Maharishi Amrit Kalash, an ayurvedic medicinal preparation, enhances cholinergic enzymes in aged guinea pig brain

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The effect of orally fed Maharishi Amrit Kalash was examined on the activities of cholinergic enzymes in the guinea pig brain. The activity of the cholinergic enzymes viz. choline-acetyltransferase and acetylcholinesterase enzymes was found to be reduced significantly (P<0.05) in the various regions of CNS of the aged guinea pigs. Oral administration of MAK (500 mg/kg body weight daily) for 2 months significantly increased (P<0.05) the activity of choline acetyltransferase and acetylcholinesterase in the older animals. The present study indicates that this food supplement can be helpful in alleviating the cholinergic deficits in the old age.

Maharishi Amrit Kalash is an Ayurvedic medicinal preparation. It is manufactured in two forms MAK-4 and MAK-5. The main components of MAK are described elsewhere. In brief it contains Terminalia chebula, Phyllanthus officinalis, Becopa monniera, Withania somnifera, Ipomea digitata, embelia officinalis, etc. The chemical analysis of MAK has shown that it contains a large number of compounds namely, tannic acid, flavinoids, catecholamines, otocopherol, polyphenols, ascorbates, riboflavin, b-carotenes, mucillage, octacosanol, saponins, sphaeranthine, asparagine, glycyrhrizin, camphecone, limonene, pinene, etc. Some of the above-mentioned chemical components are potent antioxidants.

Previous experiments have shown that MAK reduces carcinoma in up to 88% of the experimental animals and causes regression of the 60% fully formed tumors, prevents lung cancer in metastasis in up to 65% of the animal’s tested; enhances lymphoproliferative response in antigen-stimulated animals as compared to control ones; reduces platelet aggregation induced by adenosine diphosphate, arachidonic acid, collagen, and epinephrine; inhibits the opioid receptors and reduces the level of substance P, alleviates depression and stress; inhibits the microsomal lipid peroxidation; decreases free radicals and reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radical, generated both in cellular (neutrophil) and non-cellular (xanthine-xanthine oxidase systems); protects against the mitochondrial deterioration in the ageing brain; increases the mitochondrial and cytosolic antioxidant enzyme activities and prevents neuronal lipofuscin accumulation.

A cholinergic deficit in the form of changes in the activities of the cholinergic neurotransmitter enzymes choline acetyltransferase and cholinesterase is often found in the ageing brain. Alterations in the cholinergic system are often considered a prominent feature of brain ageing. These changes have often been found to underlie age-related impairment in cognitive (memory-learning) abilities. Cholinergic abnormalities have also been observed in alzheimer victims. Since MAK is believed to retard ageing, enhance memory and maximize longevity, it would be of interest to determine the MAK’s influence on cholinergic neurotransmitter enzymes in the ageing brain. The results will provide information of the mechanism by which MAK may exert its therapeutic/pharmacological effects.

Materials and Methods

Male guinea pigs (Dunkin hartley) of two age groups (8 and 30 months) were used in the present
study. Each group was subdivided into two subgroups, of 40 animals each. One subgroup served as control and other was fed a mixture of MAK-4 and MAK-5 in the ratio of 1:20 in milk. The mixture was given with the help of a canula at a dose of 500-mg/kg-body weight daily at 11.00 hours for two months. The control animals received milk only. According to Charka Samhita there is no maximum length of time for which this drug can be taken. Both the groups were fed pelleted guinea pig food (Hindustan Lever Ltd., New Delhi) and water ad libitum.

Animals (experimental and controls) were decapitated and the brains and the spinal cords were removed immediately and rinsed in chilled normal saline. The different regions of the brain, viz. the cerebral cortex, the hypothalamus, the cerebellum and the brain stem (pons and medulla) were separated. The cerebral hemisphere without the cerebral cortex and hypothalamus has been named as rest of the cerebrum in the text. Tissue samples were homogenized (10%) in 0.32 M sucrose solutions using a glass homogenizer. The homogenates were centrifuged at 1000 g for 6 min at 4°C. Supernatants were saved and a portion was used for assaying the acetylcholinesterase activity. Pellets were resuspended and homogenized again, and then centrifuged at 1500 g for 5 min. The supernatants obtained from both the centrifugation steps were combined and centrifuged at 10,000 g for 15 min.

