GEORGIA
ONCOLOGICAL CENTRE OF THE ADJARA AUTONOMOUS REPUBLIC

Clinical study of the effect of "Kamelin-M2" capsules in combination with chemotherapy on cellular immunity of oncology patients and improvement of quality of life

Adjara Batumi Cancer Centre 2011
The survey was conducted between September 2007 and January 2011.

Summary
The drug 'Kamelin-M2' capsules for oral use is a light brown powder with a characteristic odour.
Capsule content weight: 0.260 g ± 10%. Active substance "Kamelin-M" 0.0546 g and other ingredients. The medicinal substance 'Kamelin-M' is produced from a special type of bee honey. It contains highly biologically active substances: aldehydes, organic acids, ketones. Based on the results of preclinical studies of the drug, it was concluded that "Kamelin-M2" has the ability to stimulate the production of endogenous tumour necrosis factor, contributes to the normalisation of the immune background, balances the ratio of immunocompetent CD4/CD8 cells (helpers/supressors) and increases the number of natural killer (NK) cells.
Research has been carried out into two mechanisms of action of 'Kamelin-M' on tumours The first mechanism is the immunomodulatory property of 'Kamelin-M'. The mechanism of action is the stimulation of three important cytokines: IL-2, IFN-y and TNF-a.
The second mechanism of action is the cytotoxic activity of 'Kamelin-M'.
In vitro experiments on cancer cells showed that the minimum inhibitory concentrations of 'Kamelin- M' against colon cancer DLD-1 and lung cancer A-549 cells are comparable: the IC50 is 0.0063 ± 0.0006 μg/ml and 0.0078 ± 0.0005 μg/ml, respectively.
Comparison of therapeutic efficacy, cellular immune function, improvement in quality of life and change in blood toxicity when treating patients with malignant tumours at different stages of development chemotherapy in combination with the drug 'Kamelinu-M2' and in monotherapy.

Method. Randomised groups: a treatment group (58 patients) with the drug 'Kamelin-M2' and chemotherapy, and a control group (60 patients) with mono-chemotherapy.

Results. The efficacy (PR + CR) in the treatment group was 37.93%, in the control group 21.60%, the difference between the groups is significant, $p < 0.05$. Measurement of immune function in the treatment group showed that T-cell subgroup levels and NK cell activity are significantly higher in the treatment group, compared with the control group, $p < 0.0$. The Karnowski scale in the treatment group is higher than in the control group, $p < 0.05$.

Conclusion. The drug 'Kamelin-M2' in combination with chemotherapy may increase the efficacy of chemotherapy, reduce side effects, increase cellular immune function, improve quality of life, and is therefore a good drug/agent in combination with chemotherapy for the treatment of patients with intermediate- and late-stage malignancies.

Between 2007 and 2011, we used 'Kamelin-M2' capsules in combination with chemotherapy in the treatment of 58 patients with intermediate- and late-stage malignancies and compared the efficacy of the treatment with a control group (60 patients) treated with monochemotherapy during the same period in terms of parameters such as changes in cellular immune function, improvements in quality of life and blood toxicity levels.

Clinical materials

Rationale for the study.
At the current level of development of medical science, issues of cancer treatment are still relevant. Statistics in recent years show that in developed countries, 'cancer' or 'malignant neoplasm' is diagnosed in about a third of people during their lifetime, and almost a quarter die of cancer. The problem of cancer is multifaceted, with doctors from various specialities and representatives of other sciences involved in solving it: biologists, geneticists, biochemists, pharmacologists, sociologists, psychologists, ethnographers and many others. Extensive scientific and statistical material has now been gathered on various topics concerning the causes of the formation, morbidity and mortality from malignant tumours. In recent decades, medicine has been enriched by new knowledge and experience in treating this disease.

Theoretical rationale.
The treatment of cancer, despite the enormous efforts of scientists worldwide over many years, is still a largely unsolved problem. The methods used, among which the main ones, besides surgery, are chemotherapy, alone or in combination with radiation and radiotherapy treatment, are often not sufficiently effective. In addition, all these interactions themselves cause immunosuppression, resulting in inhibition of bone marrow haematopoiesis and infectious complications, as well as the development of intestinal dysbiosis.

