

## Low-dose aspirin does not affect the renal function of microalbuminuric type 2 diabetic patients

Aspirina em baixa dosagem não afeta a função renal de pacientes com diabetes melito tipo 2 microalbuminúricos

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Revista HCPA. 2011;31(4):402-406

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### Abstract

**Background:** low-grade inflammation has been implicated in the pathogenesis of diabetic nephropathy, and anti-inflammatory drugs could be potentially useful as a therapeutic tool. The aim of this study was to analyze the effect of low-dose aspirin (300 mg/d) on urinary albumin excretion (UAE) and glomerular filtration rate (GFR) levels of microalbuminuric type 2 DM patients.

**Methods:** in this randomized, double-blind, crossover, placebo-controlled study, 18 microalbuminuric (UAE=30-300 mg/24 h) type 2 DM patients received aspirin (300 mg/d) or identical placebo for 8 weeks, with a 6-week washout period. The patients were aged  $56 \pm 9$  years, had a diabetes duration of  $16 \pm 7.5$  years; 11 (61%) were female, and they were all using enalapril 10 mg bid. GFR was measured by  $^{51}\text{Cr}$ -EDTA single-injection method and UAE by immunoturbidimetry. The sample-size calculation showed that 17 patients were needed to detect a 30% change in UAE ( $\alpha = 0.05$  and  $\beta = 0.20$ ).

**Results:** after 8 weeks of treatment, there were no significant differences between placebo and aspirin, respectively, regarding UAE [ $57.7$  ( $8.9$ - $420.0$ ) vs.  $63$  ( $8.2$ - $272.0$ ) mg/24 h;  $P=0.45$ ] and GFR ( $108 \pm 34$  vs.  $111 \pm 47$  ml/min/ $1.73$  m $^2$ ;  $P=0.90$ ). Glycemic control was stable throughout the C-reactive protein levels [ $2.72$  ( $0.34$ - $10.3$ ) vs.  $2.03$  ( $0.25$ - $10.3$ )  $\mu\text{g/l}$ ;  $P=0.21$ ] were comparable after placebo and aspirin, respectively. There were no period ( $P=0.41$ ) or carry-over effects ( $P=0.49$ ).

**Conclusion:** low-dose aspirin did not affect GFR and UAE levels of microalbuminuric type 2 DM. It seems that the putative low-grade inflammation of diabetic nephropathy does not respond to these low doses of the drug.

**Keywords:** aspirin; diabetic nephropathy; GFR; glomerular filtration rate; microalbuminuria; type 2 diabetes

### Resumo

**Introdução:** a inflamação em baixo grau tem sido implicada na patogênese da nefropatia diabética e o uso de anti-inflamatórios poderia ser potencialmente útil como terapêutica.

**Objetivo:** o objetivo deste estudo foi analisar o efeito de baixas doses de aspirina sobre a excreção urinária de albumina (EUA) e taxa de filtração glomerular (TFG) de pacientes com diabetes melito (DM) tipo 2 microalbuminúricos.

**Métodos:** neste estudo randomizado, duplo-cego, cruzado, controlado com placebo, 18 pacientes DM tipo 2 microalbuminúricos (EUA = 30-300 mg/24 h) receberam aspirina (300 mg / dia) ou placebo idêntico por 8 semanas, com um período de washout de 6 semanas. Os pacientes tinham idade de  $56 \pm 9$  anos, duração diabetes de  $16 \pm 7,5$  anos, 11 (61%) eram do sexo feminino, e todos estavam usando 20 mg de enalapril/dia. A TFG foi medida pelo  $^{51}\text{Cr}$ -EDTA e a EUA por imunoturbidimetria. O cálculo do tamanho da amostra: 17 pacientes para detectar uma alteração de 30% na EUA ( $\alpha=0,05$  e  $\beta=0,20$ ).

**Resultados:** após 8 semanas, não houve diferenças significativas entre placebo e aspirina, respectivamente, em relação à EUA [ $57,7$  ( $8,9$ - $420,0$ ) vs.  $63$  ( $8,2$ - $272,0$ ) mg/24 h;  $P=0,45$ ] e TFG ( $108 \pm 34$  vs.  $111 \pm 47$  ml/min/ $1,73$  m $^2$ ;  $P=0,90$ ). O controle glicêmico manteve-se estável. Os níveis de proteína C reativa [ $2,72$  ( $0,34$ - $10,3$ ) vs.  $2,03$  ( $0,25$ - $10,3$ ) mg/l;  $P=0,21$ ] foram semelhantes após placebo e aspirina, respectivamente. Não houve efeito de período ( $P=0,41$ ) ou carry-over ( $P=0,49$ ).

