Original Article

Rectal methods of delivery of medical drugs with a protein nature in the therapies of tumor disease

Jandos Ukibayev[1], Ubaidilla Datkhayev[1], Diyas Myrzakozha[1]*, Alexander Frantsev[2], Elmira Karlova[1], Yelena Nechepurenko[1], Didara Balpanova[1], Alma Almabekova[1]

[1] Kazakh National Medical University named after S.D. Asfendiyarov, Almaty, 050010, Kazakhstan. [2] Research and production association "Fran", Almaty, 050010, Kazakhstan.

Correspondence: Diyas Myrzakozha, Kazakh National Medical University named after S.D. Asfendiyarov, Almaty, 050010, Kazakhstan.
Email: myrzakozha.d @ kaznmu.kz

ABSTRACT

The object of the study was rectal and/or vaginal suppositories (suppositories), intended for delivery to the patient's body of naturally occurring IgG antibodies of the type capable of inducing a specific antibody-complement-dependent lysis of any tumor cells (antibody phenomenon-complement-dependent lysis). Intravenous injection of protein drugs causes many negative side effects known as infusion reactions and serious consequences, such as serum sickness. This article shows the possibility of intake into the body of recipients of drug preparations of protein nature in the form of rectal suppositories. The use of rectal forms of protein preparations has a number of significant advantages compared with injectable forms. They are more effective than injectable forms since their active molecules are less exposed to the destructive action of liver enzymes. Rectal forms of protein preparations are easy to use for children and elderly patients; they are absolutely safe for nosocomial infections such as syphilis, hepatitis, HIV, etc. Moreover, they are simpler in the technology of their manufacture. The purpose of this work was to study the possibility of delivering protein compounds to the recipient organism by rectally introducing the recipient into the organism, using the example of antitumor immunoglobulins (antibodies) that are aimed at anti-genes of cells, as well as reducing the infusion reactions of the immune system.

Keywords: antibody; suppository; antitumor activity; serum

Introduction

Cancer is a national and international health problem [1, 2]. It is known that in the practice of treating tumor diseases, a large arsenal of methods and techniques that can destroy tumor cells is used. Such methods include surgery, radiotherapy, chemotherapeutic and immunobiological drugs, and various variants of their combinations. To combat tumor diseases, chemotherapeutic and immunobiological preparations are most widely used. It is believed that the creation and use of modern chemotherapeutic drugs is a dead end in the treatment of cancer due to their high toxicity and the complete absence of selective action on tumor cells when cancer cells and "normal" healthy cells of the patient's body are equally affected [3].

In comparison with chemotherapeutic drugs, immunobiological drugs are considered safer for the patient's body, the active molecules of which are monoclonal antibodies or polyclonal immunoglobulins, the antigen-binding zones (Fab1 and Fab2) of which are specific for different antigens - structures (receptors, markers, etc.) of the cell wall of cancer cells. The selectivity of such specific, "targeted" drugs is so great that a drug created against a certain type of cancer cells is absolutely useless in the treatment of other types of tumor cells. So, for example, the Herceptin preparation, designed to destroy some forms of breast cancer cells (Her 2 new - positive), is not effective against other types of breast cancer cells, and especially against cancer cells of the stomach, rectum, or any other nosologies.

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Jandos Ukibayev, Ubaidilla Datkhayev, Diyas Myrzakozha, Alexander Frantsev, Elmira Karlova, Yelena Nechepurenko, Didara Balpanova, Alma Almabekova. Rectal methods of delivery of medical drugs with a protein nature in the therapies of tumor disease . J Adv Pharm Edu Res 2021;11(1):18-22. Source of Support: Nil, Conflict of Interest: None declared.

In animal and human organisms, the process of cell division constantly occurs, associated with pregnancy, growth of the body, as well as with the regeneration and repair of damaged tissues and organs, where failures and disturbances in the processes of their division and specialization lead to the formation of atypical, pathological including tumor cells [4]. Thus, the formation of pathological cells is an inevitable physiological "norm" for any multicellular organism. However, the body's immune system recognizes and destroys emerging transformed cells. Violations of some functions of the immune system lead to the fact that individual tumor cells remain in the body and form a tumor. A common sign characteristic of all benign and malignant neoplasms is the increase, in relation to ordinary cells of the body, in their division rate. Any cell cannot enter the phase of division until it has accumulated the required number of polyunsaturated fatty acid molecules necessary for building the walls of the daughter cell.