As a result, the immune system, perhaps already weakened as a result of the development of the cancerous lesion, receives another, additional hit that inhibits its activity. It follows that the successful cure of a cancerous lesion may depend on the balance between the anti-tumour efficacy of the drugs chemotherapeutic agents and the potential of the immune system sufficient (or insufficient) to cope with the amount of cancer cells remaining after treatment.

All that has been said above leaves no doubt that the immune system is at the centre of all current attempts to improve the efficacy of anticancer therapy, and that the task of activating the anticancer potential of the immune system is paramount in modern oncology. Consequently, all attempts to mitigate the side effects (immunosuppressive) of chemotherapy are undoubtedly timely.

Based on the results of preclinical studies of the drug, it was concluded that the drug substance 'Kamelin-M1' has the ability to stimulate the production of endogenous tumour necrosis factor, contributes to the normalisation of the
immune background and balances the ratio of immunocompetent $_{CD4/CD8}$ cells (helpers/supressors) and increases the number of natural killer (NK) cells.

Two mechanisms of action of the substance 'Kamelin-M' on cancer have been investigated. The first mechanism is the immunomodulatory property of the substance 'Kamelin-M'. The mechanism of action is the stimulation of three important cytokines: IL-2, IFN-y and TNF-a.

Effect of "Kamelin-M" on cytokinins

Cytokinins	Control	"Kamelin-M"
IL-2	14.5+/- 0.7 pg/ml	45.6 +/- 4.1 pg/ml*
IFN-γ	163.4 +/-12.4 4 pg/ml	721.3 +/- 64.4 pg/ml*
TNF-(α)	362.2+/- 43.2 pg/ml	3156.2 +/- 129.9 pg/ml*

The second mechanism of action is the cytotoxic activity of 'Kamelin-M'.
In vitro experiments on cancer cells showed that the minimum inhibitory concentrations of 'Kamelin- M' against colon cancer DLD-1 and lung cancer A-549 cells are comparable: the IC50 is 0.0063 ± 0.0006 μg/ml and 0.0078 ± 0.0005 μg/ml, respectively.

Thus, it can be concluded that the substance of the drug 'Kamelin-M' has a dual action:

1. During immunobiochemical testing, the drug's ability to bind to oncofetal proteins, causing their denaturation damage, was established.

2. The anticancer effect is explained by the detergent-like action of certain molecules of the drug substance 'Kamelin-M2' on the membrane of tumour cells, thereby altering their structure and the metabolism of the tumour cells and causing their death.

Ethical and legal standards of research, instructing patients.

This clinical trial was conducted in accordance with the Law of Georgia and the principles of the Declaration of Helsinki.

The present study was initiated after approval of the clinical trial protocol by the Ethics Committee of the Pharmacology Committee of Georgia.

Patients who were potential study participants were informed about the nature of the clinical trial, the study drug, and the possible risks of taking the drug "Patient Information Sheet".

All patients included in the study signed a written agreement to participate in the study.

All documentation related to the study, and information on patients participating in the study, is strictly confidential.

Investigators and administrative structure of the study (characteristics of the clinical base).

The main contractor is the Adjaria Cancer Centre, Surgical Department

Address: Georgia, City of Batumi 118 Pushkin Street The Adjaria Cancer Centre is presented with a number of oncology departments:

I. Gynaecology Department,

II. Surgical Department,

III. Chemotherapy department

IV. Proctology Department

V. Thoracic Division

VI. Radiology Department

VII. Haematology department

The clinic has laboratories for ultrasound, gastroscopy, bronchoscopy, X-ray examinations. and laboratories: clinical, biochemical, bacteriological, histological and pathomorphological.

Ordinary materials

The study included hospitalised patients with various mid- and late-stage malignancies according to the WHO TNM criteria.

In all 118 patients, the diagnosis was confirmed pathologically or cytologically.

