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ORIGINALS

Pharmacogenetics of angiotensin system in non-diabetic nephropathy

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SUMMARY

Background: *Genetic variability could contribute to the response to pharmacological treatment in patients with nephropathy. In albuminuric diabetic patients the renoprotective effect of angiotensin I-converting enzyme (ACE) inhibition should be lower among homozygotes for the deletion allele (DD) compared to II-homozygotes.*

Methods: *A total of 71 non-diabetic chronic nephropathy patients were treated with losartan (n = 37) or amlodipine (n = 34). Blood pressure and proteinuria were determined before and after the treatment, and changes in the mean values were statistically compared. Patients were genotyped for the ACE-I/D, angiotensin I receptor type 1 (AGTR1)-1166 A/C, and angiotensinogen (AGT)-M235T polymorphims, and the reduction of blood pressure and proteinuria between the different genotypes were compared.*

Results: *The reduction in systolic or diastolic blood pressure was not found to be different between the ACE-I/D or AGT-M/T genotypes in patients treated with losartan or amlodipine. In patients treated with losartan, we found a signiticantly higher reduction of diastolic blood pressure in AGTR1-AA patients compared to AC patients (p = 0,0024). We did not find differences in proteinuria-reduction between the different genotypes in patients treated with losartan or amlodipine.*

Conclusions: *Our data show that the effects of losartan and amlodipine on the absolute mean reduction of blood pressure and proteinuria in non-diabetic nephropathy patients are similar between the different ACE or AGT genotypes. Although based on a small number of patients, the AGTR1-AA genotype was associated with a significantly higher reduction in diastolic blood pressure among losartan-treated patients. Additional studies are necessary to refute or confirm this association.*

Key words: ***Non-diabetic nephropathy. Angiotensinogen. Angiotensin converting enzyme. Angiotensin receptor. Polymorphisms. Pharmacogenetics.***

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FARMACOGENÉTICA DEL SISTEMA DE LA ANGIOTENSINA EN LA NEFROPATÍA NO DIABÉTICA

RESUMEN

Antecedentes: *La variación genética podría contribuir a la respuesta farmacológica en los pacientes con nefropatía. Así, entre los pacientes con albuminuria diabética aquellos con el genotipo DD para el gen de la enzima convertidora de la angiotensina (ECA, polimorfismo inserción/delección, I/D) tendrían una menor respuesta renoprotectora ante los inhibidores de la ECA, comparados con los pacientes con genotipo II.*

Métodos: *Estudiamos 71 pacientes con nefropatía crónica no diabética, de los cuales 37 habían sido tratados con losartán y 34 con amlodipino. Determinamos la tensión arterial y la proteinuria antes y después de ser tratados, y los valores medios se compararon estadísticamente. Todos los pacientes fueron genotipados para los polimorfismos I/D de la ECA, 1166 A/C del receptor de tipo 1 de la angiotensina I (AGTR1), y M235T del angiotensinógeno (AGT), y los valores medios de la reducción de la tensión sanguínea y la proteinuria fueron comparados entre los genotipos.*

Resultados: *No hallamos diferencias en la reducción de la presión sanguínea diastólica o sistólica entre los diferentes genotipos de los polimorfismos de la ECA y el AGT, tanto para los pacientes tratados con losartán como con amlodipino. En los pacientes tratados con losartán hubo una reducción significativa de la presión diastólica entre aquellos con genotipo AGTR1-AA comparados con los heterocigotos AC (p = 0,0024). No hallamos diferencias en el nivel de reducción de la proteinuria entre los diferentes genotipos, tanto entre los tratados con losartán como con amlodipino.*

Conclusiones: *De acuerdo con nuestros resultados, los valores medios de reducción de la presión sanguínea en los pacientes con nefropatía no diabética y tratados con losartán o amlodipino serían similares entre los diferentes genotipos de la ECA y el AGT. Aunque nuestro estudio se basó en un número reducido de pacientes, el genotipo AGTR1-AA podría estar asociado con una mayor reducción de la presión diastólica entre los pacientes tratados con losartán.*

Palabras clave: ***Nefropatía no diabética. Angiotensinógeno. Enzima conversora de la angiotensina. Receptor de la angiotensina. Polimorfismos. Farmacogenética.***

