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THE EFFECT OF MELATONIN ON ELECTROLYTE AND WATER-RETENTION RENAL DYSFUNCTION IN CHRONIC ALCOHOL INTOXICATION ASSOCIATED WITH AUTOIMMUNE NEPHRITIS: AN EXPERIMENTAL RAT MODEL

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ABSTRACT — Autoimmune nephritis represents one of the frequent types of kidney pathology. With rates of alcohol consumption being on the rise, it is crucial to study the effects of alcohol abuse on the manifestations of nephropathy. Since the activation of lipid peroxidation plays a significant role in the formation of autoimmune and alcoholic nephropathy, it appears relevant to investigate the potential positive effects of a natural antioxidant, melatonin. The aim of the study was to investigate the effect of melatonin on renal function in chronic alcohol intoxication associated with autoimmune nephritis. Material and methods. The characteristics of changes in electrolyte and water excretory functions of the kidneys in Wistar rats with chronic alcohol intoxication associated with autoimmune nephritis were examined. Experimental chronic alcohol intoxication was obtained by daily intragastric injection through an atraumatic tube of 40% ethanol solution and the administration of isovaleric acid amide solution for 30 days. The model of autoimmune nephritis was obtained via a single subcutaneous injection of an emulsion of complete Freund's adjuvant with an equal volume of a mixture of kidney cortical homogenate at five points. Melatonin was administered intragastrically at a dose of 10 mg/kg for one month.
Results. Alcohol intoxication was found to increase the degree of renal dysfunction in autoimmune nephritis. The administration of melatonin as a preventative agent led to a significant reduction in manifestations of nephropathy.
Conclusions. Alcohol intoxication may exacerbate the manifestations of autoimmune nephritis. The administration of melatonin in chronic alcohol intoxication associated with autoimmune nephritis leads to the reduction in the severity of pathological changes in the electrolyte-excretory function of the kidneys.

KEYWORDS — chronic alcohol intoxication, autoimmune nephritis, melatonin, nephrotoxicity.

INTRODUCTION

Previous studies have shown a decline in alcohol consumption in Russia since 2007, as in many other developed countries [1,2]. However, since the second quarter of 2020, when *stay-at-home* restrictions were first introduced to contain the spread of coronavirus infection, self-isolation and unemployment have been the trigger for a sharp rise in per capita ethanol consumption. Chronic alcohol intoxication causes the destruction of the normal renal microstructure, increases the distance between capillaries and renal tubular cells, and damages capillaries that supply oxygen and nutrients to renal tubular cells. Damage to renal tubular cells can lead to renal interstitial fibrosis, which in turn impairs renal function [3]. Thus, due to spread of infectious diseases, which create a favorable environment for autoimmune diseases, the growth of autoimmune disorders, including autoimmune nephritis, has been noticed. Autoimmune disorders are a major cause of chronic kidney disease, accounting for about 10% of all patients on dialysis [4].

Ways of treating multi-organ alcohol-related damage associated with autoimmune nephritis have received little attention in modern experimental studies. The prevention of chronic alcohol intoxication associated with autoimmune nephritis presents a pressing issue. The activation of lipid peroxidation plays a significant role in the pathogenesis of autoimmune nephritis and alcoholic kidney damage. In this regard, it appears relevant to study the effects of the natural antioxidant melatonin. Melatonin as a preventative medication has a positive effect in toxic nephropathies caused by heavy metals, such as those caused by long-term lead intoxication, which was confirmed by significant changes in the parameters of renal functions, systemic haemodynamics and biochemical blood parameters [6].

The aim of the present study was to investigate the effect of melatonin on renal function in chronic alcohol intoxication associated with autoimmune nephritis.