According to modern concepts of oncologists, there are about 300 types and types of cancer against which 20-25 types of anticancer targeted drugs have been created, which, due to their high selectivity, are designed to treat only one type of cancer. Another, conceptual approach for creating new immunobiological antitumor drugs is the understanding that nature has already created a reliable mechanism for protecting the body from any spontaneously emerging tumor cells, which has improved over several billion years during the evolution. This mechanism is based on the phenomenon of antibody-complement-dependent cell lysis, also known as the "complement binding reaction", which is the main mechanism for the selective destruction of cells in the body. To implement this mechanism, three participants in the reaction are necessary: a cell with certain biological structures (receptors, markers) located on the cell surface, antibodies specific to biological structures of the cell, and complement [5].

The nature of the active molecules of targeted drugs, which are glycoprotein, is new complexes, determined their injectable dosage form. It is caused by the fact that any protein molecules are destroyed by the enzyme systems of the gastrointestinal tract when you try to use these drugs "per os". For this reason, all anticancer drugs of a protein nature are introduced into the patient's body by injection methods only (intravenously, subcutaneously, etc.), in order to avoid their destruction in the intestinal tract. It is known that parenteral insertion of a solution of a foreign protein (serum, vaccine) into the human body, causes a manifestation of negative reactions from its immune system. These adverse side effects caused by any foreign proteins are called infusion reactions. Infusion reactions can manifest as chills, fever, joint pain, headache, shortness of breath and palpitations, nausea, vomiting, diarrhea, etc. To prevent or significantly reduce the manifestation of infusion reactions can reduce the rate of introduction of a solution of a foreign protein or a preliminary use of de-sensitizing drugs, such as suprastin or dexamethasone. There are non-invasive ways of delivering various biologically active compounds to the patient's body without affecting the enzymes of the gastrointestinal tract, such as vaginal or rectal induction of various medical substances in the form of enemas or suppositories [6]. The known drug "Kipferon", is developed and produced in the form of suppositories for rectal or vaginal injection, contains antibodies to the majority of urogenital viruses that circulate, to increase local immunity during infections pelvic organs of women [7].

For the rectal method of insertion of medicinal preparations, the use and safety (hospital infecting with hepatitis, syphilis, HIV, etc.) are characteristic problems associated with an unpleasant taste or the smell of drugs. Moreover, rectal induction of drugs reduces the possibility of their entry into the liver and the degradation of active molecules by hepatic enzymes, as they enter the inferior vena cava directly into the heart and spread throughout the body. This increases the therapeutic effect of the drug by 20–25% with the same dose of the administered drug as compared with intravenous injection [8]. However, the rationality and effectiveness of the rectal injection of protein compounds into the patient's body are still little studied. The purpose of this work was to study the possibility of delivering protein compounds to the recipient organism by rectally introducing the recipient into the organism, using the example of pro-tumor tumors lacto-immunoglobulins (antibodies) that are aimed at anti-genes of cells, as well as reducing the infusion reactions of the immune system.

Materials and Methods

As antibodies, "aimed" at the receptors of tumor cells, we used the finished fraction of lacto-immunoglobulins isolated from goat's milk serum, provided to us by the scientific and production association «FRAN» (Kazakhstan, Almaty). Lacto-immunoglobulins were applied as a finely dispersed, lyophilized powder, with a residual moisture content of less than 1%. The lack of moisture in the powder of lactic immunoglobulins prevents their inactivation. Adult laboratory females, outbred rats, with an average body weight of 250-280 g were used in the work. To induce a tumor process in rats, 100,000 cells of a laboratory strain of an ovarian affinity tumor of rats tropically to lung tissue were intravenously injected. As a basis for suppository mass, cocoa butter was used from the confectionery factory "Rakhat" (Kazakhstan, Almaty), used for making chocolate. Suppositories for rats were made by the method of pouring, consisting of two halves, a detachable metal form, with 30 cells with a diameter of 4 and a length of 7 mm. The volume of each cell was about 100 mm^3. General anesthesia of rats was performed by halothane vapors (Halothane).

Results and Discussion

In this work, we used lacto-immunoglobulins (antibodies) in the form of a dry powder, capable of interacting with the surface structures of tumor cells (receptors, markers) provided by «Fran». The manufacture of suppositories, with a mass of 0.1 grams, necessary to perform work on the study of the possibility of rectal administration of protein components, in particular

lacto-immunoglobulins, into the organism of recipients (rats) was performed by pouring into a detachable metal mold [9].

