and modification in purin metabolism and cell division with consequence on reproductive function during betaine supplementation were not fully explored.

The applicant reported 6 cases of pregnancy with betaine treatment without adverse effects on pregnancy or on the health of the foetus/newborn child and reported one more case which had been published in 2000 or 2003 by Gissen et al. The issue was considered acceptable by the CHMP.

Toxicokinetic data

No non-clinical data

Local tolerance

Not applicable.

Other toxicity studies

None

Ecotoxicity/environmental risk assessment

Betaine is stated to be identical to the natural constituents of mammalian tissues. After absorption, this amino acid rapidly enters the methionine/homocysteine cycle. Therefore no environmental risk can be expected from the therapeutic use of betaine. Indeed, amino acids are specifically exempted from testing in the most recent draft guideline.

Discussion on the non-clinical aspects

The effects of betaine related to the proposed therapeutic use have been investigated in several rat studies. The effects were also investigated in non-conventional species such as chick and fruit bat. The results support the use of betaine for the indication of homocystinuria.

Studies on secondary pharmacodynamics showed that betaine protected the liver against ethanol-induced injury which was related to its ability to increase hepatic SAM levels and betaine also prevented vitamin A depletion. Betaine was shown to have anti-convulsant and anti-apoptotic activity.

There are no specific studies on safety pharmacology or for pharmacodynamic reaction. It was stated that betaine occurs normally in the body, the inference being that it is unlikely to have adverse effects. After a high single dose some CNS depression signs appeared in animals (see Single dose toxicity below); it was proposed that these were consistent with the anti-convulsant effects. In view of the extensive clinical experience, the clinical data may be of greater utility in revealing potential adverse treatment related effects.

The single dose toxicity of betaine was low in rats. The CNS depressant activity only occurred at very high dose levels. This was consistent with the anti-convulsant effect of betaine reported. The irritation of the gastrointestinal tract observed is consistent with the mild gastrointestinal effects reported clinically.

Following dietary administration to rats at dose levels up to 5% of the diet for 28 or 90 days, the only adverse treatment related effects reported were small perturbations in red blood cell parameters and serum chemistry values. These were all fully reversible after 90 days recovery period. However, the information obtained for betaine from healthy animals may not be directly relevant to assessing the potential toxicity in the presence of metabolic abnormalities.

In the package of studies presented, betaine was not mutagenic. Carcinogenicity and reproduction toxicity studies were not been conducted. In view of the nature of the product, this was considered acceptable by the CHMP.

4. Clinical aspects

Introduction

The applicants own data are limited to a pharmacokinetic study conducted in 12 healthy volunteers following administration of single dose and repeated doses of betaine and to safety data gathered in EU and the USA. Clinical efficacy is based on 202 reports retrieved from literature search.

GCP

According to the applicant, the studies followed all ethical guidelines in practice at the time of conduct of the studies. No issues regarding GCP aspects have been identified during the review of the dossier.

Pharmacokinetics

The absolute bioavailability of betaine has not been determined. In healthy adult volunteers (age between 21 to 49 years), after a single oral dose of betaine (50 mg/kg), absorption was rapid ($t_{max} = 0.9 \pm 0.3$ hours and a $C_{max} = 0.9 \pm 0.2$ mM). Betaine was rapidly distributed into a relatively large volume ($V/F = 1.3$ l/kg), with a slow elimination rate (mean half life = 14 h, mean total body clearance, $CL/F, = 84$ ml/h/kg), renal clearance being negligible (5% of total body clearance), assuming 100% bioavailability. After a repeated dose regimen of 100 mg/kg/day for 5 days, the absorption kinetics did not change but the distribution half-life was prolonged significantly (up to 36 h), indicating saturable transport and redistribution processes. The pharmacokinetic data of homocystinuric patients on long-term betaine supplementation are very similar to those of healthy volunteers. This demonstrates that differences in betaine kinetics are most probably due to betaine depletion in untreated homocystinuria and are only meaningful for the initial treatment.

There is a possibility of chemical interactions with certain foodstuffs and antibiotics. These interactions, although they have not been formally investigated, may be clinically significant. Based on in vitro data, betaine might interact with amino acids mixtures and drugs like vigabatrin and GABA analogues. As mentioned in the section 4.4 of the SPC, to minimize the risk of potential drug interactions, it is advisable to leave 30 minutes between the intake of betaine and amino acids mixtures and/or medicinal products containing vigabatrin and GABA analogues.

