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Research Paper

Effects of poly (ADP–ribose) polymerase inhibitor on early peripheral neuropathy in streptozotocin–diabetic rat

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Abstract

Objective: To explore the effects and mechanisms of poly(ADP-ribose) polymerase(PARP) inhibitor 3-aminobenzamide on nerve lesions in streptozotocin-diabetic rats. **Methods**: Experimental rats were divided into normal control group(NC group), diabetic control group(DC group)and diabetic group treated with 3-aminobenzamide(DT group).Nerve conduction velocity (NCV), serum superoxide dismutase (SOD) activity and serum malondialdehyde (MDA) concentration, phosphocreatine (Pcr), creatine (Cr) concentration in sciatic nerves were evaluated after 4 weeks. **Results**: SOD, Pcr activity, and NCV were higher ($P < 0.05$) and MDA concentration were significantly lower in DT group, compared with DC group ($P < 0.01$). Meanwhile, ATP and Cr in sciatic nerves were similar in DT group, compared with DC group ($P > 0.05$). **Conclusion**: 3-aminobenzamide could alleviate the established functional and metabolic abnormalities of early DPN in the streptozotocin-induced diabetic rat models, which provided a novel approach for prevention and treatment of diabetic neuropathy.

Keywords: 3-aminobenzamide; diabetic neuropathy; streptozotocin-diabetic rat

INTRODUCTION

Under multiple factors, the nerve tissues suffer the attack of oxygen free radical after ischemia and lack of oxygen, which may cause various kinds of oxidative DNA damage and subsequently trigger the way of DNA damage recovery. As a downstream effector of free radical and oxidant-induced DNA single-strand breakage, poly(ADP-ribose)polymerase(PARP) activation plays an important role in a number of pathological conditions associated with oxidative stress, such as cardiovascular and neurodegenerative diseases, cancer and inflammation, etc. Growing evidences indicate that poly (ADP-ribose) polymerase (PARP)activation is an important mechanism in the pathogenesis of diabetes complications [1]. In the development of diabetic polyneuropathy(DPN), PARP activation is an obligatory initiator. Now we observed the preventive effects of PARP inhibitor 3-aminobenzamide (3-AB) in the early peripheral neuropathy in streptozotocin-diabetic rats.

MATERIALS AND METHODS

Materials

Thirty male Wistar rats(Tongji Medical College), streptozotocin (Sigma, America), 3-AB (Santa), 721 spectrophotometer(Shanghai), high performance liquid chromatograph (Waters, America), and Super T21 high speed centrifuge(America)were prepared.

Male Wistar rats, 8 weeks, weight from 150 - 200g, were injected with STZ (60mg·kg^{-1}). Blood samples for measurements of glucose were taken from tail veins approximately 72 h after STZ injection. The rats with blood glucose of 16.7 mmol/L or more were considered diabetic. Experimental rats were divided into normal control group(NC group, n=10), diabetic control group (DC group, n=10)and diabetic group treated with 3-aminobenzamide (DT

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group, n=10). After 2 weeks, DT group was administrated with 3-AB and the other two groups were administrated with distilled water every day for 2 weeks. Nerve conduction velocity was measured before the experiment was over. And SOD activity and the concentration of MDA, Pcr, cr were deteded after 4 weeks.

Determination of nerve conduction velocity

Rats were anaesthetized by chloral hydrate (6%, 300 mg·kg^{-1}) before fixation.The skin between musculus biceps femoris and semimembranous muscle was splitted and dissected bluntly to expose sciatic nerve. Sciatic nerves were stimulated at the sciatic notch proximally and at the ankle distally via bipolar electrodes (stimulus frequency 1Hz, stimulus width 0.3ms, time lag 1ms, stimulus interval 1500ms, stimulus intensity 2~3V). Incubations of action potential (T, from stimulation onset to primary negative wave onset) were recorded. Electrical stimulation was repeated 3 times with an intervald 1 minute to obtain general average, and the results were divided by time intervals between the stimulating and recording electrode(S) to calculate nerve conduction velocity.

Nerve sampling

The segments of sciatic nerves, which were preserved in liquid nitrogen to assay the content of ATP, Pcr and cr by use of high performance liquid chromatograph , were prepared as mentioned[2].

