Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 152-157

EFFECTS OF CHRONIC ALLOPURINOL THERAPY ON PURINE METABOLISM IN DUCHENNE MUSCULAR DYSTROPHY

M. Castro-Gago(1), S. Lojo(2), I. Novo(1),
R. del Rio(2), J. Peña(1), and S. Rodriguez-Segade(2)

(1) Departamento de Pediatría,
(Servicio de Neuropediatría),
(2) Servicio de Laboratorio Central,Hospital General de Galicia,
Facultad de Medicina,Universidad de Santiago de Compostela,
Santiago de Compostela, SPAIN

Received June 2, 1987

Adenine,adenosine,inosine,hypoxanthine,xanthosine,xanthine,guanine and guanosine blood levels in 11 Duchenne muscular dystrophy patients treated with allopurinol,10 untreated patients and 8 healthy controls,were determined by HPLC.Serum ADA,PNP and 5'-NT were also determined. Untreated patients showed lower adenine ($p<0.001$) and higher adenosine,xanthine,ADA and PNP levels ($p<0.01$) than controls. Treated patients had lower adenine and higher xanthine levels ($p<0.001$),but higher hypoxanthine,xanthosine and guanine levels ($p<0.001$),than controls,with normal ADA and PNP.The changes observed in ADA and PNP levels suggest an involvement of these enzymes in accelerated degradation of purines in Duchenne dystrophy.

The pathology of Duchenne muscular dystrophy is not yet clear.Some of the hypotheses that have been put forward postulate structural defects in the cell membrane (1-5), while others explain the disease in terms of an accelerated breakdown of muscle protein (6,7).It is also possible that both these conditions occur,and that both may be the result of some as yet unknown deficiency in dystrophic muscle cells.

Some research findings suggest that this type of muscular dystrophy is associated with abnormalities in the metabolism of adenine nucleotides (8-16),and allopurinol therapy is found both to increase the levels of these nucleotides in muscle and blood and to produce clinical improvement in the patient's condition (12,14,15), although the latter effect has -

0006-291X/87 $1.50

been questioned (17-23). This article presents the results of a study aimed at establishing whether Duchenne muscular dystrophy is or not associated with abnormalities in purine metabolism.

MATERIALS AND METHODS

The study involved 11 Duchenne muscular dystrophy patients under treatment with allopurinol,10 untreated patients and 8 healthy children in the same age range (4-12 years).The diagnosis was established for each patient by clinical examination,serum enzymology,electromyography and muscle biopsy history. According to the classification of Zellweger and Hanson (24), 5 patients were clinical stage I,6 in stage II, 3 in stage III, 3 in stage IV, 3 in stage VI and 1 in stage VII.Tablets containing 100mg of allopurinol each were administered orally to the treated patients as follows: For children of less than 2 years of age,2 tablets daily;for 3-5 year-olds,3 tablets daily;for 6-12 year-olds,4 tablets daily;for 13-16 year-olds,5 tablets daily. Treatment took place over periods ranging from 2.5 to 6 years.

Blood samples were taken from the fasting subjects and adenine,adenosine,inosine,hypoxanthine,xanthosine,xanthine,guanine and guanosine determined. Serum levels of adenosine deaminase (ADA), purin-nucleotide phosphorilase (PNP) and 5'-nucleotidase (5'-NT) were also measured.

Purines and their derivatives were determined by high performance liquid chromatography (HPLC) following McBurney and Gibson (25). A Beckman Ultrasphere ODS column were used with 100% methanol as organic modifier eluent,10mg/L KH_2PO_4 (pH= 5.8) as low ionic strength eluent and an elution rate of 1mL/min. Detection was performed at 254nm by UV detector,The range of absorbance being 0.040AUFS. The various purines were quantified by comparison of the areas of the corresponding peaks with those of peaks obtained using pure standards.

ADA was determined as per Galanti (26),PNP as per Kalker (27) and 5'-NT as per Arkesteijn (28).

The statistical significance of the differences among the three groups of subjects was examined using Student's test.