Acetylthiocholine iodide and acetyl CO A were purchased from Sigma Chemical Co., USA. Dithio-bisnitrobenzoic acid was purchased from Fluka Chemical Co., Switzerland. Other chemicals were purchased from the CSIR center for biochemicals, New Delhi, SRL or SD-line chemical Co. Mumbai, India and were of analytical grade. The Ayurvedic preparation MAK was a generous gift from the Maharishi Ayurveda Corporation Ltd., Faridabad, India.

Choline acetyltransferase (EC 3.1.1.7) was assayed in 10,000g supernatant by a standard method and the enzyme activity was expressed as µmole of coenzyme A(CO.ASH) formed/min/mg protein. Acetylcholinesterase was assayed in 1,000 g supernatant according to the method of Ellman et al. The enzyme activity was expressed as µmole of acetylthiocholine hydrolyzed/min/mg protein. Protein was assayed by the method of Lowry et al. The results were statistically analyzed by Student’s t test.

Results

Results are presented in Tables 1 and 2. The activity of both the enzymes ChAT and AchE, decreased significantly with age in the brain regions studied. The decline in the activity of ChAT followed a negative rostrocaudal pattern as the maximum decline of the activity was in the cerebral cortex (63.21%) and the lowest in the spinal cord (36.51%) followed by the cerebellum (25.39%). The treatment with MAK effectively increased the ChAT activity in all the regions of old animals. The maximum increase was in the cerebral cortex (88.19%) and hypothalamus (58.43%). In the case of young animals, MAK effect was seen only in the brain stem. The activity of AchE also declined with age in the old animals and the minimum decline was observed in cerebellum (15.92%) followed by spinal cord (25.11%). The treatment with MAK increased the AChE activity in the brain regions of old animals only. The highest increase in the activity was found in the hypothalamus (61.18%). Interestingly MAK increased ChAT activity only in brain stem of the young animals, rest of the regions did not show any effect of MAK on either of the enzymes activities. We have used a non-radioactive assay method for choline acetyltransferase. Our results do not deviate much from the activity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Young animals (10 months)</th>
<th>Old animals (32 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>±0.40 ±0.28</td>
<td>±0.25 ±0.36</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>±0.35 ±1.10</td>
<td>±0.30 ±0.30</td>
</tr>
<tr>
<td>Rest of the Cerebrum</td>
<td>±0.41 ±1.07</td>
<td>±0.14 ±0.50</td>
</tr>
<tr>
<td>Brain-stem</td>
<td>±0.43 ±0.17</td>
<td>±0.21 ±0.21</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>±0.20 ±0.21</td>
<td>±0.33 ±0.31</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>±0.33 ±0.31</td>
<td>±0.16 ±0.41</td>
</tr>
</tbody>
</table>

*Values are significantly higher than those of controls within the same age group (P<0.05)
**Values are significantly lower than those of young animals (P<0.05)
ChAT and AchE both with age in cerebellum of guinea pigs but the others have reported no change in explained by the studies on fish brain, in which ChAT activity declined in all the due to the different techniques but may be due to different animal model than ours. This can further be explained by the studies on fish brain, in which ChAT activity declined in all the regions.

Discussion
Data obtained in the present study showed that ageing decreased the activity of cholinergic neurotransmitter enzymes in the guinea pig brain. This is in agreement with studies on other animals. Dysfunction of cholinergic system plays a major role in memory loss in aged individual and many workers have established a positive correlation between the loss of cholinergic enzyme activities and decrement in learning and memory. The loss of cholinergic neurons is well documented in Alzheimer’s disease and other behavioral deficits that are more directly responsible for cognitive impairments. Thus the present findings are in agreement with the reports that a decrease in the cholinergic enzymes in CNS is a significant event in aging.

