Trace Elements & Toxic Metals

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Lecture outline

• Which elements?
• Essential elements: Cu, Zn, Se
• Iatrogenic elements: Al, Mn
• Elemental poisoning: Pb
• Elemental analysis: atomic spectroscopy

ESSENTIAL METALS

Na K Ca Mg Fe Cu Zn Se Co Cr Mn Mo

METALS USED IN THERAPY

Ag Al As Au B Bi Li Pt Ba Gd I Co Ti Mo V Hg Cr
INDUSTRIAL & ‘TOXIC’ METALS

Pb
Cd
Ni
As
Hg
Tl
Sb

Which elements? – the historical repertoire

• **Essential:** Cu, Zn, Se (serum); Cu (urine)
• **‘Toxic’:**
  - Al (plasma, water) – renal dialysis patients
  - Pb, Cd, Cr, As, Hg, Ni (blood, urine):
    - Industrial monitoring / occupational exposure
    - Asian & Far Eastern traditional medicines
    - Pica – primarily paediatric
  - Acute poisoning from accidental or deliberate ingestion of metals solutions or salts is uncommon

Which elements? – the more recent

• Cr, Co, Ni (blood, serum, urine, hip joint fluid)
• B (blood, plasma, brain, urine) – cancer chemotherapy
• Mn (blood) – overload during PN
• Pt (serum) – cancer chemotherapy
• Li (post mortem blood) – H.M. Coroner
• Li – blood spot analysis
• Br (serum) – therapeutic monitoring
• Ga, In, Te (urine) – occupational monitoring
• Tl (blood, urine, liver, faeces) – Iraqi poisoning episode
• Sb (blood) – treatment of Leishmaniasis
• I (serum) – betadine overdose
• Mo (serum, urine) – concern over dental implant.

Copper Function

• Catalytic co-factor & structural component for metalloproteins (~ 24 enzymes):
  - Cytochrome-c oxidase
  - Ferroxidases (in caeruloplasmain)
  - Cu-Zn superoxide dismutase
  - Amine oxidases
  - Tyrosinase (catechol oxidase)
• Post-absorption transport attached to albumin (not caeruloplasmin)
### Copper investigation

**Disease:** inherited disorder of Cu-transporting P-type ATPase

**Menkes:** gene expressed in all tissues except liver
defective intestinal absorption of Cu
deficiency of numerous Cu-containing enzymes

**Wilson’s:** gene expression liver, kidney to lesser extent
impairs: biliary excretion of Cu
incorporation of Cu into caeruloplasmin

**Nutrition:** malnutrition, malabsorption, burns
medical diets, parenteral nutrition
excess Zn intake

### Assessing copper status

| Serum/plasma [Cu]: | neonates – 4 months: | 1.6 – 7.9 μmol/L |
| | 4 – 6 months: | 4.7 – 17 μmol/L |
| | 7 – 12 months: | 7.9 – 20 μmol/L |
| | children > 6/12 & adults: | 11 – 25 μmol/L |
| | pregnancy > 15/40: | 25 – 39 μmol/L |

Influenced by:
- steroid hormones (inc HRT)
- acute phase response

Complemented by:
- caeruloplasmin
- 24 hour urine Cu for Wilson’s Disease
  (not affected by IUD’s)

Alternatives:
- Liver biopsy; ‘free’ copper

### Zinc Function

- Catalytic co-factor & structural component for metalloproteins (~ 200 enzymes):
  - Alkaline phosphatase
  - Alcohol dehydrogenase
  - RNA polymerase
  - Carbonic anhydrase

- Zinc finger proteins:
  - DNA transcription
  - Hormone receptors

### Zinc - clinical relevance

1. **Acrodermatitis enteropathica:** (Moynihan; 1974)
   - Rare, autosomal, recessive inherited disease of Zn absorption.
   - Identification of SLC39A4, a gene involved in AC
     Kury S et al. *Nat Gen* 2002; Jun 17
     - A novel member of a zinc transporter family is defective in AC

2. **Most common circumstances:**
   - Malnourishment; malabsorption; burns.
   - Special ‘medical’ diets; enteral & parenteral feeding.
   - Wound healing; loss of taste
Assessing zinc status

Serum/plasma:
- < 7 μmol/L: deficiency
- 7 – 11 μmol/L: Not significant
- 11 – 24 μmol/L: normal for all ages
- > 24 μmol/L: supplementation

Influenced by:
- Diurnal variation, fasting
- Pregnancy, malignancy, renal failure
- Low albumin, acute phase response

Complemented by: CRP, alkaline phosphatase

Alternatives: leucocyte zinc; serum ‘free’ zinc

Biological role of selenium

- Structural component of two amino acids:
  - Selenocysteine (animals)
  - Selenomethionine (plants)

- In animals (inc humans) all forms of ingested selenium can only be utilised following reduction to Se⁻ and metabolic incorporation into selenocysteine

Human selenoproteins

- Glutathione peroxidase: cytosolic, RBC
  (GSH-Px)
  - Plasma
  - Gastro-intestinal
  - Phospholipidhydroperoxide

- Iodothyronine 5’-deiodinase

- Thioredoxin reductases

- Selenoprotein P

- Selenoprotein N

Role of selenium in human health

- Deficiency linked to:
  - Heart disease
  - Cancer
  - Infertility
  - Just about everything!