Antibodies capable of interacting with the surface structures of tumor cells (receptors, markers) were isolated from goat's milk serum. To obtain whey, milk was degreased, and caseins were curdled by the addition of a small amount of citric acid. To remove particles of suspended casein and other mechanical impurities, the whey was centrifuged. Subsequently, the whey was fractionated. The first step in the separation of whey proteins was the fractionation of it with ammonium sulfate. A fraction of proteins containing immunoglobulins was precipitated in the range from 20% to 40% with ammonium sulfate. The precipitation of milk whey proteins obtained by salting out was redissolved in physiological saline and applied onto a Sephadex G-10 column balanced with a 0.9% sodium chloride solution to separate them from ammonium sulfate ions and other low molecular weight components. The obtained protein eluates from milk serum containing the sum of immunoglobulins used for selective sorption of antibodies by the surface structures of tumor cells. The manufacture of suppositories, with a mass of 0.1 grams, necessary to perform work on the study of the possibility of rectal administration of protein components, in particular lacto-immunoglobulins, into the organism of recipients (rats) was performed by pouring into a detachable metal mold [9].

Cocoa butter was used as a basis for suppository mass. The classic lipophilic base is cocoa butter. Cocoa butter is currently in the pharmacopeia of several countries remains the official pharmacopoeial basis. It consists of a mixture of triglycerides: tristearin, tripalmetin, triolein, trilaurin, and triarachine. Cocoa butter has positive properties as a base, namely: it releases well the substance included in it, has a pronounced melting point (31-36 °C), has good plasticity (you can prepare suppositories by three methods), and mixes well with various medicinal substances. Cocoa butter at room temperature is a solid, light yellow brittle, with a faint chocolate smell and a pleasant taste. In molten form, it has a saturated yellow color. This color is given to it by carotenes. It melts at a temperature of 31–36 ° C, turning into a transparent liquid. An important parameter of cocoa butter is the absence of moisture in it (less than 1%), which contributes to the stability of the protein molecules introduced into it (lacto-immunoglobulins).

With rectal introduction of a suppository, the drug substance is absorbed through the hemorrhoidal veins, through which blood enters the lower vena cava, passes through the venous and lymphatic systems of the pelvis. About 75% of the injected drug enters the general bloodstream bypassing the liver barrier. Due to this, biotransforming and destructive factors affect the medicinal substance to a lesser extent, which contributes to an increase in circulation time in a large circle of blood circulation.

Candlestick mass is a suspension of finely dispersed powder of lacto-immunoglobulins in cocoa butter. Lacto-immunoglobulin powder was injected into melted cocoa butter at 39°C. A mixture of lactic immunoglobulin powder and oil was subjected to homogenization in a blender, for 1 minute at 5000 rpm, for uniform distribution of fine particles of lacto-immunoglobulins in the candle mass. The lactic immunoglobulin content in the candle mass was 5% in a weight ratio. The molten, homogenized candlestick mass was poured into the cells of a metal mold, which was placed in a refrigerator to form suppositories. Figure 1 shows a photograph of a metal mold for pouring molten, homogenized candlestick mass. To extract the suppositories, the metal form was briefly immersed in water heated to 40°C, and then it was removed from half. The resulting candles were dried with filter paper to remove moisture from them, compressed with a strip of aluminum foil that divided the suppositories among themselves, preventing them from sticking together, and placed in storage in the freezer compartment of the refrigerator. Figure 2 shows a photograph of the obtained candles on aluminum foil.

