International Journal of Pharmaceutics, 55 (1989) 99–103
Elsevier

IJP 01857

Stability and behaviour of selenium in total parenteral nutrition solutions

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(Received 10 February 1989)
(Modified version received 13 April 1989)
(Accepted 19 April 1989)

Key words: Parenteral nutrition; Stability; Selenium; Nutritive mixture; Redox reaction; Complexation reaction; Trace element; Amino acid

Summary

Selenium stability in amino acid/dextrose/electrolytes/ascorbic acid/trace elements was evaluated by a specific and sensitive fluorimetric method of selenium(IV) determination. Ascorbic acid, particularly with copper(II) ions, reduced selenium(IV) in selenium metal (Se). Cysteine present in amino acids solution induces complexation with selenium. The complex has been identified by high-performance liquid chromatography when cysteine and selenite are mixed in 4/1 molar ratio. The complex is releasing cystine and selenium(II). These reactions are responsible for the complete loss of selenium(IV) in total parenteral nutrition solutions.

Introduction

Selenium (Se), an essential trace element, is a cofactor of glutathione peroxidase (this enzyme catalyses peroxides reduction). It is an essential element and is present in food: organic selenium as seleniocystine or seleniomethionine, inorganic selenium as selenite (Se in the +IV oxidation state) or selenate (Se in the +VI oxidation state).

In 1979, Van Rij et al. proved the essential function of selenium during total parenteral nutrition (TPN). Later, deficiencies in selenium have been described in patients receiving long-term TPN. They presented clinical symptomatology as cardiomyopathy and osteoarthropathy (Lane et al., 1982). Then, Lane et al. showed that selenium supplementation in TPN solutions increases plasma selenium levels as well as erythrocyte and platelet glutathione-peroxidase activities (Lane et al., 1987).

As for the stability of selenium, the data found in literature are contradictory. McGee et al. (1985) concluded that selenium was stable as selenious acid (Se(IV)) while Shils and Levander (1982) described its reduction in selenium metal (Se) which is insoluble in parenteral solutions.

Two kinds of reactions may occur to explain a diminution of selenium concentration or a modification of its availability: (1) Redox reactions are

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TABLE 1

Standard potential values of some components of TPN solutions

			ΔE_0
$Fe^{3+} + e^-$	↔	Fe^{2+}	+0.771 V
$H_2SeO_3 + 4H^+ + 4e^-$	↔	$Se + 3H_2O$	+0.740 V
$Cu^+ + e^-$	↔	Cu	+0.521 V
$Cu^{2+} + 2e^-$	↔	Cu	+0.337 V
$Cu^{2+} + e^-$	↔	Cu^+	+0.153 V
* $DHA + 2H^+ + 2e^-$	↔	Ascorbic acid	+0.127 V (pH = 5.0)
$SeO_2 + e^-$	↔	SeO^{2-}	+0.022 V to 0.028 V
$Cystine + 2H^+ + 2e^-$	↔	Cysteine	−0.05 V
$Fe^{2+} + 2e^-$	↔	Fe	−0.440 V
$Zn^{2+} + 2e^-$	↔	Zn	−0.0763 V
$Se + 2e^-$	↔	Se^{2-}	−0.920 V
$H_2SeO_3 + H_2O$	↔	$HSeO_4^- + 3H^+ + 2e^-$	−1.091 V

* DHA = Dehydroascorbic acid

TABLE 2

Nutrients contained in 1000 ml of TPN used for study of Se(IV) stability

Dextrose	150 g
Amino acids (PRIMENE *)	20 g
Sodium	30 mmol
Potassium	25 mmol
Phosphate	8 mmol
Calcium	4 mmol
Magnesium	1 mmol
Zinc(II)	7.5 mg
Iron(II)	1.5 mg
Copper(II)	300 μg
Manganese(II)	150 μg
Chromium(III)	30 μg
Selenium(IV)	45 μg

multiple. The standard potential values of some components of nutritive mixtures are reported in Table 1. It is quite difficult to specify the exact oxidation state of selenium in these conditions. Consequently, a specific and sensitive method of selenite determination (Se + IV) has been chosen. (2) Complexation reactions between trace elements and amino acids have been shown. A simple method using high performance liquid chromatography (HPLC) has been developed to measure free and complex amino acids, especially the seleniocystine complex.

The aim of this work was to study the stability and the behaviour of selenium in nutritive solutions.