- Recently, excessive supplementation linked to:
  - Increased incidence of Prostate cancer
  - Increased incidence of Type 2 diabetes
**Serum Selenium Reference Range (U.K.)**

- < 18 months: 0.4 – 0.7 μmol/L
- 18 months - 4 years: 0.6 - 1.1 μmol/L
- 5 - 16 years: 0.7 – 1.5 μmol/L
- Adults (> 16 years): 0.9 – 1.7 μmol/L

- ? Definition of reference range:
  - Empirical data for ‘normal healthy’ population or
  - Values related to functioning of biochemical expression

**ALUMINIUM TOXICITY IN CHRONIC RENAL FAILURE**

- Consequences:
  - Dementia
  - Osteomalacia
  - Anaemia

- Sources of aluminium:
  - Dialysis fluid & tap water
  - Phosphate binders

**NORMAL’ PLASMA [Al]-μg/L: 1964-1985**

- 1964 400 ± 277 Arch Env H; 8: 52 - 57
- 1970 200 - 300 Lancet; ii: 494 - 496
- 1977 6.0 ± 3.0 N Eng J Med; 296: 1389 - 1390
- 1979 140 ± 60 N Eng J Med; 301: 728 - 729
- 1985 17.3 ± 6.1 Analyst; 110: 19 - 20
- 1985 5 - 8 Clin Chim Acta; 147: 247 - 254
- (After Taylor et all, 1986)

**PLASMA [Al] REFERENCE VALUES FOR DIALYSIS PATIENTS (C.E.C)**

- < 10 μg/L Normal (no history of C.R.F.)
- < 60 μg/L Desirable in C.R.F. patients
- > 60 μg/L Excessive accumulation
- > 100 μg/L Cause for concern; high risk for children
- > 200 μg/L Urgent action required; high risk for all
- *C.E.C Upper limit for dialysis fluid: 30 μg/L*
Manganese

- Enzyme component (xanthine oxidase, Mn S.O.D.)
- Estimated Safe & Adequate Daily Intake: (USNRC)
  - Infants: 0.3 - 1.0 mg
  - Children: 1 - 3 mg
  - Adults: 2 - 5 mg
- Nervous system disorders & ? cholestatic disease related to Mn supplementation in patients on long-term PN.
- Paediatric Mn PN recommendations:
  1994: 55 µg/Kg/day (550 µg for 10 Kg child)
  1996: 1 µg/Kg/day (10 µg for 10 Kg child)
- ? Remove supplemented Mn altogether

Lead ingot ‘prescribed’ for treatment of stammer.
(‘Suck for 3 hours daily’)

PAEDIATRIC Pb POISONING FROM PICA
(female; 2.5 yrs)

- Date   Pb-µg/dL  Hb-g/dL  ZPP- µg/gHb  Pb- µg/g Hb
- norm   <10      11.5 - 13.5 <3.2     <0.7
- 9/9/98  48       8.5       42   5.65
- 9/2/99  60       7.9       61   7.59
- 11/5/99  27      7.4       58   3.65
- 20/7/99  26      10.9      13   2.39
- 12/10/99  20     11.3       8   1.77
Contamination

This is a major consideration in trace element investigation, particularly for:

• Al – glass, everything!
• Zn – gel tubes, O-rings, glove-powder
• Cr, Co, Mn – stainless steel, needles, tubes

Atomic Spectroscopy Definitions

• Atomic emission:
The process whereby an atom excited by thermal, chemical or electrical energy emits light of a characteristic wavelength during its return to a lower energy level.
• Atomic absorption:
The process that occurs when a ground-state atom absorbs energy in the form of light of a specific wavelength and is elevated to an excited state.
• Ionisation:
The process by which one or more electrons are removed from an atom yielding positively-charged ions.

Analytical Instrumentation

• Up to 2003: Flame AA (serum Cu, Zn; urine iron)
  CVG-AA (blood, urine Hg)
  Furnace AA (everything else)
• April 2003: ICP-MS acquired (7500c)
• April 2009: 7500cx acquired
• April 2010: Mass Hunter software for both instruments
  All analyses now by ICP-MS
Inductively-Coupled Plasma

A plasma is a volume of partially ionised gas (Ar) coupled to a high frequency electromagnetic radiation.

The plasma is initiated by seeding from a high voltage spark. Electrons interact with the magnetic field, gain energy and ionise the flowing argon stream by collisional excitation. Power applied via the induction coil allows the plasma to become self-supporting.

Inductively-Coupled Plasma - 2

Very high temperatures:

6000°K  ------------------------------10,000°K
atomic emission
ionisation
ICP-AES
sensitivity – medium to high
specificity – high
multi-element
ICP-MS
sensitivity – very high
specificity – very high
multi-element
isotopic analysis

ICP-MS: an isotopic technique

- Al: 27 (100%)
- As: 75 (100%)
- Cu: 63 (69%), 65 (31%)
- Zn: 64 (49%), 66 (28%), 67 (4%), 68 (19%), 70 (<1%)
- Se: 74 (<1%), 76 (9%), 77 (8%), 78 (24%), 80 (50%), 82 (9%)
- Pb: 204 (1%), 206 (24%), 207 (22%), 208 (52%)