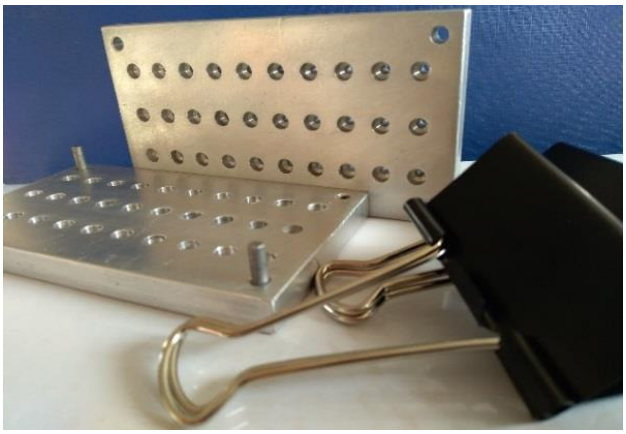

Figure 1. Form for the manufacture of suppositories by the method of pouring

Figure 2. Suppositories for rats on aluminum foil

The animals used in the work were selected adults, laboratory, female rats, with an average body weight of 250-280 g. The choice of adult rats for the experiment is due to two factors. First, the size of the anus of animals allows them to enter miniature suppositories. The researchers had a very aggressive laboratory strain of an ovarian affinity tumor of rats tropically to lung tissue resistant to chemotherapeutic drugs and radiation. The physiological characteristic of rats is a constant, with the regularity of 25-30 minutes defecation, which complicates the use of suppositories in the experiment. In addition, involuntary defecation in rats occurs when they are in a stressful state (when they are picked up, fixed, etc.). To eliminate bowel movements

for the time needed to melt the candle mass and absorb lacto-immunoglobulins of the intestinal mucosa, the rats were subjected to general inhalation anesthesia using bromine (Br) vapors containing the volatile anesthetic Halothane.

8 individuals were divided into two groups: "control" (3 rats) and "experimental" (5 animals). The choice of a small number of animals participating in the experiment is due to methodological difficulties when working with suppositories in a large group of rats, also due to the fact that the task of the study was not to study the quantitative characteristics of the action of suppositories with lacto-immunoglobulins on the immune system of animals. The possibility of preserving the therapeutic properties of antitumor lactic immunoglobulin molecules for systemic effects on the recipient's organism upon their rectal administration was studied. Candles with a mass of 0.1 grams containing lacto-immunoglobulins were introduced to rats daily for 10 days, every day 1 candle, to block the immune response of the animal's immune system to the drug molecules, using a well-known method, an anti-idiotypic suppression of the synthesis of neutralizing antibodies with high doses of antigen, in this case, lacto-immunoglobulin molecules. After 10 days, the rats of the "control" and "experimental" groups were killed and their lungs were examined for the presence of tumor clones. The results of the experiment are shown in figures 3 and 4.

Figure 3 shows the lungs of 3 rats from the control group. The photograph shows that the lung tissues of all the rats in the control group are affected by multiple clones of the solid tumor of an ovarian affinity tumor of rats tropically to the lung.

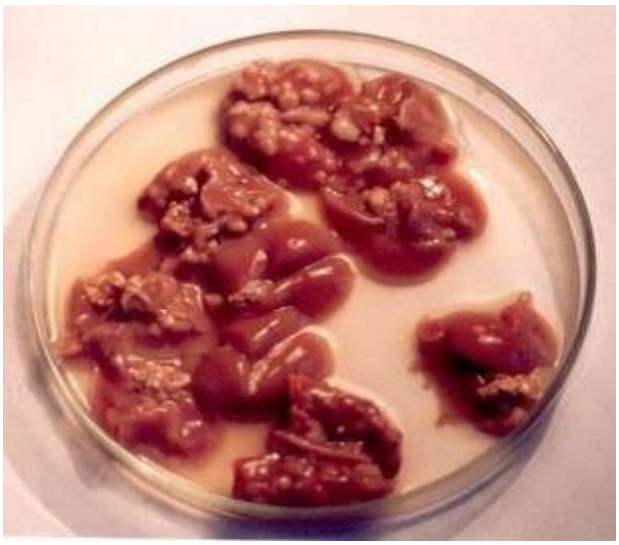

Figure 3. Lungs of rats of the control group.

100% damage to the tissues of the lung clones of a solid tumor of an ovarian affinity tumor of rats tropically to the lung.

Figure 4 shows the lungs of 5 rats from the experimental group injected with suppositories preparation for 10 days. Figure 4 shows that more than 60% of rats' lungs are free of an ovarian affinity tumor of rats tropically to lung clones, while 40% of the lungs of animals remained affected by multiple clones of the tumor.

Figure 4. Lungs of rats of the experimental group.

60% of rat lungs free from tumor clones after the use of the suppositories.

This experiment of the possibility of delivering proteinaceous compounds to the recipient's organism by rectal insertion, using the example of the antitumor drug lacto-immunoglobulins, showed that this route of injection ensures the release and availability of active molecules from the mass of the candle and retains their biological activity. The experiment was repeated three times, with the same result [9].

