

## REHABILITATION OF PATIENTS AFTER STROKE: NEW REMEDY?

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### Summary

Neuroprotective effects of a new composite protein preparation, named X-proteins (XP; chromatographically purified from extracts of pig's placentas) were investigated in model experiments in laboratory rats. Animals were exposed by short-time ischemization of their brains by ligation of both *aa.carotis*. Administered of XP 2 hours later of brain ischemization lead to prominent rise of viability of animals. Besides, administration of XP lead to more complete and more quick rehabilitation of experimental animals after ischemic injury of the brain (rehabilitation of motor skills, rise of effectiveness of long-term and short-term memory and learning). Evident positive effects of XP were revealed in 15 patients with severe and steady-state neurology defects induced by ischemic stroke or other causes. Daily applications of XP to nasal mucosa of patients during 12-14 days leads to diminution of motor, mental, speech defects and to normalization of emotional state. We suppose, preliminary XP may be characterized as preparation with prominent antiapoptotic effects revealed upon injured nervous cells and inductor of regenerative/reparative processes in reversible damaged nervous tissue.

Key words: Stroke, neurology defects, rehabilitation, X-proteins

It is not the secret that modern medicine has not really effective remedies for rehabilitation of neurologically and/or mentally complicated patients after stroke [1]. That state of the problem is powerful stimulus for searches of new approaches in prophylactic and treatment this severe pathology.

In this paper we describe some biologic and clinical effects of new complex protein preparation with prominent neuroprotective/rehabilitative activities which was separated and tested by Dr. Alexander Anikin. He was the first.

### *To kind memory of Alexander Anikin*

*One of authors of this publication (A.P.) has had good fortunes to be in circle of friends Dr. Alexander Anikin, who passed away so prematurely in 1998. After scrupulous analysis thousands of publications Dr. Anikin successfully managed purification and preparation of principally new biologically active factor with prominent neuroprotective properties. I'd had possibility to observe few patients of Dr. Anikin, which suffered with severe neurology dysfunctions (after stroke mainly), which literally returned to normal life after using of Anilin's preparation. Memory about Dr. Anikin, as well as real possibility to help many and many patients obliges us to continue of the work begins by Dr. Anikin.*

## Methods

**XP preparation.** Separation of complex of neuroprotective proteins, named X-proteins (XP) was executed in accordance to Dr. Anikin method (personal communications): Fresh swine placenta (1 kg) was homogenized in 5 volumes of 0.01 M Tris-HCl pH 8.0 with 0.1 mM PMSF, and centrifugated 30 min. at 20 000 g. Obtained extract was applied to chromatography column (100 ml) filled with DE-52 cellulose (Whatmann), equilibrated by 0.01 M Tris-HCl pH 8.0. Ion-exchange column was washed by 0,2 M NaCl, and active protein fraction (XP) was eluted by 0,4 M NaCl and concentrated by ultrafiltration (limit of exclusion 100 kD). Obtained material was separated by gel-filtration with using of calibrated Toyopearl-65 (Toyo-Soda) 2 x 50 cm column and fraction of XP with molecular mass between 100 kD - 200 kD was collected. Obtained fraction of XP consists of nearly 10 main protein bands (in accordance to electrophoresis in 7.5% PAAG with DDS-Na; staining by Coomassie R-250). Further separation of XP did not execute because diminution of neuroprotective activity – therefore activity of XP proteins probably has depends from synergic action of separate components. Obtained fraction of XP was diluted to 0,5 mg/ml of total protein by 0,1 M glycine-NaOH buffer pH 7,5, filtrated through 0,2 mkm filters (Millipore) and stored at 2...4° C before using.

**Model experiments.** 3-months male rats (Wistar) 200-220 body weight were used. Animals were kept at 14/10 hours light/dark cycle at 21° C, with free access to water and meals. Brain's ischemia was modeling by tie of both *aa. carotis* round oneself at level of 4<sup>th</sup> cervical vertebra (under ether narcosis) for 10 minutes. Passive control rats were undergone operation without tied up of inserted ligatures around of *aa. carotis*. Active control rats were undergone operation with ligaturatin of both *aa. carotis*, but without administration of XP. Next group of animals were used:

- 1 – Passive control operated rats (n = 15)
- 2 – Active control operated rats; Brain's ischemia only (n = 28)
- 3 – Brain's ischemia + XP (0.2 mg/kg weight 24 h before tied up of *aa. carotis*; n = 23)
- 4 – Brain's ischemia + XP (0.4 mg/kg weight 24 h before tied up of *aa. carotis*; n = 23)
- 5 – Brain's ischemia + XP (0.2 mg/kg weight 3 h after tied up of *aa. carotis*; n = 23)
- 6 – Brain's ischemia + XP (0.4 mg/kg weight 3 h after tied up of *aa. carotis*; n = 23)

Solution of XP was administrated as a single i.p. injection.

