The effects of a food product containing lactic acid on the activity of acetylcholinesterase

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Abstract
Objective: Patients reported that a food product containing lactic acid improved their memory and thought processes. The ingredients of the tested food product are compound substances and smooth muscle fibre, the appropriate medium in which to analyse their effects. Acetylcholinesterase inhibitors are used to treat memory loss and failing thought performance. The aim of this study was to compare the effects of the lactic acid food product with the effects of acetylcholinesterase inhibitors.

Methods: In this in vitro study the effects of the food product containing lactic acid on smooth muscle fibres of guinea pig stomach were investigated.

Results: The results show that the lactic acid food product contains substances that can inhibit the activity of both acetylcholinesterase and butyrylcholinesterase. This inhibitory effect was compared to the inhibitory effects of galantamine (Nivalin®), pyridostigmine bromide (Kalymin®) and donepezil hydrochloride (Donepezil®), which are clinically used for the pharmacological treatment of dementia. We observed a 5% to 20% less potency factor difference with the lactic acid food product compared to that of the pharmaceutical drugs.

Conclusions: This proves that the lactic acid food product can also have an impact on memory and thought performance and that these results should promote clinical trials to test efficacy.

Introduction
The clinical effects of the lactic acid food product (Kanne Brottrunk® GmbH & Co. Betriegsgesellschaft KG, Selm-Bork, Germany) have been described in a wide range of articles and publications. This lactic acid food product (LAF), which we have also examined in our research, is composed of compounds similar to that of different extracts and tinctures of plant origin. As is well-known, compounds often have multiple effects on the organism.

In a randomised cohort study e.g., the LAF was used successfully as a prophylactic against colds and flu [1]. In an observational study with cross-over design, the use of the LAF in treating non-insulin dependent diabetes mellitus resulted in a significant decrease in the HbA1c value [2]. Furthermore, two observational studies showed that, with the application of the LAF, the symptoms of psoriasis [3] and of hay fever [4] can be significantly reduced. As mentioned above, there is clinical evidence that the LAF improves memory and the thought processes [5].

For some diseases, such as senile dementia or Alzheimer’s, there are special drugs that induce the inhibition of acetylcholinesterase (AChE). Parasympathomimetic substances derived from cholinesterase inhibitors, i.e. galantamine (Nivalin®) as an indirect parasympathomimetic, pyridostigmine bromide (Kalymin®) and donepezil hydrochloride (Donepezil®), are all used therapeutically to suppress the effects of acetylcholine (ACh)[6].

It was therefore also the aim of this study to examine whether substances which suppress the activity of acetylcholinesterase and butyrylcholinesterase are to be found in the LAF. Smooth muscle fibre is a suitable medium for impact analysis of biologically active substances, especially of multi-component mixtures, because it not only possesses almost all the receptors for hormones and mediators but also releases biologically active substances such as different forms of prostaglandins.

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Materials and methods

Substances

Food product containing lactic acid

Table 1 shows an overview of the nutritional value of the tested LAF product. The investigations were carried out in the Institute for Food and Environment, Ahrensburg, Germany, in 2010 [7].

Substances used in in vitro testing

- Tris Buffer, 100 mM, pH 7.5, molecular biology grade (Merk KgaA, Darmstadt, Germany)
- Butyrylcholine jodide (Sigma-Aldrich Corp., St.Louis, MO, USA)
- Acetylcholine jodide (Sigma-Aldrich Corp., St.Louis, MO, USA)
- Substances for the Krebs solution: NaCl, KCl, MgCl, 6H2O, glucose, KH2PO4, NaHCO3, CaCl2 (Merck, Darmstadt, Germany)
- Galantamine (Nivalin®, Sofarma AD, Sofia, Bulgaria)
- Donepezile hydrochloride (Donepezil®, Actavis EAD, Sofia, Bulgaria)
- Pyridostigmine bromide (Kalymin®, AWD Pharma, Radebeul, Germany)
- Atropine (Berlin-Chemie AG, Germany)

Determination of acetylcholinesterase and butyrylcholinesterase activity

The smooth muscle fibres (SMF) of the guinea pig stomach (GPS) was incubated in a 20 ml organ bath with 50 and 100 µl of the LAF respectively for 20 min at 35°C. The control samples were then incubated under the same conditions but without the addition of the LAF. Next, the SMF was homogenized in a Tris-HCl-buffer (5·10⁻³ M EDTA, 10⁻¹ M ethylmaleimide, 1 M NaCl, 1% Triton X-100) in a ratio of 1:25 mg/ml buffer in an ice bath at 0°C. The extent of acetylcholinesterase activity was determined by the Ellman method using a Cary 1 spectrophotometer (Varian, Melbourne, VIC, Australia) [8].

