PRECLINICAL AND CLINICAL EVIDENCE OF SAFETY OF ANTIVIRAL DRUG WITH IMMUNOMODULATORY ACTIVITY

Tatiana Gennadievna Borovskaya
E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia

PRETKLINIČKI I KLINIČKI DOKAZI O BEZBEDNOSTI ANTIVIRUSNOG LEKA SA IMUNOMODULATORNOM AKTIVNOŠĆU

Tatjana Gennadievna Borovskaja
E. D. Goldberg Istražicački Institut za Farmakologiju i Regenerativnu medicinu Tomsk Nacionalni Istraživački Medicinski Centar, Ruska Akademija Nauke, Tomsk, Rusija

Received / Primljen: 11. 08. 2018. Accepted / Prihvaćen: 12. 09. 2018.

ABSTRACT

Antiviral drug Kagocel is widely used in Russia for prevention and treatment of acute respiratory infection, influenza, and herpes. The drug belongs to the group of interferon inductors. The article contains the review and analytical evaluation of safety of antiviral drug Kagocel. Kagocel is registered in the Russian Federation and some CIS countries and refers to the group of interferon inducers. This is a chemical compound of carboxymethyl cellulose and low-molecular natural polyphenol gossypol common in cotton-plant (Gossypium spp.) which protects the plant from depredators and diseases. Authors pay a special attention to the analysis and generalization of data from preclinical and clinical studies including the control of related substances. Absence of free gossypol impurities guaranteed by highly sensitive and specific quality control methods. Preclinical studies data was analyzed and the results were presented with focus on reproductive safety of Kagocel® in immature and mature animals.. No negative effect on animals' reproductive function was revealed including spermatogenesis and generative function. No long-term product effect on reproductive system or next generations of animals was recorded both at therapeutic doses and at doses 10 times their exceeding. The safety of the drug demonstrated on data obtained from numerous clinical trials, including those involving children aged 2 years and older. This confirms the safety of antiviral drug Kagocel usage in clinical practice, including pediatrics.

Keywords: *antiviral drug, interferon inducer, Kagocel, reproductive safety, pediatrics, preclinical and clinical studies*

SAŽETAK

Antivirusni lek Kagocel ima široku primenu u Rusiji za prevenciju i lečenje akutne respiratorne infekcije, gripa i herpesne infekcije. Ovaj pregledni članak sadrži pregled i analitičku procenu bezbednosti antivirusnog leka Kagocel-a. Kagocel je registrovan u Ruskoj Federaciji i nekim zemljama ZND. Lek pripada grupi induktora interferona i predstavlja hemijsko jedinjenje karboksimetil-celuloze i niskomolekularnog prirodnog polifenola gosipola, čestog u biljci pamuk (Gossipium spp.), koji štiti biljku od predatora i bolesti.

Autori posvećuju posebnu pažnju analizi i generalizaciji podataka iz pretkliničkih i kliničkih ispitivanja uključujući kontrolu srodnih supstanci. Odsustvo slobodnih gošipolnih nečistoća garantovano visoko osetljivim i specifičnim metodama kontrole kvaliteta. Podaci o pretkliničkim studijama su analizirani i rezultati su predstavljeni sa fokusom na reproduktivnu bezbednost Kagocela® kod mladih i adultnih životinja. Nije otkriven negativan uticaj na reproduktivnu funkciju životinja, uključujući spermatogenezu i generativnu funkciju. Pored toga, kod primene u terapijskim dozama i u dozama deset puta većim od terapijske nije zabelezen dugoročan efekat Kagocel®-a na reproduktivni sistem ili naredne generacije životinja Bezbednost leka zasnovana je i na podacima dobijenim iz brojnih kliničkih ispitivanja, uključujući i one koji su uključivali decu uzrasta od 2 godine i više. Ovo potvrđuje sigurnost upotrebe Kagocela protiv virusa u kliničkoj praksi, uključujući i pedijatriju.