Model experiments in rats were conducted by collaborators of The Research Institute of The Normal Physiology RAMS, Moscow, under professor V.V.Sherstnirov guidance in accordance to experimental models used in Institute.

**Open-field experiments.** Each animal was placed for 2.5 min twice (with 24 hours interval) in round arena (120 cm in diameter, 45 cm high walls; the field was separated to 20 sectors with equal area). The numbers of visited/crossed sectors (horizontal activity/mobility), and amount of vertical lifting (uprising activity/mobility) were recorded. Consequent declining of horizontal and vertical activity/mobility between first and second trials was considered as characteristic of effectiveness of long-term situational memory.

**Passive avoidance test.** Passive-avoidance box has been consist from two equal compartments 25 x 40 x 25 cm with metal roods floor. Compartments were separated by wall with square entrance 8 x 8 cm. One compartment was illuminated by lamp, and other was dark. Each animal was placed into bright compartment and period of passing to dark one was evaluated. Just after passing of rat to dark compartment an electrocutaneous stimuli (0.8 mA, 1 sec.) were strikes, and just after stimuli the rat was transferred to home-box. Rats were repeatedly placed into the box 24 h latter, and time of passing from bright compartment to the dark one was recorded (preservation of acquired passive avoidance skill).

**Working spatial memory** was analyzed in a round water-maze 120 cm in diameter, 60 cm high white walls, filled by water with 800 g of dry milk up to 40 cm. Plastic rest-platform 10 x 10 cm was asymmetrically placed in one sector of maze 2 cm under water. Water-maze was placed in room with a few spatial orientation marks (window, posters, furniture). Each animal was placed in water from 4 different points and the rates of platform reaching were recorded. Each time rat has had possibility to stay at rest-platform for 30 sec., and was returned to home-boxes for 10 min. Training was executed during 3 days, with changing position of the rest-platform each day.

Open-field tests were executed 6-7 days later of experimental acute brain's ischemia, passive avoidance test – 8-9 days latter, and water-maze training – 12-14 days latter.

**Patients.** The work was approved by the Ethical Committee of The Medical Research Center “Immunculus” (Moscow, Russia). Each patient (7 female + 8 male) and/or patient's relatives underwent the informed consent process and reserved the right to withdraw from the study at any stage and/or refuse any procedure during any stage of the investigation or treatment. Ten patients with after stroke neurological and/or mental deviations (episodes of acute stroke takes place 4-6 months before in basin of *aa. carotis* or *aa. vertebro-basilaris*), and five patients with neurology dysfunctions other etiology were observed (Table 3). Patient ages varies from 26 to 84 y.o. (57,3 in average);. Recommended scheme of XP using was: 1 drop (nearly 0.07 ml) of XP solution was drip in each nostril daily during 14 days. In one patient XP was cancel after 3 days using because allergy-like reactions (rhinitis, sneezing, itching). Any other unwilling effects not been observed nor during period of XP using nor 4-6 months.

Dispersion analysis ANOVA/MANOVA and non-parametric Mann-Whitney U-test were uses for evaluation of data obtained.

## Results

Obtained data indicates administration of XP three hours after operation (ligaturation of both *aa. carotis*) leads to decreasing of rats mortality ( $p < 0.05$ ), especially if XP were used in dosage 0.4 mg/kg body weight was used. Preliminary (24 h before operation) administration of XP was ineffective (Table 1).

Table 1. XP influences upon rats survival after experimental brain's ischemia.