METHODS

The research was performed in white male Wistar rats weighing 200–300g split into 12 groups (n=120): 1) background (intact) animals; 2) the group subjected to intragastric administration of melatonin at a dose of 10 mg/kg 3) the group subjected to intragastric administration of 40% ethanol at a dose of 3.0 g/kg for a month; 4) the group subjected to intragastric administration of 40% ethanol at a dose of 3.0 g/kg and melatonin at a dose of 10 mg/kg for a month 5) the group subjected to intragastric administration of isovaleric acid amide solution (alcohol dehydrogenase inhibitor) at a dose of 500 mg/kg for a month; 6) the group subjected to intragastric administration of isovaleric acid amide solution at a dose of 500 mg/kg and melatonin at a dose of 10 mg/kg for a month; 7) the group subjected to intragastric administration of isovalerian acid amide solution at a dose of 500 mg/kg together with 40% ethanol at a dose of 3.0 g/kg every day for 30 days (a model of chronic alcohol intoxication) [7]; 8) the group with the model of chronic alcohol intoxication subjected to intragastric administration of melatonin at a dose of 10 mg/kg every day for 30 days 9) the group of rats subjected to subcutaneous injection of Freund's complete adjuvant emulsion with an equal volume of a mixture of kidney cortex homogenate, pre-diluted with saline, at the rate of 1.0 ml of saline per 100.0 mg of tissue. The solution was injected once subcutaneously into five locations: axillary and inguinal areas, intraperitoneally 0.1 ml of the active solution per 100 g of animal weight (a model of autoimmune nephritis) [8]; 10) the model of autoimmune nephritis with intragastric administration of melatonin at a dose of 10 mg/kg for a month; 11) a model of chronic alcohol intoxication against autoimmune nephritis for 30 days; 12) the model of chronic alcohol intoxication associated with autoimmune nephritis subjected to intragastric administration of melatonin at a dose of 10 mg/kg for a month.

During the experiment, the animals were given a standard diet and had free access to water and food throughout the day. Light conditions were natural. The electrolyte- and water-excretory functions of the kidneys during spontaneous diuresis were studied. To assess renal function, animals were placed in exchange cages, where urine was collected for 6 hours. The following parameters were assessed: urine output volume, glomerular filtration rate, relative tubular water reabsorption, cations excretion level (potassium, sodium), protein content in urine. Contents of sodium and potassium in serum and urine were determined by ion-selective method using the electrolyte analyzer AEK-1 ("Kverti-Med").

Functional renal condition was assessed with conventional research methods [9] involving the application of biochemical reagent kits ("Olvex" and "Agat-Med") for the analysis of urine and blood plasma and further sample processing with the "Solar" spectrophotometer.

Experiments were performed in accordance with "International Guiding Principles for Biomedical Research Involving Animals" (1985), the 11th article of the WMA Declaration of Helsinki and the rules of laboratory practice in the Russian Federation (order of 01.04.2016 № 199).

The results obtained were statistically processed with consideration of the features of distribution within the groups using the Shapiro-Wilk criterion. Nonparametric Mann-Whitney U-criterion was applied to compare the data across the groups. Statistical analysis was performed using the standard Microsoft Excel 2016 and Statistica 10.0 software packages. Differences were considered reliable at $p \leq 0.05$.

RESULTS

The study of water-excretory function during 6 hours of spontaneous diuresis revealed no reliable changes in the functional renal condition in the control groups 2–6.

A decrease in spontaneous 6-hour diuresis ($p \leq 0.01$) was observed in Group 7 (a model of chronic alcohol intoxication), which is associated with a decrease in glomerular filtration rate ($p \leq 0.01$), contrary to the trend towards reduction in tubular water reabsorption (Table 1). We also observed an increase in urinary protein concentration ($p \leq 0.001$) (Table 1). In Group 8 (prophylactic administration of melatonin in a model of chronic alcohol intoxication), we observed an increase in spontaneous diuresis and tubular water reabsorption ($p \leq 0.05$) compared to Group 7; as well as a decrease in urinary protein content ($p \leq 0.001$) (Table 1).

In Group 9 (a model of autoimmune nephritis), a decrease in 6-hour spontaneous diuresis ($p \leq 0.05$), and a decrease in tubular water reabsorption ($p \leq 0.05$) and glomerular filtration rate ($p \leq 0.05$) relative to intact values were observed. In the Group 10 (prophylactic administration of melatonin in a model of autoimmune nephritis) we observed an increase in tubular water reabsorption ($p \leq 0.05$), as well as a decrease in urinary protein concentration ($p \leq 0.01$) compared to Group 9.