TABLE I

LEVELS OF PURINE METABOLITES IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS

Metabolite (nmol/dL)	Untreated patients(n=10)	Treated patients(n=11)	Controls (n=8)
Adenine	0.32(0.04)	0.21(0.02)	4.76(1.21)
Adenosine	10.34(4.71)	4.33(3.12)	7.51(3.46)
Inosine	0.24(0.03)	0.24(0.04)	2.13(2.73)
Hypoxanthine	0.32(0.05)	0.94(0.16)	0.37(0.12)
Xanthine	3.77(1.49)	4.46(1.60)	1.59(0.67)
Xanthosine	3.14(1.49)	14.23(4.41)	2.22(1.69)
Guanine	0.33(0.09)	0.91(0.32)	0.31(0.20)
Guanosine	0.16(0.02)	0.17(0.03)	0.25(0.14)

Numbers in parentehsis are 2*S.D.

RESULTS

Table I lists the results concerning the products of purine metabolism. With respect to the controls,untreated patients had far lower adenine levels ($p<0.001$) and much higher adenosine and xanthine levels ($p<0.01$).The levels of the other purine metabolites did not differ significantly between the two groups. The patients treated with the xanthine oxidase inhibitor likewise had significantly lower adenine levels than the controls ($p<0.001$),and higher levels of hypoxanthine,xanthosine,xanthine and guanine ($p<0.001$).

Table II shows that untreated patients had ADA and PNP levels more twice those of controls or treated patients ($p<0.01$). Levels of 5'-NT were also slightly higher in untreated

TABLE II

ADENOSINE DEAMINASE (ADA),PURINE-NUCLEOSIDE PHOSPHORILASE (PNP) AND 5'-NUCLEOTIDASE (5'-NT) ACTIVITY IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS

Subjects	ADA (U/L)	PNP (U/L)	5'-NT (U/L)
Controls(n=8)	7.3(0.5)	50.5(10.1)	7.3(0.6)
Untreated patients(n=10)	15.8(1.7)	124.3(9.0)	8.5(1.5)
Treated patients(n=11)	3.4(0.5)	43.2(10.0)	7.2(0.5)

Numbers in parenthesis are 2*S.D.

patients than in the other groups,but the differences were not statistically significant.

DISCUSSION

Though the pathogeny of Duchenne muscular dystrophy is not yet known for sure,at present all the facts seem to point to a possible alteration of primary or secondary purine metabolism (7-16). The suggestion (9) that the syndrome involves accelerated degradation of purines is supported by the present finding that untreated patients had high levels of adenosine, xanthine,ADA (Which converts adenosine to inosine) and PNP (Which converts inosine to hypoxanthine),for these results suggest that either inhibition of these enzymes may be deficient or these is stimulated due to an excessive adenosine supply (An accelerated degradation of purines or an AMP conversion failure could produce these high levels). This hypothesis is also in keeping with the low levels of adenine observed,for it is reasonable to suppose that deplenishment of ATP levels by accelerated degradation to adenosine would stimulate increased synthesis of AMP,ADP and ATP from adenine,whose levels significantly decrease (12,14).

In a previous study (12) of Duchenne muscular dystrophy patients we found that administration of allopurinol brought about a recovery of ATP levels in blood. Thoghether with the present results for treated patients,this suggests that the inhibition of xanthine-oxidase favours resynthesis of ATP from hypoxanthine via IMP route. HGPRT (Hypoxanthine-guanine phosphoribosyl transferase) is stimulated by allopurinol (29) and its activity is already increased about threefold in dystrophic's muscle (30) and for which gene is likewise located in X-chromosome (31). High levels of guanine in treated patients seem to confirm that idea.

Normal or low levels of ADA,PNP and adenosine in treated patients suggest that allopurinol affects these enzymes (Not only ATP synthesis is helped by this drug but also reduces its rate of degradation;normal or low levels of adenine in treated patients confirm it).

In view of the above findings,it seems likely that Duchenne muscular dystrophy involves an alteration of primary purine metabolism consisting in the accelerated degradation of purines.

Possible alterations in the myofibrille membranes of muscle cells (1-5) would not appear to be the cause of the disease,since allopurinol treatment,not only increases ATP levels in blood and muscle,but also normalizes the morphology of the erythrocyte membrane (12). The next step will be to investigate IMP behaviour in the three groups of children studied.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Heinz Koch Foundation.

