Moderate drinking and the ageing brain; a potential fit for smart ageing? Effects of the consumption of non-alcoholic beer

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ALZHEIMER'S DISEASE

The most common form of dementia in the elderly

There is no clear consensus on the preventive measures available to prevent the development of this disease
AMYLOID HYPOTHESIS:

Accumulation of amyloid-ß peptides
Highly toxic

Formation:

- Neurofibrillary tangles
- Amyloid plaques

APP Molecüla
Enzimas
Placa Beta-Amiloide
ALZHEIMER'S DISEASE

CHOLINERGIC HYPOTHESIS

Correlation between cholinergic deficiency and loss of patients' cognitive abilities

INHIBIDORES DE LA COLINESTERASA CEREBRAL

Mejoran el rendimiento cognitivo, los defectos funcionales y los trastornos de la conducta. Indicados en la fase leve-moderada.

- Tacrina: en desuso por RAM
- Donezepilo: inhibidor de la acetilcolinesterasa
- Rivastigmina: inhibidor de la acetilcolinesterasa y de la butilrilcolinesterasa
- Galantamina: inhibidor de la acetilcolinesterasa y modulador de receptores nicotínicos de la Ach
ALZHEIMER'S DISEASE

FREE RADICAL HYPOTHESIS

ROS $\rightarrow$ LIPIDS PROTEINS DNA

Brain: limited antioxidant capacity
ALZHEIMER'S DISEASE

ANTIOXIDANT ENZYMES
- Superoxide dismutase
- Catalase
- Glutathione peroxidase

NON-ENZYMATIC ANTIOXIDANT AGENTS
- Albumin, bilirubin
- Uric acid, lycopene
- Vitamin A, vitamin C, vitamin E

NEUROINFLAMMATORY FACTORS
- Immunoproteins and cytokines: generated by neurons, astrocytes and microglia
ALZHEIMER'S DISEASE

New development strategies

Antioxidant drugs

Free radical uptake drugs

Neuronal anti-inflammatory drugs
Neurotoxicity mechanisms

Cell damage
Free radicals
Oxidative stress

Biphasic action
Pro-oxidant (Fe)
Antioxidant enzyme inhibition
ROS: peroxidation

Cholinergic system
Increased AchE activity
ALZHEIMER'S DISEASE

Protective factors

IMPACT ON THE DISEASE BY

- Preventing its development
- Delaying the onset
- Slowing its progression
- Reducing its symptoms
**BEER**

One of the most consumed alcoholic beverages worldwide

<table>
<thead>
<tr>
<th>Rich in nutrients</th>
<th>Link with AD: intake could provide an alternative for preventing this pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Carbohydrates, amino acids, minerals, vitamins, polyphenols</td>
<td>• Silicon: one of the main sources of this element in the diet</td>
</tr>
<tr>
<td>• Different in vitro biological activities: antioxidant, anti-carcinogenic, anti-inflammatory, estrogenic and antiviral</td>
<td>• Antioxidant substances contained in hop</td>
</tr>
</tbody>
</table>

Antioxidant substances contained in hop

![Image of beer]
SILICON

Possible therapeutic effects

- Maintaining the structural integrity of the nails, hair and skin
- Global collagen synthesis
- Mineralisation and bone health
- Reducing the accumulation of metals in Alzheimer's disease
- Immune system health
- Reducing the risk of atherosclerosis
Orthosilicic acid-Aluminium interaction

The simultaneous administration of silicic acid and Al reduces the absorption of Al and increases its clearance from the body.

Formation of highly insoluble and inabsorbable hydroxialuminisilicates.

Clinical human trials: administration of mineral water rich in silicic acid.

Increased urinary excretion of aluminium.

Significant reduction in the aluminium body burden.

Possible use of silicic acid as a long-term non-invasive therapy to reduce the body burden of Al, decreasing the possibility of suffering Alzheimer’s disease.

Exley et al., 2002; Jurkič et al., 2013.
Hypothesis

Si could limit the bioavailability of Al by decreasing absorption in the gastrointestinal tract.

Objective

To determine the effect of beer consumption on the bioavailability of Al, and the possible link in preventing its neurotoxicity.
Acute study: 3 days
- **Types**: Alcoholic and non-alcoholic beer
- **Dose**: Moderate-low (0.5 l/day)
  Moderate-high (1 l/day)

Chronic study: 3 months
- Type and dose of the most effective beer

**Work plan**
- NMRI male mice
- Urine, faeces and brain collection
- Intracardiac blood extraction
- Wet mineralisation of organic matter
  - Determination of Al ➔ ICP-MS
  - Determination of Si ➔ ICP-OES
Intake of alcoholic beer, due to its silicic acid content, interferes with Al absorption and excretion kinetics by decreasing its bioavailability

González-Muñoz et al., 2007
CHRONIC STUDY: EXPERIMENTAL PROTOCOL

**Negative control**
- Deionised water

**Positive control**
- 450 mg/L Al(NO₃)₃

**Positive group with alcoholic beer**
- 450 mg/L Al(NO₃)₃ + Beer

**Positive group with silicic acid**
- 450 mg/L Al(NO₃)₃ + 50 mg/L of silicic acid
CHRONIC STUDY: EXPERIMENTAL PROTOCOL

EXPERIMENTAL DESIGN

- Metals in brain and blood
- Gene expression of inflammatory mediators
- Inflammatory factors
- Enzyme activity
- Lipid peroxidation

- TNFα
- IL-6
- SOD
- GPX
- CAT
- GR

- Al → ICP-MS
- Si → ICP-OE

- TBARS
- GSH
Beer consumption reduces cerebral oxidation caused by aluminum toxicity by normalizing gene expression of tumor necrotic factor alpha and several antioxidant enzymes


