PRELIMINARY REPORT

Adenosine Deaminase and Body Mass Index in Non–Insulin-Dependent Diabetes Mellitus

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We studied 273 subjects with non–insulin-dependent diabetes mellitus (NIDDM) from the population of Penne, Italy. A low proportion of the adenosine deaminase (ADA)*2 allele is observed in NIDDM subjects with a body mass index (BMI) of 25 kg/m^2 or less. On the contrary, a high proportion of this allele is observed in NIDDM patients with a BMI higher than 34 kg/m^2. In the intermediate BMI class, the proportion of ADA*2 alleles does not differ significantly from that of normal subjects from the same population. No significant effect on the relation between ADA and BMI has been observed for the following variables:sex, age at the time of study, age at onset, therapy with insulin, and dyslipidemia. A borderline effect has been observed for the duration of disease. Several lines of experimental evidence suggest that an excess of adenosine A1 receptor activity may contribute to adiposity in NIDDM. ADA is a polymorphic enzyme that irreversibly deaminates adenosine to inosine, contributing to the regulation of intracellular and extracellular concentrations of adenosine. Since the activity of genotypes carrying the ADA*2 allele is lower than that of the more common genotype ADA*1/*1, genetic variability of the enzyme could contribute to degree of obesity in NIDDM. Our data also support attempts to ameliorate the metabolic control of diabetes through pharmacological modulation of adenosine receptors.

SEVERAL LINES of experimental evidence suggest that an excess of adenosine A1 receptor activity may contribute to adiposity in non–insulin-dependent diabetes mellitus (NIDDM).[1] Adenosine deaminase (ADA) is a polymorphic enzyme that irreversibly deaminates adenosine to inosine, contributing to the regulation of intracellular and extracellular concentrations of adenosine. Since the activity of genotypes carrying the ADA*2 allele is lower than that of the more common genotype ADA*1/*1, genetic variability of the enzyme could contribute to the degree of obesity in NIDDM.

In this report, we show that such an effect may indeed exist, thus supporting current attempts to ameliorate the metabolic control of diabetes through pharmacological modulation of adenosine receptors.[2]

SUBJECTS AND METHODS

Two hundred seventy-three NIDDM subjects from the population of Penne, a small rural town in southeastern Italy, were studied. The sample was chosen randomly from a population of about 2.000 subjects under care at the Center of Diabetology of the local hospital. Samples were collected over a period of about 18 months from patients scheduled for metabolic control on a previously fixed day of the week. The sample includes males and females aged 24 to 91 years. Reliable information on disease duration was obtained in 266 patients.

ADA genotype was determined by starch gel electrophoresis on red blood cell hemolysates according to the method of Spencer et al.[3] Inosine produced at the sites of ADA activity is converted to hypoxanthine in the presence of nucleoside phosphorylase and phosphate. The hypoxanthine is then oxidized by the action of xanthine oxidase, and during this reaction, the tetrazolium salt MTT is reduced in the presence of phenazine methosulfate to a blue-insoluble formazan. In the ADA*1/*1 type, there are three regularly spaced components that exhibit decreasing staining intensity in order of their anodal electrophoretic mobilities. In the ADA*2/*2 type, there are also three isozymes, and their relative intensities and relative electrophoretic mobilities are similar to those of the ADA*1/*1 pattern. The difference between ADA*1/*1 and ADA*2/*2 is that the ADA*2/*2 pattern is appreciably slower than the ADA*1/*1 pattern. The pattern exhibiting four isozymes, designated ADA2-1, has the appearance of a mixture of ADA*1/*1 and ADA*2/*2 patterns.

Variance analysis was performed with SPSS programs.[4] For subdivision into discrete categories of BMI, we used the criterion of between 40 and 50 subjects included in each category.

RESULTS

Table 1 shows the proportion of ADA*2 allele carriers in relation to BMI. There were no ADA*2/*2 subjects in our sample. This genotype is rare: in our sample, the expected number is less than two. There is a highly significant association between ADA and BMI. A very low proportion of the ADA*2 allele is observed in NIDDM subjects with a BMI of 25 kg/m^2 or less. On the contrary, a high proportion of this allele is observed in NIDDM patients with a BMI higher than 34. In the intermediate classes of BMI, the proportion of the ADA*2 allele does not differ significantly from that of normal subjects from the same population.

