Remedy with coronavirus inactivating properties.

The present invention relates to the medicinal and pharmaceutical industries and may be utilized for discovery, production and application of medical and prophylactic drugs.

Liposolvents, detergents and antiseptics are known as remedies for inactivation of coronaviruses (L. Ya. Zakstel'skaya, A. V. Scheboldov // Human and animal coronaviruses; Medicine, Moscow, 1977, vol. 2, p. 221; General and particular virology, under edition of V. M. Jdanov and S. Ya. Gaidamovich, Medicine, Moscow, 1982, vol. 2, p. 316-339; A. I. Korotyaev, S. A. Babichev// Medicinal microbiology, virology and immunology, Saint-Petersburg: «Special literature», 1998, p.273.)

We propose the use of myristamidopropyldimethylbenzylammonium chloride ("Myramistin", inventor certificate 1832496) for inactivation of coronaviruses. Previously this compound was known as a preventative for traditional venereal diseases such as syphilis, trichomoniasis, gonorrhoea and as an antiseptic (R.F. Patent 2164135) for pyoinflammatory cornea diseases.

The claimed technical solution presents an increase in the number of drugs for prophylaxis and medical treatment of diseases caused by coronaviruses.

The inactivating effect of the present compound is shown in the examples given below (wherein "Myramistin" is used as a compound name for descriptive simplification).

Example 1: Myramistin minimal inhibitory concentration (MIC) was measured for coronaviruses test culture (human coronavirus OC43) on human embryo kidney cell culture (in vitro) according to following scheme:

1st stage	Test-virus + Myramistin (in corresponding concentration).
2nd stage	Incubation (2 hours).
3rd stage	Addition of neutralizer (for example, 25 % cow embryonic serum) to reaction mixture and incubation for 10-20 minutes.
4th stage	Infection of sensitive cell culture (for example, human embryo kidney cell culture).
5th stage	Incubation (5-8 days).
6th stage	Registering of results on basis of hemadsorption phenomena and cytophatic activity - symplastoformation.

Three series of experiments for the four compound preparations were carried out. Minimal inhibitory concentration of myramistin for coronaviruses was calculated on the basis of obtained results. Experimental data are given below in Table 1.

Table 1. MIC of myramistin and other antiseptics for human coronavirus OC43, in vitro.

Antiseptic	MIC (%)
Myramistin	0.01±0.005
Decamethoxin	0.1±0.05
Chlorhexidine	0.05±0.005
Etonium	0.5±0.2
Dioxydine	R
Rockal	0.1±0.05
Nonoxinol-9	0.2±0.09
Twin-20	2.0±0.8

Note: R – coronavirus is resistant to antiseptic concentration higher than 2.5%.

As can be seen from the results obtained (Table 1), myramistin is more effective as compared to all known and presently used antiseptics in terms of coronavirus inactivation.

Example 2: Measurement of myramistin MIC for human coronavirus OC43 in vivo on baby mice was carried out according to following scheme:

1st stage	Test-virus + Myramistin (in corresponding concentration).
2nd stage	Incubation (30 minutes).
3rd stage	Infection of baby mice into brain or abdominal cavity.
4th stage	Registering of results on 2-3 day after infection (hyperemia of coverlet, tremor, rigidity of muscles, absence of sucking, death of animals).

Three series of experiments for the four compound preparations with different concentrations (6 mice for each preparation) were carried out. Infectious development of virus for baby mice was evaluated in $lgLD_{50}$. Average experimental numbers are given in Table 2.

Table 2. Results of myramistin influence (MIC concentrations) on infectious activity of human coronavirus OC43 (in vivo, baby mice).

№ of series	Infectious activity of virus ($lgLD_{50}$).				
	Before treatment	After treatment with corresponding dose (%).			
		0.0001	0.001	0.005	0.01
1.	6.4	3.6	1.0	0	0
2.	6.0	3.2	1.0	0	0
3.	6.3	3.0	1.2	0	0

The results obtained clearly shows, that the presence of myramistin in 0.0001% concentration decreases virus activity almost in half and fully neutralizes virus activity with 0.005% and higher concentrations.

Example 3: Myramistin influence on hemagglutinating activity of coronavirus OC43 was evaluated on a basis of hemagglutination reaction (HGR) with chicken erythrocytes.

Myramistin solutions with concentration from 0.000001 to 0.0.001% were added to culture broth containing coronavirus OC43 with hemagglutination titre not less than 1:512. After 30 minutes of incubation, 25 % cow embryonic serum was added to neutralize myramistin and then, after 10-20 minutes HGR with chicken erythrocytes was performed. Four series of experiments for the four myramistin preparations with different concentrations (3 examples for each concentration) were carried out. Average experimental numbers are given in Table 3.

Table 3. Experimental results for myramistin influence on hemagglutination activity of coronavirus OC43.

№ of series	Hemagglutination activity in titre:				
	Before treatment	After treatment with corresponding dose (%).			
		0.000001	0.00001	0.0001	0.001
1.	512	8	4	0	0
2.	2048	16	8	0	0
3.	1024	8	4	0	0
4.	2560	16	8	0	0

As can be seen from the above experimental results, the influence of myramistin on coronavurus hemagglutinin (which provides its adsorption on sensitive cells) is achieved in lower concentrations (0.000001%) than on its infectious activity (0.0001%).

The experimental data obtained clearly shows that the proposed technical solution allows application of myristamidopropyldimethylbenzylammonium chloride («myramistin») for use in the inactivation of coronaviruses and may be used for discovery of new preventatives and medical drugs to cure diseases caused by coronaviruses.