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Diabetes Mellitus: a risk factor for drug toxicity

Diabetes Mellitus: fator de risco para toxicidade de medicamentos

Diabetes Mellitus: factor de riesgo para toxicidad de fármacos

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ABSTRACT

Objective: To assess the effect of the antibiotic Gentamicin in an experimental model in the presence of Diabetes Mellitus through renal function and oxidative profile. **Method:** Adult male Wistar rats were distributed into groups: Citrate; Gentamicin (Genta), (intraperitoneal, i.p. gentamicin, 100 mg/kg of body weight, once a day,5 days); DM (60 mg/kg of STZ (*Streptozotocin*), single dose, intravenously, i.v., diluted in citrate buffer); and DM+Genta. Physiological parameters, renal function (creatinine clearance), oxidative damage (peroxides and thiobarbituric acid reactive substances – urinary TBARS) and renal hemodynamics were evaluated. **Results:** The Diabetes Mellitus group presented chronic hyperglycemia associated with loss of body weight, polyphagia, polydipsia and polyuria, in addition to reduced renal function and with an increase in oxidative metabolite excretion. Administration of gentamicin induced a reduction in renal blood flow and increased renal vascular resistance in healthy rats. The association of Diabetes Mellitus with gentamicin resulted in an additional reduction in renal function and elevation of oxidative metabolites, with increased renal vascular resistance. **Conclusion:** The existence of Diabetes Mellitus resulted in an elevation of gentamicin nephrotoxicity, thus confirming the risk factor for drug nephrotoxicity.

DESCRIPTORS

Acute Kidney Injury; Diabetes Mellitus; Gentamicins; Oxidative Stress.

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INTRODUCTION

Acute kidney injury (AKI) consists of an abrupt reduction of renal function (RF), clinically signaled by the elevation of serum creatinine to levels greater than or equal to 0.3 mg/dL in 48 hours, or a 1.5-fold increase in this marker over a 7-dayperiod[1]. The main causes of AKI are drug nephrotoxicity or renal ischemic events. The high mortality of patients affected by this syndrome, between 15% to 60%[2], justifies scientific investments to elucidate the mechanisms that precipitate it, as well as a proposition of therapeutic interventions that can mitigate its evolution.

AKI is less frequent in the general community than in hospitalized patients, and its prevalence is significant in Intensive Care Units (ICUs), affecting 20% to 40% of the patients hospitalized in these units[2]. Gentamicin is an aminoglycoside antibiotic, widely used in the fight against gram-negative bacterial infections and has nephrotoxicity as one of its main side effects. This is because its excretion predominantly occurs by glomerular filtration. The pathophysiology of gentamicin nephrotoxicity is characterized by damage to tubular and glomerular renal cells[3-4],associated with oxidative stress, generation of reactive oxygen species (ROS) and reduced antioxidant enzymes in the kidney[5].

Chronic diseases are described as risk factors for the occurrence of AKI in critically ill patients or those undergoing diagnostic procedures involving the administration of nephrotoxic drugs. Among this group of morbidities, we can highlight Diabetes Mellitus (DM), being the subject of interest in this study. Little is known about the cellular and functional principles that act as facilitators in the occurrence of nephrotoxicity of drugs in the occasion of comorbidities such as DM[6].

The absence of data on the mechanisms that predispose individuals with DM to the development of AKI due to nephrotoxicity of drugs, especially gentamicin, supports implementing investigations aimed at elucidating the co-interference of these factors in the patient's clinical evolution and that seek to clarify this knowledge gap in the area of intensive care, in which the entire health team is involved. The aim of this study was to evaluate the effect of gentamicin in diabetic rats through an evaluation of renal function and the oxidative profile.

METHOD

This was a quantitative and experimental study developed with rats. The rats from the various groups were kept with free access to water and food and remained under adequate thermal conditions with alternating cycles of day and night. All procedures performed in this study are in accordance with the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation (*COBEA – Colégio Brasileiro de Experimentação Animal*) and were approved by the Commission of Ethics in Animal Experimentation (*Comissão de Ética em Experimentação Animal – CEEA* 155/15).

