INCREASED URINARY EXCRETION OF A GLUCOSE-CONTAINING TETRASACCHARIDE IN PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY

Arne LUNDBLAD, Sigfrid SVENSSON, Ikuo YAMASHINA[+] and Mitsuhiro OHTA*

Department of Clinical Chemistry, University Hospital, S-221 85 Lund, Sweden, [+]Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, and **Research Laboratory, National Utano Hospital, Kyoto, Japan*

Received 20 October 1978

1. Introduction

Duchenne muscular dystrophy is inherited as a sex-linked recessive trait. The primary cause of the disease is unknown, but several hypotheses exist, suggesting myopathic [1], neuronal [2], vascular [3], and auto-immune [4] mechanisms. Evidence in favour of abnormal functioning of cellular membranes has been put forward and also that this membrane defect may be generalized [5–7]. Several reports about structural alterations of membranes [8–10], alterations of different membrane enzymes [11–13], and other membrane proteins [13–15] have appeared. Elevated levels of muscle enzyme activities (e.g., creatine kinase in serum) and their depletion in the muscle tissue reflect a leakage in the muscle-cell membrane.

Urinary excretion of various peptides in Duchenne muscular dystrophy was described in [16]. It was also observed that some of these peptide fractions differed in their ability to affect the growth of chick embryo-muscle fibroblasts in culture as compared with similar fractions from urine of normal individuals [16,17].

Urinary oligosaccharide excretion has been studied under different physiological conditions and in patients with various diseases [18]. It was recently reported that urine of a patient with glycogen-storage disease type II (Pompe's disease) had a dramatically increased excretion of a glucose-containing tetrasaccharide (89.6 mg/24 h urine) [19,20]: α-D-Glc-(1→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc$_4$, referred to here as (Glc)$_4$.

This oligosaccharide, which has been regarded as a 'limit' oligosaccharide in the extracellular breakdown of glycogen, is present in small amounts in normal urine. Greatly increased excretion of the oligosaccharide was also observed in several cases with glycogen-storage disease type III (9.4–45.2 mg/24 h urine) [20]. Since muscular dystrophy is a predominant symptom in Pompe's disease it was decided to investigate some individuals with muscular dystrophies of different etiology. A moderately increased excretion was found in two patients with Duchenne muscular dystrophy and in three patients with unclassified muscular disease [20]. This investigation has now been extended to include 20 Japanese patients of different ages with Duchenne muscular dystrophy of varying severity.

2. Experimental

Urine samples (24 h) were collected from each of the patients studied. The urines were freeze-dried and transported to Sweden where they were reconstituted and passed through a mixed-bed ion-exchange resin. To 1/10th of the desalted 24 h urine portions 200 μg isomaltotetraose (IM$_4$) were added as an internal standard and the sample was reduced and methylated. The permethylated oligosaccharides were analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS). Details concerning this procedure have been published in [20]. The identification was based on identical retention time and mass spectrum as compared with those of an authentic compound. Quantitation of individual oligosaccharides was done by comparing the GLC-peak areas with those of the internal standard.

3. Results and discussion

Figure 1 shows a typical GLC-tracing obtained from subject SH. The dietary-dependent B-trisaccharide (B-tri) and B-pentasaccharide (B-penta) [18] demonstrate the subject SH is a B-secretor. The tetrasaccharide region is dominated by 3-sialyllactose (SL), $(Glc)_4$ and the internal standard (IM_4).

Apart from the regularly increased level of $(Glc)_4$, no other abnormalities were observed in these patterns. The excretion rate of $(Glc)_4$ in the 20 patients is given in table 1. The mean excretion rate was 4.9 mg/24 h and the range 3.3–12.0 mg/24 h, which in all cases is above normal. No certain correlation to age, ABO-blood group or severity of the disease was observed. The highest value (12 mg) was seen in a patient with relatively mild symptoms (severity level 4), whereas the most severe cases studied (severity level 7) had excretion rates lower than the mean value for the whole group.

