Pre 1990 animal and human tests with Arbidol

Arbidol was studied in an animal test and clinically on human beings. The antiviral effect of the preparation against influenza A/B viruses was tested on a hen embryo fabroblast cell culture.

Arbidol was added to a well culture in the amount of 5 .mu.m/ml in different periods before and after infecting the culture with influenza viruses to study the effect of the preparation on accumulation of an infectious virus in a tissue cell culture during a single-stage infection.

In another series of experiments the effect of Arbidol on influenza virus reproduction depending on the time of adding the preparation before and after infection of a cell culture, in other words on the dynamics of influenza virus reproduction, was tested.

The results of experiments showed that the virus-inhibiting effect in reducing the infectivity titer and suppressing the plague-forming activity during a single-stage infection of cells of Arbidol was expressed mainly during periods, corresponding to early stages of influenza viruses reproduction in cells: adsorption, penetration and probably deproteinization.

The antiviral effect of Arbidol was studied as compared to a known drug--remantadine.

Arbidol is superior over remantadine as regards a wide antiviral spectrum, shows a pronounced chemotherapeutical effect against influenza A/B viruses in a cell culture and on an experimental influenzal pneumonia, induced by A/B viruses. Remantadine is efficient only against influenza A virus, but it is inefficient against influenza B virus. In addition, in the experiment, the resistivity of influenza viruses to the preparation develops much slower as compared to remantadine.

A comparative characteristic of the antiviral activities of the preparation according to the invention and remantadine is given in Table 1.

The experimental study of harmfulness of the preparation according to the invention showed that the preparation in a single oral administration was low toxic (LD.sub.50 for mice is of 340 mg/kg, for rats--3.000 mg/kg, for guinea pigs -4.000 mg/kg).

A long-term per os administration of Arbidol in a dose of 100 to 125 mg/kg to rats during 6 months and guinea pigs over 3 months, in the dose of 50 mg/kg during 2 months to rabbits and in the dose of 25 mg/kg over 6 months to dogs did not cause any pathological changes in animals which is supported by a clinical, hematological, biochemical and pathomorphological evidence. A prolonged administration of the preparation may cause in animals the development of non-specific degenerative alternations in parenchymatous organs. Arbidol has no localized irritant action in oral administration which is evidenced by hystological tests.

TABLE 1

Arbidol	Remantadine
Inhibits the reproduction of influenza A/B viruses in a cell culture and chick embryos.	Inhibits the reproduction of influenza A/B viruses in a cell culture and chick embryos. It is inefficient against virus B.
Exerts a medicinal effect on an influenzal mice pneumonia induced by influenza A/B viruses.	Exert a medicinal effect on an influenzal mice pneumonia induced by influenza A/B viruses.
In passivating the influenza A virus in a cell culture in the presence of the preparation during 6 passes there is no virus resistance to the preparation.	In passivating the influenza A virus in a cell culture in the presence of remantadine there occur the resistance to the preparation beginning from a 2-3 passes.
No virucidal action on influenza viruses.	No virucidal action on influenza viruses.
The maximum tolerable concentration for a cell culture - chick embrio fibroblasts - 20 μg/ml.	The maximum tolerable concentration for a cell culture - chick embryo fibroblasts - 20 μg/ml.
LD_{50} for mice 340 mg/kg with per os administrating.	LD_{50} for mice 340 mg/kg with per os administrating.

The allergizing effect of Arbidol was tested on guinea pigs and rabbits. In carrying out subcutaneous injections and repeated skin applications, the preparation did not exert an allergizing action.

Arbidol has no teratogenic activity. In a non-toxic dose of 250 mg/kg for pregnant female animals (20-30 times as high as a maximum human daily dose)

the preparation did not cause abnormalities in embryogenesis as well as in a postnatal development of albino rats. A complex analysis of an embryo state and the descent of the first generation, using microanatomical, histological, physiological, and behavioral tests was accomplished.

Arbidol has no mutagenic activity. The study was carried out on the test object Salmonella typhimurium using Arbidol in a amount of 50 to 1000 .mu.m per disk.

Arbidol passed a clinical trials as an antiviral drug agent for influenza A/B virus infection on 1650 patients.