Ključne reči: *antivirusni lek, induktor interferona, Kagocel, reproduktivna bezbednost, pedijatrija, pretkliničke i kliničke studije*

sciendo

UDK: 615.281.03 / Ser J Exp Clin Res 2018; 19 (3): 271-276
DOI: 10.2478/SJECR-2018-0040

Corresponding author: Tatiana Gennadievna Borovskaya, PhD, MD.,
Structural unit Research Institute of Pharmacology and Regenerative Medicine named after E.D. Goldberg of Federal State Budgetary Scientific Institution Tomsk National Research Medical Center of Russian Academy of Sciences, Lenina pr., Tomsk, 634028, Russia; Mail: repropharm@yandex.ru

INTRODUCTION

Prevention and therapy of influenza and other acute respiratory viral infections (ARVIs) is a critical current issue. Thus, the data obtained from October 2015 to March 2016 for 10 Russian cities evidence that the most affected were children aged 3-6 years old, while hospitalization rate reached maximum in 15-65-year-old age group (65 %). Moderate and severe forms with high hospitalization rate prevailed, viral pneumonia including bilateral ones were found in 10 % hospitalized children and 30 % hospitalized adults (1, 2). World Health Organization currently recommends vaccination to fight influenza, however its efficacy may vary due to extreme influenza virus variability (3). For example, it has been shown that repeated annual vaccinations with the same vaccine strain of the H1N1 virus improve cellular and humoral immunity (4). Therefore, development of an effective antiviral product is of vital importance, and its safety based on comparative analysis of its efficacy and health risk is relevant as well. Safety assessment should be carried out throughout the whole life cycle of the product from development until recall from market (5). Meanwhile, while at preauthorization stage special attention is paid to pharmaceutical safety aspects (quality and stability of the substance and formulation), investigation of toxicity on laboratory animals, efficacy and tolerability in clinical studies, safety evaluation after the product implementation into clinical practice is made within post-marketing clinical studies and pharmacovigilance system. Therefore, safety assessment shall be performed both at preclinical and clinical stages and during clinical use of the product.

HISTORY

Starting from the second half of the last century, interferon inducers were the most demanded medicinal products meeting criteria of safety, multifunctional nature and lack of viral resistance with minimum adverse effects. Interferon (IFN) system forms part of immune system responsible for antiviral defense (6). Medicinal products belonging to interferon inducers possess wide range of antiviral effect (etiotropic effect) and expressed immunomodulating activity. It should be stressed that synthesis of endogenous interferons is balanced and regulated by the body itself providing lack of adverse effects typical for their exogenous administration. Most viruses are known to have no resistance to endogenous interferons (7).

Long-term targeted screening by national researching virologists managed to form a group of original interferon inducers with high therapeutic index suitable for both prevention and therapy of a number of viral infections. Russian drug Kagocel belongs to this class of the products (8). Its single use was associated with longer (120 h) interferon blood circulation as compared to other similar products (9). Oral administration provided maximum production of interferons (alpha/beta) as soon as 4 hours post dosing, and their blood circulation was observed for 4-5 days. The founder of school for development of national interferon inducers, academician of RF RAMS F.I. Ershov reports that Kagocel is one of the most well-studied antiviral agents (10). It causes production of "late" interferons representing a mixture of alpha- and beta-interferons and stimulates production of physiological amounts of gamma-interferons. A certain advantage of Kagocel is its verified efficacy against various viral pathogens. Kagocel was found to be effective against A(H1N1)v, H1N1, H5N1, H3N2 viruses, herpes type 1 and 2 virus (9-11).

Most scientific data on Kagocel are well-known in medical practice. These researches predominantly cover the studies of clinical efficacy of the product. It should be noted that specific weight of publications on safety is limited since the available works are frequently printed in extremely specialized experimental medicine editions. The purpose of the review was to provide data on Kagocel safety to a broad population of practitioners.