Determination of the concentrations of ATP, Pcr and cr in sciatic nerves

Standard solutions of ATP, Pcr and cr were prepared. (1) Chromatorraphic condition of Pcr and cr. Chromatographic column: column C18 Novapak 5u (3.9×150mm, Co.Waters); moving phase: liquid A: 20mmol/L solution of KH_2PO_4, 600ml (pH5.8, 2.9 mmol/LIPR-A was inside), water 350ml, methanol 50 ml; liquid B: 20 mmol/L solution of KH_2PO_4, 600ml (pH5.8, 2.9 mmol/LIPR-A was inside), water 180ml, methanol 220 ml. As mentioned above, liquid A and B were filtered by filter membrane of 0.45um and degassed, and then they were eluted gradiently with the flow rate of 1.2ml/min; detection wavelength was 215nm. (2)Chromatographic condition of ATP. Chromatographic column: column C18 Novapak 5u (3.9×150mm, Co.Waters); moving phase: 30mmol/L solution of $NH_4H_2PO_4$ 600ml, ammonia water was added to make the moving phase pH5.3, the flow rate was 0.9ml/min and the detection wave length was 254nm .Sciatic nerves were weight after nitrogen liquid was taken out , and then perchloric acid (0.5mol/L) was added according to 1:20 ratio before homogenated by tissue homogenizer at 4℃. Then the sciatic nerves were centrifugated (9000r/min) for 12minutes. After supernate was obtained and pH adjusted to 7.0 with solution of K_2CO_3, they were centrifugated (6000 r/min) for 5 min. Afterwards, 20ml supernate was obtained to carry out sample introduction via high efficiency liquid chromatography.

Determination of SOD activity and MDA concentration

Nitrite colouration method was used to determine SOD activity with wavelength 550nm to determine absorbance. Thiobarbital method was used to determine MDA concentration with wavelength 532nm to determine absorbance. The details were operated according to instructions.

Statistical analysis

Statistical software SPSS10.0 was used for data processing. All results were expressed as means ± SD. Variance analysis and *q*-test were used.

RESULTS

Body mass variation and blood glucose of rats

Body mass variation and blood glucose were similar in DT group, compared with DC group ($P > 0.05$), body mass variation was significantly lower in DT group, compared with NC group($P < 0.01$), and blood glucose was significantly higher in DT group, compared with NC group ($P < 0.01$) (***Tab 1***).

Result of NCV and the concentrations of ATP, Pcr, Cr, MDA and SOD activity

SOD, Pcr activity and NCV were higher($P < 0.05$) and MDA concentration were significantly lower in DT group, compared with DC group ($P < 0.01$). Meanwhile, ATP and Cr in sciatic nerve were similar in DT group compared with DC group($P > 0.05$) (***Tab 2***).

DISCUSSION

There is a great deal of theories on the pathogenesis of DPN, including ischemia, hypoxia, sorbitol accumulation, inositol reducement, oxidative stress and so on. Nowadays investigations indicate that oxidative stress plays an important role in the pathogenesis of DPN .Growing evidences indicate that oxidative stress which results in the production of lipid peroxidate is an early pivotal mechanism in the production of glycosylation dead end product and the activation of PKC via the induction of high glucose,

Tab 1 **Comparison of body mass and blood glucose among three groups** ($\bar{x} \pm s$,n=10)

Groups	Number	body mass(g)		blood glucose(mmol/l)	
		commence	4 weeks later	commence	4 weeks later
DC	10	169.32 ± 23.44	140.26 ± 14.72	25.85 ± 5.51	25.75 ± 4.56
DT	10	172.51 ± 21.22	144.21 ± 11.45	26.62 ± 3.74	26.39 ± 3.98
NC	10	166.74 ± 29.40	248.17 ± 32.93	5.29 ± 2.11	5.63 ± 1.29
F		3.10	5.24*	128.62**	120.5**
q	DC:DT	0.34	0.85	0.84	0.56
	DC:NC	0.42	3.78*	25.64**	24.32**
	DT:NC	0.64	3.64*	27.52**	28.61**

*$P < 0.05$, **$P < 0.01$

Tab 2 **Comparison of NCV and the concentration of ATP, Pcr, Cr, MDA and SOD activity among three rats groups** ($\bar{x} \pm s$,n=10)

