ChemoProtective effects of new iodine coordinated compound in benzo[a]pyrene-induced lung cancer in BALB/c mice

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Abstract. Iodine preparations are widely used in medicine as antiseptic agents. One of the new directions for the use of iodine is the prevention and therapy of certain tumor diseases, particularly breast cancer. The main mechanism of action is the induction of apoptosis. Iodine also has anti-inflammatory activity and affects the polarization of Th1/Th2 lymphocytes and M1/M2 macrophages. The newly developed iodine complex KC-144 includes both dextrin and polypeptides. In this case, lithium enhances the polarization of triiodide. We studied the acute toxicity and prophylactic activity on a model of benz(a)pyrene-induced (BaP) tumor in BALB/c mice. The average lethal dose (LD_{50}) of KC-144 when administered orally was more than 2500 mg/kg, classifying KC-144 as a low-toxicity substance. Intraperitoneal administration of benz(a)pyrene at a dose of 100 mg/kg for two weeks induces tumor development in mice. Oral administration of KC-144 at doses of 2.5 and 25 mg/kg for one week and throughout the entire treatment period increases the lifespan of BaP-induced mice.

1 Introduction

Molecular iodine exhibits protective activity against chemically induced breast cancer [1]. Antitumor activity of aqueous iodine solution in the form of potassium triiodide was demonstrated on a dimethylbenz[a]anthracene (DMBA)-induced breast cancer model [2]. After metabolic activation, DMBA and other polycyclic aromatic hydrocarbons (PAHs) metabolites interact with DNA in rapidly proliferating cells, forming adducts followed by mutations. Therefore, many PAHs are potent carcinogens [3]. Among the well-studied PAHs, benzo(a)pyrene (BaP) possesses the highest activity. Reactive metabolites are formed from BaP metabolism in the liver, the ultimate of which, BP-7,8-dihydrodiol-9,10-epoxide, predominantly forms adducts with DNA in the tumor suppressor gene p53 and the KRAS oncogene in the lungs [4]. It was also shown that instillation of BaP in rat lungs inhibits apoptosis [5]. This may explain why cigarette smoke is one of the causes of human lung cancer [6].

This study investigated the acute toxicity and protective action of a new iodine coordination compound (KC-144) on a BaP-induced tumor model in BALB/c mice.

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2 Materials and Methods

2.1. Materials

Benzo(a)pyrene was purchased from Sigma (USA). All manipulations with BaP were carried out in laboratory coats, gloves and masks. Materials in contact with BaP were considered highly hazardous waste and disposed of appropriately.

Iodine compound KC-144, CAS No.: 39392-86-4, was produced and synthesized at JSC "Scientific Center for Anti-Infectious Drugs," Almaty, Kazakhstan. KC-144 is a dark brown powder with a faint odor of iodine. The concentration of molecular iodine in KC-144 is 200 g/kg [7].

2.2. Animals

BALB/c specific pathogen-free mice (male, 9–11 weeks, n = 10), were purchased from Aikimbayev's National Scientific Center for Especially Dangerous Infections (Almaty, Kazakhstan). Each mouse, approximately weighing 25 g ± 10 %, was kept in individual ventilated cages (Tecniplast, Italy) with a 12/12 light cycle, at 23°C ± 2°C, and 60 % ± 5 % humidity. They had access to standard rodent pellet feed (Ssniff, Germany) and water ad libitum. All procedures followed the NIH Guide for the Care and Use of Laboratory Animals, with approval from the Bioethics Committee of the Scientific Center for Anti-Infectious Drugs (Protocol No. 15/2, dated 14.06.19).

2.3. Acute toxicity

Following the OECD (Organization for Economic Cooperation and Development) guidelines #425 (2001), a single animal was administered 2500 mg/kg of KC-144 intragastrically and observed over 48 hours. Control animals received water. If death did not occur or signs of severe poisoning were not evident, the same dose was administered to the remaining four animals. Each group consisted of 10 animals. The animals were weighed daily. On the 14th day, the animals were euthanized by cervical dislocation and necropsied.