An increase in free radical attack during ageing may be responsible for the dysfunction of the cholinergic neurotransmitter enzyme system. Increase in membrane peroxidation adversely affects the release of acetylcholine in rats. Lipid peroxidation decreases membrane fluidity which, in turn, reduces choline uptake by 60-70% in the neurons of aged rats. The transport of choline across the blood-brain barrier is also decreased in aged rats. Thus, both the diminished availability of the precursor and the decreased activity of free choline acetyltransferase may be responsible for the diminished neurotransmitter synthesis in the ageing brain. Antioxidant treatments enhance the activity of cholinergic enzymes. We have earlier reported higher lipid peroxidation product accumulation in brain regions of guinea pigs, and MAK because of its antioxidant properties lowered the risk of lipid peroxidation in the brain of guinea pigs. Therefore, the enhanced activity of ChAT and AchE in the aged guinea pigs after MAK treatment may be attributed to the antioxidant action of MAK.

Many other herbal and synthetic formulations have been reported to be effective in enhancing the cholinergic enzyme activities in aged animals. For example Kami-Untan-To(KUT), a Japanese herbal drug increases the ChAT activity in aged mice in cortex, striatum and hippocampus only, but it was not effective in Cerebellum and spinal cord. Nimodipine enhanced the activities of both ChAT and AchE in the brain of 3 months as well as 11 months old mice, whereas MAK was very specific for its action on the aged animals only. Centrophenoxine another synthetic drug has been found to increase the AchE activity in the brain stem but not in cerebellum of the aged rats. Similarly N(5-hydroxyxicotinol)glutamic acid(ONK) also increases the cholinergic enzyme activity in both the young and older animals. Acetyl-L-carnitine treatment also increased the ChAT activity in the aged rat brains. The mechanism of action of MAK seems to be similar to the action of another herbal formulation Sho-sai-to-go-keishi-ka-shakuyaku-to (TJ-960), which has scavenging action against hydroxyl radicals, superoxide radicals and carbon centered radicals, decreases thiobarbituric acid reactive substance and increases SOD and cholineacetyltransferase activities in the hypothalamus and hippocampus.

Table 2—Effect of MAK on the activity of acetyl cholinesterase

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Young animals (10 months)</th>
<th>Old animals (32 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>28.90</td>
<td>±1.62</td>
</tr>
<tr>
<td></td>
<td>31.23</td>
<td>±0.69</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>33.52</td>
<td>±0.83</td>
</tr>
<tr>
<td></td>
<td>35.22</td>
<td>±1.08</td>
</tr>
<tr>
<td>Rest of cerebrum</td>
<td>30.04</td>
<td>±0.55</td>
</tr>
<tr>
<td></td>
<td>31.55</td>
<td>±0.40</td>
</tr>
<tr>
<td>Brain-stem</td>
<td>25.90</td>
<td>±0.61</td>
</tr>
<tr>
<td></td>
<td>27.65</td>
<td>±0.06</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>26.05</td>
<td>±1.00</td>
</tr>
<tr>
<td></td>
<td>29.48</td>
<td>±0.62</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>5.22</td>
<td>±0.99</td>
</tr>
<tr>
<td></td>
<td>27.79</td>
<td>±1.18</td>
</tr>
</tbody>
</table>

*Values are significantly higher than those of controls within the same age group (P<0.05)
**Values are significantly lower than those of young animals (P<0.05)
of aged rats. Similar effects for MAK on various antioxidants properties have been observed. MAK appears unique in its action as it increases the cholinergic activity in all the regions of brain tested in the older animals where cholinergic activity was significantly reduced. This appears to be a special property of MAK that it was effective only in the aged regions where normal homeostasis of the body is disturbed.

References