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RESEARCH ARTICLE

Anti-Inflammatory, Antiviral Veterinary Medicine with Immuno-Modulating Activity

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ABSTRACT:

The aim of this development is an estimation of the efficacy of veterinary medicine "Trifuzol 2,5% Solution for Injection" and comparative characteristics according to available domestic analogues. "Trifuzol 2,5% Solution for Injection" has an antioxidant, immuno-modulating, anti-inflammatory activity. The medicine increases resistance to the diseases with bacterial, viral, parasitic etiology. It is planned ahead to manufacture and sale the medicine on the internal and external pharmaceutical markets.

KEYWORDS: Solution, trifuzol, efficacy, prevention, treatment.

INTRODUCTION:

Treatment of the widely spreaded diseases of the respiratory, hematopoietic system etc. of the livestock and pets with present medications is not always sufficiently effective. The store of the

domestic veterinary medicines needs to be widened owing to creation of the new effective drugs in different dosage forms with antiviral, anti-inflammatory, immunomodulating activity and low toxicity.

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MATERIALS AND METHODS:

"Anti-inflammatory and antiviral solution for injection" (Ukrainian patent № 90228 from 12.05.2014, published in bulletin № 9) containing morpholine 2- [5- (pyridin-4-yl) -1,2,4- triazol-3-ylthio] acetate, also known as "Avesstim", is presented on the national pharmaceutical market of veterinary medicines [1].

Common characteristics of "Avesstim" and proposed medicine are the next: they have derivative of 1,2,4-triazol as an active substance, the anti-inflammatory and antiviral activity, solution for injection as a dosage form, presence of excipients.

But it must be taken into account that the active substance of "Avesstim" morpholine 2- [5- (pyridin-4-yl) -1,2,4- triazol-3-ylthio] acetate is unstable and has been destroyed in solution with formation of the insoluble components. Also, a rise in the dose of

morpholine 2- [5- (pyridin-4-yl) -1,2,4- triazol-3-ylthio] acetate leads to lowering the functions of immune system and body's resistance. In addition, the medicine does not have any immunomodulating effect.

A task of improving of anti-inflammatory and antiviral medication by the way of using the more effective active substance for providing with the higher efficacy and additional immunomodulating effect was placed into the base of the development of the new dosage form.

In order to achieve this objective, the anti-inflammatory and antiviral veterinary medication in the form of the solution for injection with derivative of 1,2,4-triazol as the active substance was improved by the new characteristics: the drug is 2,5% solution for injection of piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate and has additionally immuno-modulating effect.

Piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate as the active substance in the form of solution for injection provides with increasing of therapeutical efficacy in livestock, pets, birds and is used as the anti-inflammatory medicine for concomitant pathology in comparison with well-known drugs.

"Trifuzol 2,5% Solution for Injection" is a multifunctional drug influencing on the biochemical markers. In particular, it causes further decrease of an aspartate aminotransferase, lactate dehydrogenase, gamma-glutamyl transpeptidase activity, uric acid, cholesterol and triglyceride in animals' blood serum [2].

Medication is appropriated for livestock, piglets, birds, dogs and cats, it provides with prevention and treatment of gastrointestinal and respiratory diseases with viral and mixed etiology (viral on the base of bacterial), improves general body's resistance, activates the specific vaccine-challenged immune response and reduces the postvaccinal complications. It is effective in combined therapy of the pyoinflammatory diseases (wound, abscess etc.) because of increasing the reparative processes and activation of the individual's immune defenses.

Mechanism of action is based on the activation of biochemical processes in cells.

Medication has an additional immuno-modulating effect, provides further improvement in restoration of factors of specific and nonspecific resistance, rising amount of T-cell and B-cells in blood [3-6].

RESULTS AND DISCUSSION:

Estimation of anti-inflammatory activity of "Trifuzol 2,5% Solution for Injection"

Anti-inflammatory activity had been studied on the 10 sheep. Animals had been influenced with experimentally modelled inflammation in soft tissues complicated by pyogenic infection. Pyo-inflammatory process in soft tissues was caused through the making cut on the left side of croup with scalpel followed a special pattern. The wounds had a length of 10 sm, a width of 1 sm, a depth 2 sm. These injuries were infected by the contaminated with faeces plug and litter. Before modelling of the experimental pyogenic inflammation the samples of blood were taken and essential tests were performed. These data were taken as clinical norm.

