DECREASED CEREBROSPINAL FLUID MONOAMINE METABOLITES IN PATIENTS WITH ALZHEIMER'S DISEASE

J.C. García y A. González-Quevedo*

*División de Biotecnología y Biomedicina del Ministerio de Ciencia, Tecnología y Medio Ambiente, Avenida 17, No. 5008 entre 50 y 52, Playa, *Instituto de Neurología y Neurocirugía, Calle 29 y D, Vedado, Plaza de la Revolución, Ciudad de La Habana, Cuba.*

Recibido: 16 de agosto de 1996.

RESUMEN. Los estudios de las monoaminas en el líquido cefalorraquídeo (LCR) de pacientes con Enfermedad de Alzheimer (EA) han aportado resultados contradictorios. En el presente trabajo se midieron las concentraciones en LCR de 3-metoxi-4-hidroxifeniletilglicol (MHPG), ácido 5-hidroxindolacético (5-HIAA), ácido homovanílico (HVA) y ácido dihidroxifenilacético (DOPAC), metabolitos de la noradrenalina, serotonina y dopamina, por cromatografía líquida de alta resolución (HPLC) con detección electroquímica. Se obtuvo LCR de 18 pacientes con EA y de 16 sujetos sin enfermedad neurológica o psiquiátrica, a los que se les realizó punción lumbar para anestesia raquídea. Se observaron valores significativamente más bajos de MHPG, 5-HIAA y HVA en el LCR de pacientes con EA con respecto al grupo control. No se encontraron cambios en la concentración de DOPAC. Los niveles de monoaminas en el LCR no estuvieron significativamente asociadas con la severidad de la demencia. El estudio de las monoaminas en el LCR en la EA pudiera brindar más información acerca de la fisiopatología de la enfermedad y ofrece la posibilidad de controlar las terapias sustitutivas de neurotransmisores.

ABSTRACT. Studies on cerebrospinal fluid (CSF) monoamines in patients with Alzheimer's disease (AD) are controversial. In this work, CSF concentrations of 3-methoxi-4-hydroxi-phenylethylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and dihydroxyphenylacetic acid (DOPAC), metabolites of noradrenaline, serotonine and dopamine were measured by high performance liquid chromatography (HPLC) with electrochemical detection. CSF was obtained from 18 patients with AD and 16 subjects without neurological or psychiatric diseases, who underwent spinal anesthesia. Significantly lower levels of CSF MHPG, HVA and 5-HIAA were observed in AD patients with respect to the control group. No changes in CSF DOPAC concentration were demonstrated. CSF monoamine levels were not significantly associated with the severity of dementia. Studies of CSF monoamines in AD may provide further information for the pathophysiology of this disorder and offers the possibility of monitoring neurotransmitter replacement therapies.

INTRODUCTION

The etiology and pathogenesis of Alzheimer's Disease (AD) have not been elucidated, although many factors have been involved: abnormal protein processing, genetic aspects, infectious agents, neurotransmitter and neuropeptide changes, toxins and others.[1-3] Impairment of catecholaminergic, serotonergic[4] and cholinergic[5] activity have been reported in AD. Abnormalities of monoamine neurotransmitters and their metabolites have been encountered in the cerebrospinal fluid (CSF) of AD patients.[6-8] As neurotransmitter replacement therapies represent a short term hope for treating these patients, the establishment of CSF monoamine metabolite pattern is of importance as neurochemical markers for the follow up of pharmacological trials.

The purpose of this work was to study indirectly monoamine metabolism and its relation with the severity of the disease in a group of patients with AD, through CSF measurements of homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylethylglycol (MHPG), catabolites of dopamine, serotonine and noradrenaline.

MATERIALS AND METHODS

Eighteen patients with AD, diagnosed according to NINCDS-ADRDA criteria[9] (58.7 ± 10.7) years ; 8 women and 10me n) were studied. The severity of dementia was assessed with the Mini Mental State Examination and evaluated as slight, moderate, severe and profound. An age matched control group (5 women and 11 men) was randomly selected from a large control group previously reported.[10]