Antiviral drug Kagocel is widely used in Russia for prevention and treatment of acute respiratory infection, influenza, and herpes. The drug belongs to the group of interferon inductors.The active ingredient of drug Kagocel is a highly molecular compound synthesized using carboxymethyl cellulose sodium salt and low molecular natural polyphenol, i.e. gossypol (12-15). Carboxymethyl cellulose is a polymeric carrier, a macromolecule conventionally used in alimentary and medical industries. Gossypol is contained in cotton plant, predominantly in free form, and defends the plant from pests and diseases. Numerous multinational studies demonstrated that gossypol produces antitumour, antioxidant and immunomodulating effect. (8-11). However, it should be noted that gossypol use as a medicinal product is limited by low therapeutic breadth of the doses applied, especially systemic ones. Gossypol is known to exert negative effect on erythro- and myelopoiesis, have hepatotoxicity, suppress spermatogenesis, therefore being limited in medicine for long time (16-18). It caused interest due to identification of new properties of its derivatives. A number of scientific studies demonstrated that, by molecular cross-links with polymeric carriers, gossypol loses its toxic properties while retaining antiviral and immunomodulating effects (19-22). It is this approach upon which Kagocel development is based. Kagocel shows expressed antiviral and immunomodulating effects being absolutely safe. Kagocel manufacturers pay special attention to quality control, especially control of impurities of natural polyphenol. So, throughout the life cycle of the product, investigation of its safety at both chemical and biological levels are ongoing.

MANUFACTURING QUALITY CONTROL

Highly sensitive spectrophotometric assay has been generally used to ensure reliable control of impurities of residual gossypol in the substance at safe level. It is noteworthy that the current RF State Pharmacopoeia based on global prac-

tice recommends integrating more specific methods into control and quality systems including high-performance liquid chromatography (HPLC). To implement the method in the manufacturing of pharmaceutical substance of Kagocel, the current HPLC method with spectrophotometric detection was developed and validated to ensure accurate and selective determination of gossypol in Kagocel substance. This method ensures reliable detection of free gossypol impurity with high accuracy and precision at minimum amounts starting from 1.56•10-5 mg/mL. The specified method sensitivity is in line with the international publications on HPLC assay of gossypol (11, 23). It should be mentioned that the method validation demonstrated that whole gossypol amount added to the substance from outside is reliably and accurately identified in this complex evidencing lack of nonspecific sorption of its free molecules on polymeric matrix. HPLC with UV detection revealed that the contents of residual impurities of free gossypol in the product, both after manufacturing and after storage during the established shelf life, is at 0.0002-0.0030 % of the substance weight being 20-100 times lower vs. minimum thresholds for trace constituents in drug substances defined by international pharmacopoeias including the RF State Pharmacopoeia XIII. Such a low level of gossypol impurity guarantees lack of any untoward effect of Kagocel on humans. It should be noted that unfavorable physiological effects of free gossypol are observed when it is administered per os by humans in free form at doses > 0.12 mg/kg (16). Currently, HPLC method is used for Kagocel manufacturing ensuring effective complete elimination of unbound residues of free gossypol. Each batch of the substance is subject to monitoring.

Research unit of the company jointly with researchers from Federal Research Center of Biotechnology of RAS considered potential dissociation of molecules of bound gossypol due to long-term exposure by the components model medium simulating gastrointestinal media. These simulating systems may modify dissolution parameters or cause changes in the proper pharmacological active ingredient. The study results demonstrated that long-term incubation of Kagocel (24-hour) in the model media specified above as well as in a special medium containing microbial cellulase capable of destroying cellulose and its derivatives do not increase levels of free gossypol (19). Therefore, the current methods of purification of the substance from gossypol impurities, hydrolytic stability of its molecule secondary to gastric and intestinal juice exposure and in the medium containing microbial cellulose suggest no toxicity of Kagocel typical of natural polyphenol. However, the information ensuring Kagocel safety may only be obtained in experimental *in vivo* and *in vitro* studies as per current RF MoH regulations.