Materials and Methods

Solution preparation

In the first trial, selenium (Se(IV)) was determined in TPN solutions containing dextrose, amino acids (Primene, Cernep Synthelabo, Le Plessis Robinson, France), electrolytes and trace elements (Table 2), then in simple solutions containing one or more nutrients (Table 3).

In the second trial, amino acid selenium complex was detected in a mixture of sodium selenite (Prolabo, Paris, France) and cysteine (Fluka, Bucks, Switzerland) in a molar ratio of 1/4.

TABLE 3

Composition of mixture used for study of Se(IV) stability

Mixture 1	Se(IV)		45 μg/l
Mixture 2	Se(IV)		
	Trace element	Zn(II)	7.5 mg/l
		Fe(II)	1.5 mg/l
		Cu(II)	300 μg/l
		Mn(II)	150 μg/l
		Cr(III)	30 μg/l
Mixture 3	Se(IV)		
	Ascorbic acid		200 mg/l
Mixture 4	Se(IV) + Ascorbic acid + Trace Element (without Cu)		
Mixture 5	Se(IV) + Ascorbic acid + Trace Element (with Cu)		
Mixture 6	Se(IV)		
	Dextrose		150 g/l
Mixture 7	Se(IV) + Dextrose Electrolytes (Phosphate Buffer pH = 6.5)		
Mixture 8	Se(IV)		
	Amino acids (PRIMENE *)		20 g/l
Mixture 9	Se(IV)		
	Methionine		0.48 g/l
Mixture 10	Se(IV)		
	Cysteine		0.38 g/l

Analysis

Selenium. Selenite (Se(IV)) was measured according to the fluorometric method proposed by the Analytical Methods Committee with some modifications (Analytical Methods Committee, 1979). Calibration was based on standard solutions prepared from reagent grade sodium selenite with a concentration range from 10 to 80 μg/l in selenite. Samples, 2 ml, were directly mixed without preliminary mineralisation with 2 ml of hydroxylammonium EDTA solution. The pH of each solution was adjusted to 1.8 with HCl 2.5 N. 2 ml of DAN solution (2,3 diamino naphtalene solution: 1% in HCl 0.1 N; DAN, Sigma, Saint-Louis, U.S.A.) were added. After 30 min, the complex of selenium with DAN was extracted by 3 ml cyclohexane (for fluorimetry, Merck, Darmstadt, F.R.G.) and measured in fluorimetry (excitation wavelength = 375 nm and emission wavelength = 520 nm). The spectrofluorometer was a Perkin Elmer 204 (Bois d'Arcy, France).

Free cysteine and complex cysteine/selenium. Assay of cysteine, cystine and complex was performed by HPLC with direct ultraviolet detection at 210 nm (Martinez et al., 1988). Standard solutions were prepared using crystalline amino acid with a concentration range from 0.18 to 0.72 μmol/ml. The chromatograph was a Hewlett Packard 1090 (Waldbronn, USA) equipped with a diode array detector. The column was a 5 μm, 250 × 4.6 mm ID, Rosil C8 cartridge (Altech, Eke, Belgium). Elution of cysteine and complex was obtained using an elution gradient (Table 4) in acetonitrile/water with sodium heptane sulfonate (4 g/l) as a counter ion, sodium sulfate (5 g/l) and sulfuric acid to pH 2.5 with a flow rate at 0.7 ml/min. Injection volume was 20 μl.

TABLE 4

Elution gradient used for the determination of amino acids by HPLC

t (min)	*A* (%)	*B* (%)
0	100	0
10	95	5
30	80	20
60	60	40

A = Water + heptane sulfonate + sodium sulfate
B = Water/acetonitrile (50/50) + heptane sulfonate + sodium sulfate
pH (A; B) = 2.5 (sulfuric acid)

TABLE 5

Se(IV) recovery for fluorimetric method (%) (n = 10) mean ± S.D.

TPN solutions	0
Se	98 ± 1.9
Se + trace element	105 ± 2.1
Se + ascorbic acid	43 ± 0.9
Se + ascorbic acid + trace element (without Cu(II))	41 ± 0.8
Se + ascorbic acid + trace element (with Cu(II))	20 ± 0.4
Se + dextrose	87 ± 1.7
Se + dextrose + electrolytes	62 ± 1.2
Se + amino acids	0
Se + methionine	105 ± 2.1
Se + cysteine	0

Limit of detection of Se(IV) = 2 μg/ml.