Animals: Twenty-one adult male Wistar rats weighing 250-300 grams distributed into the following 4 groups were used: *Citrate (Control):* administration of citrate buffer (0.01 M pH 4.2, i.v. in the caudal vein); *Gentamicin:* animals who received gentamicin (gentamicin, 100 mg/kg of body weight, once a day, intraperitonealy, i.p., for 5 days); *DM:* induction of DM by administration of streptozotocin (STZ) (60 mg/kg, diluted in 0.01 M citrate buffer pH 4.2, i.v)[7-8]; and *Gentamicin+DM:* animals with DM who received gentamicin after 4 weeks of DM induction.

The animals submitted to the DM model had their glycemic blood levels evaluated by blood glucose test 48 hours after induction using Advantage reagent strips (Advantage – Roche®, Brazil). Animals that presented glycemic blood levels above 250 mg/dl in this period were considered diabetic. All diabetic animals had their body weight and blood glucose monitored for 4 weeks (28 days) once a week.

Obtaining the biological material: At the end of the protocol, the animals were placed in individual metabolic cages for the collection of 24-hour urine to evaluate renal function and oxidative metabolites. After this period, the animals were anesthetized with thiopental sodium (40-50 mg/kg, i.p.) for collection of terminal blood through puncturing the abdominal aorta and subsequent evaluation of renal function. Finally, euthanasia of the animal was performed at the end of the experiment according to the ethical standards for the handling of animals in research.

Renal function: Renal function was assessed by creatinine clearance. The Jaffé colorimetric method was used to determine serum and urinary creatinine values. Creatinine clearance was calculated by the formula[9]: creatinine clearance = urinary creatinine x 24-hour urinary flow / serum creatinine.

Oxidative metabolites: Oxidative metabolites were evaluated by measuring urinary peroxides and thiobarbituric acid reactive substances (TBARS).The evaluation of urinary peroxides was performed by the FOX-2 method, in which the use of an orange iron-xylenol oxidizes the Fe^{2+} ion producing a complex of purplish-blue coloration (α= 4.3 x 10^4 M^{-1} cm^{-1})[10-11]. An evaluation of urinary TBARS allows identification of end products of the lipid peroxidation cascade that react in the presence of thiobarbituric acid in organic fluids (α= 1.56 x 10^5 M^{-1} cm^{-1})[12-13].

Renal Hemodynamics: Renal hemodynamics were assessed by measuring renal blood flow (RBF), mean arterial pressure and renal vascular resistance. For renal blood flow, the left renal artery was isolated and involved by ultrasonic probes to measure the blood flow of the vessel. Mean arterial pressure (MAP) was determined by carotid artery catheterization, while renal vascular resistance (RVR) was calculated according to the following formula: RVR = MAP / RBF[14].

Statistical analysis: The results were presented as mean ± standard deviation. The statistical analysis of the results was performed by analysis of variance (ANOVA), followed by Newman-Keuls multiple comparisons in the statistical program Graph-Pad Prism version-3 for Windows®. The considered significance level was 5%.

RESULTS

Physiological parameters

The physiological parameters of the groups were demonstrated by food and water intake, animal body weight, kidney weight/animal weight ratio and glycemia, as shown in Table 1. It was possible to observe an increase in the weight of the Citrate group (control) when compared to the groups Genta, DM and DM+Genta. All treated groups presented a renal hypertrophy coefficient, represented by an increase in the kidney weight/animal weight ratio, with the highest indices being observed for groups of diabetic animals. Other characteristics that confirm the standardization of the DM model are polyphagia and polydipsia, indicated by the increase in food and water intake by diabetic rats, and hyperglycemia in the DM and DM+Genta groups.

Table 1 – Physiological parameters of the Citrate, Genta, DM and DM+Genta groups – São Paulo, SP, Brazil, 2017.

