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54 **Pharmaceutical preparations for lowering homocysteine levels, containing vitamin B6, folic acid and vitamin B12.**

57 Pharmaceutical preparations for lowering blood and tissue levels of homocysteine are disclosed, comprising:  
a) vitamin B6;  
b) folate or a suitable active metabolite of folate or a substance which releases folate in vivo;  
c) vitamin B12, with or without intrinsic factor  
and optionally antioxidants, choline and/or betaine. a) and b) are provided in slow release form (2-8 hours) and c) is to be released immediately (within 20 minutes).

**EP 0 595 005 A1**

The present invention relates to pharmaceutical preparations for lowering levels of homocysteine or for the prophylaxis or treatment of elevated levels of homocysteine in patients and for counteracting the harmful effects associated with homocysteine.

Elevated homocysteine levels can be correlated with some of the principle causes of morbidity and mortality in the Western world, the so-called "Western" diseases, including such conditions as myocardial and cerebral infarction. Precocious vascular disease is the main single cause of death accounting for the majority of these deaths (New Eng J Med 1986, 314 : 488). It is generally agreed that nutritional factors play an important role in the etiology of this and the other Western diseases. The precise nature of the nutritional factors responsible for these diseases, is difficult to define but it can be stated with certainty that these are multi-factorial. Briefly, in the affluent Western societies, there is an overconsumption on the one hand of macro nutrients such as proteins, fats and refined carbohydrates, which are normally underconsumed in the Third World countries. Due to food refinement and all the other facets of food processing necessitated by increased urbanisation in the West, much of the micro-nutrients (vitamins, minerals) are lost. This results in a metabolic imbalance between macro-nutrients (especially proteins and fats) on the one hand and the essential micro-nutrients on the other hand which are necessary for the normal metabolism of the former. Under these conditions, abnormal metabolic pathways may be activated leading to the production of toxic and harmful intermediary products which in many cases are the cause of disease and which normally are not produced at all or only in very small quantities. The metabolism of the amino acid methionine is a good example, in which case excessive quantities of the toxic and unnatural amino acid homocysteine are produced.

Elevated homocysteine levels also occur in certain patients due to genetic causes and may also be caused by certain drugs, including certain vitamin B6 antagonistic drugs.

Normally, methionine is metabolised by the transmethylation and transsulfuration pathways to produce cysteine.

Three pathways exist by means of which blood and tissue levels of homocysteine are controlled to ensure homocysteine homeostasis:

1. Conversion into cysteine by means of the vitamin B6 dependent enzyme cystathionine  $\beta$ -synthase (CBS)
2. Remethylation to methionine which requires folate (as substrate) and vitamin B12 as co-factor.
3. Remethylation to methionine in which other methyl donors such as betaine participate.

Elaborate provision therefore exists in the healthy body to keep homocysteine levels in check. The reason for this is that homocysteine is a very toxic compound which in the chronic situation may affect a variety of systems and tissues in the body.

A pathological condition due to one or more of several hereditary enzyme defects wherein homocysteine levels are abnormally high, is known as homocysteinuria. This condition is often associated with high blood levels of homocysteine (often 200  $\mu$  mole/l or higher) and the associated clinical defects include the following:-

1. Disintegration of the vascular elastic interna due to binding of homocysteine to allysine residues of tropoelastin.
2. Inhibition of the process of polymerisation and cross linking in the formation of elastin and collagen.
3. Hyperplasia of arterial smooth muscle cells and synthesis of extracellular collective tissue.
4. Degradation of vascular glycocalyx and synthesis of extracellular connective tissue.
5. Pro-thrombotic effects (activation of Hagemann factor and stimulation of thromboxane 2 production by platelets).
6. Progressive premature arteriosclerosis.
7. Accelerated osteoporosis (Metabolism 1985, 34 : 1073).
8. Precocious occlusive vascular disease frequently manifested clinically as myocardial infarction, stroke, pulmonary embolism (Am.J.Med.Sc. 1977, 273: 120) and peripheral vascular occlusion.
9. Abnormalities in eyes, skeletal system, central nervous and vascular systems.
10. Occlusive disease of cerebral, carotid and aorto-iliac vessels.
11. Occlusion or stenosis of renal arteries which often results in renovascular hypertension. (See for example: Metabolism 1985, 34 : 1073, Am J. Med. Sc. 1977, 273 : 120, Stroke 1984, 15 : 1014, Atherosclerosis 1988, 71 : 227.
12. The sex and age related variations in plasma homocysteine parallel well-established age and sex-related risk factors in atherosclerotic disease.