PRECLINICAL STUDIES

Toxicological characteristics of Kagocel are well-studied, both preclinically and clinically. Pre-authorisation preclinical studies included experiments investigating acute, subchronic and chronic toxicity and specific types of toxicities in the leading research centers of Russia and CIS countries. The experiments involved mice, rats, rabbits and dogs. Acute toxicity study allows to determine tolerable, toxic and lethal doses of test product administered inragastrically, establish the causes of animal mortality during 14-day follow-up period, investigate its effect on general condition and a number of functional and morphological parameters. It should be noted that intoxication signs in acute toxicity study of free gossypol has been investigated thoroughly including respiratory distress, body weight changes, anorexia, weakness, apathy, signs of cardiac failure and death several days later (11). At that, the data from acute toxicity study of Kagocel revealed no animal mortality and no signs of acute intoxication were detected either. In subchronic and chronic toxicity studies, all the study parameters were not different from the ones in control group of animals. The results of studies of specific toxicities revealed that the product has no immunotoxic, genotoxic, allergenic, mutagenic or carcinogenic potential. The experiments showed that Kagocel does not produce negative effect on any aspects of reproductive system of animals and does not affect generative function of males or females, does not cause teratogenic or embryotoxic effect, does not have fetotoxic potential in postnatal or antenatal periods. Therefore, preclinical experiments demonstrated that Kagocel is absolutely safe. Meanwhile, free gossypol in toxicological experiments proved to be a highly toxic compound.

Preclinical studies investigated reproductive toxicity (at the stage of germ cell development) of Kagocel on mature animals (19). Such experiments are mandatory stage of preclinical studies. The results demonstrated that daily 70-day Kagocel administration at therapeutic dose and at the dose exceeding therapeutic one 25-fold does not deteriorate reproductive function of male rats. Embryonic loss parameters in intact female rats coupled to them did not exceed the control values. The litter of male rats receiving Kagocel did not show abnormal changes or retarded physical development; at that, the litter had high survival rate. Investigation of morphological and functional spermatogenesis parameters revealed that weight factor of testicles and tail region of epididymis as well as average sperm count, relative count of its immobile forms, maximum mobility time and number of its abnormal forms were similar to control values. Morphological examination of testicles of rats receiving Kagocel did not detect reduced spermatogenesis index. No suppression of proliferative activity was observed in testicular tissue. The number of sources of proliferative pool of spermatogenesis (normal spermatogonia) was in line with the values in control group. Therefore, Kagocel did not exert negative effect on rat spermatogenesis, while free gossypol at high doses inhibits maturation of male germ cells (24). The data obtained evidence that Kagocel does not exert toxic effect on reproductive system of mature male rats. Given that Kagocel is indicated for prevention and therapy of pediatric viral infections, evalu-