On the 3-d day according to plan of the study sheep were divided on two groups (experimental and control) by the five heads in each group. Sick animals in control group had been treated by "Avesstim" from the 3d day of pyogenic inflammation. Animals in experimental group were treated by the proposed method. For this purpose after preliminary removal of necrotic tissue and irrigation of inflammation by solution of potassium permanganate (1:1000) "Avesstim" and "Trifuzol 2,5% Solution for Injection" were injected intramuscularly based on 1 ml/10 kg of body weight with interval 72 hours. During the treatment the common clinical tests and local checkup were performed [7-9]. Also, the tests of blood samples taken from each sheep in experimental and control group were carried out before, on the 3-d and 10-th days of study. As a material for laboratory tests a serum and blood plasma, stabilized by addition of trilon B, and tissue sampling were used. Markers were determined for the healthy and experimentally sick animals on the 3-d and 10-th days of illness. The morphological (calculating amount of erythrocytes, leucocytes, leukograms) and biochemical blood parameters (determination of hemoglobin, total blood protein and protein fractions) were determined (table 1).

On the 3-d day the maximum manifestation of clinical symptoms of acute pyogenic inflammation were fixed in all 10 sheep. General condition was depressed. Partial loss of appetite was observed. General temperature was 40,3-40,7°C, pulse rate was 79-86, respiration rate was 18-23 per minute. Scar contractures were 3-6 per two minutes. Visible mucous membranes were colored in pale pink. After touching in the area of wound the muco-purulent discharge was noted.

On the 10th day of inflammatory process (7th day of treatment) gradual improving in general condition of sheep in both groups was observed. General condition of the animals in experimental group was satisfactory. Sheep could feed on actively. Body temperature was

38,6-39,4°C, pulse rate was 75-77, respiration rate was 17-18 per minute. Wound size decreased to 11,7±0,77 sм2. Surface area dried out. After removal of dry scabs the serous and bloody discharge was observed. The wound became granulated.

General condition of animals in control group was satisfactory. Sheep could feed on actively. Body temperature was 39,2-39,8°C, pulse rate was 74-78, respiration rate was 16-18 per minute. Wound size decreased to 14,8±1,07 $см^2$. Surface area dried out. After removal of dry scabs the serous and bloody discharge was observed. Wound became full mainly with healthy granulation.

Table 1: Morphological and biochemical blood parameters

Parameter	Clinically healthy animals (clinical norm)	Clinical course, day	
		3d	10th
Hematology			
Hemoglobin, g/L (experimental group)	106,06±2,96	79,22±8,82	88,69±2,97
Hemoglobin, g/L (control group)	106,06±2,96	72,94±2,97	80,64±6,77
Erythrocytes, g/L (experimental group)	7,94±0,36	6,73±0,42	7,44±0,17
Erythrocytes, g/L (control group)	7,94±0,36	6,77±0,59	6,88±0,37
Leucocytes, g/L (experimental group)	8,41±0,35	16,65±1,71	17,07±1,69
Leucocytes, g/L (control group)	8,41±0,35	20,43±1,93	18,85±1,24
Total blood protein and protein fractions			
Total blood protein, g/L (experimental group)	64,94±1,16	60,55±2,86	68,75±2,17
Total blood protein, g/L (control group)	64,94±1,16	66,55±1,07	73,15±1,35
Albumin, % (experimental group)	39,05±1,95	31,27±1,58	36,43±1,67
Albumin, % (control group)	39,05±1,95	34,39±1,23	40,67±1,57
α-globulin, % (experimental group)	11,57±0,87	14,64±1,02	12,95±1,17
α- globulin, % (control group)	11,57±0,87	11,44±0,96	10,95±1,05
β- globulin, % (experimental group)	6,09±0,85	23,64±2,63	12,96±0,98
β - globulin, % (control group)	6,09±0,85	25,25±0,87	12,68±0,77
γ- globulin, % (experimental group)	43,37±1,75	30,45±2,43	37,74±1,53
γ- globulin, % (control group)	43,37±1,75	28,79±2,04	35,73±1,14

Positive changes in clinical status of animals in experimental group were proved by the more optimal laboratory blood and tissue tests than in control. So, administration of solution for injection with piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate (experimental group) leads to the faster healing of purulent wounds in comparison with morpholine 2- [5-(pyridin-4-yl) -1,2,4- triazol-3-ylthio] acetate (control group). Difference of injury size on the 10-11th day was 16,7%. Clinical tests are confirmed by the laboratory blood and tissue tests.