Sample preparation and biochemical analysis

The CSF was obtained by lumbar puncture (LP) between 8:00a nd 9:30a. m. The first 2 mL were immediately frozen at -70 °C . At the moment of assay, CSF was thawed, 200 µL were deproteinized with 60 µL of 0.4 mol/L perchloric acid and centrifuged at 4 °C and 10 000 r/min for 10 min . In CSF specimens from free patients of neuroleptic drug and controls from elective raquideal operation, simultaneous determination of MHPG, DOPAC, HVA and 5-HIAA was carried out with high performance liquid chromatography (HPLC) and electrochemical detection [10]. In brief, the HPLC apparatus consisted of a Knauer HPLC pump and injection valve, a Hypersyl ODS (120X4) mm I.D.5mp article size column, aM etrohm 641-VA electrochemical detector and a Shimadzu C-R6A Chromatopac integrator. The mobile phase was a buffer solution composed of 0.1 mol/L NaH_2PO_4, 0.46 mmol/L octanesulphonic acid and 0.6 mmol/L $EDTANa_2$ anda djustedt op H4 .3 with phosphoric acid. Flow rate was 1.0mL /min, and the electrochemical detector was set at a potential of 0.7 V and a sensitivity of 1 nA/V .

Data analysis

CSF monoamine metabolite levels were compared with the control group and according to the severity rating, employing the Kruskal-Wallis test.

RESULTS

Statistical analysis of the data demonstrated lower levels of MHPG, HVA and 5-HIAA ($p < 0.001$) in AD patients with respect to the control group and no changes in DOPAC (Table I).

TABLE I
CSF monoamine metabolites in patients with Alzheimer's Disease

Metabolites	Alzheimer's Disease (n=1 8)	Control (n=1 6)
	(ng/mL)	
MHPG	3.8 ± 2.1*	6.7 ± 1.8
5-HIAA	8.1 ± 4.1*	15.9 ± 5.0
DOPAC	O.6 ± 0.2	0.5 ± 0.2
HVA	15.0 ± 8.6*	24.0 ± 7.8

*p<0. 001.

CSF monoamine concentration was not significantly associated with disease severity (Table II).

DISCUSSION

AD is a chronic, slowly progressing, irreversible disorder that involves, in addition to the noted changes in presynaptic cholinergic function, substantial pathology of other neurotransmitter systems.[4,5]

Decreases in cathecolaminergic activity have been reported in AD, and data from postmortem brain and CSF also indicate impairment of the serotonergic system.[4]

For the evaluation of monoamine activity in man, the closest we can get is studying CSF monoamines and their metabolites.

Thus the importance of establishing the CSF monoamine pattern in this disease.

TABLE II
CSF monoamine metabolites in patients with Alzheimer's Disease according to severity

Severity	MHPG	5-HIAA	DOPAC	HVA
		(ng/ mL)		
Slight	3.2 ± 1.9	5.4 ± 1.7	0.9 ± 0.02	15.6 ± 1.2
Moderate	3.4 ± 1.7	9.7 ± 4.7	0.6 ± 0.2	17.7 ± 8.7
Severe	5.8 ± 2.7	9.5 ± 3.2	0.6 ± 0.4	17.3 ± 9.1
Profound	3.3 ± 1.4	5.8 ± 2.5	0.9 ± 0.4	15.3 ± 8.0
p	NSN	S	NSN	S

Kawakatsu et al[4] found lower 5-HIAA and HVA levels in the CSF of patients with AD, while other authors detected low HVA levels with normal 5-HIAA[5,6] and MHPG.[6] Elevated CSF MHPG has also been encountered.[11,12] Nevertheless, various authors have reported that CSF monoamine metabolite levels have no variation in AD.[13,14] Thus, the CSF monoamine status in AD is still controversial. Differences in experimental conditions could explain this. Indeed, several factors are known to influence CSF monoamine metabolite concentrations: age, sex, diet, motor activity, site of LP and CSF volume collected, body height, weight, time of the day, season.[10]

In the present study a decrease of CSF MHPG, 5-HIAA and HVA was found within a group of demented patients. These results suggest impaired monoamine function in these patients, as Kawakatsu et al also previously reported.[6] The differing results observed in AD by various authors may also reflect the problems that surround its diagnosis.

AD is currently diagnosed by excluding other possible causes of the observed cognitive and behavioral manifestations, as early diagnostic markers are still lacking. Thus, the possibility of including patients which may not be suffering AD exists.

Leee ta l.[7] reported that the CSF levels of HVA decreased in severe dementia, while 5-HIAA did not correlate with dementia severity. Other investigators[12] found that CSF MHPG correlated positively with ratings of dementia severity in AD patients. In our study no differences of CSF monoamine metabolite concentrations were observed according to the severity of dementia in AD patients. Similar results were obtained by Kawakatsu et al.[6] This could indicate that when clinical symptoms of AD appear, the neurochemical abnormalities, which accompany the neuronal degeneration have already been previously established, as in Parkinson's Disease.

Further studies of CSF monoamines in AD may provide potential implications for the pathophysiology and offers the possibility of monitoring neurotransmitter replacement therapies, which may be useful in treating the symptoms of AD.