The mean BMI (mean ± SE) is 29.31 ± 0.33 in *1/*1 subjects and 30.87 ± 0.59 in *1/*2 subjects ($P < .025$). We also examined the relationship between BMI (six categories) and ADA activity (assigned to each genotype according to Battistuzzi et al[5]). The results indicate the presence of both a linear ($P = .01$) and a nonlinear ($P = .04$) component. These results agree with the analysis in Table 1 showing that most of the heterogeneity is due to differences in the ADA*1/*2 proportion between the extreme classes of BMI, while no appreciable difference is detectable among intermediate classes.

No significant effect on the relation between ADA and BMI was observed for the following variables: sex, age at the time of

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Table 1. Proportion of ADA*2 Allele Carriers According to BMI in NIDDM

Parameter	BMI (kg/m²) (A) ≤25	(B) >25 ≤27	(C) >27 ≤29	(D) >29 ≤31	(E) >31 ≤34	(F) >34	Chi-Square Test of Independence	*df*	*P*	Mantel-Haenszel Test for Linear Association	*df*	*P*
ADA*2 allele carriers												
%	9.5	18.8	17.0	15.2	12.8	39.5						
No.	42	48	47	46	47	43						
All classes of BMI							16.1591	5	.0064	6.4123	1	.0113
A *v* B + C + D + E *v* F							15.5459	2	.0004	12.6684	1	.0004

study, age at onset, therapy with insulin, and dyslipidemia (data not shown). A borderline effect was observed for the duration of disease.

Table 2 shows the duration of disease in relation to BMI and ADA genotype. The maximum duration (21 years) is observed in ADA*2 carriers with a BMI of 25 or less. In the other classes, the duration of disease was 8 to 14 years.

DISCUSSION

A1 adenosine receptors are more active in obese compared with lean Zucker rats, and an excess of A1 activity may contribute to adiposity in the obese line of these animals.[1,6-8] Other studies have also shown that adenosine increases the sensitivity to insulin in isolated adipocytes.[9-11]

Agonists for the A1 adenosine receptor decrease cyclic adenosine monophosphate in adipocytes, decreasing lipolysis and inhibiting glycerol release from adipose tissue. There is also evidence of higher levels of endogenous extracellular adenosine in obese animals.[1] In obese humans, a low A1 receptor number has been observed.[12] This may represent an adaptation to chronically high levels of endogenous adenosine.[1]

At present, four adenosine receptors have been identified (A1, A2a, A2b, and A3).[13,14] However, their relationship with cellular functions has not been clarified, and it is probably complex.[15]

ADA (EC 3.5.4.4) is a polymorphic enzyme that is ubiquitous in mammalian tissues.[3,16] It is controlled by a locus with two codominant alleles, ADA*1 and ADA*2, located on the long arm of chromosome 20.[17] The corresponding three common ADA genotypes have different enzymatic activity: ADA*1/*1 is 15% more active than ADA*1/*2 and 30% more active than ADA*2/*2.[5]

ADA catalyzes the irreversible deamination of adenosine to inosine. Red blood cells are in equilibrium with freely diffusing adenosine,[18] indicating an important role in regulating the adenosine concentration.

The enzyme occurs in a small molecular form (MW 33,000) called "red blood cell ADA" and a large molecular form (MW 200,000) called "tissue-specific ADA." The various ADA tissue enzymes consist of one or more molecules of red blood cell ADA and one molecule of ADA complexing protein (ADPC).[19,20]

ADPC is identical to CD26, a T-cell–activating antigen, and to a glycoprotein present in epithelial cells of various tissues. Recent data suggest that ADA and CD26 are colocalized on the T-cell surface but not inside the cells. Cells expressing ADA and CD26 on the surface are much more resistant to the inhibitory effect of adenosine. These data suggest that ADA on the cell surface is involved in an important regulatory mechanism by which released ADA binds to cell-surface CD26, and this complex is capable of reducing the local concentration of adenosine.[21]

The positive correlation between the BMI and ADA*2 allele suggests that the ADA*2 allele favors the increase of body mass, in agreement with experimental data pointing to a positive effect of adenosine on adiposity.

In another perspective, the high proportion of ADA*2 carriers (39%) among extremely obese NIDDM subjects suggests that this allele is a risk factor for severely obese NIDDM. On the contrary, the low proportion of ADA*2 carriers in lean NIDDM subjects suggests a protective role of ADA*2 against lean NIDDM. Table 2 shows that lean subjects carrying ADA*2 have a longer duration of disease, suggesting that besides having a lower susceptibility to NIDDM, these subjects may have a longer survival also.

The present data indicate that even within the relatively small "normal" variations due to genetic polymorphism, ADA activity, probably through modulation of the adenosine concentration, may have a significant effect on the BMI. This observation gives strong support to strategies based on pharmacological modulation of the adenosine receptor to improve metabolic control in diabetic disorders.