Estimation of antiviral activity of "Trifuzol 2,5% Solution for Injection"

Antiviral activity of solution for injection with piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate was studied on the reference strains of vesicular stomatitis virus, dog plague and infectious encephalomyelitis in chickens.

An efficacy of medicine towards the reference strains means an activity due to other strains.

Table 2 contains the results of study of antiviral activity of investigating medicine "Trifuzol 2,5% Solution for Injection" if administration is carried out at the same time as the cell culture HEP-2 by vesicular stomatitis virus (VSV) is infected and effect during the whole period of virus reproduction (in hours) is lasting in comparison with the same tests.

Table 2: Comparative results of the determination of the antiviral activity of the test drug with the active substance piperidin-2- [5-(furan-2-yl) -4-phenyl-1,2,4-triazol-3-yl] thioacetate

Hour	VSV, lg	VSV+medicine with piperidine 5-(furan-2-yl)-4-phenyl-1,2,4-triazol-3-ylthio acetate, lg	Δlg	VSV+ medicine with morpholine 2-[5- (pyridin-4-yl) -1,2,4-triazol-3-ylthio] acetate, lg	Δlg
24	2,6	1,7	1,0	1,7	0,8
31	3,7	2,1	1,5	2,2	1,4
48	5,0	3,5	1,5	3,4	1,7
72	>10	3,5	6,5	3,2	6,8
120	>10	>10	0	0	>10

Table 2 shows that investigating medicine decreases infectious activity of vesicular stomatitis virus in all time spans in comparison with "Avesstim" if the procedure of administration is logical. That proves the higher antiviral activity of medicine with piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate as an active substance.

Estimation of immuno-modulating activity of "Trifuzol 2,5% Solution for Injection"

Estimation of immunomodulating activity of "Trifuzol 2,5% Solution for Injection" was determined in comparison with medicine containing morpholine 2- [5-(pyridin-4-yl) -1,2,4- triazol-3-ylthio] acetate and sodium chloride by the investigation of the influence the blood parameters in different time of blood drawing (each 3 hours after administration the drugs). Results are presented in table 3.

Table 3: Evaluation of immunomodulating activity of "Trifuzol 2.5% solution for injections" in comparison with the drug containing morpholine 2- [5- (pyridin-4-yl) -1,2,4-triazol-3-ylthio] acetate and sodium chloride