Arbidol was administered orally in the dose of 0.2 g 4 times a day during 3 days. The clinical checking of the preparation was performed in comparison to control groups o patients which were taken a symptomatic treatment. The Therapeutical efficacy of Arbidol against an influenza A/B virus infection was manifest by shortening the febrile period, intoxication and catarrhal conditions as well as by shortening the illness duration. A medicinal efficacy of Arbidol was evidenced by data of serological tests--an incremental ratio of antibodies to influenza A/B viruses significantly decreased as compared to control groups of patients given a symptomatic treatment. Arbidol prevented the development of postinfluenzal complications and decreased considerably the frequency of chronic disease exacerbations in human beings who has an influenza virus infection.

The clinical trials have proven a safety of the preparation in application in doses of 1800 to 3200 mg per course; side-effects occurred neither in influenzal patients nor in healthy individuals volunteers.

The study of the interferon-inducing effect of Arbidol was carried out in experiments in vitro (on an initially trypsinized cell culture of hen embryo fibroblasts), in experiments in vitro on 340 noninbred albino mice weighing from 18 to 20 g, and on 25 volunteers (healthy young individuals of both sexes at the age of 18 to 30).

In order to induce interferon, different doses of Arbidol together with 2% diethylaminoethyl dextran in a 1:4 ratio were added to a hen embryo fibroblast cell culture in 0.2 ml per tube contained a cell culture monolayer. The incubation of treated cells was carried out at 37.degree. C. for 1 hour. The cells were then rinsed from the preparation with the medium No. 199, and 1 ml of the medium No. 199 was poured therein without adding the bovine blood serum. In 8, 24 and 48 hours after the incubation at 37.degree. C. a culture fluid was collected and titrated in the presence of interferon in a hen embryo fibroblast cell culture infected with Venezuelan encephalitis viruses using a conventional technique.

The results of titration are given in Table 2.

In studying the interferon-inducing effect of arbidole a reference standard was a highly active interferon inducer--a double-stranded RF.sub.2 phage RNA (a double-stranded ribonucleic acid of RF.sub.2 -phage).

The results given in Table 2 allow the conclusion that Arbidol is a highly active interferon inducer in a hen embryo fibroblast cell culture. At the concentration of 20 g/ml the preparation induces interferon already in 8 hours (its titer 640-1280 U/ml; the maximum titer of 2560 U/ml was found 24 hours later; by 48 hours the interferon titer decreases to 320 U/ml).

A dose-dependent effect of interferon induction in a hen embryo fibroblast cell culture under the action of the preparation was established. A two-fold reduction of the concentration of the preparation (up to 10 .mu.g/ml) leads to an 8-fold reduction of interferon induction, the dynamics, however, remains the same.

TABLE 2

Interferon-Inducing Effect of the Preparation According to the Invention in a hen Embryo Fibroblast Cell Culture

Preparation	Concentration	Interferon titer (lg/ml) after 8 h	24 h	48 h
Arbidol	10.0	80.0	160.0	40.0
Arbidol	20.0	640–1280	1280–2560	64–320
A double-stranded RF_2-phage RNA	100.0	0	160.0	not studied
A double-stranded RF_2-phage RNA	400.0	640–1280	1280–2560	640–320

A comparative study of interferon induction by the action of Arbidol and a double-stranded RF.sub.2 -phage RNA as a highly active interferon inducer standard revealed a similar induction dynamics, whereas a double-stranded RF.sub.2 phage RNA was needed 20 times as much as the preparation (400 g/ml), the concentration of 100 g/ml caused a weak (slight) interferon induction.

In the following series of experiments the capability of Arbidol to induce interferon in test animal blood serum was studied.

Arbidol was per os administered once to mice (3 mouse groups each of 100 animals in the doses of 250, 125 and 62.5 mg/kg, and 16, 24, 48 and 72 hours later blood serum was obtained to be used for interferon titrations in a transferred mouse L-cell line against test vesicular stomatitis viruses. In addition, Arbidol was once injected intraperitoneally into the group of 20 mice in the dose of 10 mg/kg, and 24 hours later blood serum was obtained in which interferon titres were also determined.