Total 88 men, 30 women, mean age - 56.5 years (34-71 years). Lung cancer -- 20 patients, stomach cancer -- 29, rectal cancer -- 23, oesophageal cancer -- 6, breast cancer -- 15, malignant lymphoma -- 8, liver cancer -- 17.
Karnowski scale
> 60 points.

Patients were randomised into a treatment group ('Kamelin-M2' + chemotherapy) and a control group (chemotherapy).

One week before and one week after treatment, all indicators were tested; during treatment, all patients took the drug according to the standard regimen, without the addition of any other drugs or special immune modulators.

Treatment

Treatment group: chemotherapy + "Kamelin-M2", 3 capsules per day, 1 treatment cycle - 60 capsules, a total of III cycles were prescribed. The interval between courses was 5-7 days.

Control group: monochemotherapy, 2 cycles (6-8 weeks).

The first- or second-line chemotherapy regimen was prescribed according to the standards adopted abroad or in Georgia, depending on the type of cancerous lesion.

Observation indicators and evaluation of therapeutic effectiveness

Observational indicators: tumour lesion reduction, change in immune function (T-lymphocyte subgroup measurement, NK cell activity), change in body weight, Karnowski scale, peripheral haemogram.

Tumour lesion reduction was determined according to the WHO's indicators for assessing the therapeutic effect of cancer:

CR (complete remission) - complete disappearance of a visible outbreak of disease, lasting at least more than 4 weeks;

PR (partial remission) - the product of the largest perpendicular diameters of the visible tumour decreased by more than 50% and this lasted for more than 4 weeks, without the development of any foci of disease or the appearance of new foci;

MR (improvement) - tumour has shrunk by 25% or more, no new foci have appeared; SD (stabilisation) - tumour has shrunk or enlarged by less than 25%, no new foci have appeared;

PD (progression) - the tumour has enlarged by 25% or more or new foci of disease have appeared. Effects Side effects were assessed according to WHO standards. Patients' quality of life was assessed by comparing the Karnowski scale (increase or decrease) and body weight (increase or decrease) before and after treatment.

Before treatment, they assessed patients' quality of life based on activity status using the Karnowski scale. "Increasing" or "increasing": if the scale increased or decreased by more than 10 points after treatment in patients. "Stabilisation": an increase or decrease in the scale of less than 10 points.

At the same time, weight changes were assessed. "Gain" or "loss": if, after treatment, weight increased or decreased by more than 1 kg, 'stabilisation': if the patients' weight increased or decreased by less than 1 kg.

Before and after chemotherapy, fasting patients had their blood drawn from a vein early in the morning for T-lymphocyte subgroup analysis and NK cell activity.

Results

Therapeutic efficacy

In the treatment group (58 patients) CR - 5 patients, PR - 17, MR - 10, SD - 22, PD - 4, efficacy (PR+CR) - 37.93%. In the control group (60 patients) CR - 2 patients, PR - 11, MR - 16, SD - 25, PD - 6, efficacy (PR+CR) - 21.66%. The difference between the groups is significant, $P < 0.05$.

Changes in immune function

In a comparison of pre- and post-treatment results, CD_3, CD_4, CD_4/CD_8 and NK cells increased to varying degrees after treatment, the difference between groups being significant, $P < 0.05$. CD_8 increased slightly.

A comparison in the two groups after treatment showed that CD_3, CD_4, CD_4/CD_8 and NK cell levels increased significantly in the treatment group, $P < 0.01$ (see table 1).

Table 1. Changes in immune function in the two groups ($\bar{x} \pm s$)

Group	CD_3	CD_4	CD_8	CD_4/CD_8	NK cells
Therapeutic					
Before treatment	50,13 ± 9,51	31,07 ± 8,55	23,83 ± 7,65	1,40 ± 0,54	37,58 ± 10,60
After treatment	51,88 ± 10,66[b]	35,58 ± 8,18[a,b]	23,80 ± 6,68	1,53 ± 0,57[a,b]	40,28 ± 9,20[b]
Control					
Before treatment	50,18 ± 8,53	33,03 ± 7,36	22,45 ± 5,64	1,46 ± 0,75	36,72 ± 11,2
After treatment	45,43 ± 6,63	30,72 ± 7,50	22,44 ± 6,0	1,31 ± 0,61	30,18 ± 7,8

a - vs before treatment $P < 0.05$. b vs control group, $P < 0.01$.