Recently, some authors have launched new treatment schedules for patients with AD, in which the biochemical follow-up has proved to be very useful for the evaluation of the therapeutical approach.[15-17]

BIBLIOGRAPHY

1.

Davis R.E., Emmerling M.R., Jaen J.C., Moos W.H. and Spiegel K. Therapeutic intervention in Dementia. **Critical Reviews in Neurobiology, 7**, 41, 1993.

2.

Mullan M. and Crawford F. The molecular genetics of Alzheimer's Disease**. Mol. Neurobiol., 9**, 15, 1994.

3.

Itzhaki R.F. Possible factors in the etiology of Alzheimer's Disease. **Mol. Neurobiol., 9**,1 ,1 994.

4.

Nazarali A.J., Reynolds G.P. Monoamine neurotransmitters and their metabolites in brain regions in Alzheimer's Disease: A postmortem study. **Cellular and Molecular Neurobiology, 12**,5 81,1 992.

5.

Fibiger H.C. Cholinergic mechanisms in learning, memory and dementia: a recent review of recent evidence. **TINS, 14**,2 20,1 991.
6.

Kawakatsu S., Morinobu S., Shinohara M., Totsuka S. and Kobashi K. Acetylcholinesterase activities and monoamine metabolite levels in the cerebrospinal fluid of patients with Alzheimer's Disease. **Biol-Psychiatry, 28**, 387, 1990.

7. Lee S., Chiba T., Kitahama T., Kaieda R., Hagiwara M., Nagazumi A. and Terashi A. CSF beta-endorphin, HVA and 5-HIAA of dementia of the Alzheimer type and Binswanger's disease in the elderly. **J. Neural. Trans.**, Suppl., **30**, 45, 1990.
8. Martignoni E., Bono G., Blandini F., Sinforiani E., Merlo P. and Nappi G. Monoamines and relative metabolite levels in the cerebrospinal fluid in patients with dementia of Alzheimer's type. Influence of treatment with L-deprenyl. **J. Neural Transm. Park. Dis. Dement. Sect., 3**, 15, 1991.
9. Mckhann G. Clinical diagnosis of disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Services Task. Force on Alzheimer's Disease. **Neurol, 34**, 939, 1984.
10. González-Quevedo A., García J.C., Fernández R. and Hernández. Monoamine metabolites in normal human cerebrospinal fluid and in degenerative diseases of the central nervous system. **Bol. Estud. Méd. Biol. Méx., XLI**, I3, 1993.
11. Tohgi H., Ueno M., Abe T., Takahashi S. and Nozaki Y. Concentrations of monoamines and their metabolites in the cerebrospinal fluid from patients with senile dementia of the Alzheimer type and vascular dementia of the Binswager type. **J. Neural Transm. Park. Dis. Dementia** Sect., 4, 69-77, 1992.
12. Molchan S.E., Hill J.L., Mellow A.M., Lawlor S.A., Martínez R. and Sunderland T. The dexamethasone suppression test in Alzheimer's disease and major depression: relationship to dementia severity, depression and CSF monoamines. **Int. Psychogeriat, 2**, 99, 1990.
13. Hartikainen P., Reinikainen K.J., Soininen H., Sirvio J., Soikkeli R., Riekkinen P.J. Neurochemical markers in the cerebrospinal fluid of patients with Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis and normal controls. **J. Neural. Transm. Park. Dis. Dement**. Sect, 4, 53-68, 1992.
14. Nyback H., Nyman H., Blangvist G., Sjogren I. and Stone-Elander S. Brain metabolism in Alzheimer's dementia: studies of 11c-deoxyglucose accumulation, CSF monoamine metabolites and neuropsychological test performance in patients and healthy subjects. **J. Neurol. Neurosurg. Psychiatry, 54**, 672, 1991.
15. Bruno G., Scaccianoce S., Bonamini M., Patacchioli F.R.Cesarino F., Grassini P., Angelucci L. and Lenzi G.L. Acetyl-L-carnitine in Alzheimer's Disease: a short term study on CSF neurotransmitters and neuropeptides. **Alzheimer. Dis. Assoc. Disord., 9**, 128, 1995.
16. Pomara N., Stanley M., Lewitt P.A., Galloway M. Singh R. and Deptula D. Increased CSF HVA response to arecoline challenge in Alzheimer's Disease. **J. Neural Transm., 90**, 53, 1992.
17. Golden R. Dementia and Alzheimer's disease. Indications, diagnosis and treatment. **Minn. Med., 78**, 25, 1995.