Table 2. Duration of Disease in Relation to BMI and ADA Genotype

Parameter	BMI ≤25 *1/*1	BMI ≤25 ADA*2 Carrier	BMI >25 ≤34 *1/*1	BMI >25 ≤34 ADA*2 Carrier	BMI >34 *1/*1	BMI >34 ADA*2 Carrier
Duration of disease (yr)						
Mean	12.10	21.00	12.11	13.30	8.36	9.94
SE	1.10	4.52	0.63	1.92	1.11	1 88
No. of subjects	40	4	151	30	25	16

NOTE. $P = .029$ (variance analysis).

REFERENCES

1. Xu B, Berkich DA, Crist GH, et al: A1 adenosine receptor antagonism improves glucose tolerance in Zucker rats. Am J Physiol 274:E271-E279, 1998

2. Heseltine L, Webster JM, Taylor R: Adenosine effects upon insulin action on lipolysis and glucose transport in human adipocytes. Mol Cell Biochem 144:147-151, 1995

3. Spencer N, Hopkinson D, Harris H: Adenosine deaminase polymorphism in man. Ann Hum Genet 32:9-14, 1968

4. Nie NH, Hull HC, Jenkins JG, et al: Statistical Package for the Social Sciences (ed 2). New York, NY, McGraw-Hill, 1975

5. Battistuzzi C, Scozzari R, Santolamazza P, et al: Comparative

activity of red cell adenosine deaminase allelic form. Nature 251:712, 1974

6. Berkich DA, Luthin DR, Woodard RL, et al: Evidence for regulated coupling of A1 adenosine receptors by phosphorylation in Zucker rats. Am J Physiol 268:E693-E704, 1995

7. LaNoue KF, Martin LF: Abnormal A1 adenosine receptor function in genetic obesity. FASEB J 8:72-80, 1994

8. Vannucci SJ, Klim CM, Martin LF, et al: A1-adenosine receptor–mediated inhibition of adipocyte adenylate cyclase and lipolysis in Zucker rats. Am J Physiol 257:E871-E878, 1989

9. Londos C, Honnor RC, Dhillon GS cAMP dependent protein kinase and lipolysis in rat adipocytes. III. Multiple modes of insulin regulation of lipolysis and regulation of insulin responses by adenylate cyclase regulators. J Biol Chem 260:15139-15145, 1985

10. Ohisalo JJ, Strandberg H, Kostiainen E, et al: Stimulation of lipoprotein lipase activity of rat adipose tissue and post-heparin plasma by N6-(phenylisopropyl)adenosine. FEBS Lett 132:121-123, 1981

11. Vannucci SJ, Nishimura H, Satoh S, et al: Cell surface accessibility of GLUT4 glucose transporters in insulin-stimulated rat adipose cells. Modulation by isoprenaline and adenosine. Biochem J 288:325-330, 1992

12. Kaartinen JM, Hreniuk SP, Martin LF, et al: Attenuated adenosine sensitivity and decreased adenosine receptor number in adipocyte plasma membranes in human obesity. Biochem J 279:17-22, 1991

13. Olah ME, Ren H, Stiles GL: Adenosine receptors. Protein and gene structure. Arch Int Pharmacodyn Ther 329:135-150, 1995

14. Snyder SH: Knockouts anxious for new therapy. Nature 388:624, 1997

15. Richardson PJ: Blocking adenosine with antisense. Nature 385:684-685, 1997

16. Edwards YH, Hopkinson DA, Harris H: Adenosine deaminase isozymes in human tissues. Ann Hum Genet 35:207-218, 1971

17. Hening J, Martinik F, D'Eustachio P, et al: Confirmation of the regional localization of the genes for human acid-glucosidase and adenosine deaminase by somatic cell hybridization. Ann Hum Genet 48:49-56, 1984

18. McKusik VA: Mendelian Inheritance in Man (ed 11). Baltimore, MD, The Johns Hopkins University Press, 1994

19. Herbschleb-Voogt E, Grzeschk KH, Pearson PL, et al: Assignment of adenosine deaminase complexing protein (ADPC) gene(s) to human chromosome 2 in rodent-human somatic cell hybrids. Hum Genet 59:317-323, 1991

20. Kameoka J, Tanaka T, Nojima Y, et al: Direct association of adenosine deaminase with T cell activation antigen, CD26. Science 261:466-469, 1993

21. Dong RP, Kameoka J, Hegen M, et al: Characterization of adenosine deaminase binding to human CD26 on T cells and its biologic role in immune response. J Immunol 156:1349-1355, 1996