Group 11 (a model of chronic alcohol intoxication in autoimmune nephritis) revealed a marked decrease in 6-hour spontaneous diuresis ($p \leq 0.01$) relative to baseline values, associated with decreased glomerular filtration rate ($p \leq 0.001$), despite the trend

Table 1. *Basic urine formation and urine protein content indicators in rats under conditions of spontaneous diuresis in chronic alcohol intoxication in the setting of autoimmune nephritis*

Experimental conditions	Urine output, (ml/h/100 g)	Glomerular filtration rate, (ml/h/100 g)	Relative tubular water reabsorption (%)	Urine protein concentration (mg/ml)
Intact animals	0,092±0,004	19,8±1,41	99,53±0,012	0,137±0,012
Group 2 (melatonin administered at 10 mg/kg)	0,098±0,0031	20,3±1,122	99,54±0,014	0,0987±0,0044
	-	-	-	-
Group 3 (40% ethanol at 3.0g/kg administered for 1 month)	0,087±0,007	17,9±1,5	99,51±0,010	0,156±0,007
	-	-	-	-
Group 4 (40% ethanol at 3.0g/kg and melatonin at 10mg/kg administered for 1 month)	0,090±0,0034	18,422±1,212	99,523±0,012	0,155±0,0086
	-	-	-	-
Group 5 (isovaleric acid amide solution administered at 500 mg/kg for a month)	0,085±0,008	18,6±1,65	99,54±0,011	0,156±0,013
	-	-	-	-
Group 6 (isovaleric acid amide solution at 500 mg/kg and melatonin at 10 mg/kg administered for a month)	0,081±0,0052	18,425±1,322	99,548±0,014	0,114±0,0102
	-	-	-	-
Group 7 (model of chronic alcohol intoxication)	0,073±0,005	14,3±0,9	99,49±0,009	0,401±0,023
	**)	**)	**)	***)
Group 8 (model of chronic alcohol intoxication with daily administration of melatonin at 10 mg/kg for a month)	0,087±0,0021	16,835±0,873	99,517±0,011	0,221±0,0145
	#)	-	#)	***)###)
Group 9 (model of autoimmune nephritis)	0,076±0,004	15,4±1,0	99,5±0,009	0,394±0,033
	*)	*)	*)	***)
Group 10 (model of autoimmune nephritis with melatonin administered at 10 mg/kg for a month)	0,086±0,0024	17,842±0,755	99,527±0,007	0,266±0,0176
	-	-	!)	***)!!)
Group 10 (model of chronic alcohol intoxication against autoimmune nephritis)	0,061±0,003	11,6±0,75	99,46±0,011	0,647±0,01
	***)	***)	***)	***)
Group 12 (model of chronic alcohol intoxication against autoimmune nephritis with melatonin administered at 10 mg/kg for a month)	0,079±0,0026	16,844±0,657	99,513±0,010	0,343±0,0339
	*)♣♣♣)▲▲)	♣♣♣)	♣♣)	***)♣♣♣)▲▲▲)

Notes: (*) –p≤0,05; (**) –p≤0,01; (***) –p≤0,001 vs background
(#) –p≤0,05; (##) –p≤0,01; (###) – p≤0,001 vs Group 7
(▲) –p≤0,05; (▲▲) –p≤0,01; (▲▲▲) – p≤0,001 vs Group 8
(!) –p≤0,05; (!!) –p≤0,01; (!!!) – p≤0,001 vs Group 9
(♣) –p≤0,05; (♣♣) –p≤0,01; (♣♣♣) – p≤0,001 vs Group 13

towards a reduction in tubular water reabsorption (p≤0.001). Urine protein concentration was significantly increased compared to background (intact animal) values (p ≤0.001).

In Group 12 (prophylactic administration of melatonin in a model of chronic alcohol intoxication in autoimmune nephritis), an increase in spontaneous diuresis (p≤0.001) and glomerular filtration rate (p≤0.001) compared to the melatonin-free Group 11 was observed. Tubular reabsorption of water also increased (p≤0.01; Table 1) compared to Group 11.

The study of electrolyte excretion function under the conditions of 6-hour spontaneous diuresis revealed no reliable changes in renal functions in the control groups 2-6 (see Table 2).