One can only speculate about the origin of $(Glc)_4$ in these patients, but it seems likely that in analogy with muscular enzymes, glycogen is leaking out from muscle cells to a higher extent in the patients than in normals. Patients with the largest remaining muscular mass would then be expected to have the highest excretion levels. The glycogen which is released to the circulation is then degraded by serum and/or urine amylase to give this 'limit' tetrasaccharide. The amylase action on glycogen has been demonstrated in vitro and under certain conditions $(Glc)_4$ can be formed [21]. The increased level of $(Glc)_4$ is most likely

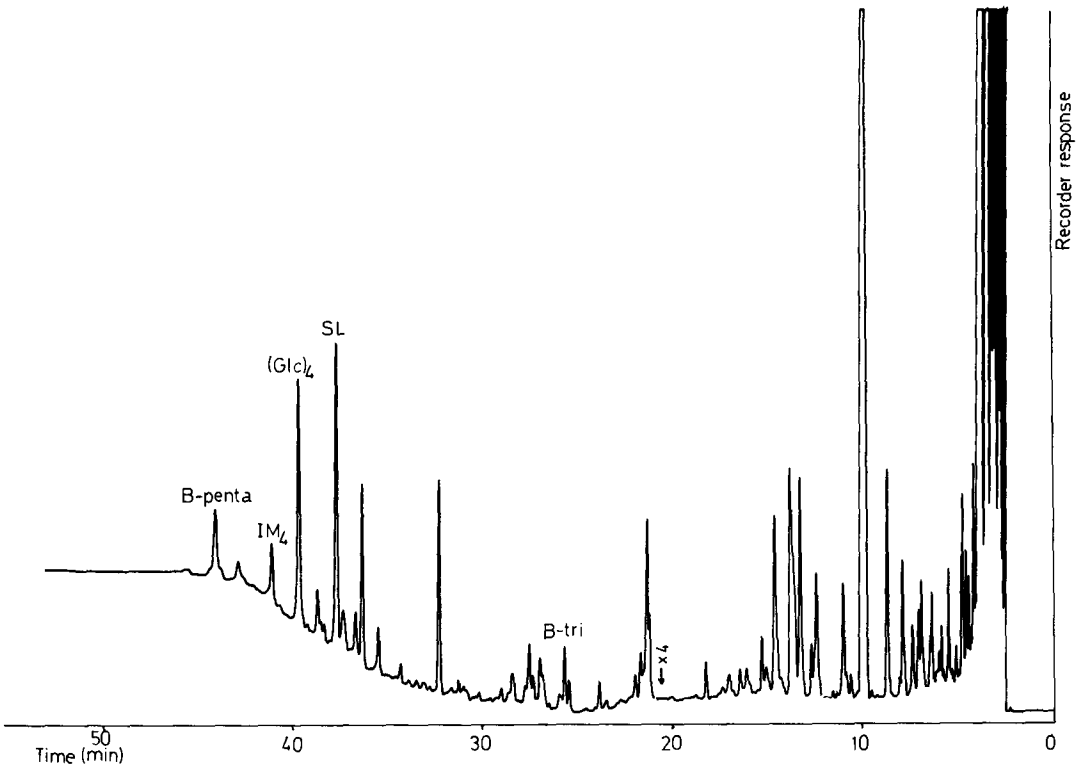

Fig.1. GLC-tracing of oligosaccharides, as their permethylated alditols, in the urine of subject SH. The separation was performed on a Perkin-Elmer 3920 gas chromatograph fitted with a glass capillary column (25 m × 0.25 mm) wall-coated with SE-30 (LKB-Produkter, Sweden) and a flame ionisation detector. A temperature program of 4°C/min from 180–330°C and then isothermal run at 330°C were employed. The injection was split with an all-glass splitter so that 10% of the injection (1 μl) was introduced onto the column. For abbreviations see text. At the point indicated : ×4, detector sensitivity was increased by a factor of 4.