INTRODUCTION

Pharmacogenetics refers to variability in pharmacological response due to genetic factors[1]. This variation would explain some of the differences observed among patients treated with the same drug, and would be due to the existence of polymorphisms within the genes that encode for enzymes related with the metabolism of these drugs, or within the genes that encode for the proteins of the physiological pathway on which the drug acts. The insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme I (ACE) relates to ACE blood levels, the individuals with the DD genotype having the highest levels and with II genotype the lowest ones[2]. This polymorphism could be implicated in the anti-proteinuric response regulation with ACE inhibitors treatment (ACEI)[3-6]. Among other published studies, a lesser anti-proteinuric response has been described in diabetic patients

with the DD and ID genotypes, as compared to those with the II genotype.[5,6] The renoprotective effect has also been observed among patients with non-diabetic nephropathy[4]. Besides variability within the ACE gene, polymorphisms within the genes of angiotensinogen I (AGT) and angiotensin II type 1 receptor (AGTR1) may also contribute to modulate the response to several drugs that act on the renin-angiotensin system, such as ACEI and receptor antagonists.

In this study, we evaluate the role of three polymorphisms within the ACE, AGT, and AGTR1 genes on the renoprotective capability of losartan (a receptor antagonist) and of amlodipine (a calcium channel blocker) among patients with non-diabetic nephropathy.

METHODS

Study design and patients

We studied 71 patients older than 18 years, with non-diabetic nephropathy and that met the following criteria: chronic nephropathy with 24-hour proteinuria > 1.5 g, a systolic blood pressure (SP) of 140-170 mmHg and/or diastolic blood pressure (DP) of 90-105 mmHg, and serum creatinine < 2.5 mg/dL. None of the patients was diabetic, received more than one antihypertensive drug, or had suffered from cardiovascular events. All of these patients were comprised in a broader study in which the role of losartan and amlodipine was assessed in 97 patients.[7] In the study of the pharmacogenetics, 26 patients could not be included because of refusal to participate (17 patients) or inability to obtain DNA (9 cases).

The 71 patients were submitted to a 7-weeks period during which they received no antihypertensive treatment, which was substituted by a placebo. After completion of this "wash-out" period, 37 patients were treated with losartan (50 mg) and 34 with amlodipine (5 mg), qd. After 4 weeks of treatment, hydrochlorotiazide (HCTZ 12.5 mg qd.) was added if blood pressure was not maintained within the desired range (SP > 140 mmHg and/or DP > 90 mmHg). After 8 weeks of treatment (week 8), the daily dose of losartan or amlodipine was increased to 100 mg and 10 mg qd., respectively, if blood pressure was above the desired levels. If these values were kept above the desired levels after 4 additional weeks (week 12), the HCTZ dose was increased to 25 mg qd.[7] The SP, DP, and proteinuria values were measured on the last day of week 16 of treatment.

Genetic analysis

ACE I/D, AGT M235T (a change C/T at the codon 235 of the AGT gene), and ATR1 1166 A/C polymorphisms were analyzed following previously described procedures[8,9]. In the case of the ACE I/D polymorphism, fragments of 490 (allele I) or 190 (allele D) base pairs were amplified by means of polymerase chain reaction (PCR). The reaction mixture contained the primers CTGGAGACCACTCCCATCCTTTCT and GATGTGGCCATCACATTCGTCAGAT, and after 32 amplifications cycles at 95º C-30 s, 58º C-45 s, and 72º C-90 s, 10 µL of each reaction were submitted to 2% agarose gel electrophoresis in order to visualize the alleles and determine each patient's genotype (DD, ID, or II). Since the insertion allele may be amplified less efficiently than the deletion allele, some ID individuals could be mistakenly be genotyped as DD. For that reason, each DD genotype was checked by means of verification of absence of the amplification product with a PCR containing a specific primer for the I allele, according to a previously described protocol[10].

In order to determine the AGT polymorphisms, each patient's DNA was amplified with the GATGAGCACAACGTCCTG and CAGGGTGCTGTCCACACTGGCTCGC primers (priming at 62º C). After 32 PCR cycles, each reaction was digested with the BstUI restriction enzyme and submitted to 3% agarose gel electrophoresis. Both alleles were visualized as bands of 303 bases (235 M) and 279 bases (235 T).

The 1166 A/C polymorphisms at the non-translated 3' region of the AGTR1 gene was analyzed according to the previously described protocol in which the DNA was amplified with the GCAGCACTTCACTACCAAATGAT and TGTTCTTCGAGCAGCCGT primers (priming at 58º C).[8] Each reaction was then digested with the Bcl I restriction enzyme, and then put on 3% agarose gel electrophoresis to visualized the alleles as bands of 176 bases (1166 C) or 156 bases (1166A).

Statistical analysis

We compared the mean values of SP, DP and proteinuria decrease between the different ghenotypes for each polymorphism by Student's t test. The 95% confidence interval values for the mean decrease were also calculated for each phenotype. For the statistical calculations, we used a statistical software for Windows.