An increase in sodium (p≤0.01) and potassium excretion (p≤0,001) compared to background (intact animals) values was observed in Group 7 (a model of chronic alcohol intoxication). Sodium filtration charge (p≤0.001) and tubular cation reabsorption (p≤0.001) showed a decrease. Blood sodium concentration (p≤0.05) decreased, whereas blood potassium concentration significantly increased (p≤0.01). In Group 8 (prophylactic use of melatonin in a model of chronic alcohol intoxication), we observed an increase in sodium (p≤0.05) and potassium (p≤0.05) excretion compared to Group 7. Tubular reabsorption of sodium (p≤0.05) was shown to increase; there was also an increase in blood sodium concentration (p≤0.05) and a decrease in plasma potassium concentration

Table 2. *Renal electrolyte processing indicators (sodium, potassium) and their concentrations in blood plasma in rats in a model of chronic alcohol intoxication in autoimmune nephritis*

Experimental conditions	Sodium excretion (μmol/hr/100g)	Sodium filtration charge (μmol/hr/100g)	Sodium channel reabsorption (%)	Blood sodium levels (mmol/l)	Potassium excretion (μmol/hr/100g)	Potassium filtration charge (μmol/hr/100g)	Blood potassium levels (mmol/l)
Intact animals	7,84±0,37	2696±164	99,7±0,032	145±1,69	4,4±0,39	85,7±7,58	4,45±0,28
Group 2 (melatonin administered at 10 mg/kg)	7,77±0,6556	2746,44±142	99,717±0,028	144,656±1,53	4,290±0,41	87,39±7,15	4,570±0,31
	-	-	-	-	-	-	-
Group 3 (40% ethanol at 3.0g/kg administered for 1 month)	8,93±1,09	2420±202	99,6±0,035	143±0,75	5,2±0,45	80,2±6,7	4,7±0,17
	-	-	-	-	-	-	-
Group 4 (40% ethanol at 3.0g/kg and melatonin at 10mg/kg administered for 1 month)	7,81±0,6435	2610,42±213	99,701±0,0312	143,843±0,523	4,864±0,3334	84,32±7,1332	4,622±0,142
	-	-	-	-	-	-	-
Group 5 (isovaleric acid amide solution administered at 500 mg/kg for a month)	7,83±0,61	2518±223	99,7±0,02	144±0,8	5,1±0,5	92,6±8,2	4,79±0,1
	-	-	-	-	-	-	-
Group 6 (isovaleric acid amide solution at 500 mg/kg and melatonin at 10 mg/kg administered for a month)	7,92±0,8051	2575,11±210	99,692±0,0267	145,456±0,643	5,142±0,4233	94,85±7,3340	4,335±0,213
	-	-	-	-	-	-	-
Group 7 (model of chronic alcohol intoxication)	9,24±0,31	1891±118	99,5±0,03	140±1,28	6,4±0,34	66,9±5,9	5,44±0,25
	**)	***)	***)	*)	***)	-	**)
Group 8 (model of chronic alcohol intoxication with daily administration of melatonin at 10 mg/kg for a month)	8,11±0,4340	2247,14±104	99,639±0,0264	143,755±0,835	5,414±0,3022	82,46±4,5330	4,327±0,266
	#)	-	*)#)	#)	#)	-	##)
Group 9 (model of autoimmune nephritis)	9,09±0,43	2074±132	99,6±0,032	141±1,09	5,9±0,48	67,7±4,3	5,2±0,15
	*)	**)	**)	*)	**)	*)	**)
Group 10 (model of autoimmune nephritis with melatonin administered at 10 mg/kg for a month)	7,89±0,4055	2534,12±185	99,689±0,0232	144,344±1,134	4,456±0,4253	78,59±3,3430	4,533±0,185
	-	-	!)	!)	!)	-	!)
Group 10 (model of chronic alcohol intoxication against autoimmune nephritis)	10,22±0,35	1530±139	99,3±0,054	139±1,2	7,5±0,34	53,4±5,2	5,7±0,13
	***)	***)	***)	**)	***)	**)	***)
Group 12 (model of chronic alcohol intoxication against autoimmune nephritis with melatonin administered at 10 mg/kg for a month)	8,58±0,4013	2136,76±154	99,599±0,0425	142,235±0,755	5,653±0,2554	69,66±4,7440	4,752±0,135
	♣♣)	♣♣)	♣♣♣)	♣♣)	*)♣♣♣)	*)♣♣)	♣♣♣)

Notes: see Table 1.

