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Results of Study on Effect of Antischemin Preparation on Antioxidant System and Vascular Endothelial During the Cerebral Ischemia Model

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Abstract— *Self-regulating mechanism and driving process of the organism has been a subject matter for the science for many years. Then, very simple determination of antioxidant system which is one of the above regulation is that it is unique biological system that keeps alive nature of the cell, origin for the humanity existence and studying changes and activity of the system on Antischemin is one set of the experiment. Research was carried out on ischemia model through methodology of Farкes E et al., 2007 with in the purpose to study effects of Antischemin on superoxidismutase, glutathione, glutathione peroxidase (GSH-Px pmol/ml), GABA /γ-aminobutiric acid/ and Vascular endothelial growth factor (VEGF) which indicate antioxidant activity. According to the results of the study glutathione (GSH-ng/L) level in cerebral tissue homogenate has decreased by 26-40.1%, superoxide dismutase (SOD) by 25.8-41%, glutathione peroxidase (GSH-px-pmol/ml) 15.4 by 44.5% and by 57.3-60.9% in the plasma respectively on 1st to 21st days after forming cerebral ischemia on trial animals and it proves rapid reduction of endogenous antioxidants in cerebral and cardiac ischemia. And in animals on which 100 mg/kg Antischemin was used, glutathione (GSH-ng/L) has increased by 16.1-27.2% in tissue homogenate, superoxide dismutase (SOD) by 14-20.9%, glutathione peroxidase by up to 14.3-31.1% on 1st to 21sr trial days and it ensures condition to provide tissue with high energy substances such as ATP by increasing endogenous antioxidant reserve during oxygen deficiency. Also 16.7-20.95% decrease of VEGF-pg/ml in control group shows unstable metabolism in vascular and tissue causing main factor to damage vessel during cerebral hypoxia and among the group that used Antischemin VEGF-pg/ml has increased by 11.9-17.9% in brain homogenate and by 7.8-10.5% in comparative group, Bilobil, relatively and it indicates that substances influence during new vessel formation even not so strong.*

Keywords— *Antioxidant system, Superoxide Dismutase, glutathione, glutathione peroxidase, endothelial growth factor.*

I. INTRODUCTION

Antioxidant system protects against the effects of peroxidation process on fat compounds of tissue membrane, damage and degradation of membrane, tissue disfunction [1] and Antischimen which has positive effects on this system consists of roots of *Astragalus membraneceus, Scutellaria baicalensis* Georgi and leaves of *Gingko biloba* that are planted in Mongolia. Antioxidant activity of this medicine has been studied on ischemia model and main causes for vascular endothelial damage, further forunstable metabolism of vital organs including brain and heart during the ischemia include imbalance of electron and proton flows on "9 stepped cycle of proton and electron conductance with in 14 trillion tissues of human body" and oxygen deficiency. [2, 3, 4, 6]

It is important to produce medicine which provides preventive and treatment activity and is capable of activating antioxidant system which is unique biological system for the tissue to keep its alive nature, to be used separately and in combination with other medicines on the above disorder at the integrated system level of dependence between many factors.

Purpose

To study Antischemin effects on superoxidismutase, glutathione, glutathione peroxidase (GSH-Px pmol/ml), GABA /γ-aminobutiric acid/ and Vascular endothelial growth factor (VEGF) that show antioxidant activity during the cerebral ischemia model formed in rat.

II. METHODOLOGY

Disorder model of cerebral ischemia was excuted through methodology by Farкes E et al., 2007[5]. After making 220-280 gram white rat by using ketamine and attaching to the surgical table tightly entered polyethylene tube into trachea with the help of special laryngosgope, connected with small animal ventilator R407 RWD Life Science and allowed breathing by adjusting average breath 94-104 min with volume of 1.8-2.0 cc per minute. Syrgery was carried out under aseptic environment with headlamp. After cutting hair along the neck and sterilizing skin area by 5% iodine solution put out left jugular artery and bound by special suture.