RESULTS

In Table I we summarize the main features of patients with non-diabetic nephropathy treated with losartan (n = 37) or with amlodipine (n = 34). We observed a significant decrease in blood pressure with both treatments, without any significant differences in SP or DP reduction between both drugs. However, proteinuria decrease was significantly higher among patients treated with losartan (p = 0.032).

We compared the decrease in SP, DP and proteinuria depending on the genotypes of the three analyzed polymorphisms. These results are summarized in Tables II, III, and IV. We did not find any significant differences in the decrease of any of the three values for the genotypes ACE and AGT, both in losartan- and amlodipine-treated patients. Among patients treated with losartan, the decrease in SP and DP was greater in those with the AGTR1-AA genotype than in those with AC genotype (no patient had a CC homozygotic genotype), although only reduction in DP was significantly higher in AA patients (p = 0,0024; Table IV).

Table I. Main features of patients with non-diabetic nephropathy treated with losartan (37 cases) and amlodipine (34 cases). Baseline and final values of diastolic (DP) and systolic (SP) pressures and proteinuria correspond to mean values. Between brackets besides percentages are also indicated the 95% confidence interval values (95%CI)

	Losartan (n = 37)	Amlodipine (n = 34)
Men/women	26/11	26/8
Mean age ± SD	48 ± 14	46 ± 15
Types of glomerulonephritis*		
IgA	12 (32%)	8 (24%)
MGN	4 (12%)	9 (26%)
FSG	6 (16%)	4 (12%)
NS	3 (8%)	1 (3%)
Other	12 (32%)	12 (35%)
Mean SP mmHg (95% CI)		
Baseline	148 (143, 152)	148 (144, 156)
Final	130 (125, 135)	137 (132, 141)
	P = 0.0708	
Mean DP mmHg (95% CI)		
Baseline	93 (90, 96)	91 (88, 93)
Final	83 (80, 86)	85 (83, 88)
	P = 0,2762	
Mean proteinuria mmHg (95% CI)		
Baseline	3,361 (2,704, 4,180)	2,689 (2,056, 3,516)
Final	1,842 (1,452, 2,339)	2,732 (2,063, 3,619)
	P = 0.0324	

*IgA = IgA-nephropathy; MGN = membranous glomerulonephritis; FS = focal and segmental glomerulosclerosis; NS = nephrosclerosis.

DISCUSSION

The role of inherited genetic factors in the differences between people in the response to pharmacological treatments is a confirmed fact and has led to the development of pharmacogenetics. Currently, we know most of the human genome genes and many that codify for proteins implicated in drug metabolism (activation and elimination) have been identified. Other genes codify for peptides on which the active part of the drug. The variation in these and other genes may affect the level of protein expression, or the drug affinity due to changes in the amino acids in the protein itself, but in both cases the individual's ability to react to treatment would be implicated. In the face of this variability, and due to their genotype for theses genes polymorphisms, some patients may require higher doses of a drug than others in order to achieve the same effect, and in extreme cases, the drug might be completely ineffective in some people or have toxic effects at low doses in others[1].

Losartan binds to angiotensin II type 1 receptor, reducing the vasopressor response of angiotensin II by competing with it. The AGTR1 gene is polymorphic and some of these polymorphisms have been related to the risk for developing cardiovascular and renal diseases[11-15]. On its side, angiotensin II is formed after the hydrolysis of its precursor, angiotensinogen, by the angiotensin-converting enzyme, both molecules codified by polymorphic genes

Table II. SP, DP and proteinuria reduction in patients treated with losartan and amlodipine, depending on ACE genotypes. All values are showed as mean reductions (between brackets, the 95% confidence intervals values)

		ACE			
Mean reduction		II (n = 6)	ID (n = 21)	DD (n = 10)	DD *vs* II + ID
SP (mm Hg)	Los	15 (3.27)	20 (13.26)	14 (4.23)	-5 (-16.6) P = 0.36
	Am	7 (-2, 16)	11 (5, 17)	14 (7, 21)	4 (-4, 12) P = 0.3514
DP (mm Hg)	Los	8 (0, 16)	9 (6, 14)	11 (3, 16)	0,15 (-7.7) P = 0,96
	Am	5 (-1, 11)	3 (-1, 6)	9 (4, 13)	5 (0.10) P = 0.0589
Proteinuria (mg/24 h)	Los	75 (43, 129)	51 (38, 68)	53 (35, 81)	95 (58, 157) P = 0.85
(Final/Baseline) %	Am	100 (67, 148)	113 (88, 143)	89 (67, 118)	82 (58, 115) P = 0.24

Los: losartan; Am: amlodipine.