Groups of animals	Deaths: <i>n</i> (%)
Passive control operated rats (without ischemia)	0 from 15 (0%)
Active control operated rats (10 minutes brain's ischemia)	8 from 28 (28.6%)
Brain's ischemia + XP (0.2 mg/kg weight 24 h before operation)	5 from 23 (21,7%)
Brain's ischemia + XP (0.4 mg/kg weight 24 h before operation)	7 from 23 (30,4%)
Brain's ischemia + XP (0.2 mg/kg weight 3 h after operation)	3 from 23 (13%)
Brain's ischemia + XP (0.4 mg/kg weight 3 h after operation)	2 from 23 (8,7%)

XP influences upon behavioral restoration in rats after experimental brain's ischemia (Table 2)

**Open-field experiments; a) First trial:** Brain's acute experimental ischemia, as such (active control rats), does not influenced upon horizontal activity compare to passive control rats (average numbers of crossed sectors were, accordingly,  $n = 22 \pm 8$  in active control group, and  $n = 25 \pm 6$  in passive control). Administrations of XP also does not influenced upon this parameter (average numbers of crossed sectors was  $23 \pm 7$ , and  $26 \pm 8$  if XP were used in dosage 0.2 mg/kg and 0.4 mg/kg, accordingly). In opposite, vertical activity in active control rats was decreased (average liftings  $n = 6 \pm 3$ ; against  $n = 18 \pm 6$  in passive control rats;  $p < 0.05$ ).

Administration of XP 24 h before operation leads to restoration vertical lifting activity (at dosage 0.2 mg/kg average  $n = 14 \pm 5$ ; at dosage 0.4 mg/kg  $n = 17 \pm 4$ ); administration of XP after operation did not influenced this parameter ( $n = 8 \pm 3$  and  $7 \pm 3$  at XP dosages 0.2 mg/kg and 0.4 mg/kg). *b) Second trial:* Horizontal+vertical activities were reduced compare to the first trial data in any group ( $p < 0.05$ ) – most noticeably in passive control rats ( $n = 4 \pm 2$ .) and less effective in active control rats ( $n = 15 \pm 4$ ). Administration of XP leads to reduction of horizontal+vertical activities; especially if used 3 h after operation in dosage 0.4 mg/kg ( $n = 6 \pm 3$ , and  $n = 8 \pm 5$  at dosage 0.2 mg/kg).

**Passive avoidance test:** In rats of passive control group an average of the second trial time of passing to dark compartment from illuminated one was nearly 30 times longed compare to the first (“learning”) trial: 1.5 sec. against 46.5 sec and only 2 times longer In rats of active control group this parameter was nearly unchanged (first trial - 2.5 sec.; second trial - 4.5 sec.). This data indicate for prominent impairment of long-term memory as a result of acute brain’s ischemia. Administration of XP leads to effective restoration of this function. Especially effective was introduction of XP as 0.4 mg/kg 3 h after operation (1.5 sec. at first trial, against 55 sec. at second trial); if XP were introduced as 0.2 mg/kg 24 h. before operation results were 2.0 sec. against 30.5 sec., accordingly.

**Water-maze test:** Average times of reaching of the rest-platform were longest in active control group (accordingly 42/38/34 sec. in average at the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> consequent days); this data indicate to prominent impairment of spatial working memory as a result of acute brain’s ischemia. Administration of XP leads to restoration of this function. Especially effective was using XP as 0.4 mg/kg 3 hours later operation (38/25/12 sec. consequently); if XP were used as 0.2 mg/kg 24 hours before operation average times of the rest-platform reaching were 40/35/30 sec. Average times of reaching of rest-platform by passive control rats were 36/27/18 sec. in average at the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> consequent days.

Table 2. Protective and behavioral effects of XP in a model experiments

	Surviving of rats	<b>Open-field:</b> General activity (mobility)	<b>Open-field:</b> Long-term situational memory	<b>Passive avoidance box:</b> Passive avoidance learning	<b>Water-maze:</b> Working spatial memory
<b>XP: 0.2 mg/kg 24 h</b> before operation	0	+	0	+	+
<b>XP: 0.2 mg/kg 3 h</b> after operation	+	0	+	+	++
<b>XP: 0.4 mg/kg 24 h</b> before operation	0	++	+	++	+
<b>XP: 0.4 mg/kg 3 h</b> after operation	+	0	++	++	++

0 negligible effect ( $p > 0.05$ ),

+ reliable restoration ( $p < 0.05$ )

++ complete restoration up to control level ( $p < 0.01$ ),

Obtained data indicates increase the general motor activity/mobility in rats after administration of XP (this function has been impaired prominently after acute brain’s ischemization in rats). Especially effective was XP dosage 0.4 mg/kg body weight if administered 24 h before operation ( $p < 0.01$ ). Stimulating effects of XP upon long-term and working memory also were found, especially if XP were used in dosage 0.4 mg/kg body weight 3 h later operation.

