Journal of Chromatography, 494 (1989) 278-282
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 4844

Note

Analysis of fatty acids in equine cerebrospinal fluid using gas chromatography with electron-capture detection

RAYMOND W SWEENEY*, JILL BEECH, ROBERT H WHITLOCK and PATRICIA L CASTELLI

Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine, 382 W Street Road, Kennett Square, PA 19348 (U S A)

(First received March 15th, 1989, revised manuscript received May 3rd, 1989)

Gas chromatographic (GC) analysis of cerebrospinal fluid (CSF) has been shown to be an accurate and rapid method for the diagnosis of certain neurologic conditions in man [1-6]. By GC detection of metabolic waste products of infectious pathogens, or host metabolic products produced in response to these pathogens, various forms of meningitis have been distinguished in man [3,4], and GC has been used to identify pathogens in experimentally induced bacterial meningitis in dogs [5].

Several neurologic diseases of horses are indistinguishable clinically, but the treatment and prognosis for these syndromes vary depending on the disease process [7-10] The purpose of this study was to investigate the possible diagnostic use of GC analysis of equine CSF The objective was to examine the fatty acid profiles of CSF from normal horses and compare these to profiles of CSF from horses with various neurologic diseases (diagnosis confirmed by post-mortem examination)

EXPERIMENTAL

CSF was obtained by lumbosacral or atlanto-occipital puncture from horses with neurologic diseases presented to the Widener Hospital for Large Animals Only samples from cases in which the diagnosis was later confirmed at post-mortem were used. Samples for this study were selected from horses with one

0378-4347/89/$03 50 © 1989 Elsevier Science Publishers B V

of three post-mortem diagnoses: equine protozoal myelitis (EPM), a common central nervous system infection caused by an unidentified protozoan [8], spinal cord compression (SCC) due to congenital malformation and/or misalignment of the cervical vertebral bodies [9]; and equine degenerative myeloencephalopathy/neuraxonal dystrophy (EDM/NAD), an idiopathic degenerative disease [10]. Samples were also obtained from neurologically normal horses for use as controls. Samples were frozen and stored at $-70°C$ until analyzed

Samples from nine horses with EPM, eight horses with EDM/NAD, five horses with SCC, and twelve normal horses were extracted and derivatized for analysis of fatty acids according to methods described by Brooks and co-workers [1,2,11] Briefly, the CSF sample was acidified and the fatty acids were extracted with chloroform Extracted free fatty acids were then derivatized to form electron-capturing compounds. Carboxylic acids were esterified with 2,2,2-trichloroethanol (TCE) catalyzed by heptafluorobutyric anhydride (HFBA) A 2-μl volume of derivatized sample was injected on a Hewlett Packard 5790A gas chromatograph with a 3390A integrator A 5% phenyl methyl silicone cross-linked fused-silica capillary column, 25 m$\times$0 32 mm I D, was used (Hewlett Packard) The oven was programmed for an isothermal hold at 100°C for the first 12 min following sample injection, followed by a 6°C/min rise to 265°C, and then a 6 min isothermal hold at 265°C. A frequency-pulsed electron-capture detector (Hewlett Packard) at 300°C was used The column carrier gas was hydrogen (2 ml/min) and the detector auxiliary gas was nitrogen (60 ml/min). The injection port temperature was 250°C and the injection split ratio was 50 1 Peaks were tentatively identified by comparison of retention times of sample peaks to those of derivatized fatty acid standards. Relative concentration, a semi-quantitative method of comparing sample concentrations, was determined by comparing the area under the sample peaks to the area under the internal standard peak (heptanoic acid, final concentration$=3\ 94\cdot10^{-6}$ mol/l)