Groups (n)	Water intake 24 h (ml)	Food intake 24 h (g)	Animal weight (g)	Kidney weight (g)	Kidney weight/ Animal weight (%)	Glycemia (mg/dL)
Citrate (5)	30.0 ± 7.0	23.0 ± 3.5	392 ± 48	1.41 ± 0.20	0.36 ± 0.03	110±3.0
Genta (5)	33.0 ± 5.7	15.2 ± 4.9	320 ± 27[a]	1.14 ± 0.22	0.39 ± 0.09[a]	107±4.5
DM (5)	122.0 ± 29.5[ab]	36.2 ± 2.2[ab]	262 ± 36[ab]	1.47 ± 0.12	0.57 ± 0.08[a]	427±17.1[ab]
DM+Genta (5)	87.1± 34.1[c]	24.8 ± 10.1[c]	295 ± 18[a]	1.90 ± 0.40[ab]	0.54 ± 0.24[a]	374±13.0[abc]

Values represent mean ± standard deviation.
[a]p <0.05 *vs.* Citrate.
[b]p <0.05 *vs.* Genta.
[c]p <0.05 *vs.* DM.

Renal function and oxidative stress of the several groups

Table 2 shows the renal function, urinary peroxide and TBARS data of the various groups. The Citrate group presented urine flow and creatinine clearance parameters/100 g considered as normal. The Genta group presents a significant reduction in the clearance/100 g, however with urinary flow maintenance. The DM group demonstrates reduced clearance/100 g when compared to the Citrate and Genta groups, with elevated urinary flow. The DM+Genta group presented an increase in the urinary flow with an intensified reduction of renal function with high peroxides and TBARS values, indicating significant oxidative damage.

Table 2 – Renal and oxidative function of the Citrate, Genta, DM and DM+Genta groups – São Paulo, SP, Brazil, 2017.

Groups (n)	Urinary flow (ml/ min)	U. C. (mg/dL)	S.C. (mg/dL)	Clcr/100 (ml/min)	U.P. (nM/g of cr U)	TBARS (nM/g of cr U)
Citrate (5)	0.012 ± 0.001	82.36 ± 9.46	0.38 ± 0.13	0.76 ± 0.16	1.80 ± 0.94	0.19 ± 0.13
Genta (5)	0.018 ± 0.004	50.84 ± 13.23 [a]	0.90 ± 0.16	0.26 ± 0.05[a]	7.68 ± 2.65	1.80 ± 0.98
DM (5)	0.055 ± 0.010[ab]	21.52 ± 3.84 [ab]	0.99 ± 0.13[a]	0.47 ± 0.08[ab]	5.24 ± 2.56	11.73 ± 2.75[ab]
DM+Genta (5)	0.040 ± 0.018[ab]	20.33 ± 14.09[ab]	1.30 ± 0.52[a]	0.16 ± 0.06[ac]	10.38 ± 6.07[a]	21.68 ± 7.00[abc]

U.C.: urine creatinine; S.C.: serum creatinine; U.P.: urine peroxides; TBARS: thiobarbituric acid reactive substances.
Values represent mean ± standard deviation.
[a]p<0.05 *vs.* Citrate.
[b]p<0.05 *vs.* Genta.
[c]p<0.05 *vs.* DM.

Renal Hemodynamics

Figure 1A shows an increase in the heart rate of the Genta (573.2 ± 36.7) and DM+Genta (580.5 ± 20.4) groups when compared to DM (394.0 ± 96.7) and Citrate (410.5 ± 56.7) groups. The mean arterial pressure (figure 1B) remained close to citrate values for all groups (Citrate: 105.2 ± 6.8; Genta: 127.0 ± 27.9; DM: 103.6 ± 11.5; DM+Genta: 94.7 ± 4.8). Figure 1C shows that the Genta (4.00 ± 0.54) and DM+Genta (2.30 ± 0.42) groups showed decreased renal blood flow in comparison to DM (5.17 ± 2.25) and Citrate (7.08 ± 0.69) groups. However, renal vascular resistance (Figure 1D) was elevated in the Genta (32.27 ± 8.39) and DM+Genta (42.18 ± 7.29) groups, when compared to DM (22.82 ± 10.54) and Citrate (14.90 ± 0.75) groups.