Blood parameter	Time of blood drawing	Medications	
		Medicine with piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl] thio acetate	Medicine with morpholine 2- [5- (pyridin-4-yl) -1,2,4- triazol-3-ylthio] acetate
1	2	3	4
Albumin, g/L	I	27,9	26,72
	II	28,5	26,33
	III	29,4	28,34
Total blood protein, g/L	I	53,2	49,0 7
	II	60,5	56,76
	III	54,9	54,06
ALP, U/L	I	707,2	551,1
	II	640,7	650,7
	III	704,5	680,3
ALT, U/L	I	74,7	71,62
	II	80,4	79,71
	III	62,7	73,63
LD, U/L	I	1215,4	1050,34
	II	898,6	895,33
	III	693,8	534,02
AST, U/L	I	52,2	51,63
	II	55,3	51,7
	III	57,3	56,3
GGT, U/L	I	84,3	83,62
	II	102,4	101,31
	III	46,9	45,31
Creatinine, µmole/L	I	82,6	77,32
	II	84,6	83,43
	III	81,7	80,32
Urea, mmole/L	I	4,27	4,12
	II	3,83	3,68
	III	3,56	3,42
Glucose, mmole/L	I	4,87	4,63
	II	6,25	6,02
	III	5,42	4,72
Cholesterol, mmole/L	I	3,50	3,43
	II	4,17	4,03
	III	4,01	3,92
Triglycerides, mmole/L	I	1,14	1.01
	II	0,87	0,8 2
	III	0,75	0,68
Amylase, U/L	I	4620,5	4617,7
	II	4887,5	4880,2
	III	4956,6	4954,1
Bilirubin total, µmole/L	I	12,9	12,01
	II	8,47	8,33
	III	7,27	7,02
- direct, µmole/L	I	3,73	3,63
	II	3,44	3,01
	III	3,53	3,32
- indirect, µmole/L	I	8,37	8,32
	II	5,56	5,33
	III	4,86	3,7 5
Thymol test, U	I	2,74	2,56
	II	3,07	2,94
	III	2,36	2,13
Phosphorus, mmole/L	I	1,33	1,17
	II	1,14	1,05
	III	2,05	1,93
Calcium, mmole/L	I	2,41	2,35
	II	1,58	1,47
	III	1.43	1,37
Albumin / globulin	I	1,23	1,23
	II	0,92	0,89
	III	1,17	1,12
AST/ALT	I	0,84	0,73

	II	1,11	1,06
	III	0,45	0,89

The data in table 3 show that the biochemical blood parameters after administration of medicine with piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate as active substance are higher than the same ones after using the medicine with morpholine 2-[5- (pyridin-4-yl) -1,2,4- triazol-3-ylthio] acetate. Furthermore, it has additional immunomodulating activity.

Moreover, it was established experimentally that proposing medication was characterized by not only antiviral, anti-inflammatory and immuno-modulating activity but also does not have any irritating and sensitizing effect. It is intoxic in suggested dose when it is used intragastrically to laboratory animals (white mice, rats) and chickens. LD_{50} is 12000 mg/kg.

"Trifuzol 2,5% Solution for Injection" must be prepared by the next way

Firstly, weigh 25 g piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate and 4,67 sodium chloride on the handle balance and place in a 1L volumetric flask. Measure out just boiled water for injection. Dissolve piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate and sodium chloride with some amount of water in the sterile volumetric flask with stirring. Then add water to the mark and stir well.

The quantification of piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4- triazol-3-yl]thio acetate in the solution for injection was carried out by the spectrophotometric method. In a case of high concentration add water taking into account a volume of solution volume. If the concentration is lesser than it is needed, add required quantity of piperidine 2-[5-(furan-2-yl)-4-phenyl-1,2,4-triazol-3-yl]thio acetate. Filter solution for injection through the folded filter from the laboratory filter paper (State Standard 12026-76) three times into sterile chemical glass. Pour the solution into ampules in 2,1 ml portions.

Seal the ampules by the tip seal method in a gas burner flame. For this purpose put the ampule in the flame at the top of the ampule downward. Constantly spin the ampule during the sealing process to create a rounded seal. Sterilize ampules at a temperature of 120°C for 15 minutes right after the sealing. Put hot ampules in a cold solution of methylene blue and wait for their cooling. Then take the ampules out and wash with warm water. Colored ampules must be taken away. Carry out visual inspection to absence of visible particles with UK-2 device. Ampules with opacity of solution or uncovered visible particles must be rejected.

CONCLUSION:

- Domestic veterinary medicine "Trifuzol 2,5% Solution for Injection" is an effective drug.
- Trifuzol 2,5% Solution for Injection" lowers infectious activity of vesicular stomatitis virusin all periods of time in comparison with "Avesstim" if the procedure of administration is logical. That proves the higher antiviral activity of the declared medicine.
- Biochemical blood parameters after administration "Trifuzol 2,5% Solution for Injection" are higher than the ones after administration "Avesstim". Besides, additional immuno-modulating activity of the declared medicine is higher in comparison with "Avesstim".
- According to experimental data the proposed medicie "Trifuzol 2,5% Solution for Injection" has not only not only antiviral, antiinflammatory and immuno-modulating activity but also does not have any irritating and sensitizing effect. It is intoxic in suggested dose.

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