The experimental results are given in Table 3.

TABLE 3

Dynamics of Interferon Induction under the Action of **Arbidol**

Preparation and administration method	Dose, mg/kg	Interferon titer (U/ml) after 16 h	24 h	48 h	72 h
Arbidol (per os)	250	320–540	640–1280	320	160
Arbidol (per os)	125	640	320–640	640	160
Arbidol (per os)	62.5	320–640	640	160	40
Arbidol (intraperitoneally)	10	not studied	640–120	not studied	
Mice blood serum without the preparation of the invention	0	10–20	10–20	10–20	10–20

The results presented in Table 3 show that Arbidol administered per os causes interferon induction in the mouse blood serum. The effect is characterized by dose-dependence and is most strong by pronounced in a dosage range of 250 to 62.5 mg per 1 kg of an animal body weight. High interferon titers (640 U/ml) were found in the mouse blood serum in 16 hours, and persisted there during up to 48 hours. The preparation of this invention enhances the interferon induction also in case of intraperitoneal injection.

With repeated administering of Arbidol to mice for interferon induction, a depressed reactivity state condition occurs in the animals, which is pronounced in reducing the interferon titers in the blood serum.

Arbidol as an interferon inducer has a preventive effect on induced virus infections. Its preventive effect was studied on a mouse influenzal pneumonia induced by infecting the animals intranasally with influenza A virus (Bethezda) 63 (H.sub.2 N.sub.2), A (Aichi) (H.sub.3 N.sub.2) and on a generalized mouse herpes, induced by intranasal infection of them with herpes simplex virus from antigen-type I L.sub.2 -strain.

The results are given in Table 4.

Arbidol in administering it per os as prophylactic 24 and 6 hours before infecting in doses of 31.2 mg/kg to 125 mg/kg lowers lethality of mice suffering from an influenzal pneumonia by 40-50% as compared to control. A single administration of the preparation per os in the dose of 30 mg/kg 24 hours prior to infection prevents the death of 40% of animals suffering from a generalized herpetic infection.

The interferon-inducing effect of Arbidol was studied also in human beings. The blood serum of human beings give per os tablets of the preparation (0.1 g) in different dosage schemes were assayed for serum interferon. Serum titration was performed with conventional procedures using a human diploid M-22 cell culture and test mouse encephalomyocarditis viruses. The results are given in Table 5.

TABLE 4

Preventive Action of **Arbidol** According to the Invention in Experimental Virus Infections

	Mouse influenzal pneumonia				
Dose, mg/kg	Time and administration method	Lethality: absolute animal number, (*)	%	The decrease of lethality, %	P
1	2	3	4	5	6
125	per os	8/40	20	50	<0.01
62.5	24 and	8/40	20	50	<0.01
31.2	6 hours before infecting	12/40	30	40	<0.01
Control (without **Arbidol**)		28/40	70	—	
30	per os,	12/20	60	40	0.01
15	24 hours	15/20	75	25	0.01
Control (without **Arbidol**)		20/20	100	—	

*in the numerator - the number of dead animals
in the denominator - the number of mice in the group

TABLE 5

Study of Interferon-inducing Effect of **Arbidol** in Human Beings

		Interferon content in the human blood serum					
		After a single administration of Arbidol per day (100 mg)		After administering Arbidol 3 times per day (300 mg)		After administering Arbidol 6 times per day during 2 days (600 mg)	
No. group	The number of men in group	The number of positive reactions	Inteferon titer, U/ml	The number of positive reactions	Interferon titer, U/ml	The number of positive reactions	Interferon titer, U/ml
Group 1	13	8	40–80	—	—	—	—
Group 2	12	—	—	12	160–320	12	40–80

It has been found that a single per os administration of Arbidol in the amount of 100 mg causes the interferon induction in titers ranged of 40 to 80 U/ml in 8 out of 13 volunteers (61.5%). A 3-times administration of the preparation (300 mg) 1 tablet a day resulted in a rapid decrease of interferon induction (interferon was found in the blood serum in all 12 volunteers (100%) in titers contained 160-320 U/ml. With a further increase in the dose to 600 mg (3 tablets a day) during 2 days, a sharp reduction of interferon induction occurred (the interferon titer in the blood serum was not in excess of 40-80 U/ml).