RESULTS

Fig 1 shows the average relative concentration (expressed as a percentage of internal standard concentration) of the acids detected Fatty acids consistently present in CSF of all the normal and diseased horses were isobutyric, isovaleric, phenylacetic, lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), oleic ($C_{18\ 1}$), and stearic (C_{18}) Variably present were butyric (C_4), valeric (C_5), caproic (C_6), octanoic (C_8), nonanoic (C_9), decanoic (C_{10}), and pentadecanoic (C_{15}) acids. Unidentified peaks with 'equivalent chain length' (an interpolation based on retention times of standards eluted just prior and just after the unknown) of 4 4 and 7 8 were variably present Mass spectrometry was not available, but would have been helpful for identification of unknown peaks and

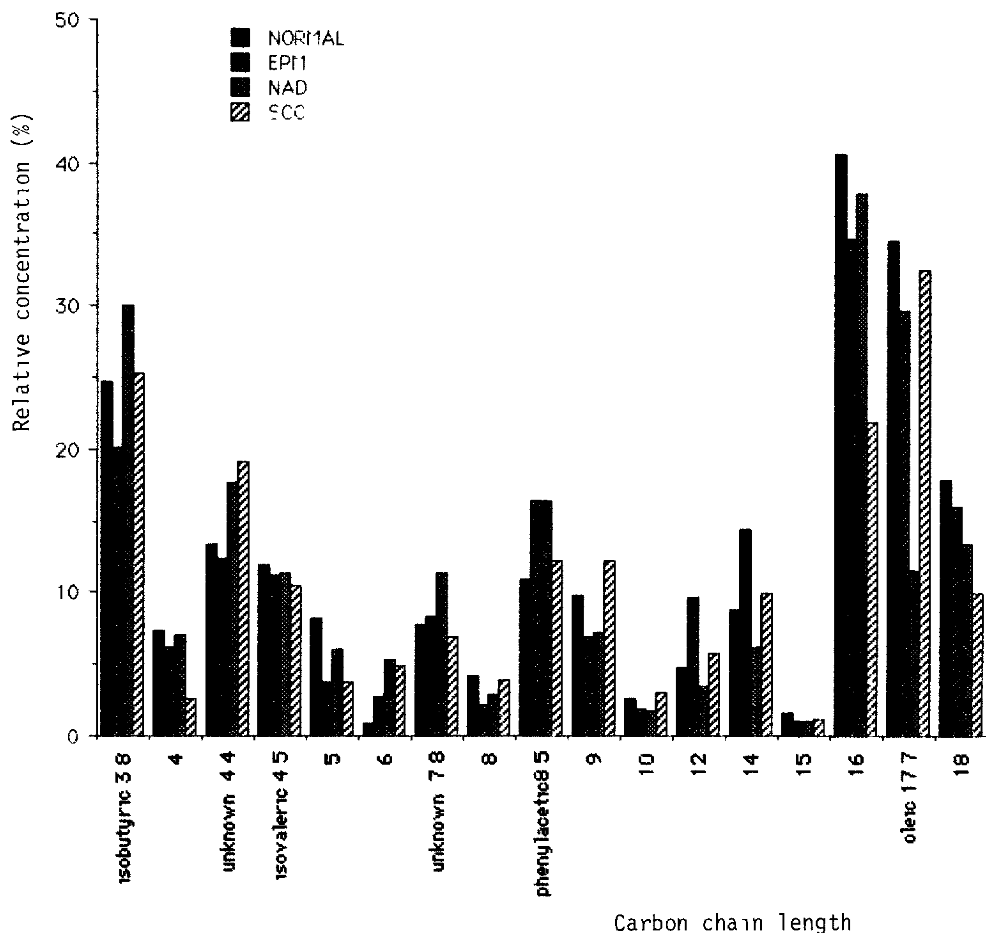

Fig 1 Relative concentrations (percentage of internal standard) of fatty acids in CSF from normal horses and horses with various neurologic diseases

confirmation of the identity of known peaks As can be seen in Fig 1, isobutyric, palmitic, and oleic acids were present in the highest concentrations. The same fatty acids were present in the CSF of normal and diseased horses, and the relative concentrations did not differ between groups