REFERENCES

1.Godin,D.V.;Bridges,M.A. and McLeod,P.M.J. (1978) Res.Commun. Chem.Pathol.Pharmacol.20:331-335.
2.Pearson,T.W. (1978) Life Sci.22:127-131.
3.Pickard,N.A.;Gruemer,H.D.;Verril,H.L.;Isaacs,E.R.;Robinow,M.; Nance,W.E.;Myers,E.C. and Goldsmith,B. (1978) N.Eng.J.Med.299: 841-845.
4.Schotland,D.L.;Bonilla,E.;Van Meter,M. (1978) Sicience 196:1005-1010.
5.Wilkerson,L.S.;Perkins,R.C.Jr.;Roelofs,R.;Swift,L.;Dalton,L.R. and Park,J.H. (1978) Proc.Nat.Acad.Sci.USA 75:838-842.
6.Somer,H.;Chien,S.;Lung,L.A. and Thurn,A. (1979) Neurology 29:519-523.
7.Kar,N.C. and Pearson,C.M. (1973) Neurology 23:478-482.
8.Berthillier,G.;Gautheron,D. and Robert,J.M. (1967) C.R.Acad. Sci.(Paris) 265:79-82.
9.Bertorini,T.E.;Palmieri,G.M.A.;Airozo,D.;Edwards,N.L. and Fox,I.H. (1981) Pediatr.Res.15:1478-1482.
10.Bertorini,T.E.;Palmieri,G.M.A.;Griffin,J.;Chesney,C.;Pifer,D.; Verung,L.;Airozo,D. and Fox,I.H. (1985) Neurology 35:61-65.
11.Bonsett,C.A. and Rudman,A. (1984) Indiana Medicine 77:446-449.
12.Castro-Gago,M.;Rodríguez-Segade,S.;Beiras Iglesias,A.;Novo,I. and Pombo,M. (1986) Clin.Chem. (in press).
13.Kar,N.C. and Perason,C.M. (1978) Muscle Nerve 1:308-312.
14.Thomson,W.H.S. and Smith,I. (1978) Metabolism 27:151-163.
15.Thomson,W.H.S. and Smith,I.(1978) N.Eng.J.Med. 299:101.
16.Vignos,P.J. and Warner,J.L. (1963) J.Lab.Clin.Med.63:579-590.

17.Bretag,A.H.;Stern,L.N.;Ballard,F.J.;Tomas,F.M.;Cooper,D.M.;Fewings,J.D. and Goldblatt,E. (1981) Lancet I:276.
18.Castro-Gago,M.;Jiménez,J.F.;Torres Colomer,M.;Fuster,M.;Paz, M.;Novo,I. and Peña,J. (1980) An.Esp.Pediat.13:865-876.
19.Castro-Gago,M.;Jiménez,J.F.;Pombo,M.;Couselo,J.M.;Tojo,R. and Peña,J. (1980) Lancet I:1358-1359.
20.Castro-Gago,M.;Novo,I. and Peña,J. (1985) The Annals (Houston) 7:71-83.
21.Kulakowski,S.;Renointe,P. and Bruyn,C.M.M. (1981) Neuropediatrics 12:92-93.
22.Mendell,J.R. and Wiechers,D.O. (1979) Muscle Nerve 2:53-56.
23.Tamari,H.;Ohtani,Y.;Higashi,A.;Miyoshino,S. and Matsuda,I. (1982) Brain Dev.4:137-143.
24.Zellweger,H. and Hanson,A. (1968) Dev.Med.Child.Neurol.9:576-581.
25.McBurney,A. and Gibson,T. (1980) Clin.Chim.Acta 102:19-25.
26.Galanti,B. and Giusti,G. (1974).In "Methods of Enzymatic Analysis" (Edited by Bergmeyer,H.U.),vol 2,pp.1092-1099.Academic Press,New York.
27.Kalckar,H. and Friedkin,M. (1961).In "The Enzymes" (Edited by Boyer,P.D.;Lardy,H.A. and Myrback,K.),vol.5,p.237.Academic Press,New York.
28.Arkesteijn,C.L.M. (1976) J.Clin.Chem.Clin.Biochem.14:155-158.
29.Edwards,W.L.;Recker,D.;Airozo,D. and Fox,I.H. (1979) J.Lab. Clin.Med. 98:673-678.
30.Necrunjun,J.S.;Allsop,J. and Dubowitz,V. (1979) Muscle Nerve 2:19-23.
31.Becker,M.A.;Yen,R.C.K.;Itkin,P.;Goss,S.J.;Seegmiller,J.E. and Bakay,B. (1979) Science 203:1016-1018.