The results obtained show a pronounced interferon-inducing effect of Arbidol in human beings in administering it per os. Maximum content of interferon in the human blood serum was found after administration of 300 mg of Arbidol (in 100 mg 3 times a day). An increase in the dose and duration of administration of the preparation resulted in the development of hyporeactivity in human beings, which was characterized by a sharp reduction of serum interferon titers.

The immunomodulatory immunopotentiating effect of Arbidol was also assayed.

The effect of Arbidol on the immune system functions was studied.

Phagocyte function under the action of Arbidol was studied on adult female hybridous mice (CBA/C 57 Bl.sub.6)F.

Arbidol was administered per os in the dose of 125 mg/kg once and once in a day during 5 days. A reference standard was levamisole given on the same scheme in the dose of 50 g per mouse. During different period after administering of the preparation mouse peritoneal macrophages were isolated in which an absorbing capacity was measured by absorbance of neutral red and was determined quantitatively from a calibration curve using spectrophotometrical techniques (at 530 nm).

The results are given in Table 6.

The administration of the preparation according to the invention and levamisole has no effect on counts of macrophages washed off the peritoneal cavity, but it enhanced their absorbing (activity) capacity. Thus, by 2-3 days after a single administration of the preparation the absorbing capacity of macrophages was remained at the same level--164.+-.13% ($p<0.05$) and 138.+-.11% ($p<0.05$), respectively.

TABLE 6

Absorbing Capacity (mg/6 × 10^6 cells) of Peritoneal Macrophages during Different Periods after a Single Administration of **Arbidol** and Levamisole

Time after administration	Control (starch)	Levamisole	**Arbidol**
The 1 day	20.8 ± 3.8	24.2 ± 4.5 (116 ± 21%)	27.7 ± 3.5 (125 ± 17%) $p < 0.01$
The 2 day	22.8 ± 2.0	27.7 ± 3.2 (121 ± 14%)	33.6 ± 4.7 (147 ± 20.6%) $p < 0.05$
The 3 day	24.1 ± 4.2	32.5 ± 2.4 (135 ± 9.9%)	37.4 ± 3.7 (155 ± 15%) $p < 0.05$)

Note:
in brackets percent of control.

Thus, administering Arbidol in vivo stimulates the absorbing capacity of macrophages to a greater extent than administration of levamisole.

The effect of Arbidol on antibody genesis was studied in comparison with levamisole and a double-stranded RF.sub.2 phage RNA. There were used hybrid mice (CBA/A 57 BL.sub.6)F as well as the tumor-carrier animals (breast cancer grafted subcutaneously into a leg), intact and totally irradiated (2 Gr.).

The antibody-producing mechanism was studied as applied to sheep erythrocytes by subperitoneal immunization of the animals in the amount of 1.times.10.sup.8 cells. Five days after immunization the animals were sacrificed by decapitation, and the content of antibody-producing cells in the spleen was determined according to Canningame. The results of the tests are summarized in Table 7.

Arbidol increased the content of antibody-producing cells at different dosage schemes up to 167-246%, and levamisole and a double-stranded RF.sub.2 phage RNA, in the range of 143 to 170%.

Only Arbidol had a pronounced immunostimulating effect on irradiated animals, and to a maximum degree when administered 3 days prior to irradiation: once irradiated, the immune response was of 230% against the control. Levamisole was of low efficacy in these animals; a double-stranded RF.sub.2 phage RNA was inefficient.

TABLE 7

Comparative Study of Effects of **Arbidol** , Levamisole and a Double-Stranded RF_2 Phage RNA on Antivody-Producing Mechanism (% of control) in intact mice, totally irradiated (2 Gr.), tumor-carriers and totally irradiated tumor-carriers (2 Gr.)