**Conclusão:** a inflamação de baixo grau descrita na patogênese da nefropatia diabética não responde a baixas doses de aspirina.

**Palavras-chave:** aspirina; nefropatia diabética; TFG; taxa de filtração glomerular

Multiple mechanisms seem to be involved in the development of diabetic nephropathy (DN), reflecting an interaction between metabolic and hemodynamic changes induced by hyperglycemia and genetic predisposition (1-3). The role of activation of cytokines, profibrotic elements, inflammation, and vascular growth factors has been extensively investigated to explain the matrix accumulation that characterizes DN (4). A number of clinical studies have implicated inflammatory mechanisms as important pathogenic factors in both glomerulosclerosis and tubulointerstitial injury in DN (2,5). Furthermore, recent papers claim that endothelial dysfunction and possibly inflammation are novel predictors of progression of kidney and cardiovascular disease in type 1 and type 2 diabetes (6,7). Therefore, it is tempting to speculate that blocking inflammatory pathways would result in renal benefits. Indeed, some papers show that anti-inflammatory drugs can reduce albuminuria levels in proteinuric diabetic patients (8-10). On the other hand, it has long been feared that these drugs could damage the kidneys (11-13). The aim of this study was to analyze the effect of low-dose aspirin (300 mg/d) on UAE and GFR levels of microalbuminuric type 2 DM patients.

## Methods

The eligible patients had type 2 DM according to American Diabetes Association (ADA) criteria, and microalbuminuria was defined as an UAE of 30 to 300 mg in at least two out of three consecutive 24-hour urine collections (14). The patients were recruited from the Endocrinology Division outpatient clinic at Hospital de Clínicas de Porto Alegre. All participants signed a written informed consent form, approved by the hospital's ethics committee. In the run-in phase, the patients were followed for a period of 8 weeks, receiving enalapril 10 mg bid plus other antihypertensive drugs if necessary (diuretics, beta-blockers, calcium channel blockers and hydralazine, either alone or in combination). Diet only, metformin alone or with sulfonylurea, and insulin were used for the treatment of DM. No patient was using lipid-lowering medications. They were excluded if blood pressure remained above 180/100 mmHg or if fasting glycemia was persistently higher than 200 mg/dL.

From the initially evaluated 78 patients with type 2 DM and microalbuminuria, patients were excluded due to cardiovascular disease (N=18), peptic ulcer diagnosis or aspirin intolerance (N=7), regular use of other anti-inflammatory (N=12), participation in other clinical trial (N=11), and other reasons (N=12) (figure 1).

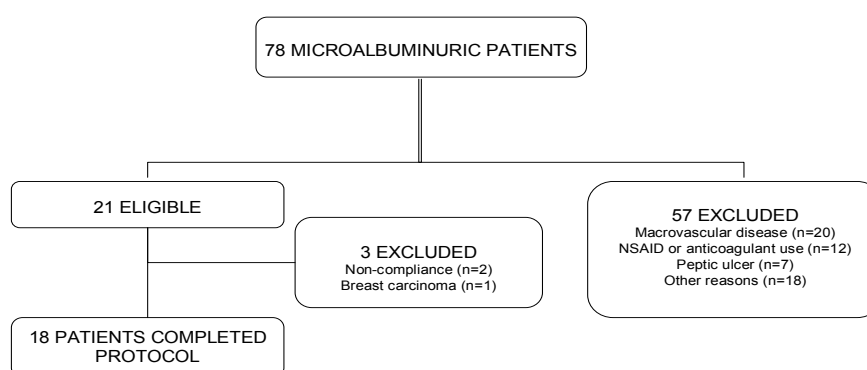


Figure 1 – Flowchart study

NSAID: non-steroidal antiinflammatory drugs

Therefore, 21 patients fulfilled the inclusion criteria. Three patients were excluded after randomization, one due to diagnosis of breast carcinoma and the others for non-compliance with the study protocol. Therefore, 18 patients completed this double-blind, crossover clinical trial, being randomized to aspirin (300 mg/d) or identical placebo for 8 weeks, after which GFR and UAE were measured, with a 4-week washout period. Randomization was performed according to CONSORT guidelines, stratified by UAE levels (15). Treatment compliance was assessed by pill count at the end of each treatment period and it was 98% in both groups.

## Clinical and laboratory evaluation

Clinical evaluation consisted of a complete medical history and physical examination. UAE was assessed in 24-hour sterile samples, by immunoturbidimetry (Microalb; Ames-Bayer, Tarrytown, NY, USA; inter- and intra-assay CV of 6.9% and 3.8%, respectively), with 3 exams per patient at each period. To check for a complete urine collection, the ratio of observed over expected urinary creatinine excretion (creatinine index) was evaluated. Expected creatinine excretion (mg/d) was derived from the observed weight of the person and calculated as:

body weight (kg) x 24 (males) or 21 (females). Urine collections with a creatinine index falling outside the range of 0.6–1.4 were discarded (16).