XP influences upon neurology state of patients. Phenomenology.

Table. 3. Clinical observations in individual patients (situation before-and-after of XP using)

1. *Patient P., age 55 y.o., m.*; been hospitalized with complains of suddenly began weakness in left arm and leg (can not keep items by left hand), face distortion. In a period of hospitalization: orientating herself in a time and place, in a contact with medical stuff, criticism to her state lowered; tongue is deviated to the left; left nose-lip wrinkle is sleek; speech is disartric; coarse left-side hemiparesis; harsh reduction of strength in left arm (more pronounced in a distal compartments); only minimal movements of left hand fingers are possible; reduction of strength in left leg to 3 points; Babinsky' symptom at left. Perceptible defects don't found. Computer tomography data (brain) – slipshod area of low density was revealed in right hemisphere of brain, about border of frontal and temporal lobes. Four months latter neurology state without positive dynamics. Motor functions and speech has been disturbed. Walking is difficult and possible only with somebody help

After XP using (1 month latter): Prominent positive dynamics in neurology state: size of movements in left extremities is nearly normal (small clumsiness makes itself feels in left humeral joint only if hand is in position above chest). Walk without outside assistance. Total regression of disartric speech deviations.

2. *Patient K., age 65 y.o., f.*; been hospitalized with complains of weakness in right arm and leg, instability during walking, speech deviations; beginning of disease was acute (suddenly in morning feels “weight of head”, giddy, nausea, vomiting, weakness and dumbness in right extremities. Was hospitalized in neurology department with diagnosis: acute cerebral thrombosis in basin of *a. cerebri medialis sinistra*. Heterogenic zone of low density in region of the left basal nuclei, and contraction of the left ventriculus were revealed by computer tomography. структуры, левый боковой желудочек незначительно поджат. Medicines used for improvement of cerebral blood circulation and nootropics were prescribed, however only minimal improvement of neurology state has been noted 3 months latter.

After XP using (3 weeks latter): speech was normalized; strength of extremities is increased. Four months latter does walking without support.

3. *Patient R., age 40 y.o., m.*; Clinical state after surgical ablation of glioblastoma (left hemisphere); post-operative period was complicated by brain's oedema with dimness of consciousness up to coma II degree. Left-side hemiparesis (movement restriction up to 2 points) has been developed after surgery and retains during 4 months before XP using.

After XP using (3 weeks latter): Evident regression of motor defects was noted (size of movements was rise up to 4 points).

4. *Patient M., age 26 y.o., m.*; Clinical state after surgical ablation of extramedullar malignant tumor (upper lumbar section); paraplegia of lower extremities, pelvic organs dysfunction (involuntary urination).

After XP using (2 weeks latter): Deep and superficial sensibility in pelvic organs and legs reappeared; the feeling of bladder filling reappeared; can to control urination; size of legs movements rise up to 1-1.5 points.

5. *Patient P., age 34 y.o., f.*; Acute intoxication by methanol was accompanied by loss of consciousness (up to coma III degree) and brain' oedema. During 4 days was used apparatus for artificial respiration because paralysis of breath center. At 8<sup>th</sup> day - level of conscious is spoor; at 10<sup>th</sup> day – in conscious; apalic syndrome was developed. One year latter: apalic syndrome, ambliopia, general hypotrophy, amenorrhea.

After XP using (3 months latter): menstrual function was restored; emotional reactions upon familiar songs were appeared (cry).

6. *Patient S., age 67 y.o., m.*; Clinical state after acute cerebral thrombosis in basin of *a. cerebri medialis dextra*.; deep autism was developed; stop practically any contacts with relatives.

After XP using (1.5 months latter): verbal contacts with relatives been reappeared; began to watch TV.

7. *Patient S., age 74 y.o., m.*; Clinical state after acute cerebral thrombosis; left-side paraplegia, intensive vertigo; be in a bed permanently (more then 5 months can not stand and walk); bedsores appear.

After XP using (2.5 months latter): Vertigo practically did not bother. Tonus and strength in left extremities notably higher. Began to stand up from bed; walk somewhat around room with support. Bedsores has been disappeared.