Groups	NCV (m/s)	ATP (μmol/g)	Pcr (nmol/g)	cr (nmol/g)	MDA (nmol/l)	SOD NU/ml
DC	40.2 ± 2.2	0.72 ± 0.10	1.65 ± 0.18	2.32 ± 0.09	7.46 ± 2.58	157.21 ± 21.48
DT	48.3 ± 3.6	0.70 ± 0.13	2.13 ± 0.11	2.26 ± 0.12	5.92 ± 2.67	172.46 ± 19.25
NC	56.9 ± 5.3	0.68 ± 0.15	2.52 ± 0.14	2.67 ± 0.16	3.47 ± 1.49	199.61 ± 15.43
F	3.47*	3.21	4.86*	3.56*	25.64**	4.27*
q						
DC:DT	3.78*	0.38	3.98*	0.41	4.42*	3.32*
DC:NC	4.01*	0.81	5.12**	3.82*	6.48**	4.02*
DT:NC	3.47*	0.46	3.74*	3.96*	5.92**	3.81*

*$P < 0.05$, **$P < 0.01$

with the main promoter poly (ADP-ribose) polymerase (PARP)[2]. According to monistical viewpoint, the pathogenesis of DPN can be explained as follows: ischemia and hypoxia of nerve tissue at the induction of high glucose can cause enhancement of oxidative stress, excessive production of free radical and increase activity of aldose reductase[3], what follows next is the activation of poly (ADP-ribose) polymerase[4], the latter mediates formation of glycosylation dead end product [5,6], which aggravates oxidative stress to result in abnormality of nerve axonal transport and neurotrophic factors. All those factors can affect schwann cells, neurons and axons and result in development of DPN via intra-cellular conduction such as activation of PKC.

PARP participates in the development of DPN via two mechanisms : PARP activation causes elevation of activity of aldose reductase at high glucose condition , cleaves NAD^+ to form nicotinamide and ADP-ribose residues[4], depletes NAD^+ and ATP (or other high-energy phosphates), and ultimately NAD^+ depletion results in cellular oxidative damage and increases inflow of Ca^{2+}; furthermore, NAD^+ depletion inhibits glyceraldehyde 3-phosphate dehydrogenase reaction of glycolysis [7], and elevates the formation of diacylglycerol and methylglyoxal (a precursor of advanced glycation end products) which are activators of protein kinase C (PKC) and mitogen activating protein kinase (MAPK)[8], and methylglyoxal interacts with its receptors after forming advanced glycation end products, which aggravates the reaction of oxidative stress [9,10] and forms infernal circle that results in genesis of DPN [11]; On the other hand, PARP controls activation of numerous transcription factors[12, 13], i.e. nuclear factor-kappa B[14], activator of transcription-1 and p53 protein via direct binding poly (ADP-ribosyl)ation or both. This, in turn, up-regulates numerous genetic expressions[14,15] including those implicated in the pathogenesis of DPN, such as endothelin-1, cyclooxygenase-2 etc. Thus, PARP activation can be a trigger of multiple mechanisms implicated in diabetes-associated neuropathic changes.

Nerve conduction is the most correlated with nerve-energy state among various kinds of metabolic parameters because nerve transmembrane electric activity demands energy support[16]. Peripheroneural ATP is not exhausted completely in early diabetic condition, but another high energy phosphate compound Pcr is correlated closely with diabetic nerve-energy state. The degression of Pcr indicates chronic depolarization of cellular membranes and deactivation of sodium channels. What follows next is depression of nervous excitability and disorders of nerve conduction. MDA concentration and SOD activity are correlated intimately with organizational metabolic state .The level of lipid peroxidative sub-

stance has increased obviously and the activity of antioxidase including SOD has depressed significantly in the condition of early diabetes before histopathology changes of nerve tissue emergence, and regulations of oxyradical metabolism and improvement of nerve-energy state in the early condition of chronic diabetic complications can decrease cellular oxidative stress damages and postpone the development of pathological changes.

PARP inhibitors include 3-aminobenzamide (3-AB) and PJ34, etc[17, 18]. Researches have shown that short-term administration of PARP inhibitors for rat models of diabetic cardiomyopathy [18], diabetic encephalopathy[19] and diabetic neuropathy[20, 21] could suppress the development of pathological changes effectively and recover cellular energy metabolism state. Long-term administration of PARP inhibitor PJ34 for rat models of diabetic retinopathy has also confirmed to gain satisfactory therapeutic effect[16].

However,it is not reported that long-term administration of PARP inhibitors for DPN has any effect on these pathological changes,such as ganglia demyelination and neurofibra loss. Furthermore, it is not known that long-term inhibition of PARP has any side effect on organism.What we can be sure is that , investigation of PARP inhibitors can provide a novel approach for prevention and treatment of DPN and related diabetic complications.

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