Improving quality of life

In the treatment group, the number of patients whose Karnowski scale increased after therapy was significantly higher than in the control group, the difference being statistically significant, $P < 0.05$ (table 2).

Table 2. Comparison of patients' quality of life in two groups after treatment (n)

Indicators	Therapy group			Control group		
	Increase	Stabilisation	Reduction	Increase	Stabilisation	Reduction
Changes "Kamelin-2M"	30	25	3	8	31	21
Changes in body weight	25	20	13	8	15	37

Note: comparison across two groups, $P<0.05$.

Changes in peripheral haemogram before and after treatment.

Comparison in the treatment and control groups (Kruskal-Wallis), the difference in groups is significant, $P < 0.05$ (table 3).

Discussion

The drug "Kamelin-M2" capsules for oral use. Contains highly biologically active substances: aldehydes, organic acids, ketones.

The results of pharmacodynamic and clinical studies have shown that 'Kamelin-M2' is effective against many cancers and has a pronounced inhibitory effect. Two mechanisms of action of the substance 'Kamelin-M' against cancer have been investigated. The first mechanism is the immunomodulatory property of the 'Kamelin-M' substance. The mechanism of action is the stimulation of three important cytokines: IL-2, IFN-y and TNF-a.

The second mechanism of action is the cytotoxic activity of 'Kamelin-M'.

In vitro experiments on cancer cells have shown that the minimum inhibitory concentrations of 'Kamelin- M' against colon cancer DLD-1 and lung cancer A-549 cells are comparable: the IC50 is 0.0063 ± 0.0006 μg/ml and 0.0078 ± 0.0005 μg/ml, respectively. At present, this dual-action, broad-spectrum drug (of natural origin) is ideal for preventive and clinical applications.

Table 3. Comparison of side effects in blood in two groups (n)

Degree of toxicity	HBG		WBC		PLT	
	Therapy group	Control group	Therapy group	Control group	Therapy group	Control group
0	24	16	6	5	38	26
I	25	23	36	18	18	23
II	9	18	10	26	2	10
III	0	3	6	*9*	0	1
IV	0	0	0	2	0	0

Materials show that the immune function of patients with malignant tumours in the middle and late stages is reduced, abnormalities in the T-lymphocyte subgroup and reduced levels of NK cell activity lead to a severe reduction in cellular immunity, the tumour evades the host immune response, leading to its growth and metastatic spread. In addition, patients develop cachexia, which makes treatment largely difficult.

The study data showed an efficacy of 37.93% in the treatment group and 21.66% in the control group, with a comparison across groups of $P < 0.05$.

"Kamelin-2 in combination with chemotherapy significantly increases the efficacy of chemotherapy in the treatment of malignant tumours in the middle and advanced stages.

In the treatment group, post-treatment results, compared to pre-treatment results, showed that $_{CD3}$, $_{CD4}$, $_{CD4}$ / $_{CD8}$ and NK cell levels increased to varying degrees, the difference being statistically significant, $P < 0.01$.

"Kamelin-2 can increase patients' immune function. The improvement in quality of life in the treatment group significantly exceeds that of the control group and, despite the reduction in quality of life of individual patients, statistical analysis showed $P > 0.05$.
When comparing blood and bone marrow toxicity in the treatment group, the behaviour of the haemogram significantly was superior to the control group and allowed timely completion of chemotherapy. No hepatic or renal dysfunction, vascular irritation, phlebitis or other side effects were observed in the treatment group.

"Kamelin-2" is safe, has no side effects, can improve the efficacy of chemotherapy and patients' immune function, especially $CD3$, $CD4$, $CD4/CD8$ T-cell subgroup and NK cell activity, improve patients' quality of life, preserve haemogram. "Kamelin-2" is an adjunctive drug used in combination with chemotherapy for the treatment of patients with malignant tumours in the middle and advanced stages.

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