ation of its effect on immature gonads sensitive to various toxic effects is certainly important (25). Thus, reproductive system laboratory under RI of Pharmacology and Regenerative Medicine named after E.D. Goldberg, National Research Medical Center, carried out a number of experiments investigating long-term reproductive safety of Kagocel on immature sex glands (24). These studies were fully in line with the requirements of FSBI "Scientific Center for Evaluation of Medical Products". The experiments included 3 series of studies. The first series investigated potential long-term toxic effect of Kagocel on reproductive system of infantile rats (males, females; aged 10 days). The product was administered for 12 days. The second series investigated reproductive system of rats after administration (48-hour) in pubertal period (aged 52-54 days). The third series investigated potential toxicity of the product after its triple course administration (for 4 days) throughout the whole process of maturation of sex glands (infantile, prepubertal, pubertal periods). In all series of the experiments Kagocel was administered at therapeutic dose and at the dose exceeding therapeutic one 10-fold. Evaluation of reproductive safety was made when the animals reached reproductive age, i.e. long after the product exposure. Kagocel did not reduce fertility of male or female rats. Kagocel does not induce cytogenetic changes in germ cells leading to embryonic loss based on embryonic loss rates. Furthermore, the product does not increase rate of DNA breaks in germ cells in DNA comet assay. Administration of the study product did not exert toxic effect on the litter of animals receiving the product. Body weight of fetuses and infant rats, survival rate, condition of visceral organs and ossification process were similar to control values. Morphometric analysis of testicles did not reveal atrophy of convoluted seminiferous tubules. Seminiferous epithelium of male rats in the study and control groups was represented by spermatogonia, spermatocytes, spermatides, spermatozoa. Thinning of spermatogenous tissue was not observed. Tubular lumens were free, and no reinforced desquamation of dead cells was reported. Spermatocytes and spermatogonia showed active processes of cell division. The number of sources of proliferative pool of spermatogenesis was similar to control values. The latter suggests that spermatogenesis suppression is unlikely within 3 months after the experiment. Lumens between spermatogonia in study and control groups contained Sertoli cells. Their cell membranes did not look damaged suggesting integrity of blood-testis barrier. Testosterone-synthesizing cells (Leydig cells) were found between convoluted seminiferous tubules of all experimental animals. Most of them had specific granularity which is known to be typical of functionally active cells. As reported previously, gossypol administration exerts toxic effect on Leydig's cells and Sertoli's cells causing spermatogenesis suppression at which maturing germ cells stop proliferating. The resulting data evidence that Kagocel does not induce any abnormal changes in testicles of immature animals after reaching maturity. Meanwhile, bibliographic sources suggest that administration of free gossypol to prepubertal and pubertal male rats results in epididymis cysts being potential cause of infertility (26). Therefore, thorough external examination of the tail region of epididymis was performed in experiments investigating potential reproductive toxicity of Kagocel, their weight was determined and weight factor was calculated. The study results demonstrated that male rats receiving Kagocel did not have any cysts. Weight factors of epididymes were in line with the control values (placebo). Three batches of the experiments also investigated morphology of female sex glands of rats receiving Kagocel. It was also similar to that in control animals. Hemodynamic changes were not reported. Glandular tissues contained follicles at various stages of maturity: primordial, with two or more layers of granular cells, Graafian vesicles. In a number of cases, the follicles were atresic. Developing yellow bodies were distinctly visualized. Thecal membranes did not look deorganized. Interstitial cells were intact. Present ovulating follicles in ovaries and the fact that rat fertility did not decrease suggest that Kagocel, unlike gossypol, does not suppress folliculogenesis.

So, administration of Kagocel to immature animals does not produce detrimental effect on their sex glands, reproductive system or litter when reaching reproductive age. The resulting data revealed no long-term sequelae of Kagocel effect on sex glands of immature animals and characterize the product as having high reproductive safety profile. It may be used in pediatrics.

CLINICAL STUDIES

Assessment of safety of a new medicinal product is not limited with experimental trials only. All adverse effects of a new product should be traced at each stage of clinical studies in accordance with the current laws and approved by state regulatory authorities. Phase I clinical studies of Kagocel established its good tolerability at the study doses in healthy volunteers, revealed no allergic reactions or toxic effect on hepatic, renal functions, homeostasis or immunocompetent cells. Further studies of therapeutic and preventive efficacy of Kagocel at specific diseases (ARVI/influenza, herpes) and simultaneously safety of the product were included in phase 2 and 3 clinical trials. Registration randomized, blind, placebo-controlled, multicenter studies of efficacy and safety of Kagocel in adult subjects in the treatment and prevention of influenza and other ARVIs were performed by the leading Russian research institutes: RI of Influenza, RAMS (Saint-Petersburg), D.I. Ivanovskiy RI of Virology, RAMS (Moscow) and S.M. Kirov Military Medical Academy (Saint-Petersburg) in 2000-2002. The study results along with efficacy findings revealed no side events. The subjects administering Kagocel for therapeutic and preventive purposes reported its good tolerability, lack of adverse events of allergic reactions, gastrointestinal complaints or other organ system complaints. According to the results of laboratory tests, Kagocel did not exert nega-