Fermentation connecting reaction: Following indicators have been determined by taking blood from heart via vacuumtainer after making the trial animal a sleep by injecting ketamine hydrochloride on 1st, 3rd, 7th, 14th and 21st days of experiment after forming cerebral ischemia on trial animal through (Enzyme-linked immunosorbent assay) ELISA device, including, SOD /superoxidismutase/, GSH /glutathione/, GSH-Px /glutathionpereoxidase/, GABA /γ-aminobutiric acid/, Vascular endothelial growth factor (VEGF) in blood plasma and brain tissue.[7].

III. RESULTS

According to our study, superoxidismutase activity contained in the brain tissue has decreased by 32.9% (7.77±0.10 pg/ml in healthy group, 5.21±0.43 pg/ml, $P<0.001$ in control group) on the 1st day or acute stage, 28.6%

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(8.04±0.14 pg/ml in healthy group, 5.74±0.17 pg/ml, P<0.001 in control group) on the 3rd trial day, 25.8% (7.13±0.18 pg/ml in healthy group, 5.29±0.12 pg/ml, P<0.05 in control group) on the 7th day, 36.5% (7.64±0.13 pg/ml in healthy group, 4.85±0.17 pg/ml, P<0.001 in control group) on the 14th day, 41% (6.89±0.28 pg/ml in healthy group, 4.06±0.12 pg/ml, P<0.001 in control group) on the 21st day after forming cerebral ischemia on the wite rat compared to the animals in the healthy group.

Table 1. Amount of superoxide dismutase (SOD) contained in the cerebral tissue homogenate and effects of Antischemin on it during the cerebral ischemia disorder model

№	Trial days	SOD containe din the cerebral tissue (pg/ml)			
		Healthy n=6	Control n=6	Antischemin-100 mg/kg n=6	Bilobil-40 mg/kg n=6
1	1 day	7.77±0.12	5.21±0.43*	6.53±0.53**	5.93±0.36
2	3days	8.04±0.13	5.74±0.17*	7.03±0.16**	7.09±0.14**
3	7days	7.13±0.18	5.29±0.12*	6.69±0.14**	6.94±0.21**
4	14 days	7.64±0.13	4.85±0.17*	5.64±0.13**	6.20±0.11**
5	21 days	6.89±0.28	4.06±0.12*	6.03±0.15**	5.86±0.17**

*- comparison of indicators of control group with healthy group P≤ 0.05, P≤ 0.001
**- comparison of indicators of treated group with control group P≤ 0.05, P≤ 0.001

For animals that were treated by Antischemin prepared by condensed infusion of *Astragalus membraneceus, Scutellaria baicalensis* Georgi and leaves of *Gingko biloba* it has increased by 20.2% (5.21±0.43 pg/ml in control group, 6.53±0.53 pg/ml, P<0.05 in Antischemin group) on the 1st day, 18.3% (5.74±0.17 pg/ml in control goup, 7.03±0.16 pg/ml, P<0.05 in Antischemin group) on the 3rd day, 20.9% (5.29±0.12 pg/ml in control group, 6.69±0.14 pg/ml, P<0.05 in Antischemin group) on the 7th day, 14% (4.85±0.17 pg/ml in control group, 5.64±0.13 pg/ml, P<0.05 in Antischemin group) on the 14th day,32.6% (4.06±0.123 in control group, 6.03±0.157 pg/ml, P<0.001 in Antischemin group) on the 21st day of the trial compared to the control group.