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Si was able to block the proinflammatory and prooxidant actions induced by Al

González-Muñoz et al., 2008

Hypothesis to use beer as an effective prophylactic measure to prevent the development of AD

However, its alcohol content, although not very high, would limit its recommendation to the entire population
EFFECT OF NON-ALCOHOLIC BEER ON GENE EXPRESSION AND ACTIVITY OF CEREBRAL INFLAMMATORY MARKERS AND ANTIOXIDANT ENZYMES

Possible effect of non-alcoholic beer and its ingredients (Si and hop)

Neuronal toxicity

Pro-inflammatory effects

Pro-oxidant effects
## Specific objectives

**To evaluate the degree of cerebral oxidative stress produced by Al and determine the effect of non-alcoholic beer and its components**
- Glutathione titration
- Levels of lipid peroxidation (TBARS)

**To quantitatively evaluate the effect of non-alcoholic beer and its components:**
- Enzyme activity, levels and gene expression of antioxidant defence mechanisms (SOD, CAT, GPx and GR) in the brain of rats intoxicated with Al

**To determine the possible involvement of inflammatory mediators**
- TNFα and IL1β in the brain tissue of rats intoxicated with Al

**To evaluate the neurological progression of animals that have received the different treatments**
- Specific behavioural tests: study of the neurological damage induced by Al and determination of the preventive capacity of beer and its components to alleviate these problems
EFFECT OF NON-ALCOHOLIC BEER ON GENE EXPRESSION AND ACTIVITY OF CEREBRAL INFLAMMATORY MARKERS AND ANTIOXIDANT ENZYMES

In vitro tests

Antioxidant capacity
FRAP
DPPH
ORAC

Polyphenol content in beer and hop

Acetylcholinesterase activity
EFFECT OF NON-ALCOHOLIC BEER ON GENE EXPRESSION AND ACTIVITY OF CEREBRAL INFLAMMATORY MARKERS AND ANTIOXIDANT ENZYMES

FRAP: Fe uptake

DPPH: DPPH radical uptake

ANTIOXIDANT CAPACITY

ORAC: free radical uptake
Si demonstrated a capacity to inhibit AChE activity *in vitro*. Possible new mechanism to reverse Al neurotoxicity.
EFFECT OF NON-ALCOHOLIC BEER ON GENE EXPRESSION AND ACTIVITY OF CEREBRAL INFLAMMATORY MARKERS AND ANTIOXIDANT ENZYMES

In vivo tests

**Negative control:** Desionized water

**Positive control:**
- Aluminum: 450 μg/kg/day Al(NO₃)₃
- AI + non-alcoholic beer: 450 μg/kg/day Al(NO₃)₃ Beer

**Aluminium + hop:**
- 450 μg/kg/day Al(NO₃)₃
- 0.4 mg hop extract

Aluminium + silicon:
- 450 μg/kg/day Al(NO₃)₃
- 50 mg/L silicic acid

Beer and Health
In vivo tests: Behavioural tests

- Hole board
- Forced swimming
In vivo tests: Behavioural tests

Variables indicative of motor activity, exploration and emotionality are recorded.

- Grooming
- Defecation index
- Immobility
- Curiosity
- Rearing
**In vivo tests: Behavioural tests**

- **Forced swimming**

  Allows the capacity for learning and memory to be evaluated.

  Swimming time and immobility time is recorded.
HOLE BOARD TEST

Non-alcoholic beer, Si and hops alleviate the behavioral changes produced by Al
Al increase the learning time of animals

Acetylcholinesterase activity

Beer and Si neutralize this effect, obtaining better results than the control animals

The acetylcholinesterase inhibitory capacity of Si
EFFECT OF NON-ALCOHOLIC BEER ON GENE EXPRESSION AND ACTIVITY OF CEREBRAL INFLAMMATORY MARKERS AND ANTIOXIDANT ENZYMES

- Gene expression of enzymes and inflammatory mediators
- Ex vivo tests
- Metals in brain and blood
- Lipid peroxidation
- Enzyme activity
  - SOD
  - GPX
  - CAT
  - GR
  - GSH
- TNFα
- IL-1β
- Nrf2
- Al → ICP-MS
- Si → ICP-OE
- TBARS
- GSH
Hop has no effect at this level: mechanism of action different from chelation

Non-alcoholic beer and silicic acid reduce the accumulation of Al in the brain

Possible use as a long-term non-invasive therapy to reduce Al in patients with AD
Effect of non-alcoholic beer and its components on lipid peroxidation: TBARS, glutathione and redox index

Pattern of oxidative stress induced by Al

Si significantly decreases lipid peroxidation: neuroprotective

Antioxidant capacity of hop: resveratrol and xanthohumol
La cerveza sin alcohol resulta más efectiva en la reducción de los niveles de TBARS y GSSG y en el incremento de GSH.

Mayor capacidad antioxidante observada in vitro.
Effect of non-alcoholic beer and its components on TNFα levels

- Increased TNFα ➔ inflammatory response of the CNS
- Interaction with the cholinergic system

- Si ➔ decreased Al body burden (HAS)

- Hop ➔ anti-inflammatory effect exerted by polyphenols
The expression and activity of antioxidant enzymes altered by the administration of Al is normalised by treatment with non-alcoholic beer and its components. Silicon, hop and beer are able to improve antioxidant defence in the brain.
Conclusions

Due to the capacity of its components to alleviate the neurodegenerative effects induced by Al intoxication

Moderate consumption of non-alcoholic beer provides an effective prophylactic measure for preventing the onset and development of AD
Gracias por su atención