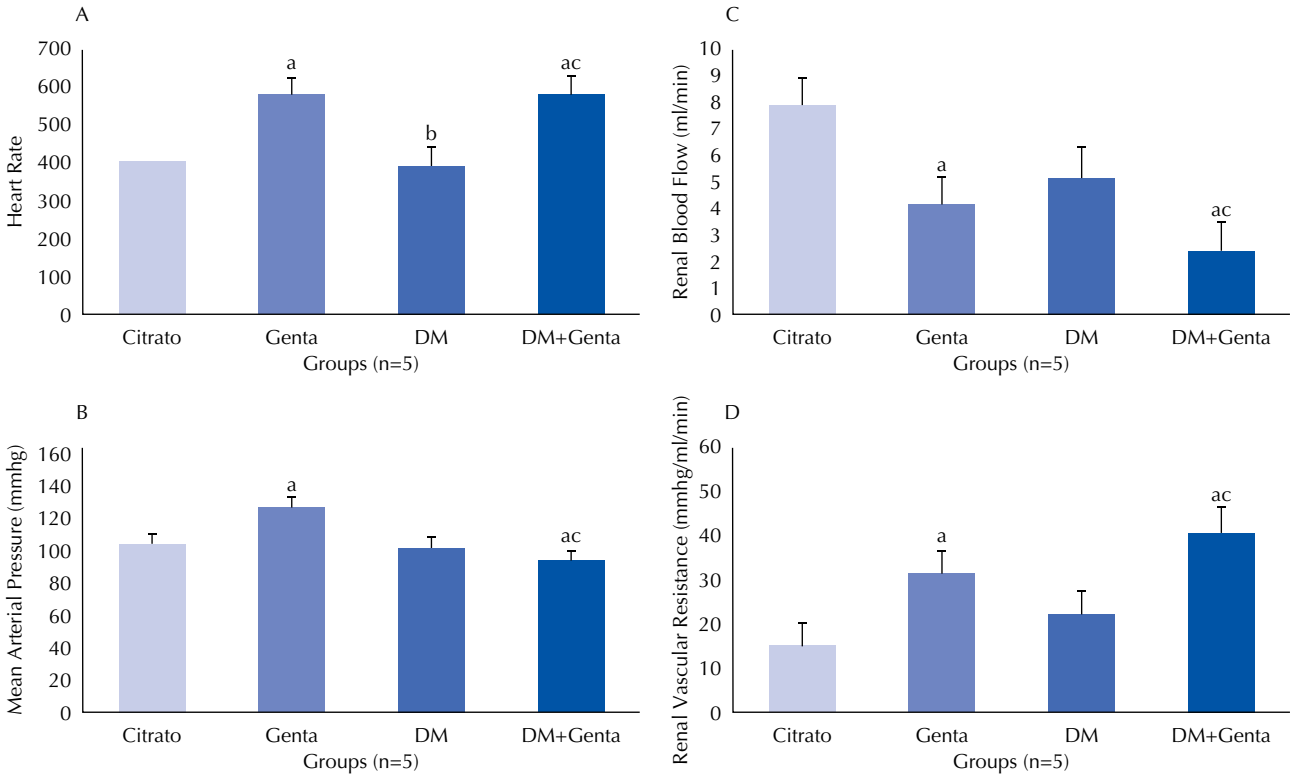

Figure 1 – Heart Rate, Mean Arterial Pressure, Renal Blood Flow and Renal Vascular Resistance. [a] p<0.05 *vs.* citrate; [b]p<0.05 *vs.* Gentamicin; [c]p<0.05 *vs.* DM. Data are shown as mean ± SD – São Paulo, SP, Brazil, 2017.

DISCUSSION

Studies describe Diabetes Mellitus (DM) as a risk factor for the development of kidney injuries. The continued use of nephrotoxic drugs is common in ICUs and becomes even more relevant in patients presenting comorbidities such as DM[6,15].

DM is a pathology that results in chronic hyperglycemia, as it triggers metabolic disorders due to defects in the synthesis and/or secretion of insulin or its action in tissues. This is an important pathology on the world scenario due to its morbidity and mortality rates, as well as its increasing incidence and prevalence rates[16].