Superoxide dismutase content was not so different for animals in comparative group on the 1st day compared to the control group and it has increased by 19% (5.74±0.17 pg/ml in control group, 7.09±0.14 pg/ml, P<0.05 in Bilobil group) on the 3rd day, by 23.77% (5.29±0.12 pg/ml in control group, 6.94±0.21 pg/ml, P<0.05 in Bilobil group) on the 7th day, by 21.77% (4.85±0.17 pg/ml in control group, 6.20±0.11 pg/ml, P<0.05 in Bilobil group) on the 14th day, by 30.7% (4.06±0.12 pg/ml in control group, 5.86±0.17 pg/ml, P<0.001 in Bilobil group) on the 21st day of the trial. Generally, it has been observed that Bilobil and Antischemin preparation are similar for increasing activity of SOD.

Table 2. Amount of glutathione (GSHng/L) brain tissue homogenate during the cerebral ischemia model and effects of Antischemin preparation on it

№	Trial days	Glutathione GSH (ng/L)			
		Healthy n=6	Control n=6	Antischemin-100 mg/kg n=6	Bilobil-40 mg/kg n=6
1	1 day	137.19±1.69	121.35±4.98	127.98±1.93	128.35±6.83
2	3 days	129.23±1.3	95.56±2.31*	113.94±2.85**	102.43±1.54**
3	7 days	119.89±1.27	77.31±2.12*	81.77±1.09	79.06±1.09
4	14 days	143.79±1.3	98.15±1.67*	134.94±10.74**	132.07±1.93**
5	21 days	118.59±1.98	65.11±0.88*	84.21±1.30**	79.78±1.01**

*- comparison of indicators of control group with healthy group P≤ 0.05, P≤ 0.001
**- comparison of indicators of treated group with control group ≤ 0.05, P≤ 0.001

On the 1st trial day, in acute period of forming cerebral ischemia on white rat, glutathione content in cerebral tissue homogenate of untreated control group animals was decreased (137.19±1.69 ng/L in healthy group, 121.35±4.98 ng/L in control group) by 11.54%, on the 3rd day by 1.35 times or 26% (129.23±1.3 ng/L in healthy group, 95.56±2.31 ng/L, P<0.05 in control group); on the 7th day by 35.5% (119.89±1.27 ng/L in healthy group, 77.31±2.12 ng/L, P<0.001 in control group); on the 14th trial day by 31.7% (143.79±1.30 ng/L in healthy group, 98.15±1.67 ng/L, P<0.001 in control group); on the 21st day by 40.1% (118.59±1.98 ng/L in healthy group, 65.11±0.88 ng/L, P<0.001 in control group) compared to the indicators of healthy group.

On the other hand, glutathione content in animals that were given 100 mg/kg Antischemin preparation has increased by 5.18% (121.35±4.98 in control group, 127.98±1.93 ng/L in treated group) on the 1st day, by 16.13% (95.56±2.31 in control group, 113.94±2.85, P<0.05 in treated group) on the 3rd day, by 5.45% (77.31±2.12 in control group, 81.77±1.09 in treated group) on the 7th day, by 27.26% (98.15±1.67 in control group, 134.94±10.74, P<0.001 in treated group) on the 14th day, by 22.68% (65.11±0.88 in control group, 84.21±1.30, P<0.001 in treated group) on the 21st trial day compared to control group.

In animals that were treated by Bilobil in comparative group this indicator was increased by 5.45-6.71% on 1st, 3rd, 7th days, by 25.68% (98.15±1.67 in control group, 132.07±1.93, P<0.001 in Bilobil group) on the 14th day, by 18.38% (65.11±0.88 in control group, 79.78±1.01, P<0.05 in Bilobil group) on the 21st daycompared to untreated control group animals.

B.Ariuntsetseg, B.Sarantsetseg, M.Ambaga, and D.Tserendagva, "Results of Study on Effect of Antischemin Preparation on Antioxidant System and Vascular Endothelial During the Cerebral Ischemia Model," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 2, Issue 4, pp. 58-62, 2019.