It has also been shown in many studies, that whereas lipid levels are not markedly different in coronary patients and controls, homocysteine levels are significantly different. (See for example J Am. Coll. Cardiol. 1990, 16:1114)

It is therefore now widely accepted that elevated plasma homocysteine is a risk factor independent of established risk factors such as cigarette smoking, hypertension and diabetes for generalised arteriosclerotic disease (Circulation 1989, 79 : 1180).

5 On the other hand, evidence exists which suggests that B6 deficiency independently of homocysteine may be associated with vascular disease stressing the prime importance of an adequate intracellular B6 status to prevent these diseases.

It is therefore now accepted in the art that elevated blood levels of homocysteine are highly undesirable. Normalisation of such elevated levels of homocysteine therefore constitutes a therapeutic goal as such without reference to any specific disease entity, possibly causally related to such elevated levels.

10 Evidence is mounting that high cholesterol levels alone are not the risk factor in atherosclerotic diseases as was previously believed. Before cholesterol contributes to vascular occlusion another form of damage occurs which is correlated with high homocysteine levels. Once that damage has occurred the beneficial effects of cholesterol-lowering drugs, in particular so-called statins become highly questionable, particularly when viewed in the light of side effects of such drugs (raising Lp(a), decreasing HDL, weakening the immune system, cataracts, GI disturbances, myositis, myocarditis). Nevertheless, the prejudice in favour of cholesterol depressants has been so strong that these adverse findings have, until now, been given inadequate coverage in the review literature.

The present invention is aimed at counteracting root causes of arteriosclerotic disease which damage the blood vessels before cholesterol becomes a problem.

20 The clinical condition of homocysteinuria, is an inborn error of metabolism which is either caused by an enzyme defect in the transsulfuration pathway or a similar defect in the 5-methyl tetrahydrofolate dependent remethylation of homocysteine to methionine. Patients with this disease usually have very high fasting blood levels of homocysteine (in excess of 200 micromolar in homozygotes) and have a limited life expectancy due to early vascular complications. This rare condition must be clearly distinguished from other milder (but chronic) forms of homocysteinemia which may arise from other causes - both external and internal - but which are clinically of much greater importance due to the vastly higher prevalence thereof. Accordingly, a need exists for reducing or preventing not only the extreme elevated homocysteine levels in cases of homocysteinuria, but also the much more moderately elevated homocysteine levels pertaining to homocysteinemia.

30 Inadequate metabolic status individually of vitamin B6, folate and vitamin B12 have been recognised as determinants of heart and peripheral occlusive disease. At the same time, deficiencies (individually) of each of these vitamins have also been known to be associated with increased homocysteine levels. Thus vitamin B6 deficient humans have a 43 % reduction in cystathionine  $\beta$ -synthase (CBS) activity and they excrete increased quantities of homocysteine in the urine, reflecting the effect of an inadequate B6 status on homocysteine blood levels. A negative correlation exists between dietary B6 intake and blood levels of protein bound homocysteine.

Similar relationships have been described between B12 and folate levels individually on the one hand and blood levels of homocysteine on the other hand. These relationships have been described by several authors and have been summarised in the following publications:-

- 40 1. Stroke, 1984, 15 : 1012
2. Metabolism 1984, 34 : 1073
3. Metabolism 1988, 37 : 175
4. Scan J Clin Lab Invest 1988, 48 : 215
5. Atherosclerosis 1988, 71 : 227
- 45 6. Circulation 1990, 81 : 2004

The present state of the art knowledge on homocysteine and its involvement in disease is well summarised and presented in a recent review article (J.Lab.Clin Med. 1989, 114 : 473). In the course of own investigations into the relationship between B6, B12 and folate metabolic status, homocysteine metabolism and occlusive vascular disease, applicant has established that in addition to the known and published information on these relationships, certain other aspects - heretofore unknown or not appreciated or not correctly interpreted - are of prime importance in connection with treatment and prevention of homocysteine related occlusive vascular disease. In addition, by the judicious application of these findings, treatment of hyperhomocysteinemia may be appreciably facilitated.