DISCUSSION

The fatty acid profiles determined for equine CSF in this study are similar to those reported for human CSF [12,13] Tichy and Skorkovska [12] reported that oleic, palmitic, and stearic acids predominate in human CSF, as was the case in this study Isobutyric and phenylacetic acids, though abundant in equine CSF, reportedly were not found in human CSF in that study, al-

though apparently the method did not permit identification of fatty acids with carbon chain length less than 12 As in this study, lauric, myristic, and pentadecanoic acids were present in human CSF in lesser quantities [12] Unsaturated fatty acids (other than oleic) were present in low quantities in human CSF and were not identified in this study Tichy and Skorkovska [12] also reported a wide variability in the fatty acid profiles of control subjects, with many of the compounds present in as few as 10% of the cases, and this was confirmed by other authors [12,14]. Similarly in the horses in this study, some of the compounds were variably present, notably butyric, valeric, caproic, octanoic, nonanoic, decanoic, and pentadecanoic acids

No 'marker' peaks were identified that would enable one to distinguish the CSF of diseased horses from that of the normal controls The inter-individual variation in fatty acid profiles for both control and diseased human patients limited the value of this procedure as a diagnostic test for certain diseases [12,15,16] However, GC has been most useful for the diagnosis of bacterial meningitis in human patients [3–6], but this is uncommon in the horse and was not investigated in this study. Also, as with certain meningitides of human beings, a more diagnostic value may be obtained from analysis of other fractions of CSF, such as amines or alcohols [1]

We conclude that the fatty acid composition of equine CSF appears similar to that reported for human CSF, but no clinically significant differences between normal horses and horses with EPM, EDM/NAD, or SCC were apparent

ACKNOWLEDGEMENTS

The authors acknowledge the generosity of Miss Margaret Gardiner for her financial support of this project. We wish to thank Linde Miller and the Hewlett Packard Co., Avondale Division for their gracious support including technical assistance and equipment, and Dr John B. Brooks of the Center for Disease Control for advice and assistance

REFERENCES

1 R B Craven, J B Brooks, D C Edman, J D Converse, J Greenlee, D Schlossberg, T Furlow, J M Gwaltney and W F Miner, J Clin Microbiol, 6 (1977) 27
2 D C Edman and J B Brooks, J Chromatogr, 274 (1983) 1
3 J B Brooks, D S Kellogg, M E Shepherd and C C Alley, J Clin Microbiol, 11 (1980) 45
4 J B Brooks, D S Kellogg, M E Shepherd and C C Alley, J Clin Microbiol, 11 (1980) 52
5 F M LaForce, J L Brice and T G Tornabene, J Infect Dis, 140 (1979) 453
6 H Thadepalli, P K Gangopadhyay, A Ansari, G D Overturf, V K Dhawan and A K Mandal, J Clin Invest, 69 (1982) 979
7 A de Lahunta, Veterinary Neuroanatomy and Clinical Neurology, W B Saunders, Philadelphia, PA, 2nd ed, 1983, p 205
8 B D Brewer, in N E Robinson (Editor), Current Therapy in Equine Medicine, W B Saunders, Philadelphia, PA, 2nd ed, 1987, p 359

9 P C Wagner, ın N E Robınson (Edıtor), Current Therapy ın Equıne Medıcıne, W B Saunders, Phıladelphıa, PA, 2nd ed , 1987, p 355
10 J Beech, ın N E Robınson (Edıtor), Current Therapy ın Equıne Medıcıne, W B Saunders, Phıladelphıa, PA, 2nd ed , 1987, p 353
11 J B Brooks, Laboratory Manual - Analytıcal Bacterıology Branch, Center for Dısease Control, Atlanta, GA, 1983
12 J Tıchy and I Skorkovska, J Chromatogr , 162 (1979) 185
13 J Tuna, J Logothetıs and R Kammereck, Neurology, 13 (1963) 331
14 M Farstad, Scand J Clın Lab Invest , 16 (1964) 139
15 J Tuna, J Logothetıs and R Kammerıck, Neurology, 13 (1963) 381
16 M Farstad, Scand J Clın Lab Invest , 16 (1964) 554