Differences in pharmacokinetics related to gender, age or race or the comorbidity have not been systematically studied. However, considering the nature and prevalence of the disease this was considered to be acceptable.

Pharmacodynamics

The pharmacodynamics of betaine are based on a review of four published studies in 161 healthy volunteers, one study with a sequential increase of dose in 34 volunteers and three studies with repeated doses and on published data in patients.

The mechanism of action of betaine in hyperhomocystinaemia has sufficiently been established. No additional data was required. Betaine supplementation appears to be effective in lowering plasma tHcy (plasma total homocysteine) concentrations in healthy subjects and in patients with homocystinuria. Betaine supplementation also seems to improve the metabolic abnormalities in the central nervous compartment of patients with homocystinuria. The extent of the effect on plasma tHcy is dependent on the absolute degree of hyperhomocysteinemia, being higher in severe hyperhomocysteinemia.

In CBS- (Cystathionine beta-synthase) deficiency the risk of an excessive accumulation of methionine and associated adverse events should be considered.

The SPC sections 4.4 and 4.5 adequately reflect the potential risk of drug interactions.

Clinical efficacy

Clinical efficacy is based on 202 reports retrieved from literature search. Most cases were reported as individual case reports and about 25% cases were pooled as groups of patients. Some of these patients have been reported several times, i.e. as a case report at diagnosis, as a case report later in life, and in a pooled review. Verified data are available in 140 patients, in which individual biochemical or clinical features as well as dose and duration of betaine therapy, and pre-existing or concomitant therapies were documented.

Biochemical efficacy of betaine treatment in homocystinuria using the widely accepted surrogate marker of plasma homocysteine appears to have been demonstrated in the published reports submitted for this application.

Regarding clinical efficacy no data from systematic studies are available. In addition a variety of doses and co-treatments have been used. Several forms of bias might have influenced the results. Therefore the clinical efficacy of betaine treatment is more difficult to assess than the biochemical efficacy. No clear improvement was expected from further studies, especially considering the heterogeneous patient population in question. Also the limited available data indicated at least an efficacy of betaine in preventing further disease progress. FUMs were not regarded to improve the situation either.

It is therefore considered that the data submitted showing biochemical efficacy and the associated improvements regarding the various disease symptoms after betaine therapy compared with historical data of untreated patients provide sufficient evidence of betaine's effectiveness. However, it is likely that due to the multiple nature of therapy (dietary, pharmaceutical, supportive) in these patients, there may be an element of overestimation in the clinical effects of betaine treatment. The seemingly late detection in symptoms leads to irreversible damage, especially to the nervous system and connective tissue. Such damage cannot be corrected by further therapy. These issues are reflected in the section 5.1 of the SPC.

The available clinical data do not allow correlating dosage and clinical efficacy. There is no evidence of development of tolerance. For example, there were no reported rises in blood levels of homocysteine during long-term betaine treatment.

In conclusion, although there are only a few systematic studies available, data presented in this application appear to show both biochemical and clinical benefits of betaine supplementation in the management of homocystinuria.

Clinical safety

In general, adverse effects seen with betaine therapy appear to be not serious and are mainly related to the gastrointestinal system. Study OMC-BETR-1 indicates that these AEs are dose-related. Betaine has already been supplemented in many patients over many years. Many of the available data concerning safety of betaine have been obtained from neonates, infants, and children of different age groups. In this regard, no age-related difference in toxicity of betaine has been observed. However data from controlled trials are sparse.

The occurrence of five cases of potentially life-threatening cerebral oedema while receiving the usual dose of betaine raises concern. It is not clear whether these reported cases occurred due to accumulation of methionine, or betaine, or both, or independently of therapy. In this regard special attention should be paid in patients with poor dietary control of hypermethioninemia. It is necessary to advise prescribing physicians to monitor methionine plasma concentrations especially in patients with CBS-deficiency receiving betaine and to pay attention to early clinical signs of cerebral oedema. Plasma methionine level should be monitored, at start of treatment and periodically thereafter. The plasma methionine concentrations should be kept below 1000 μM . If any symptoms of cerebral oedema like morning headaches with vomiting or visual changes appear, plasma methionine level and

compliance to the diet should be checked and treatment with Cystadane interrupted. If symptoms of cerebral oedema recur after re-introduction of treatment then betaine therapy should be discontinued indefinitely. This information is reflected in the SPC.

Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan.