55 Regarding the treatment and prophylaxis of hyperhomocysteinemia, it is known that vitamin B6, vitamin B12 and folate play a role in regulating the methionine - homocysteine pathway and controlling levels of homocysteine (David E L Wilken, Nicholas P P Dudman, Haemostasis 1989; 19 (supplement 1) : 14 - 23; Per Magne Ueland and Helga Refsum, J.Lab.Clin.Med. November 1989, 473 - 501. However, it was previously not recognised, that many patients develop hyperhomocysteinemia not primarily because of a

lack of the relevant vitamins, but often because of absorption problems, especially in the case of vitamin B12.

Accordingly there is a need for improvement in pharmaceutical compositions for lowering elevated homocysteine levels in plasma and counteracting adverse clinical conditions associated therewith, especially with respect to those patients in whom elevated plasma homocysteine levels are primarily related to absorption problems such as occur in many elderly patients. It is precisely in such patients that the problem of hyperhomocysteinaemia with accompanying vascular pathology is often a serious one.

In particular, there is a need to provide pharmaceutical compositions and dosage regimens which achieve adequate lowering of plasma homocysteine levels and counteracting adverse clinical conditions associated therewith in the greatest number of patients suffering from elevated plasma homocysteine levels covering substantially all age groups and preferably with relatively low dosages of active ingredients.

More particularly, there is a need for pharmaceutical compositions and dosage regimens which attain the foregoing with surprisingly low dosage rates of folate as compared with the prior art.

In the present invention, special provision is made to overcome such problems. These preferably include the following galenical and biochemical variations:-

- a) the use of pyridoxal instead of pyridoxine as a source of B6 activity;
- b) the galenical presentation of the vitamins concerned in such a form that the rate of release of each vitamin is compatible with maximum absorption and utilisation;
- c) the use of transdermal vitamin formulations which allows direct absorption through the skin of small quantities over prolonged periods. This is accomplished either through the use of appropriately formulated vitamin plasters or through the use of sub-lingual tablets.

Reference is made to applicant's copending patent application entitled "Compositions for the Treatment and Prophylaxis of Metabolic Disturbances in Infants", claiming priority of ZA-PA 92/6989.

Here pharmaceutical and dietary preparations are disclosed for the treatment or prophylaxis of elevated homocysteine and/or methionine levels in the blood of human infants and pathological disturbances connected therewith, said preparation comprising in combination:-

- a) vitamin B 6 as such or in the form of a pharmaceutically acceptable acid salt, at least in part in the form of pyridoxal (PL) or a compound which in vivo readily releases PL without the intervention of oxidase enzyme or oxygen.
- b) folate or a precursor of folate which releases folate in vivo, and
- c) vitamin B12, with or without intrinsic factor, in the following ratios:-
  - a) . b) from 1:25 to 10 000 : 1
  - b) : c) from 1:1 to 50 000 : 1

The preparations are to be incorporated in infant bone feed mixes. That disclosure, by cross-reference, forms part of the present disclosure. The same applies to the contents of a study performed on behalf of the applicant and published after the priority date hereof in Am.J.Clinical Nutrition (1993), 57, pp 47-53.

In accordance with the invention there is provided the use in the manufacture of a pharmaceutical preparation for lowering levels of homocysteine or for the prophylaxis or treatment of elevated levels of homocysteine in a patient of a combination which comprises

- a) vitamin B6;
- b) folate or a suitable active metabolite of folate or a substance which releases folate in vivo;
- c) vitamin B12, with or without intrinsic factor.

The invention is applicable to the lowering of total homocysteine blood levels if elevated by any known cause, including genetic causes (e.g. enzyme polymorphism) diets, drugs or depressed activity levels of folate, vitamin B6, vitamin B12 or any combination of these due to whatever cause, pregnancy, chronic renal failure, psoriasis, occlusive vascular disease, chronic liver disease, homocysteine-associated psychiatric problems. Drugs which induce elevated homocysteine levels include anticonvulsant drugs, xanthine bronchodilators (e.g. theophylline), methotrexate, nitrous oxide, and many others.