($p \leq 0.01$; Table 2). Obtained results are consistent with previously published literature [12].

In Group 9 (a model of autoimmune nephritis), we observed an increase in sodium ($p \leq 0.05$) and potassium ($p \leq 0.01$) excretion compared to baseline values. Sodium filtration charge ($p \leq 0.01$) and tubular cation reabsorption ($p \leq 0.001$) decreased. Blood sodium concentration ($p \leq 0.05$) decreased, whereas blood potassium concentration significantly increased ($p \leq 0.01$) (Table 2). We observed a decrease in potassium excretion ($p \leq 0.05$) in Group 10 (prophylactic administration of melatonin in autoimmune nephritis) compared to Group 9, as well as an increase in blood sodium concentration ($p \leq 0.05$) and a decrease in plasma potassium concentration ($p \leq 0.05$; Table 2).

In Group 11 (chronic alcohol intoxication in autoimmune nephritis), we observed an increase in urine excretion of sodium ($p \leq 0.01$) and potassium

(p≤0.001). The decrease in sodium levels (p≤0.05) was associated with high urinary sodium losses due to renal tubular damage and decreased tubular cation reabsorption (p≤0.001; Table 2), despite a significant decrease in cation filtration charge. Potassium filtration charge tended to decrease due to decreased glomerular water filtration rate (p≤0.01), but cation excretion was still higher than that in controls (p≤0.001), probably due to changes in tubular cation processing under hyperpotassemia (p≤0.05). In the Group 12 (prophylactic use of melatonin in a model of chronic alcohol intoxication in autoimmune nephritis), we observed a decrease in sodium (p≤0.01) and potassium excretion (p≤0.001). Filtration charge of potassium (p≤0.01) and sodium (p≤0.01;Table 2) showed an increase compared to the group without prophylactic administration of melatonin.

DISCUSSION

Thus, it was found that chronic alcohol intoxication against the background of autoimmune nephritis was associated with a more pronounced decrease in the volume of 6-hour spontaneous diuresis than alcohol intoxication without concomitant renal pathology. This is probably due to dystrophic changes in the tubular epithelium and linear deposits of IgG and complement component C3 along the glomerular basal membranes, which occurs in autoimmune renal damage [10]. The increase in protein concentration in urine was also more pronounced than in chronic alcohol intoxication without concomitant pathology. In the experimental group with simulated chronic alcohol intoxication in autoimmune nephritis, we observed a decrease in sodium levels related to high urinary sodium losses due to renal tubular apparatus damage and decreased tubular reabsorption of cations. Potassium filtration charge was significantly decreased due to decreased glomerular water filtration rate, but cation excretion was still higher than in controls, probably due to changes in tubular cation processing under the conditions of hyperpotassemia. These changes are most likely related to the deposition of circulating immune complexes in renal structures, which is accompanied by the activation of monocytes and macrophages, resulting in increased damage to the endothelium of the glomerular vessels, apoptosis and disruption of blood flow in the microcirculatory channel [11]. Administration of melatonin as a preventative agent in chronic alcohol intoxication associated with autoimmune nephritis contributed to the restoration of spontaneous diuresis indicators and glomerular filtration rate compared to the melatonin-free group; we also observed an increase in tubular water reabsorption, which is probably associated with one of the main preventive effects of melatonin manifesting in the increased sensitivity of the renal tubular apparatus to endogenous regulators. The positive effect of melatonin in toxic nephropathies has also been confirmed in the literature, where it is reported that it may reduce the severity of lipid peroxidation processes in renal cellular structures and the amount of reactive oxygen species, and also may exert immunomodulatory effects [12].

CONCLUSIONS AND KEY POINTS

1. Alcohol intoxication intensifies the manifestations of autoallergic nephritis. 2. The use of melatonin in chronic alcohol intoxication associated with autoimmune nephritis leads to a decrease in the severity of pathological changes in renal function.

Contributors

The aim, the objectives and planning of research — V.Brin; the experiments — V.Zemlianoy.

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