In this study it was possible to verify the triad of polyuria, polydipsia and polyphagia, in addition to increased capillary glycemia (hyperglycemia) in animals chemically induced to develop DM. This finding confirms that the model standardization was successful[7,14].

One of the pathophysiological characteristics of the chronic hyperglycemia condition is the development of renal hypertrophy resulting from intense glomerular hyperfiltration, which leads to the thickening of the glomerular basement membrane and expansion of the mesangial matrix[17]. As precipitating mechanisms, some studies point out an increase in the polyol pathway, non-enzymatic glycosylation, with the production of advanced glycation end-products (AGEs) and oxidative stress resulting in increased free radicals and altered kinase C protein to justify vascular damage caused by high blood glucose concentration[18,19]. The higher kidney weight/body weight ratio found in diabetic animals in this study confirms the occurrence of renal hypertrophy.

Renal function in this study was assessed by creatinine clearance, which is considered the gold standard for measuring the glomerular filtration rate (GFR). The Genta group presented reduced creatinine clearance when compared to the Citrate group. These findings confirm the nephrotoxic potential of this drug, which acts in the proximal tubule, being transported into the cells connecting to the lysosomes, where it forms myeloid bodies and consequently causes interference in the phosphatidyl inositol cascade, blocking the hydrolysis of phospholipase C, affecting all intracellular signaling and its homeostasis, thereby compromising one of the main tubular functions which is the absorption of water and sodium to the concentration of urine, and thus characterizing renal injury by gentamicin[20-21]. The DM group additionally presented a 50% reduction in renal function when compared to citrate. Renal injury by DM is clinically and initially characterized by an increased glomerular filtration rate (glomerular hyperfiltration) and microalbuminuria. However, with progression of the disease, a reduction in GFR is confirmed as demonstrated. This pathophysiological mechanism of DM enhances the effect of other injury factors such as drug nephrotoxicity. In this study, we found an even more pronounced reduction of RF when gentamicin was administered to diabetic rats. This finding confirms the diabetic animal's vulnerability to other renal exposures.

Although the mechanisms by which gentamicin alters glomerular filtration are not fully understood, many studies have revealed the involvement of renal vasoconstriction and direct tubular toxicity as important agents[22-24]. In this study, assessment of renal blood flow and renal vascular resistance demonstrated significant changes in animals treated with gentamicin alone and with gentamicin in combination with chronic hyperglycemia, evidencing intense hypoperfusion; this differs from what was observed in the DM group, in which the values remained similar to those of the Citrate group. A study found normal parameters of hemodynamics in DM groups which changed when other nephrotoxic substances were associated[14]. Measuring renal hemodynamics contributes to understanding the action mechanism of the vasodilator and vasopressin agents involved in renal injury models.

The pathophysiology of oxidative renal injury is described based on the exacerbated consumption of ATP, which occurs in the case of hypoxia in the renal medulla, which in turn induces abnormalities in oxygen metabolism such as the generation of ROS with nitrogen compounds (peroxide nitrite), oxygen (hydroxyl radical), glycation and formation of adenosine and its metabolite hypoxanthine[25-26]. The present study evaluated oxidative stress through measuring urinary peroxides and TBARS. The isolated use of gentamicin presented increased yet not significant values of oxidative metabolites. On the other hand, in the DM groups and especially the DM+Genta group, the significant increase of the markers demonstrated free radicals and oxidizing agents participating in the associated model of diabetic nephropathy with drug nephropathy. Studies of isolated analyzes have demonstrated oxidative damage caused by advanced oxidation protein products (AOPP) and catalase antioxidant enzyme in gentamicin nephrotoxicity models[27],and by lipid peroxidation and analysis of thiols in diabetes[26]. Association of chronic hyperglycemia and treatment with the nephrotoxic aminoglycoside gentamicin reveals more aggressive oxidative renal lesions, with cellular apoptosis which results in a chronic renal injury model, as reproduced in this study.

Experimental studies with animals that reproduce clinical situations aiming for elucidating individualized pathophysiological factors facilitate clinical reasoning and help in the identification of patients at risk for drug nephropathy by the multiprofessional team, such as those induced by gentamicin.