Table III. SP, DP and proteinuria reduction in patients treated with losartan and amlodipine, depending on angiotensinogen genotypes. All values are showed as mean reductions (between bkacets, the 95% confidence intervals values)

		AGT			
Mean reduction		**MM (n = 11)**	**MT (n = 22)**	**TT (n = 4)**	**MM *vs* MT + TT** **TT *vs* MM + MT**
SP (mm Hg)	Los	19 (11, 28)	16 (10, 22)	18 (3, 33)	-3 (-14, 7) P = 0.53 0.55 (-15, 16) P = 0.94
	Am	13 (7, 20)	14 (8, 21)	5 (-2, 12)	-3 (-11, 6) P = 0.48 -9 (-17, 1) P = 0.063
DP (mm Hg)	Los	9 (3, 14)	10 (6, 14)	9 (0, 18)	1 (-6, 7) P = 0.80 -0.58 (-11, 9) P = 0.90
	Am	8 (4, 12)	7 (3, 11)	-0.3 (-4.6, 4)	-4 (-10, 1) P = 0.11 -8 (-13, -3) P = 0.053
Proteinuria (mg/24 h)	Los	44 (30, 63)	65 (49,86)	43 (23, 81)	76 (38, 152) P = 0.43 138 (88, 216) P = 0.15
(Final/Baseline)%	Am	113 (85, 152)	92 (70, 121)	102 (75, 139)	100 (70, 145) P = 0.98 85 (60, 121) P = 0.35

Los : losartan; Am: amlodipine.

Table IV. SP, DP and proteinuria reduction in patients treated with losartan and amlodipine, depending on angiotensin II receptor 1 genotypes. All values are showed as mean reductions (between brackets, the 95% confidence intervals values)

		AGTR1		
Mean reduction		**AC (n = 15)**	**AA (n = 22)**	**AA *vs* AC**
SP (mm Hg)	Los	13 (5, 20)	20 (14, 26)	7 (-18, 2) P = 0.13
	Am	9 (2, 16)	13 (8, 19)	4 (5, 14) P = 0.51
DP (mm Hg)	Los	4 (0, 8)	13 (10, 17)	9 (3, 15) P = 0.0024
	Am	2 (-2, 7)	8 (4, 11)	6 (4, 17) P = 0.13
Proteinuria (mg/24 h)	Los	54 (39, 76)	56 (42, 75)	104 (65, 162) P = 0.88
(Final/Baseline) %	Am	-6 (-41, 21)	6 (-16, 24)	88 (-33, 41) P = 0.79

Los: losartan; Am: Amlodipine.

as well. Polymorphisms within the AGT and ACE genes have been related to ACE blood levels and may significantly contribute to the risk of suffering from cardiovascular and renal diseases, or modulate its course once the pathological process has been initiated[11,16-20]. Since the AGTR1 antagonists act on a component of the renin-angiotensin system, polymorphisms within the genes that encode for proteins in this physiological pathway may contribute to modulate the response to losartan. Our data from patients with non-diabetic nephropathy treated with losartan showed a significant decrease in blood pressure and albuminuria, but this decrease was similar between the different ACE and AGT genotypes. With regards to the AGTR1 polymorphism, we observed a greater diastolic pressure reduction in patients with the 1166AA genotype, as compared to the AC genotype. However, although the difference was statistically significant, this comparison was based upon a limited number of patients (22 AA, and 15 AC), and no patient had the rare CC genotype. Thus, other studies with larger series of patients are needed in order to confirm or refuse the relationship between this polymorphism and the response to losartan in non-diabetic nephropathy.

The absence of a significant association between the response to losartan and the ACE I/D polymorphism is in agreement with the results described by Andersen y cols[21]. These authors analyzed hypertensive patients with nephropathy due to type 1 diabetes, 28 II homozygotic and 28 DD homozygotic, and they did not find differences in blood pressure or proteinuria decrease between both genotypes. However, albuminuria reduction in patients treated with an ACE inhibitor might be influenced by the genotype, and II patients would have a significantly higher reduction than DD patients[5,6].

In conclusion, we have not found significant differences in blood pressure or proteinuria reduction between the different ACE or AGT genotypes in patients with non-diabetic nephropathy treated with losartan or amlodipine. We did find a significant reduction in diastolic pressure among patients with the AGTR1-AA genotype treated with losartan. However, these results were based on a limited number of patients and, thus, should be cautiously taken. More studies with larger samples are needed to confirm or refuse the relationship between this polymorphism and the response to losartan in non-diabetic nephropathy before genotype determination can be applied to predict the pharmacological response in these patients.

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