Table 3. Amount of glutathione peroxidase (GSH-Px pmol/ml) brain tissue homogenate during the cerebral ischemia model and effects of Antischemin preparation on it

№	Trial days	Glutathione peroxidase GSH-Px pmol/ml			
		Healthy n=6	Control n=6	Antischemin-100 mg/kg n=6	Bilobil-40 mg/kg n=6
1	1 day	9.46±0.2	5.57±0.14*	6.80±0.22**	6.42±0.25**
2	3 days	9.06±0.06	5.46±0.16*	7.06±0.11**	7.23±0.24**
3	7 days	9.14±0.06	7.73±0.16*	9.03±0.19**	9.20±0.22**
4	14 days	9.72±0.14	6.64±0.08*	9.65±0.18**	9.20±0.22**
5	21 days	10.26±0.12	5.69±0.19*	10.16±0.07**	9.76±0.08**

*- comparison of indicators of control group with healthy group P≤ 0.05, P≤ 0.001
**- comparison of indicators of treated group with control group ≤ 0.05, P≤ 0.001

According to the study results, activity of glutathione peroxidase contained in the cerebral tissue homogenate has decreased by 41.1% (P<0.001) in untrated control group animals (5.57±0.14 pmol/ml) compared to healthy group (9.46±0.20 pmol/ml) on the 1st day after forming cerebral ischemia, on the 3rd day by 39.7% (5.46±0.16 pmol/ml in control group, 9.06±0.06 pmol/ml, P<0.001 in healthy group), on the 7th day by 15.4% (7.73±0.16 pmol/ml in control group, 9.14±0.06 pmol/ml, P<0.05 in healthy group), on the 14th day by 31.6% (6.64±0.08 pmol/ml in control group, 9.72±0.14 pmol/ml, P<0.001 in healthy group), on the 21st day by 44.5% (5.69±0.19 pmol/ml in control group, 10.26±0.12 pmol/ml, P<0.001 in healthy group).

And for animals that used Antischemin preparation it has increased by 18.08% (5.57±0.14 pmol/ml in control group, 6.80±0.22 pmol/ml, P<0.05 Antischemin) on the 1st trial day, by 22.66 % (5.46±0.16 pmol/ml control, 7.06±0.11 pmol/ml, P<0.001 Antischemin) on the 3rd day, by 14.39% (7.73±0.161control, 9.03±0.19 pmol/ml, P<0.05 Antischemin) on the 7th day, by 31.19% (6.64±0.08 pmol/ml control, 9.65±0.18 pmol/ml, P<0.001 Antischemin) on the 14th day, by 43.99 % (5.69±0.19 pmol/ml control, 10.16±0.07 pmol/ml, P<0.001 Antischemin) on the 21st trial day compared to the control group.

When using comparative preparation Bilobil with dose of 40 mg/kg, glutathione peroxidase level in the cerebral tissue homogenate has increased by 13.23% (control 5.57±0.14 pmol/ml, Bilobil 6.42±0.258 pmol/ml) on the 1st day, by 24.48% (control l5.46±0.16 pmol/ml, Bilobil 7.23±0.24 pmol/ml, P<0.001) on the 3rd day, by 15.97% on the 7th day by 27.8% on the 14th day, by 41.7 % (P<0.001) on the 21st day, respectively compared to the control group and it has proven that both Antischemin preparation and Bilobil has similar function to increase level of glutathione peroxidase.

Table 4. Test results determined by Vascular endothelial growth factor (VEGF)-r Elisa rat kit in rat plasma and cerebral tissue during/on the cerebral ischemia model

Group	3 days pg/ml		7 days pg/ml	
	Blood plasma	Cerebral tissue homogenate (1:10)	Blood plasma	Cerebral tissue homogenate (1:10)
Healthy n=6	93.36±3.15	84.76±0.79	93.36±3.15	84.76±0.79
Control n=9	67.03±0.47	67.04±8.49*	67.39±0.77	70.54±0.97*
Antischemin n=9	79.76±0.76	75.35±0.89	81.66±1.51	80.08±1.30
Bilobil n=9	83.26±1.87	72.78±0.66	80.69±0.94	78.89±1.31