Considering that in recent years sedentary lifestyles coupled with obesity have triggered an increase in the number of individuals with Diabetes Mellitus, and that a lack of adherence to chronic hyperglycemia treatment may make them more susceptible to side effects of medications such as nephrotoxicity[28], this study implemented an animal model of diabetes and nephrotoxicity and confirmed that hyperglycemia associated with the toxic insult of gentamicin resulted in severe acute kidney injury, characterizing DM as a risk factor for drug nephropathy.

CONCLUSION

DM animals show signs of diabetic nephropathy with reduced RF and elevated lipid peroxidation. Gentamicin induced a reduction in RF and increased generation of oxidizing agents, with an elevation in RVR and RBF. Treatment with gentamicin in rats with DM induced worsening of RF, lipid peroxidation with significant elevation in RVR and reduced RBF.

RESUMO

Objetivo: Avaliar o efeito do antibiótico gentamicina em modelo experimental na presença de Diabetes Mellitus por meio da função renal e perfil oxidativo. **Método:** Ratos Wistar, adultos, machos, foram distribuídos nos grupos: Citrato; Gentamicina (Genta), (gentamicina 100 mg/kg de peso corporal, 1 vez ao dia, intraperitoneal, i.p., 5 dias); DM (60 mg/kg de STZ, intravenosa, i.v., dose única, diluída em tampão citrato) e DM+Genta. Foram avaliados os parâmetros fisiológicos, a função renal (*clearance* de creatinina), a lesão oxidativa (peróxidos e substâncias reativas ao ácido tiobarbitúrico – TBARS urinários) e a hemodinâmica renal. **Resultados:** O grupo Diabetes Mellitus apresentou hiperglicemia crônica, associada à perda de peso corporal, polifagia, polidipsia e poliúria, além de redução da função renal, com aumento na excreção de metabólitos oxidativos. A administração de gentamicina induziu a redução do fluxo sanguíneo renal e o aumento da resistência vascular renal em ratos saudáveis. A associação do Diabetes Mellitus com gentamicina resultou em redução adicional na função renal e elevação de metabólitos oxidativos, com aumento de resistência vascular renal. **Conclusão:** A existência de Diabetes Mellitus determinou a elevação da nefrotoxicidade da gentamicina e se confirmou como fator de risco para nefrotoxicidade de medicamentos.

DESCRITORES

Lesão Renal Aguda; Diabetes Mellitus; Gentamicina; Estresse oxidativo.

RESUMEN

Objetivo: Evaluar el efecto del antibiótico gentamicina en modelo experimental en la presencia de Diabetes Mellitus mediante la función renal y el perfil oxidativo. **Método:** Ratas Wistar, adultas, machos, fueron distribuidas en los grupos: Citrato; Gentamicina (Genta), (gentamicina 100 mg/kg de peso corporal, 1 vez al día, intraperitoneal, i.p., 5 días); DM (60 mg/kg de STZ, intravenosa, i.v., dosis única, diluida en tampón citrato) y DM+Genta. Fueron evaluados los parámetros fisiológicos, la función renal (aclaramiento de creatinina), la lesión oxidativa (peróxidos y sustancias reactivas al ácido tiobarbitúrico – TBARS urinarios) y la hemodinámica renal. **Resultados:** El grupo Diabetes Mellitus presentó hiperglucemia crónica, asociada con pérdida de peso corporal, polifagia, polidipsia y poliuria, además de reducción de la función renal, con aumento en la secreción de metabolitos oxidativos. La administración de

gentamicina indujo a la reducción del flujo sanguíneo renal y al incremento de la resistencia vascular renal en ratas sanas. La asociación del Diabetes Mellitus con gentamicina resultó en reducción adicional en la función renal y elevación de metabolitos oxidativos, con aumento de resistencia vascular renal. **Conclusión:** La existencia de Diabetes Mellitus determinó la elevación de la nefrotoxicidad de la gentamicina y se confirmó como factor de riesgo para nefrotoxicidad de fármacos.

DESCRIPTORES

Lesión Renal Aguda; Diabetes Mellitus; Gentamicina; Estrés Oxidativo.

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