Results

In Fig.1, the effects of 50 µl LAF and 10⁻⁵ M ACh are shown on the SCA of the SMF guinea pig stomach. The effect of 10⁻⁵ M ACh shows the
maximum contractile activity of the SMF. We see that what was achieved with 50 µl LAF (in 20 ml organ bath) was an activation of the SCA of the SMF of more than 50% of the maximal contractile activity of the SMF. Again in Fig.1, we also see the effects of various concentrations of LAF on the SCA of the SMF and that the maximum stimulating effect attained at a concentration of 100 µl is about 80% of the maximum concentration of the SMF.

Fig.2 shows the stimulating effects of LAF and 15 µl 10⁻⁵ M ACh on the SCA of the SMF. Also in Fig.2, the same effects with the addition of 10⁻⁵ M atropine are shown. We see that with the prior suppression of the ACh receptors with atropine 10⁻⁵ M, the stimulating effects of LAF are completely inhibited.

In our preliminary experiments with electrical field stimulation, we demonstrated that the stimulating effects of the LAF on the SCA of the SMF are most likely a consequence of the suppression of the AChE. Based on these considerations, we first examined the effects of the LAF on AChE activity and then followed by comparing the effects of galantamine (Nivalin®), pyridostigmine bromide (Kalymin®) and donepezil hydrochloride (Donepezil®).

In Fig.3, the effects on the activity of the AChE of 10 µl LAF and 6.10⁻⁶ M galantamine (Nivalin®) (below) are shown. We see that both substances cause an inhibition of AChE. The inhibition of galantamine (Nivalin®) is more pronounced.

Fig.4 demonstrates the comparison of the suppressive effects on AChE activity of 5.10⁻⁶ M pyridostigmine bromide (Kalymin®) and 6.10⁻⁶ M donepezil hydrochloride (Donepezil®).
Figure 3. Change of activity of acetylcholinesterase under normal conditions after prior addition of 10 μL LAF (above) and after prior addition of 6.10^-6 M galantamine (Nivalin®)(below).

Figure 4. Change of activity of acetylcholinesterase under normal conditions with prior addition of 5.10^-6 M pyridostigmine bromide (Kalymin®)(above) and after prior addition of 6.10^-6 M donepezil hydrochloride (Donepezil®)(below).

Table 2. Change of activity of acetylcholinesterase with different inhibitors

<table>
<thead>
<tr>
<th></th>
<th>Control value</th>
<th>10 μL LAF</th>
<th>Galantamine (Nivalin®) 6.10^-6 M</th>
<th>Pyridostigmine bromide (Kalymin®) 5.10^-6 M</th>
<th>Donepezil hydrochloride (Donepezil®) 6.10^-6 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>[μg]</td>
<td>0</td>
<td>340</td>
<td>35</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td>dA/min</td>
<td>0.4598</td>
<td>0.2622</td>
<td>0.2085</td>
<td>0.2179</td>
<td>0.1923</td>
</tr>
<tr>
<td>Specific activity of AChE [U/mg]</td>
<td>8.1225</td>
<td>4.5312</td>
<td>3.6905</td>
<td>3.8568</td>
<td>3.4037</td>
</tr>
<tr>
<td>Decrease of AChE activity [%]</td>
<td>42.89</td>
<td>54.57</td>
<td>52.51</td>
<td>58.09</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows the tabulated results shown in Figs.3&4. We see that the inhibitory effects of LAF on AChE activity are only 10% to 16% less than that of other AChE inhibitors.

In Fig.5 we see the results of the inhibitory effects on the activity of butyrylcholinesterase with the addition of 10 μL of LAF and 6.10^-6 M galantamine (Nivalin®). We see, that with the addition of LAF, the suppression of the activity of butyrylcholinesterase is more pronounced.