Conclusions

The fundamental difference between a new immunobiological antitumor drug and existing and currently developing immunochemical drugs is that it uses natural antibodies specific for any tumor cells and not immunoglobulins artificially created by biotechnological methods that are "aimed" at certain types of cancer cells [10, 11].

This executed research, using the example of antitumor lacto-immunoglobulins, showed the possibility of rectal induction of protein drugs into the body of recipients, without losing their biological activity. The use of cocoa butter-based suppositories for these purposes showed a good release and availability of active protein molecules, despite the fact that they were present in the candle mass in the form of fine powder [12].

Thus, rectal administration of protein preparations into the patient's body is a simpler and safer and more effective method of creating a systemic effect on the patient's body of medicinal compounds than is achieved with invasive methods.

In addition, a simpler technology for the production of rectal forms of drugs compared with injectable forms makes them promising for production [13].

Cancer is a form of manifestation of functional insufficiency of certain parts of the body's immune system. The presence of tumor cells in the body are deadly symptoms of such a defect.

Treatment of neoplastic diseases should be aimed at restoring the protective functions of the immune system, and not just at combating the cancer cells themselves. Otherwise, relapse of tumor diseases is inevitable even after the seemingly "successful" treatment [14].

Acknowledgment

The authors wish to thank research centers personnel who participated in the experiments.

References

1. Moldogazieva N.T., Terentyev A.A. Alpha-fetoprotein - new approaches to the study of the structure and function of GOU VPO Russian State Medical University of Roszdrav //Materials of the conference "Fundamental Research", Dominican Republic on April 10-20, 2006.
2. Frantsev A.P., Frantseva I.A., Medvedeva E.M. Patent of the Republic of Kazakhstan No. 14142 "Anti-idiotypic antibodies to alpha-fetoprotein used for cell lysis" dated March 15, 2000; bull. Number 3.
3. G.S. Vasiliev, I.A. Frantseva. Studies of the action of Normogen on the metastatic ability of affinity ovarian tumor (AfOT) in rats with tropism for lung tissue. Oncology and radiology of Kazakhstan, 2005; 2(11): 27 - 31.
4. Melis, J.P., Strumane, K., Ruuls, S.R., Beurskens, F.J., Schuurman, J. and Parren, P.W. Complement in therapy and disease Regulating the complement system with antibody-based. Molecular Immunology, 2015; 67: 117–130.
5. Frantseva I.A., Frantsev A.P. A method for the treatment of neoplastic diseases. Patent of the Republic of Kazakhstan No. 21098 dated 15.06. 2011; bul. № 6.
6. Clinical Immunology and Allergology, edited by L. Yeager in two volumes, Moscow, Medicine, 1990; 1: 287–288.
7. Kipferon suppositories for rectal or vaginal insertion. Moscow Research Institute Epidemiology and Microbiology. G.N. Gabrichevsky. Instructions for use.
8. Bronovitskaya Z.S., Goretov V.P. Salting out proteins with ammonium sulfate at low temperatures. Tables and nomogram. Applied Biochemistry and Microbiology, 1967; 3: 707-709.
9. Frantsev A.P., Tolmachev V.S., Frantseva I.A., Medvedeva E.M. Use of antiidiotypical antibodies to alpha-fetoprotein for selective destruction of transformed cells, International Journal on Immunorehabilitation, 2004; 6(2): 258.
10. Moiseenko V.M. Monoclonal antibodies in the treatment of malignant tumors - M, Practical Oncology. 2003; T.4, No 3: 148-156.
11. Rectal drug administration: clinical pharmacokinetic considerations.de Boer AG, Moolenaar F, de Leede LG, Breimer DD. Clin Pharmacokinet. 1982 Jul-Aug;7(4):285-311.
12. Lipp, M. Review of cocoa butter and alternative fats for use in chocolate. M. Lipp, E. Adam, Food Chemistry. 1998; 62(1): 73-97.
13. Development of a mucoadhesive dosage form for vaginal administration/G.C. Ceschel [et al]//Drug Dev. Ind. Pharm. 2001. 6(4):541-547.
14. Pokorny, R.M., Wrightson, W.R., Lewis, R.K. et al. Suppository administration of chemotherapeutic drugs with concomitant radiation for rectal cancer. Dis Colon Rectum 1997; 40: 1414-1420.