GFR was measured by  $^{51}\text{Cr}$ -EDTA single-injection method (CV 11%). Glycated hemoglobin (HbA1c) was measured by HPLC (Merck-Hitachi L-9100 Analyzer, Tokyo, Japan; inter- and intra-assay CV of 2.4% and 0.5%, respectively); reference values 4.1 to 6.0%. Glycemia was measured by UV glucose oxidase enzymatic analysis, and capillary glycemia was recorded twice daily. Total cholesterol and triglycerides were analyzed by enzymatic methods.

### Statistical analysis

UAE was expressed as median (range) and was log transformed before analysis. Other results were expressed as either mean  $\pm$  standard deviation or 95% confidence interval. For assessment of treatment period and carry-over effects, t-tests were used, according to Bland & Altman analyses (17). A 30% coefficient of variation was previously observed for urinary albumin, and it was calculated that 17 patients would be necessary to demonstrate a change greater than this (log UAE:  $4.09 \pm 0.87$ ;  $\alpha = 0.05$  e  $\beta = 0.20$ ). Statistical Package for Social Science (SPSS 14.0-Professional Statistics<sup>TM</sup>, SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

### Results

The clinical characteristics of the 18 selected patients were: age  $56 \pm 9$  years, diabetes duration of  $16 \pm 7.5$  years, and 11 (61%) female (table 1).

Table 1 - Clinical characteristics of the type 2 diabetes patients.

Clinical characteristics (N=18)	
Age (years)	$56 \pm 9$
Gender (M/F)	7/11
Race (White/No white)	12/6
DM duration (years)	$16 \pm 7.5$
Smoking habit	3 (16)
Arterial hypertension	12 (65)
Retinopathy	7 (38)
Body mass index (kg/m <sup>2</sup> )	$29 \pm 4$
Treatment (D /OD/ I)	1 /14/ 3

Data expressed as mean  $\pm$  SD or number of cases (%).

DM: diabetes mellitus; M=male, F= female, D: diet, OD: oral drugs, I:insulin

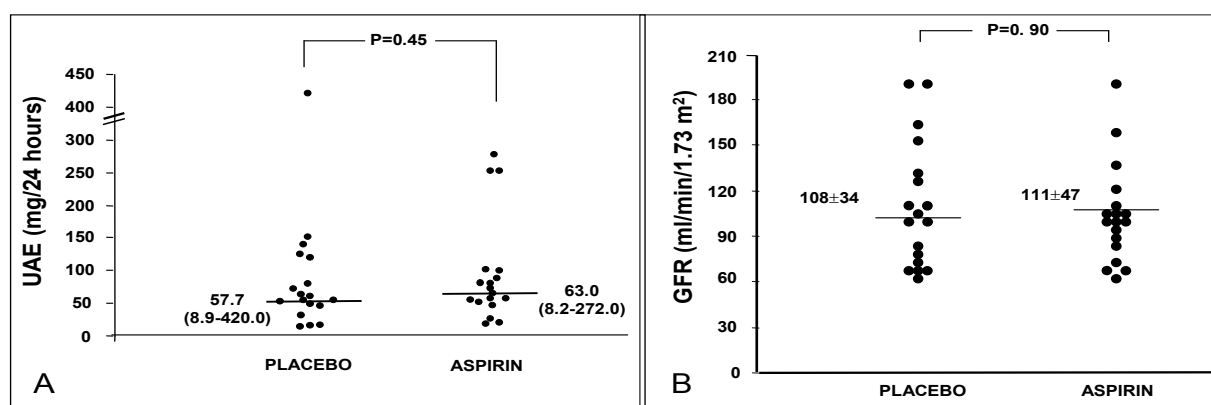


Figure 2 – Effect of aspirin and placebo on urinary albumin excretion (UAE) (panel A) and on glomerular filtration rate (GFR) (panel B) in 18 microalbuminuric type 2 diabetic patients.

After 8 weeks of treatment, there were no significant differences between placebo and aspirin, respectively, regarding GFR ( $108 \pm 34$  vs.  $111 \pm 47$  ml/min/1.73 m<sup>2</sup>;  $P=0.90$ ), and UAE [ $57.7$  ( $8.9-420.0$ ) vs.  $63$  ( $8.2-272.0$ ) mg/24 h;  $P=0.45$ ] (figure 2).

