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Effect of "Antischemin" Upon Lipid Peroxidation Process on Brain Ischemic Disorder Induced on Experimental Rat Model

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Abstract

In recent years, wide range drug development research that prevents cerebral tissue and cell necrosis, degradation are being carried out. The purpose of our research is to determine effect of "Antischemin" during stimulation of lipid peroxidation on white rat brain ischemic disorder model. We have performed using Farkes E and his colleagues 2007 methodology. Our study of brain ischemia induced model in experimental white rat shows that Antischemin inhibits lipid peroxidation and has neuro-protective effects from neuron necrosis, degradation.

Keywords: Lipid peroxidation; brain ischemia; malondialdehyde; sialic acid; lipoperoxidase; cytochrome C.

1. Introduction

Lipid peroxidation is continuous process that takes place in every organ, tissue and cells of mammal animal and if within normal range, with participation of unsaturated fatty acid peroxidation products essential phenomena that are necessary to body including prostaglandin synthesis, generation cholesterol hydroperoxide leading to steroid hormone synthesis, microsomal membrane enzyme activity regulation takes place [1,2,3]. In recent years, wide range drug development research that prevents cerebral tissue and cell necrosis, degradation are being carried out [4].

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In relation to this trend, researchers are largely focusing on brain cell injury, necrosis preventive drug development from domestic herbal plants [5]. Several researchers have concluded that Antischemin mixture is made of *Scutellaria baicalensis* which has antioxidant [6], central nervous system depressive [7], anti-platelet [8] effects, *Astragalus membranaceus* which has antioxidant [9], immune system boosting [10], stress relieving effects, maidenhair tree *Ginko Biloba* which has immune system boosting [12], central nervous system depressive, anti-ischemic, antioxidant, memory enhancing [13], anti-platlet [14], cholesterol reducing effect [15] effects.

2. Material and methods

Global brain ischemia model induction: Unilateral permanent occlusion – Common carotid artery

We have performed using Farkes E and his colleagues 2007 methodology[16]. On white rat with weight of 220-280 gram, we started sedation with ketamine, and have secured rat to immobile which after attached rat to ventilator. We have maintained experimental animal body temperature between 36.7-37^{o}C and reinforced immobile rat to surgery table. With help of laryngoscopy, we have introduced polyethylene tube to trachea, and attached it to ventilator (small animal ventilator R407 RWD Life Science/ and have ventilated 112-114/min, 1.8-2.0 cc volume and performed surgical procedure in aseptic condition with help of surgical headlight. After cutting hair of lateral neck we have cleaned surgical site using 5% iodine solution and have occluded artery by suture. Gave water to control group, Bilobil to comparison group, 100 mg/kg dose of Antischemin to experimental group for 28 days. On 1, 3, 7, 14, 21 days of experiment animals were sedated with ketamine hydrochloride and we have taken sample blood for heart and measured MDA, SA, Cyt C concentration, and from brain tissue LPO concentration were measured by using Enzyme-linked immunosorbent assay kit.

3. Results

Plasma MDA concentration of control group animals had increased 50.5-74.5% during 1-21 days, and have been maintained stable during either acute and chronic stage. ($P \leq 0.001$)

Table 1: Antischemin effects on plasma MDA in brain ischemia induced model of white rat

№	Days of experiment	Plasma MDA nmol/L			
		Healthy	**Control**	**Antischemin 100 mg/kg**	**Bilobil 40 mg/kg**
1	Day 1	0.87±0.03	1.76±0.07*	1.04±0.03**	0.98±0.03
2	Day 3	1.07±0.03	4.19±0.34*	3.14±013**	2.79±0.16**
3	Day 7	1.14±0.04	3.05±0.06*	2.32±0.04**	2.70±0.34**
4	Day 14	1.11±0.03	3.64±0.10*	2.76±0.10**	2.94±0.17**
5	Day 21	1.09±0.03	3.21±0.13*	2.43±0.08**	2.41±0.05**

*- When compared control group measurements with healthy group $P \leq 0.05$, $P \leq 0.001$

**- When compared treatment group measurements with control group P≤ 0.05, P≤ 0.001

Compared to control group, treatment group animals plasma MDA concentration had decreased following sequence of 40.2%, 25%, 36.3%, 24.2%, 24.3% and effect of neutralizing peroxidation intermediate stage damaging compounds and inhibition of chain reaction are being observed.