8. *Patient K., age 64 y.o., f.*; Clinical state after acute cerebral thrombosis; agnosia, alexia.

After XP using (1.5 months latter): Partial restoration of speech functions; may recognize and call objects.

9. *Patient L., age 54 y.o., m.*; Clinical state after acute cerebral thrombosis; prominent emotional inadequacy; pathological aggressiveness, irritability, tearfulness.

After XP using (2 months latter): notable normalization of emotional state (in accordance to his wife remark – "... he became a normal person again")

10. *Patient K., age 62 y.o., m.*; Clinical state after acute cerebral hemorrhage; during 4 months can not stand (fall); constant intensive vertigo.

After XP using (20 days latter): Vertigo practically did not bother; walk somewhat around room with support, did not fall any more.

11. *Patient N., age 68 y.o., f.*; Clinical state after acute cerebral thrombosis; disartric speech (blurred, inarticulate).

After XP using (1 month latter): Notable improvement of speech functions (clear pronunciation).

12. *Patient P., age 59 y.o., f.*; Arterial hypertension II; cerebral atherosclerosis; in accordance to neurologist opinion – have had small (sub-clinical) cerebral thromboses earlier. Complains of prominent impairment of memory, especially to recent events; in accordance to her daughter remark – "...she remember nothing what she promise to make".

After XP using (3 months latter): Notable restoration of memory functions was noted by patient herself, and her daughter; she easily remembers any person which were meet last week.

13. *Patient F., age 86 y.o., f.*; Post-stroke dementia; more 8 months be in a bed (can rise with support of relatives only); can't walk; practically did not speak; does not recognize of her children and grandchildren.

After XP using (3 months latter): Partial restoration of motor functions - 12 days after beginning of therapy she left her bed with help of daughter, and made few steps around bed; 20 days latter walk out home (first time during last year). Speech and mental functions – without positive changes.

14. *Patient T., age 34 y.o., m.*; Diagnosis – logo-neurosis; complains of severe stammering from early childhood, especially at nerveless or when he ought to be in contact with new person. Topical neurological symptoms did not found.

After XP using (1 month latter): Negligible (minimal) stammering only. During one year observation situation has been stabile.

15. *Patient M., age 38 y.o., f.*; Diagnosis – posttraumatic logo-neurosis; nearly 1 years ago she was under automobile crush and has had concussion of the brain with loose consciousness; complains of severe stammering after crush (can not use phone for communications because stammering).

After XP using (2 weeks latter): Stammering is nearly absent (legible speech, can use phone easily for contacts with relatives and strangers).

**Note:** XP was used as nasal drops (1 drop in each nostril), daily, during 12-14 days.

## Discussion

Common opinion about central neurons was attribution this cells to elements with genuine lacking of regenerative abilities. Accordingly, any harsh injury of the brain should obligatory leads to irreversible consequences. However, during last 10-20 years there were presented many experimental data indicates for constant generation of neuroblasts in some regions of adult brain in vertebrata [2]. Such newly formed pre-neurons probably may replace injured mature neurons at least potentially. Reparative properties of nervous fibers (axons) also were underestimated [3]. Some authors suppose that any structural elements of mature nervous tissue keep some regenerative and reparative potential, but realization of this potentiality require lot of additional factors and conditions [4]. Probably, one of important condition of neuronal regeneration is availability cocktails some of scanty investigated trophic factors. It could not be exclude that placenta may be important source for separation such kind of biologically active products, as well as biological function of this organ is directly related to trophical maintenance of different tissue and organs of forming fetus, nervous tube included.

This kind of logic discourse became the ground for investigations by Dr. Anikin. Separation and experimental investigation of fraction of X-proteins directly confirm his ideas.

Results described there are no more than phenomenological data, insufficient for conclusion about mechanisms of biologic activity of X-proteins. None the less, we suppose, X-proteins could be characterized very preliminary by the next

- XP preparation does reveal general trophic and tonic effects concerning brain's neurons.
- XP preparation show stimulating influences upon brain' integrative functions, memory and learning including.
- Probably XP did influences mostly upon injured (but not dead) neurons in parabiosis state.
- Probably XP does reveal anti-apoptotic activity and stimulates regeneration of reversibly injured nervous structures.
- Neuroprotective effects of XP may partly be depends from positive influences upon blood circulation in the brain.

We suppose data obtained and describes there indicates for evident promise of further clinical and experimental studies of XP.

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