Preferably, in the preparation, the ingredients a) - c) are present in the following ratios by weight calculated on the basis of pure unphosphorylated pyridoxal (PL), pure vitamin B12 and pure folic acid:

- a):b) from 100:1 to 1:10 and
- b):c) from 100:1 to 1:50

The preferred ratios are:

- a):b) from 50:1 to 1:1,5
- b):c) from 15:1 to 1:2

more preferred ratios are:-

- a):b) from 20:1 to 2,5:1
- b):c) from 4:1 to 1:1

and in particular:-

- a):b) from 20:1 to 5:1
- b):c) from 2:1 to 1:2

The scope of the invention is intended to include a pharmaceutical preparation as such as aforesaid, wherein if the preparation is for oral use and any of the vitamin B6 is represented by pyridoxine (PN), such PN is formulated in slow-release form. This is particularly advantageous in the context of PN, because of the limited capacity of the liver to convert PN into PLP and the resultant risks of excess PN in plasma leading to poor utilisation and undesirable entrance of PN into peripheral cells and erythrocytes.

Pharmaceutical preparations containing the aforesaid active ingredients have been described, albeit for totally different purposes and mostly in ratios differing from the aforesaid ratios or at least from the preferred or more preferred ratios. In GB-PS 1201 014 (examples 6 and 7) the ratio of a):b) = 3:1 and that of b):c) = 1000:5. No indication is disclosed for these dragees. GB-PS 2254 556, published after the priority date of the present disclosure, also discloses compositions, only some of which contain in combination folic acid, vitamin B12, vitamin B6. No distinction is drawn between pyridoxine, pyridoxal and pyridoxamine. These compositions are intended for adolescent girls. GB-PS 149 3993 discloses compositions for treating obesity. Pyridoxal is not disclosed. GB-PS 2145 331 discloses all these ingredients but in quite different ratios and in dosages which are partly too high and partly too low for the purposes of the present invention. GB-PS 2197 587 describes a "blood conditioning tonic" for race horses. Pyridoxal is not disclosed. GB-PS 1431 841 discloses preparations for cataract treatment. The ratios are different from those according to the invention and pyridoxal is not disclosed. GB 101 3939 discloses compositions for paediatric purposes in ratios which overlap the broadest ratio according to the invention, but the important feature that the vitamin B6 must be in the form of pyridoxal or suitable precursor thereof is not disclosed. This also applies to EP-0144051, EP 0121 036 or PCT WO 83/00085.

Not one of the aforesaid references discloses such combinations for the treatment or prophylaxis of elevated homocysteine levels in plasma, or the crucial role of pyridoxal in that context, which is the only useful form which when it enters peripheral cells or erythrocytes is directly converted there into active pyridoxal phosphate (PLP).

It is now realised that such intracellular PLP is the sole form in which vitamin B6 controls homocysteine levels in plasma.

The preparations in accordance with the invention are formulated to provide approximate daily dosages as follows ( $\mu\text{g}/\text{d}/\text{kg}$  body weight).

	a) Vitamin B6	b) Folic Acid	c) Vitamin B12
Broadest range	15-750	1,5-150	1,5-75
preferred range	30-400	7,5-50	3-15
more preferred range	75-250	10-30	7-10
typical example	150	15	7,5

These dosages may be exceeded somewhat for short durations, e.g. at the beginning of the treatment. Also, where the daily dosages are divided into several dosage units to be administered at different times of the day, the compositions may differ to provide optimum effect in accordance with circadian variations in homocysteine production. The latter may fluctuate in a manner depending on time, on meal intake, its quantity and composition. The dosage regimen may be programmed to be optionally adapted to a predetermined daily dietary programme.

Preferably the preparation is formulated to make available to the patient the vitamin B6 and preferably also the folate over a period of more than 1 hour and to make available an effective dosage of the vitamin B12 in less than 1 hour after administration. This feature is considered to contribute materially to the efficacy of the invention and is considered to be novel and inventive *per se*.

The preparation may be galenically formulated for parenteral administration, preferably by infusion or by intramuscular injection. The latter form inherently provides for a retarded availability of the ingredients, which effect may be further enhanced by depot forms of formulation.

Preferably the preparation combines all three essential ingredients in a single dosage form, which except for very drastic cases of elevated homocysteine levels is preferably designed for oral administration.

However, it is possible within the scope of the invention, to provide separate ingredients of the preparation in separate distinctive dosage forms, e.g. capsules, tablets or coated tablets, preferably

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