Animal groups (mice) 1	Levamisole during 3 days, daily 2	Levamisole 5 times at a 5-day interval 3	A double-stranded RF_2 phage at a 3-day interval 4
Intact	164 ± 6.1	170 ± 15.1*	143 ± 7.5
Totally irradiated (2 Gr.)	174 ± 24.0	104 ± 6.0	80 ± 6.6
Tumor-carriers	14 ± 23.0	91 ± 14.0	74 ± 31.0
Irradiated	86.0 ± 35.0	402 ± 12.5	92 ± 21.0
Arbidol	**Arbidol**	**Arbidol**	**Arbidol**
after a single use 5	during 3 days, daily 6	**during 5 days daily** 7	5 times in 3 days 8
246 ± 6.0*	141 ± 13	192 ± 14.0*	167 ± 12.3*
230 ± 12.0*	129 ± 19	181 ± 11.3	96 ± 20.0
211 ± 10.0*	99 ± 19	154 ± 16.6*	110 ± 11.0
175 ± 24.6*	121 ± 25	128 ± 28.5	250 ± 21.5*

Note:
*Differences from control values are confident ($p < 0.05$).

Arbidol has also the immunopotentiating effect on tumor-carrier animals (the 7-17 day of tumor development) after a single (3 days prior to immunization) or a 5-times daily administration. Levamisole and a double-stranded RF.sub.2 RNA in these experiments were inefficient.

Arbidol was active only in totally irradiated tumor-carrier animals and had a pronounced immunopotentiating effect even in cases when the level was decreased to about 50 times as low as compared to intact animals. Levamisole also had the immunopotentiating effect on these animals; a double-stranded RF.sub.2 phage RNA was inefficient.

Thus, Arbidol is capable of stimulating (enhancing) the antibody genesis in all mouse breeds tested, both intact and irradiated, and by efficiency it is superior over reference standards--levamisole and a double-stranded RF.sub.2 phage RNA.

The effect of Arbidol on cell-mediated immunity responses was studied using the graft versus host technique.

Hybridous male mice (CBA/C 57 BL.sub.6)F.sub.1 at the age of 3 months were irradiated in the dose of 5 Gr. One day after irradiation the animals were given injections intravenously with lymphocytes prepared from lymphadens of a parent mouse CBA breed aged 3 months, in the concentrations of 1.25, 2.5 and 5.0.times.10.sup.6 kariocytes. Once lymphocytes were intravenously injected in the test groups, Arbidol was administered orally in a dose of 100 mg per 1 kg of a body weight, and levamisole in a dose of 5 mg per 1 kg in a 2% starch solution. Each group had 15 mice. Eight days after irradiation the mice were killed, the spleens were fixed, and the count of colonies having a diameter of at least 0.2 mm was determined.

The data obtained are given in Table 8. Adding the allogenic lymphocytes in high concentrations results in a decrease in the yield of the spleen colonies in control, and in the minimum concentration used some increase in the yield of the spleen colonies occurred. The administration of Arbidol reduces both observed deviations as compared to control values if the concentration of introduced cells is not the highest (5.times.10.sup.6). Levamisole has identical but a less pronounced effect. Furthermore, in the case when allogenic lymphocytes are not to be added, levamisole somewhat decreases the yield of the spleen colonies, whereas Arbidol does not have such an effect.

TABLE 8

Effect of **Arbidol** and Levamisole on Inactivation of the Stem Cells with Allogenic Lymphocytes

The preparation	The number of given lymphocytes $\times 10^6$	Administration of preparation per os	The counts of spleen endocolonies (M ± m)
Control	5.0	—	0.3 ± 0.1
"	2.5	—	0.9 ± 0.3
"	1.25	—	4.1 ± 0.6
"	—	—	2.2 ± 0.5
Arbidol	5.0	+	0.3 ± 0.1
Arbidol	2.5	+	1.6 ± 0.2
Arbidol	1.25	+	2.5 ± 0.4
Arbidol	—	+	2.2 ± 0.4
Levamisole	5.0	+	0.7 ± 0.2
"	2.5	+	1.1 ± 0.2
"	1.25	+	2.3 ± 0.5
"	—	+	1.1 ± 0.3

Thus, administering Arbidol under induced graft versus host reaction conditions normalizes the proliferative activity of target cells and their ability of producing a vital colony, both in case of inhibition (with high lymphocyte concentrations). Arbidol by its efficacy is superior over a known drug levamisole.