Table Summary of the risk management plan

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities (see the section referred below in the Product Information)
Cerebral oedema	routine pharmacovigilance patient registry to gather data on demographics, drug utilisation and safety profile.	<p>4.4 Special warnings and precautions for use</p> <p>Uncommon cases of severe cerebral oedema and hypermethioninemia were reported within 2 weeks to 6 months of starting betaine therapy (see section 4.8). Complete recovery was seen after treatment discontinuation:</p> <ul style="list-style-type: none"> - Plasma methionine level should be monitored, at start of treatment and periodically thereafter. The plasma methionine concentrations should be kept below 1000 µM. - If any symptoms of cerebral oedema like morning headaches with vomiting and/or visual changes appear, plasma methionine level and compliance to the diet should be checked and treatment with Cystadane interrupted. - If symptoms of cerebral oedema recur after re-introduction of treatment then betaine therapy should be discontinued indefinitely. <p>4.8 Undesirable effects</p> <p>Uncommon cases of severe cerebral oedema and hypermethioninemia were reported within 2 weeks to 6 months of starting betaine therapy, with complete recovery after treatment discontinuation. High increases in plasma methionine</p>

		<p>levels in a range from 1,000 to 3,000 μM were noted in these patients. As cerebral oedema has also been reported in patients with hypermethioninemia, secondary hypermethioninemia due to betaine therapy has been postulated as a possible mechanism of action.</p> <p>For specific recommendations, refer to section 4.4.</p>
Limited number of patients treated with betaine	<p>routine pharmacovigilance</p> <p>patient registry to gather data on demographics, drug utilisation and safety profile</p>	

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. There are no unresolved quality issues, which have a negative impact on the Benefit Risk balance of the product.

Efficacy

The data submitted showing biochemical efficacy and the associated improvements regarding the various disease symptoms after betaine therapy compared with historical data of untreated patients provide sufficient evidence of betaine's effectiveness. Betaine in the treatment of homocystinuria has been investigated in different age groups and both male and females. No differences in pharmacokinetics or dose-response have been reported. In addition, long-term treatment demonstrated constant effects over time. However, it is likely that due to the multiple nature of therapy (dietary, pharmaceutical, supportive) in these patients, there may be an element of overestimation in the clinical effects of betaine treatment.

Since no patient has been treated with betaine alone, it is difficult to distinguish the clinical benefits attributable to betaine from those affected by other treatment options. Nonetheless, the addition of betaine to pre-existing therapies appears to lower homocysteine plasma concentrations and leads to improved disease symptoms in a substantial group of patients, particularly those with cardiovascular symptoms. Homocysteine plasma concentrations are generally accepted as a surrogate marker for the severity of disease. Therefore, it can be considered that betaine offers benefit to patients with homocystinuria. It is however important to emphasise that betaine is no substitute for the currently available therapies such as relevant vitamin or dietary preparations. In cases where biochemical markers are controlled by restricted diet and vitamin supplementation (B6, B12, folate) no advantage is to be expected for betaine co-treatment. Even though the dosage is not critical, its use should be clinically and, where appropriate, also biochemically monitored by an experienced physician on a regular basis.

Safety

With regards to safety, betaine appears to be well tolerated. Many of the available data concerning safety of betaine have been obtained from neonates, infants, and children of different age groups.

In general, adverse effects seen with betaine therapy appeared to be not serious and were mainly related to the gastrointestinal system. The occurrence of cases of potentially life-threatening cerebral oedema while receiving the usual dose of betaine raised concern. However this issue was adequately dealt with in the SPC and the provided RMP.

User consultation

Results of assessments carried out in cooperation with target patient groups on the package leaflet ('User Consultation' of the package leaflet according to (Art 59(3) and 61(1) of the amended Directive) were presented. Data included the method, background information and results of the testing procedures.

The proposed product information submitted was considered satisfactory as the leaflet tested accounted for the changes as requested in the Day 120 LOQ for the product and as such additional testing was not considered necessary.

Risk-benefit assessment

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- Apart registry no additional pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed.
- no additional risk minimisation activities were required beyond those included in the product information.

Betaine was shown to be useful as an adjunctive therapy to the existing treatment of homocystinuria. It is no substitute for other currently available therapies such vitamin supplementation or dietary intervention. When used as indicated, betaine seems to have a favourable benefit to risk ratio. Although there were only a few systematic studies available, data presented in this application showed both biochemical and clinical benefits of betaine supplementation in the management of homocystinuria and that Cystadane has a positive risk-benefit ratio in the treatment of these patients.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus decision that the risk-benefit balance of Cystadane in the adjunctive treatment of homocystinuria was favourable and therefore recommended the granting of the marketing authorisation.