Results

Complete loss of Se(IV) was observed in TPN solutions. The influence of different nutrients has been studied.

The presence of trace elements such as copper (Cu(II)), chromium (Cr(III)), zinc (Zn(II)), iron (Fe(II)), manganese (Mn(II)) induced a little excess in Se(IV) (Table 5).

With ascorbic acid, important change of Se(IV) was found. Addition of Cu(II) ions to selenite solution containing ascorbic acid increased the loss of Se(IV) (Table 5).

Loss of Se(IV) occurred also with dextrose, and the results indicate a detectable change in Se(IV) recovery when the solution contains electrolytes as phosphate salts (Table 5).

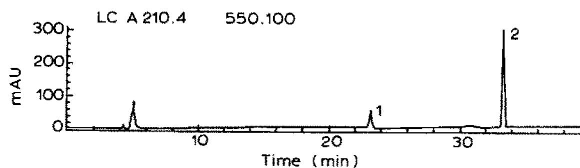


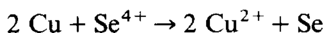
Fig. 1. Chromatogram selenite:cysteine mixture (1:4). 1, Cystine; 2, Seleniocystine complex.

Lastly, Se(IV) disappeared in amino acid and in cysteine solutions when it was fully recovered with methionine (Table 5).

Secondly trial mixtures of sodium selenite and cysteine in a molar ratio 1/4 were analyzed by HPLC. The chromatogram showed a cystine peak (retention time = 23 min) and an unknown peak (retention time = 33 min) but no cysteine peak (retention time = 18 min) (Fig. 1).

Discussion

Results indicate that selenium(IV) as selenite or selenious acid is not stable in TPN solutions. Ascorbic acid, by its strong reducing power, especially in the presence of Cu(II) as trace element, partly converted Se(IV) to Se. Influence of Cu(II) ions can be explained by the production of copper metal by reduction with ascorbic acid. The new compound itself can reduce selenium(IV) to selenium metal following the reaction:



$$2\ Cu + Se^{4+} \rightarrow 2\ Cu^{2+} + Se$$

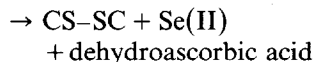
Shills and Levander (1982) also showed the influence of copper ions and ascorbic acid on the reduction of selenium. However, our results are not in agreement with the data of Mc Gee et al. (1985). According to the method used, selenium is converted into selenium(IV) by boiling with HCl 4 N. All chemical forms of selenium are determined like selenium by atomic absorption spectrometry. The method proposed here determines specifically selenium(IV).

Cysteine, present in amino acids solution, induces complexation process with selenium following the reaction:

$$\underset{\text{Cysteine}}{4\ CSH} + Se(IV) \rightarrow \underset{\text{Seleniocystine}}{CS{-}Se{-}SC} + \underset{\text{Cystine}}{CS{-}CS}$$

The complex is unstable, releasing cystine and selenium (lower state oxidation).

We can think that selenium present in the complex is reduced to selenium(II) analogically with amperometric assay of cysteine by copper ions (Pointeau and Bonaste, 1970).



$$CS{-}Se{-}SC + \text{Ascorbic acid} \rightarrow CS{-}SC + Se(II) + \text{dehydroascorbic acid}$$

Our method determines neither selenium in seleniocystine, nor Se(II). Interaction between selenium and cysteine leads first to the formation of seleniocystine complex and cystine, and then to the reduction of Se(IV) in Se(II) with supplementary cystine formation.

The selenium status of patients receiving total parenteral nutrition was soon widely described (Davis et al., 1987).

Plasma selenium level, erythrocyte and platelet glutathione peroxidase activity are achieved with selenium supplementation. A discordance should exist between complete loss of Se(IV) in total parenteral nutrition solutions and adequate selenium status. In fact, the incorporation pathways of Se into proteins is still under debate. However, most enzymes containing selenium, as glutathione peroxidase, include a residue of seleniocystine at their active site (Ursini and Bindoli, 1987).

The complex seleniocystine releases Se(II), a presumed intermediate of other metabolisms (Combs and Combs, 1988). The formation and the reduction of this complex do not have clear clinical or biological consequences. Another form of selenium is not used (e.g. selenomethionine).

Further investigations are required to evaluate the real biodisposition of seleniocystine, Se(II) and seleniomethionine in the body by parenteral injection.

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