Table 1
Excretion rate of $(Glc)_4$ in 20 normals and in 20 patients with Duchenne muscular dystrophy

Subject	Age (years)	ABO-blood group	Severity level[a]	mg $(Glc)_4$/ 24 h urine
controls (n = 20)	5–20	n.d.		Mean = 1.0 Range = 0.1–2.5
TF	9	B	6	3.5
HY	9	B	2	5.6
YI	9	O	4	7.3
MY	9	O	5	5.2
KK	10	A	6	6.6
KM	11	AB	4	5.4
NH	12	B	5	4.8
MT	12	B	6	3.5
YN	13	O	6	3.3
MH	13	O	6	4.1
KK	13	A	6	8.4
MM	14	A	4	12.0
YH	14	O	7	4.8
HK	15	B	6	6.4
KN	15	A	6	4.2
KM	15	B	6	4.4
KY	17	O	7	4.4
YK	17	O	7	3.4
SH	18	B	6	6.9
TO	20	O	6	4.2
			Mean =	4.9
			Range =	3.3–12.0

[a] Severity levels are defined as follows:
1. Can walk and climb up stairs without any help
2. Can walk, but cannot climb up
3. Can walk only on flat ground
4. Can walk, but cannot stand up from a chair
5. Cannot walk but can move on all fours
6. Cannot move on all fours, but can crawl on knees
7. Cannot crawl, but can sit
8. Stays in bed all the time

secondary to a generalized membrane defect, but of course it cannot be ruled out that it is reflecting some unknown disturbance in the glycogen metabolism in these patients.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (03X-2 and 03X-4956). The authors are indebted to Mrs Lisa Palm-Svensson and Miss Ewa Persson for skilful technical assistance.

References

[1] Thompson, E. J., Yasin, R., Van Beers, G., Nurse, K. and Al-Ani, S. (1977) Nature 268, 241–243.
[2] Gallup, B. and Dubowitz, V. (1973) Nature 243, 287–289.
[3] Hathaway, P. W., Engel, W. K. and Zellweger, H. (1970) Arch. Neurol. 22, 365–378.

[4] Jasmin, G. and Bokdawola, F. (1970) Rev. Can. Biol. 29, 197–201.
[5] Das, P. K., Watts, R. L. and Watts, D. C. (1971) Biochem. J. 123, 24P-25P.
[6] Howland, J. L. (1974) Nature 251, 724.
[7] Howland, J. L. and Iyer, S. L. (1977) Science 198, 309–310.
[8] Matheson, D. W. and Howland, J. L. (1974) Science 184, 165.
[9] Percy, A. K. and Miller, M. E. (1975) Nature 258, 147.
[10] Schotland, D. L., Bonilla, E. and Van Meter, M. (1977) Science 196, 1005–1007.
[11] Roses, A. D., Herbstreith, M. H. and Appel, S. H. (1975) Nature 254, 350–351.
[12] Mawatari, S., Schonberg, M. and Olarte, M. (1976) Arch. Neurol. 33, 489–493.
[13] Das, P. K., Graesslin, D. and Goedde, H. W. (1976) Biochem. Soc. Trans. 4, 723–724.
[14] Roses, A. D. and Appel, S. H. (1976) J. Neurol. Sci. 29, 185–193.
[15] Roses, A. D., Herbstreith, M., Metcalf, B. and Appel, S. H. (1976) J. Neurol. Sci. 30, 167–178.
[16] Gross, S., Galicka, N., Burzynski, S. R. and Stolzmann, Z. (1976) Physiol. Chem. Phys. 8, 161.
[17] Gross, S. (1977) Clin. Chem. 23, 299.
[18] Lundblad, A. (1978) Methods Enzymol. 50, 226–235.
[19] Hallgren, P., Hansson, G., Henriksson, K. G., Häger, A., Lundblad, A. and Svensson, S. (1974) Eur. J. Clin. Invest. 4, 429–433.
[20] Lennartson, G., Lundblad, A., Sjöblad, S., Svensson, S. and Öckerman, P. A. (1976) Biomed. Mass Spectrom. 3, 51–54.
[21] Walker, G. J. and Whelan, W. J. (1960) Biochem. J. 76, 257.