Table 2: Antischemin effects on SA measurements in brain ischemia induced model of white rat

№	Days of experiment	Plasma SA nmol/L			
		Healthy	Control	Antischemin 100 mg/kg	Bilobil 40 mg/kg
1	Day 1	18.51±0.27	31.66±1.30*	25.16±0.98**	23.44±0.85**
2	Day 3	19.22±0.12	45.15±1.22*	37.32±0.54**	37.66±0.39**
3	Day 7	20.08±0.23	64.25±0.73*	48.78±1.03**	53.75±0.89**
4	Day 14	19.98±0.20	63.55±1.09*	47.79±1.06**	39.97±1.09**
5	Day 21	20.12±0.09	44.28±0.22*	34.05±0.22**	33.95±0.15**

*- When compared control group measurements with healthy group P≤ 0.05, P≤ 0.001

**- When compared treatment group measurements with control group P≤ 0.05, P≤ 0.001

Plasma SA concentration in non-treatment control group during subacute stage of 7-14 days of experiment were increased by 68% were attraction attention. (P≤0.001) During aforementioned stages of experiment, plasma SA concentration of Antischemin treatment group decreased by 24%, which shows that it protects from brain neuron necrosis and decomposition. (P≤0.001) When comparison group with dose of 40mg/kg Bilobil, compared to non-treatment control group, during all of experimental days statistically significant reduction of SA concentration were concluded as result. (P≤0.001)

Table 3: Antischemin effects on LPO measurements in brain ischemia induced model of white rat

№	Days of experiment	Brain tissue LPO nmol/L			
		Healthy	Control	Antischemin 100 mg/kg	Bilobil 40 mg/kg
1	Day 1	65.87±1.13	98.68±2.48*	87.27±2.45	85.10±2.05**
2	Day 3	66.2±0.97	98.37±2.13	85.64±1.61**	76.15±0.71**
3	Day 7	61.89±1.2	80.29 ±0.92*	69.32±0.76**	69.52±1.16**
4	Day 14	64.32±0.92	117.80±1.88*	100.38±1.7**	93.03±1.86**
5	Day 21	63.45±0.64	130.63±2.19*	115.29±1.64	109.77±1.55**

*- When compared control group measurements with healthy group P≤ 0.05, P≤ 0.001

**- When compared treatment group measurements with control group P≤ 0.05, P≤ 0.001

During 1-25 days of experiment, non-treatment control group brain tissue LPO enzyme concentration activity have increased by following sequence of 33.2%, 32.7%, 22.91%, 45.4%, 51.4%. Whereas Antischemin used treatment group 1-21 days measurements have decreased by 11.7-14.8%. Antioxidant, membrane stabilizing effects and limiting hydroperoxide generation of lipid compounds of properties of biological active plant compounds within Antischemic composition have been observed.

Table 4: Antischemin effects on Cyt C measurements in brain ischemia induced model of white rat

№	Days of experiment	Plasma Cyt C nmol/L			
		Healthy	Control	Antischemin 100 mg/kg	Bilobil 40 mg/kg
1	Day 1	6.06±0.14	9.68±0.42*	8.41±0.46	7.21±0.65**
2	Day 3	5.97±0.09	9.05±0.54*	5.98±0.35**	7.37±0.40**
3	Day 7	7.08±0.09	15.41±0.29*	14.32±0.25	14.69±0.21
4	Day 14	6.32±0.09	17.35±0.20*	15.27±0.21**	15.37±0.29**
5	Day 21	7.36±0.1	16.82±0.17*	15.14±0.45**	15.85±0.21**