*- comparison of indicators of control group with healthy group P≤ 0.05, P≤ 0.001
**- comparison of indicators of treated group with control group ≤ 0.05, P≤ 0.001

On the 3rd trial day after forming cerebral ischemia on rat Vascular endothelial growth factor (VEGF) level in the cerebral tissue has decreased by 20.9% (in healthy group 84.76±0.79, in control group 67.04±0.49 pg/ml, P<0.001) on the 1st trial day, by 16.7% (in healthy group 84.76±0.79, in control group 70.54±0.97 pg/ml, P<0.001) on the 7th day, for animals treated by Antischemin preparation it has increased by 11% (in control group 67.04±0.49., in Antischemin group 75.35±0.89 pg/ml, P<0.05) on the 3rd trial day, by 11.9% (in control group70.54±0.97, in Antischemin group 80.08±1.3 pg/ml, P<0.05) on the 7th trial day, respectively and for animals in Bilobil group it has increased by 7.8% (in control group 67.04±0.49 pg/ml, in Bilobil group 72.78±0.66 pg/ml, P<0.05) on the 3rd trial day, by 10.5% (in control group 70.54±0.97 pg/ml, in Bilobil group 78.89±1.31 pg/ml, P<0.05) on the 7th day, respectively.

And Vascular endothelial growth factor (VEGF) level in the serum has decreased 28.2% (in healthy group 93.36±3.15, in control group 67.03±0.47 pg/ml, P<0.001) on th 3rd trial day, by 27.8% (in healthy group 93.36±3.15 in control group 67.39±0.77 pg/ml, P<0.001) on the 7th day, for animals treated by Antischemin preparation it has increased by 15.9% (in control group 67.03±0.47., in Antischemin group 79.76±0.76 pg/ml, P<0.05) on the 3rd trial day, by 17.5% (in control group 67.39±0.77, in Antischemin group 81.66±1.5 pg/ml, P<0.05) on the 7th trial day, respectively and for animals in Bilobil group it has increased by 19.5% (in control group 67.03±0.47 pg/ml, in Bilobil group 83.26±1.87 pg/ml, P<0.05) on the 3rd trial day, by 16.48% (in control group 67.39±0.77 pg/ml, in Bilobil group 80.69±0.94 pg/ml, P<0.05) on the 7th trial day, respectively.

B.Ariuntsetseg, B.Sarantsetseg, M.Ambaga, and D.Tserendagva, "Results of Study on Effect of Antischemin Preparation on Antioxidant System and Vascular Endothelial During the Cerebral Ischemia Model," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 2, Issue 4, pp. 58-62, 2019.

Table 5. Y-aminobutyric acid (gamma-ABA μmol/L) level in cerebral tissue homogenate during/on the cerebral ischemia model and effects of Antischemin preparation on it

№	Trial days	Gamma-ABAin cerebral tissue (μmol/L)			
		Healthy n=6	Controln=6	Antischemin-100 мг/кг n=6	Bilobil 40 мг/кг n=6
1	1 day	2.47±0.06	1.92±0.04	1.90±0.04	1.92±0.03
2	3 days	2.31±0.12	1.52±0.06	1.55±0.09	1.58±0.01
3	7 days	2.07±0.15	1.78±0.07	1.88±0.06**	1.92±0.02
4	14 days	2.18±0.11	1.90±0.10	1.19±0.09	1.47±0.14
5	21 days	1.98±0.09	1.52±0.12*	1.55±0.15	1.58±0.05

*- comparison of indicators of control group with healthy group P≤ 0.05, P≤ 0.001
**- comparison of indicators of treated group with control group ≤ 0.05, P≤ 0.001

During the in acute, subacute and chronic phaseof the cerebral ischemia model gamma-ABA contained in the cerebral tissue of animals in the untreated control group reached to 1.52-1.92 μmol/L and it has decreased by 12.8-34.2% compared to the animals in the healthy group and it is observed that all motivation and protection system in the body is lost during the oxygen deficiency of the cerebral tissue.