There were also no significant differences between blood pressure ( $133 \pm 16/79 \pm 7$  vs.  $134 \pm 11/77 \pm 7$  mmHg;  $P=0.41$ ), capillary glycemia ( $127 \pm 46$  vs.  $131 \pm 39$  mg/l;  $P=0.67$ ) and HbA1c ( $8.0 \pm 1.7$  vs.  $8.6 \pm 1.7\%$ ), respectively, after placebo and aspirin. Total cholesterol ( $208 \pm 69$  vs.  $223 \pm 81$  mg/dl;  $P=0.55$ ), triglycerides [ $112$  ( $58-849$ ) vs.  $122$  ( $59-850$ ) mg/dl;  $P=0.21$ ], and urinary urea ( $28 \pm 14$  vs.  $27 \pm 12$  g/24 hours;  $P=0.82$ ) were similar at the end of each period,

after use of placebo and aspirin, respectively. Likewise, C-reactive protein levels [ $2.72$  ( $0.34-10.3$ ) vs.  $2.03$  ( $0.25-10.3$ )  $\mu\text{g/l}$ ;  $P=0.21$ ] were comparable after placebo and aspirin, respectively. No treatment period ( $P=0.41$ ) or carry-over effects ( $P=0.49$ ) were observed.

### Discussion

This randomized, double-blind trial did not demonstrate any change in GFR and UAE levels after 8 weeks of low-dose aspirin (300 mg/d) treatment in microalbuminuric type 2 diabetic patients. Inflammatory activity and endothelial dysfunction seem to be interrelated and involved in the increased UAE in diabetic patients (6,18). Therefore, the

use of aspirin could theoretically result in renal benefits. In previous studies, the employment of large doses of aspirin (>500 mg/d) resulted in a significant reduction of proteinuria, supporting this hypothesis (8-10). In a prospective study, Donadio et al. demonstrated that higher-doses of aspirin (975 mg/d) significantly reduced the UAE of type 1 DM patients with proteinuria (UAE >1g/d) (8). In another study, the use of a similar aspirin dose (1g/d) was associated with a significant reduction of proteinuria in type 2 DM with DN (11). These findings reinforced the concept that aspirin could be useful in the treatment of DN. However, in our study, we did not observe any significant effect of aspirin on UAE levels. Likewise, the anti-inflammatory marker C-reactive protein was not reduced after 8 weeks of low-dose aspirin. Possibly higher aspirin doses, achieving the anti-inflammatory range (>500 mg/day), would be associated with a more remarkable effect.

At the other end of the spectrum, aspirin has been linked to the development of renal failure, particularly in patients with DN (11,12). This is a real concern, because diabetic patients need to receive the cardiovascular protection of the antiplatelet agent, and according to the Primary Prevention Project (PPP) study, doses higher than 100 mg/day are probably needed (19). In patients with chronic kidney disease, renal prostaglandins may be partly responsible for maintaining renal blood flow and GFR among the surviving nephrons. Thus, inhibition of prostaglandin synthesis by aspirin might adversely affect renal function. The possibly involved mechanisms include the decrease of vasodilatory renal prostaglandins, interstitial nephritis or papillary necrosis, and these effects have been related to dose and duration of use of aspirin. However, aspirin is a relatively weak inhibitor of renal prostaglandin synthesis and it has recently been demonstrated that the regular use of anti-inflammatory was not associated with renal injury (20-22). Likewise, in our study,

the short-time use of low-dose aspirin did not adversely affect GFR or UAE, supporting the safety of the drug in this respect.

In our patients, blood pressure levels, metabolic profile and protein intake were kept stable throughout each period of the 8-week trial to ensure no changes in these parameters, which could otherwise influence kidney function. The main pitfalls of this trial are the relatively small number of patients and short-term duration of the trial. However, the number of patients was calculated and allowed the detection of a relevant UAE change. Longer follow-up periods are still necessary to prove the safety of long-term aspirin in small doses.

Aspirin in a dose of 300 mg/d is higher than the currently recommended preventive dose of 75-162 mg/d (14,23). Nonetheless, the Primary Prevention Project suggested that DM patients gained less benefits from 100 mg/d (18) and possibly a higher daily dose of aspirin (150-300 mg/d) would be necessary for these patients. We have intentionally chosen the highest dose yet recommended to cardiovascular prevention to challenge the interaction of aspirin with the UAE and to test its potential anti-inflammatory properties.

In conclusion, low-dose aspirin can not be considered as an anti-albuminuric drug, but, on the other hand, no impairment in renal function of microalbuminuric type 2 DM patients was observed.

### Acknowledgments

This study was supported by grants from Programa de Apoio a Núcleos de Excelência (PRONEX) and Fundo de Incentivo à Pesquisa e Eventos (FIPE) – Hospital de Clínicas de Porto Alegre.

We have no conflicts of interest regarding our article.

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*Recebido: 24/11/2011*

*Aceito: 29/12/2011*