*- When compared control group measurements with healthy group P≤ 0.05, P≤ 0.001

**- When compared treatment group measurements with control group P≤ 0.05, P≤ 0.001

During experimental days of 1-21 composition of Cyt C have increased 34-63.5% in non-treatment control group shows that during either acute, subacute and chronic stages of brain ischemic disorder due to disruption of normal electron and proton flow through mitochondrial internal membrane leads to decreased generation of high energy compounds such as ATP and leads to Ca^{2+} induced phospholipase C enzyme over-activation which in turn leads to strong membrane degradation [5]. (P≤0.001) During aforementioned period of experiment, Cyt C concentration in treatment group used Anti-ischemin compared to control group decreased in following sequence of 13%, 33.9%, 7.2%, 12%, 10%. With effects of total polyphenols and flavonoids in the composition of Antischemin reduces ATP deficiency during brain ischemia and neutralizes Ca induced phospholipase C overactivation, reduces cytochrome C loss from internal mitochondrial membrane, and form condition where normal cellular respiration takes place.

Abbreviation: MDA=malondialdehyde, SA=sialic acid, LPO=Lipoperoxidase, Cyt C=Cytochrome C

4. Discussion

During brain ischemia on 3 state line of cell membrane reduction potential, primary deficiency of oxygen as donator occurs and because of this ATP, NADPH synthesis decrease follows. On the membrane antioxidant system weakens, stimulation lipid peroxidation occurs, membrane degrades and cytochrome C, sialic acid will

get freed from mitochondrial inner membrane and gets into plasma. Due to this, on the 3 typical lines of mitochondrial inner membrane – membrane reduction potential proton flow decreases and conditional metabolic water generation from it decreases and H_2O+CO_2, H_2CO_3, HCO_3+H^+ generation in somatic cells and plasma weakens and entry of HCO_3 in the form of bicarbonate form decreases leading to red blood cell internal membrane $HbO_2+HCO_3^-$ exchange disruption occurs further leading to decreased oxygen offloading and secondary oxygen deficiency of brain cells occurs. Cytochrome C is protein that participates synthesis of most important and major energy source, high energetic compound ATP from NADH, FADH compounds formed by food and nutrition metabolism in humans and animal body and by transporting electrons from low potential to high potential environment through 4 types of electron transporter protein complexes on internal mitochondrial membrane [5]. From general results of experiment, during each stages of global brain ischemia induced in white rat, oxygen deficiency dominance in brain tissue leads to disruption of one directional electron and proton flow on mitochondrial membrane, and ATP generation on cellular internal membrane inhibition occurs, leading to high energy compound deficiency that leads to Ca^{2+} induced phospholipase C enzyme overactivation which furher leading to strong stimulation of cell degradation and necrosis which is based outflow intensity of proteins such as one Cytochrome C is being observed and we have proven that Antischemin mixture protects from this phenomenon. Cytochrome C outflow preventive activity of Antischemin mixture is superior to Bilobil which were comparative mixture have been observed. During our study, 1-21 days of experiment non-treated animal control group plasma MDA concentration increased by 50.5-74.5%, SA concentration increased by 41.5-68.7%, Cyt C concentration increased by 34-63.5%, in brain tissue LPO concentration increased by 22.9-51.4% shows that large amounts of intermediate products of oxidation builds up in blood plasma, during all stages of brain ischemia acute, subacute and chronic, disruption of electron, proton normal flow occurs in cellular respiration chain reaction, and large amounts of reduced forms of NADH, FADH reacts each other leading to stimulation of peroxidation, and overstimulation of peroxidation in bilayer fatty acid membrane of brain cells some parts of membrane structure, in particular parts of glycoprotein such as sialic acid breaks off and accumulate in plasma by large amounts have been revealed and our study results were in line with Liau PR, Wu MS, Lee CK and his colleagues study of antioxidants activity and lipid peroxidation preventive effects of *Scutellaria baicalensis* plant. Whereas in treatment group used Antischemin during experimental period plasma concentration of MDA decreased by 24.17-40.45%, SA concentration decreased by 14.79-24.07%, Cyt C concentration decreased by 17-24.4%, LPO concentration in brain tissue decreased by 11.5-14.7 respectively, which shows that Antischemin has suppressive effects in lipid peroxidation, brain neuron necrosis and degradation, degeneration. When neuro-protective effect of Antischemin were close Bilobil when compared to it.

6. Conclusion

Our study of brain ischemia induced model in experimental white rat shows that Antischemin inhibits lipid peroxidation and has neuro-protective effects from neuron necrosis, degradation.

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