Fig.6 shows the comparative effects of pyridostigmine bromide 5.10^-6 M (Kalymin®) and 6.10^-6 M donepezil hydrochloride (Donepezil®) (below) on the activity of butyrylcholinesterase. It can be noted that both substances suppress the activity of butyrylcholinesterase. Although the effect of the donepezil hydrochloride (Donepezil®) is slightly more pronounced.
Figure 5. Change of activity of butyrylcholinesterase under normal conditions with prior addition of 5.10^{-6} M LAF (above) and after prior addition of 6.10^{-6} M galantamine (Nivalin®) (below).

Figure 6. Change of activity of butyrylcholinesterase under normal conditions with prior addition of 5.10^{-6} M pyridostigmine bromide (Kalymin®) (above) and after prior addition of 6.10^{-6} M donepezil hydrochloride (Donepezil®) (below).

Table 3. Change in activity of butyrylcholinesterase with the addition of different inhibitors

<table>
<thead>
<tr>
<th></th>
<th>Control value</th>
<th>10 μl LAF (Nivalin®) 6.10^{-6} M</th>
<th>Pyridostigmine bromide (Kalymin®) 5.10^{-6} M</th>
<th>Donepezil hydrochloride (Donepezil®) 6.10^{-6} M</th>
</tr>
</thead>
<tbody>
<tr>
<td>[μg]</td>
<td>0</td>
<td>340</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>[dA/min]</td>
<td>0.0298</td>
<td>0.0178</td>
<td>0.0218</td>
<td>0.0224</td>
</tr>
<tr>
<td>Specific activity of butyrylcholinesterase [U/mg]</td>
<td>0.5274</td>
<td>0.3151</td>
<td>0.3858</td>
<td>0.3964</td>
</tr>
<tr>
<td>Decrease in butyrylcholinesterase activity [%]</td>
<td>40.25</td>
<td>26.84</td>
<td>24.83</td>
<td>38.26</td>
</tr>
</tbody>
</table>

The results for butyrylcholinesterase activity (Figs.5&6) are shown in Table 3. It can be seen that the inhibitory effects are more with LAF although the differences amount to no more than about 16%.

Discussion

The results in Fig.1 show that LAF possesses very high biological activity. Even at concentrations as low as 50 μl/l, there is still a significant stimulating effect occurring in the SCA of the SMF of the guinea pig stomach. The maximum stimulating effects were registered at a concentration of 5 ml/l. The stimulating effects reach about 80% of maximum contractile activity of the SMF, which can be obtained with 10^{-5} M ACh.

The results shown in Fig.1, demonstrates that for realization of the stimulating effects of LAF on the SCA sound structures of ACh receptors are required.
In case of inactivating the ACh receptors by use of $10^{-5}$ M atropine, the stimulating effects of LAF will be suppressed up to 100%. Therefore we can assume, that there are two modes of actions of these stimulating effects of LAF on the SCA of the SMF. Either LAF contains substances which can show agonistic effects on the ACh receptors or there are substances which increase the level of ACh in the extracellular space by suppressing the activity of AChE.

Our assumption that the stimulating effects of LAF on the SCA of the SMF are a result of the suppression of AChE have been confirmed by our present research. Our study of the comparative inhibitory effects of LAF, galantamine (Nivalin®), pyridostigmine bromide (Kalymin®) and donepezil hydrochloride (Donepezil®)(Table 2) on AChE showed that LAF produces a very pronounced inhibitory reaction on AChE, with a difference of a maximum of 16%. Butyrylcholine, which is found in the central nervous system, and it’s suppression by LAF was more than 14% when compared to that of pyridostigmine bromide (Kalymin®).

In light of these results further discussion must be promoted, in terms of clinical therapy, whether LAF can play a role as an AChE activity inhibitor. Only clinical studies can show whether and to what extent the in vitro effects of LAF have on clinical efficacy and in which stage of the disease. Moreover, it has to be proven whether LAF, in the future, can complement current esterase inhibitor treatment for senile dementia, degenerative diseases of the skeletal muscles and Alzheimer’s.

Acknowledgements/Competing Interests
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References