tive effect on hepatic, renal function or hematopoiesis (27). The safety data obtained in clinical studies on adults (lack of adverse effects and good tolerability) as well as lack of adverse reactions in clinical studies according to pharmacovigilance service of the company in addition to preclinical data justified initiation and performance of clinical studies in children. Pediatric studies investigating therapeutic and preventive efficacy of the product in influenza and ARVI and safety were carried out consecutively in 2 steps obtaining data on safety with gradual reduction of children's age: at ≥ 6 years old (2007-2009) and at 2-6 years old (2010-2011). Multicenter, blind, randomized, placebo-controlled clinical studies were performed by test facilities of .I. Ivanovskiy RI of Virology, RAMS, Russian State Medical University, Institute of Immunology, FMBA, Moscow RI of Pediatrics and Pediatric Surgery of Rosmedtechnologies. Adverse events, toxic or allergic reactions to Kagocel in clinical studies were not reported. The children receiving therapy showed good tolerability of Kagocel with no adverse reactions verified by lack of negative changes in peripheral blood, urinalysis and biochemistry over time (27-30). Despite the fact that clinical study in children > 2 years of age was authorized and safety data were obtained for children of this age, patient information leaflet for Kagocel authorizes its use in children > 3 years of age. This is due to the fact that Kagocel is manufactured in tablets which are approved for use in children > 3 years old only. Therefore, starting from 2008, the product is recommended for the treatment of influenza and ARVI in children > 6 years old, from 2011 – in children > 3 years old.

More than 2000 subjects including adults and children > 2 years old were enrolled in blind randomized placebo-controlled clinical studies in 2000-2011. The results of these studies demonstrated high safety of Kagocel along with its high therapeutic and preventive efficacy, both for influenza caused by various types and subtypes of virus (including pandemic one) and ARVI. The data obtained in clinical studies on populations with specific nosologies selected in accordance with strict inclusion/exclusion criteria and performed on a small sample (hundreds, thousands of patients) are critical. However, they do not provide the full pattern of peculiarities of use and tolerability of the product in various populations who may have co-morbidities which are found commonly in actual clinical practice. For this purpose, phase IV studies are carried out which are initiated after the product has obtained state registration for use in broad practice. These post-marketing studies pursue a number of purposes, one of them being to identify previously unknown or potential side effects of the product and risk factors.

In 2016, the results of a large-scale international prospective observational study were published in which therapy of ARVI and influenza with Kagocel was investigated for the first time in outpatient practice involving large number of adult patients (17,266) from 262 medical centers of several countries: Russia, Armenia, Moldova, Georgia. These studies demonstrated good tolerability and efficacy of Kagocel over time, regardless of the time of therapy prescription. The product was found to be combined well with other medicinal products including those for ARVI and influenza therapy, and arising complications were detected (30, 31). The information on adverse effects was obtained in 14 subjects receiving Kagocel. At that, mild to moderate allergic reactions were most common. Based on the composition of excipients of Kagocel tablets, contraindications include individual hypersensitivity to its components, lactase deficiency, lactose intolerance and glucose-galactose malabsorption as well as pregnancy and lactation, pediatric use < 3 years of age (31, 32).

CONCLUSION

Therefore, the published materials suggest that the Russian antiviral medicinal product Kagocel has high safety profile evidenced by experiments on animals and results of clinical studies. Special attention is paid by the manufacturer to quality control of the substance using innovative methods of the substance purification from impurities including gossypol as well as advanced control techniques. The data available suggest that the Russian drug Kagocel is a highly effective and safe antiviral product both for adults and children > 3 years old.