The effects of Arbidol as an immunopotentiator on the development of a transferred tumor, and on thermal skin lesions were studied.

The effect of Arbidol on the development of sarcoma-45 (Rous sarcoma) grafted subcutaneously to the right leg of adult female mice of the Vistar breed was studied. Arbidol was administered peritoneally as a suspension in a 1% starch solution in the dose of 125 mg/kg every day during 5 days two weeks after grafting the tumor. Control animals were given only a starch solution. The dynamic development of the tumor was monitored by intravital measurements of its mass. During a 12 month period of observation the inhibition of the development of tumor was found in the group (10 animals) which were given Arbidol.

Thus Arbidol has the inhibitory effect on the development of a massive tumor induced by grafting, probably, via the activation of immunological reactivity.

The results of experiments are given in Table 9.

The study of the effect of the preparation according to the invention on a thermal skin lesion revealed the ability of the preparation to exert a thermoprotective action and reduce a thermal skin lesion.

The effect of the preparation according to the invention on an immunological human status was studied.

Healthy human young volunteers, at the age of 18 to 30 (25 individuals) were under observation.

The immunological status was evaluated on the basis of T-cellular immunity analysis: the state of lymphocytes in the blood of the peripheral vessels, an absolute and relative count of T-cell by Jondal, the state of receptor stability on the outer lymphocyte membrane.

TABLE 9

Effect of **Arbidol** (125 mg/kg daily during 5 days) on the Development of Sarcoma-45 in Rats

Time after grafting the tumor	Sarcoma growth, cm^3		
	without **Arbidol**	administration of **Arbidol**	
1 month	11.9 ± 0.86	8.2 ± 1.4	$p < 0.05$
1 month 10 days	35.0 ± 3.4	21.0 ± 3.9	$p < 0.05$
1 month 16 days	59.8 ± 5.0	38.0 ± 7.5	$p < 0.05$
2 months	70.1 ± 2.6	39.9 ± 9.7	$p < 0.05$)

Using different doses of Arbidol an immunological status was checked in healthy young human beings without a preliminary antigenic enhancement and under vaccinal influenza conditions produced by immunizing them intravenously with influenza A virus vaccine in a 1:1 dilutions in 1 ml.

The experiment was performed by using a double blind method wherein together with tablets of Arbidol, each of 100 mg, an appropriate placebo was used. The results were statistically handled.

The data obtained show that Arbidol in a single per os administration of 100 mg and a 300 mg daily

(100 mg three times a day) fails to change the counts of T-populations, but leads to changes in bonding receptors with the outer lymphocyte membrane, which persisted during 14 days (the time of observation).

The vaccination which was performed 24 hours after administering Arbidol had no effect on the nature of changes of bonding receptors with lymphocyte membranes.

The change in the surface properties of lymphocyte membranes under the action of Arbidol is likely to result in changes in functional properties of these cells leading to enhancment of function of the immunity system, which is evidenced by observed protective effect against the development of influenza virus infections. Arbidol prevents from post-influenzal complications, and considerably decreases the frequency of chronic disease exacerbations in persons which had an influenza virus infection.

The results of the clinical trials show that Arbidol is non-toxic, is well tolerated by patients, has the therapeutical effect on influenza A/B virus infections, prevents from the development of postinfluenzal complications and chronic disease exacerbations after influenza virus infections. In addition, Arbidol is an efficient interferonogene and immunopotentiator having neither toxic nor side effects on the body. Arbidol may be preferably used as an immunopotentiator in secondary immunodeficiency conditions including radiation and thermoradiation in the treatment of oncologic (tumor) patients, and in chronic and relapsing virus infections.

Arbidol is preferably used 1-2 times a week during the whole course of a specific antineoplastic therapy. A single dose is in the range of 300 to 400 mg (about of 250 mg/m.sup.2), a therapeutic dose per course (a curattive course of tumor patients lasts from 1 to 2 months) is of 3 to 6 g of the preparation.

The results of a pharmacokinetic study of a C.sup.14 -labelled preparation of the invention show that the preparation is rapidly evacuated from the body, which allows one to remove all fears against its possible accumulation in the body.