This indicator has quite different in animals of treated group used Antischemin preparation and comparative group compared to the indicator during the normal condition. And it has increased slightly on the 3rd trial day compared to the untreated control group but no statistically true difference has been observed. There was no major difference between the 2 groups during the other days of the trial.

IV. Discussion

During the subacute and chronic phases formed cerebral ischemia in rat, multiple stepped occurrences of disease are occurred in the cerebral tissue causing oxygen deficiency dependent on ischemia inside the cell, messy peroxidation of fat, substantial formation of oil hydroperoxyl, activation of antioxidant and much consumption of dispensed compounds such as glutathione which keeps peroxidation. Thus, quantity of dispensed compounds are observed as being reduced during the cerebral ischemia model.

Glutathione reserve is reduced as the sickness becomes chronic which in turn fat peroxidation is activated strongly, free radicals are increased withinthe cerebral tissue, resulting in reduction of dispensed compounds such as glutathione due to high activation of endogenous antioxidant protection system in the body during the cerebral and cardial ischemia.

Flavonoid and fenolt compounds contained in *Astragalus membraneceus, Scutellaria baicalensis* Georgi and leaves of *Gingko biloba* dilutes blood with in the micro circulation of blood, decrease peroxidation and positively effect on reserves of internal endogenous antioxidant system during the ischemia. Based on functions of *Astragalus membraneceus, Scutellaria baicalensis* Georgi and leaves of *Gingko biloba* to protect nerve cells, [8, 9] eliminate blood stasis, [10, 15] anti-hypoxia activation, [11] effect on cell protection, [12, 16] protection against blood and cerebral loss caused by cerebral edema, [13, 17] potential to be used for inflammation, immune regulation, kidney and heart support, long life expansion as well as many treatments for disease, we have selected the above three plants for our study object and studied antioxidant activation of the preparation.

V. Conclusion

1. During the 1st to 21st days after forming cerebral ischemia on the trial rat, glutathione (GSH-ng/L) level in cerebral tissue homogenate has decreased by 26-40.1%, superoxide dismutase (SOD) level in the cerebral tissue homogenate by 25.8-41%, glutathione peroxidase (GSH-px-pmol/ml) level in the cerebral tissue homogenate by 15.4-44.5%, by 57.3-60.9% in the plasma in the animals of untreated control group and reserves of endogenous antioxidants rapidly decrease in cerebral and cardiac ischemia. In animals that used 100 mg/kg dose of Antischemin preparation glutathione (GSH-ng/L) level in the tissue homogenate has increased by 16.1-27.2%, superoxide dismutase (SOD) by 14-20.9%, glutathione peroxidase level by 14.3-31.1% during the 1st to 21st days of formation of cerebral ischemia model and it ensures condition to provide cell with high energy compounds such as ATP by increasing reserves of endogenous antioxidants during the oxygen deficiency in the cerebral tissue.
2. 16.7-20.95% decrease of vascular endothelial growth factor (VEGF-pg/ml) in cerebral tissue homogenate of the control group proves creation of many factors for vascular damage following unstable metabolism with in the vascular and tissue environment during the oxygen deficiency in the cerebral tissue and vascular endothelial growth factor (VEGF-pg/ml) in the cerebral tissue homogenate has increased by 11.9-17.9% in the group that used Antischemin preparation, by 7.8-10.5% in comparative or Bilobil group, respectively proving the preparations effect formation of new vein slightly.

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B.Ariuntsetseg, B.Sarantsetseg, M.Ambaga, and D.Tserendagva, “Results of Study on Effect of Antischemin Preparation on Antioxidant System and Vascular Endothelial During the Cerebral Ischemia Model,” *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 2, Issue 4, pp. 58-62, 2019.