REFERENCES

1. Lvov, D. et. al. (2016) Virological, epidemiological, clinic, and molecular genetic features of the influenza epidemic in 2015-2016: prevailing of the influenza A(H1N1)09 pdm virus in Russia and countries of the Northern hemisphere. Voprosy Virusologii, 61(4), 159-166
2. Goncharova, O. V. (2016). Phytotherapy in treatment and rehabilitation of children recovering from acute respiratory viral infections. Medical Council, 1(1), 58–62.
3. Ulasevich, A., Jacobs, S., Mbangdadji, D., van Over, M., & Steffens, L. (2017). Understanding Flu Vaccination in a Competitive Context. Social Marketing Quarterly, 23(4), 320–334.
4. Trieu, M. C., Zhou, F., Lartey, S. L., Sridhar, S., Mjaaland, S., & Cox, R. J. (2018) Augmented CD4(+) T-cell and humoral responses after repeated annual influenza vaccination with the same vaccine component A/H1N1pdm09 over 5 years. NPJ. Vaccines. 3, 37.
5. Blakely, S. A. R. (n.d.). Research Approaches to Special Preclinical Safety and Toxicological Evaluations. Nutraceuticals, 45–53.
6. Mishra, S., & Kumar, H. (2017). Balancing anti-viral innate immunity and immune homeostasis. Cellular and Molecular Immunology. doi:10.1038/cmi.2017.98
7. Mänz, B., Dornfeld, D., Götz, V., Zell, R., Zimmermann, P., Haller, O. Schwemmle, M. (2013). Pandemic Influenza A Viruses Escape from Restriction by Human MxA through Adaptive Mutations in the Nucleoprotein. PLoS Pathogens, 9(3), e1003279. doi:10.1371/journal.ppat.1003279

8. Hamming, O. J., Terczyńska-Dyla, E., Vieyres, G., Dijkman, R., Jørgensen, S. E., Akhtar, H. Hartmann, R. (2013). Interferon lambda 4 signals via the IFNλ receptor to regulate antiviral activity against HCV and coronaviruses. The EMBO Journal, 32(23), 3055–3065. doi:10.1038/emboj.2013.232
9. Ershov F.I. Narovlyansky A.N. (2015) Use of interferon inducers in viral infections. Voprosi virusologii; 60 (2): 54-10. PMID:26182650;
10. Galegov, G. A., et al. (2002) The effect of Kagocel on herpes virus reproduction. Voprosy virusologii 47(4) 42-44.
11. Sologub, T. V., & Tsvetkov, V. V. (2017). Kagocel in the therapy of influenza and acute respiratory viral infections: Data analysis and systematization from the results of preclinical and clinical trials. Terapevticheskii Arkhiv, 89(8), 113.
12. Myat, T. S., & Tetsuka, M. (2016). Gossypol inhibits LH-induced steroidogenesis in bovine theca cells. Animal Science Journal, 88(1), 63–71.
13. Warnsmann, V., Meyer, N., Hamann, A., Kögel, D., & Osiewacz, H. D. (2017). A novel role of the mitochondrial permeability transition pore in (–)-gossypol-induced mitochondrial dysfunction. Mechanisms of Ageing and Development. doi:10.1016/j.mad.2017.06.004
14. Keshmiri-Neghab, H., & Goliaei, B. (2013). Therapeutic potential of gossypol: An overview. Pharmaceutical Biology, 52(1), 124–128. doi: 10.3109/13880209.2013.832776
15. Yang, Z.-J., Ye, W.-S., Cui, G.-H., Guo, Y., & Xue, S.-P. (2004). Combined administration of low-dose gossypol acetic acid with desogestrel/mini-dose ethinylestradiol/testosterone undecanoate as an oral contraceptive for men. Contraception, 70(3), 203–211.
16. Schwartze, E. W. (1926). Gossypol and cottonseed meal poisoning. Journal of Oil & Fat Industries, 3(5), 173–178.
17. Lu, Y., Li, J., Dong, C.-E., Huang, J., Zhou, H.-B., & Wang, W. (2017). Recent advances in gossypol derivatives and analogs: a chemistry and biology view. Future Medicinal Chemistry, 9(11), 1243–1275.
18. Xu, L., Yang, D., Wang, S., Tang, W., Liu, M., Chen, J., Lippman, M. E. (2004). 643 Radiosensitization of human prostate cancer by natural polyphenol inhibitor of Bcl-2/XL, (–)-gossypol, results in tumor regression. European Journal of Cancer Supplements, 2(8), 194. doi:10.1016/s1359-6349(04)80651-7
19. Borovskaya, T. G. (2017). Safety of the Russian antiviral drug Kagocel. Terapevticheskii Arkhiv, 89(11), 93.
20. Ye W et al. (2010). Induction of apoptosis by (-)-gossypol-enriched cottonseed oil in human breast cancer cells. International Journal of Molecular Medicine, 26(1), 113-119.
21. Yang, J., Li, L.-L., Li, J.-R., Yang, J.-X., Zhang, F., Chen, G., Wu, S.-W. (2018). Synthesis and biological evaluation of water-soluble derivatives of chiral gossypol as HIV fusion inhibitors targeting gp41. Bioorganic & Medicinal Chemistry Letters, 28(1), 49–52. doi:10.1016/j.bmcl.2017.08.049
22. Tilyabaev, K. Z., Kamaev, F. G., Vypova, N. L., Yuldashev, A. M., Ibragimov, B. T., & Talipov, S. A. (2010). Synthesis, structures, and acute toxicity of gossypol nonsymmetrical aldehyde derivatives. Russian Journal of Bioorganic Chemistry, 36(3), 390–395.
23. Aoyama, K. (2008). Determination of Gossypol in Feeds by HPLC. Journal of the Food Hygienic Society of Japan (Shokuhin Eiseigaku Zasshi), 49(4), 303–307.
24. Habas, K., Brinkworth, M. H., & Anderson, D. (2017). In vitroresponses to knownin vivogenotoxic agents in mouse germ cells. Environmental and Molecular Mutagenesis, 58(2), 99–107.
25. Gossypol as undesirable substance in animal feed - Scientific Opinion of the Panel on Contaminants in the Food Chain. EFSA Journal, 7(1), 908.
26. Zhang, W., Huang, C., Xie, W., & Shen, L. (2014). Determination of gossypol in edible vegetable oil with high performance liquid chromatography-tandem mass spectrometry. Chinese Journal of Chromatography, 32(6), 582.
27. Borovskaya, T. G., Poluektova, M. E., Vychuzhanina, A. V., Mashanova, V. A., & Shchemerova, Y. A. (2017). Experimental Assessment of the Effect of Kagocel on Reproductive Function in Pubertal Male Rats. Bulletin of Experimental Biology and Medicine, 163(2), 214–217.
28. Vinokurov, A. A. Hodgkin's lymphoma and male fertility disorders. Klin. Onkogematol. 2013;6(3):258-273.
29. Durnev, A. D., Smol'nikova, N. M., Skosureva, A. M., Nemova, E. P., Solomina, A. S., Shrede O. V., Gus'kova, T. A., Verstakova, O. L., & Syubaev, R.D. Methodical recommendations for preclinical study of reproductive toxicity of new drugs. Manual for Preclinical Studies of New Pharmacological Substances. Mironov AN, Bunatyan ND. Moscow, 2013. Part I. P. 80-93.
30. Rybalkin, S. P., Konovalova, E. V., Guskova, T. A., & Savinova, T. B. Experimental evaluation of the effect of Kagocel on generative function in animals. Toksikol. Vestnik. 2013. 2: 33-38.
31. Harlamov, F. S., Kladova, O. V., Uchaykin, V. F., Cheshik, S. G., Vartanyan, R. V., & Yablonskaya, K. P. (2015). Metapneumovirus and bokavirus respiratory infections in the structure of sars in children. Children infections, 14(2), 5–11.
32. De Peyster, A., & Wang, Y. Y. (1993) Genetic toxicity studies of gossypol. Mutat. Res. 297(3), 293-312.