2.4. Cancer induced model

Experimental animals were organized into three groups, each comprising ten mice. All animals received an intraperitoneal injection of BaP (benzo[a]pyrene) dissolved in 100 µL of sterile olive oil, once a week for two weeks, at a dosage of 100 mg/kg. The treatment with KC-144 began one week before the first dose of BaP was administered and continued for two weeks. The experimental design is outlined in Table 1 below:

Table 1. Design of the experiment KC-144

Groups	Treatment	Dose (mg/kg)	Treatment schedule
1	BaP control	-	-
2	BaP+KC-144, low dose	2,5	oral gavage
3	BaP+KC-144, high dose	25	oral gavage

Table 1 summarizes the grouping, treatments, dosages, and the method of administration (oral gavage) for the experiment involving KC-144 and its evaluation in the context of BaP (benzo[a]pyrene) exposure.

2.5. Evaluation of antitumor activity *in vivo*

Effects of KC-144 treatment on survival time with cancer bearing of mice were analyzed by using the mean survival time (MST), which is the average survival time of mice in a particular group (either treated or control). The percent increase in life span (ILS) of cancer-bearing mice treated with KC-144. The formula used for calculating ILS is as showed in article [8].

2.6. Statistical analysis

Mean values (m) and standard deviations (SD) were calculated for all quantitative indices. The data were compared by an analysis of the Kruskal-Wallis with a posteriori comparison of each group to the non-parametric Dunn's criterion. MST data were analyzed using one-way ANOVA followed by Holm-Sidak's multiple comparisons test. All calculations are carried out with the GraphPad Prism6 software (GraphPad Software, Inc., San Diego, CA, USA). The p-value < 0.05 was considered as significant difference.

3 Results and Discussion

Many iodine coordination compounds exhibit relatively low acute toxicity when administered orally, as reported in various scientific sources [9, 10]. Therefore, to assess the acute toxicity of KC-144, half of the maximum recommended dose of 2500 mg/kg KC-144 according to the OECD guideline 423 was used. Following the administration of KC-144, the animals were weighed daily (Fig. 1).

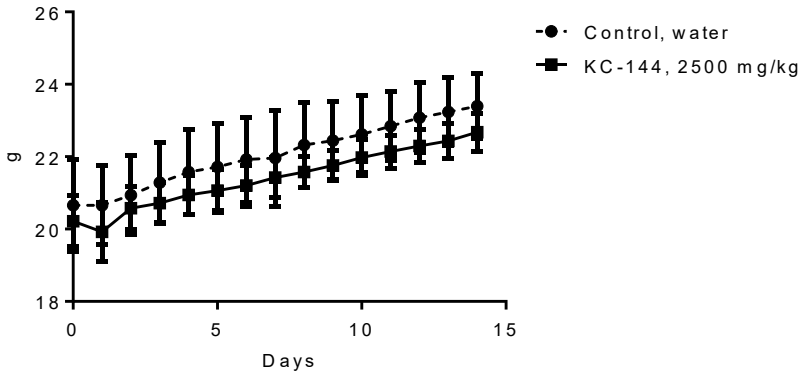

Fig. 1. Illustrates the weight dynamics of the animals after the treatment with KC-144

One sensitive biomarker of toxicity is weight loss in experimental animals. However, KC-144 did not have a detrimental effect on the weight of the animals. No signs of poisoning were observed in the animals. After euthanizing the animals, necropsy was conducted, which also revealed no visible damage to internal organs [11]. A single exposure to high doses of iodine complexes in mice without the development of clinical signs of poisoning typically

does not induce pathological changes in the tissues of internal organs. The LD_{50} of KC-144 in mice exceeds 2500 mg/kg, classifying this new iodine complex into toxicity category 5.

In the subsequent phase of the study, the prophylactic properties of KC-144 were examined using a model of chemically-induced tumor in mice. Fig. 2 presents the data on the efficacy of KC-144's prophylactic application at various doses of 2.5 and 25 mg/kg.

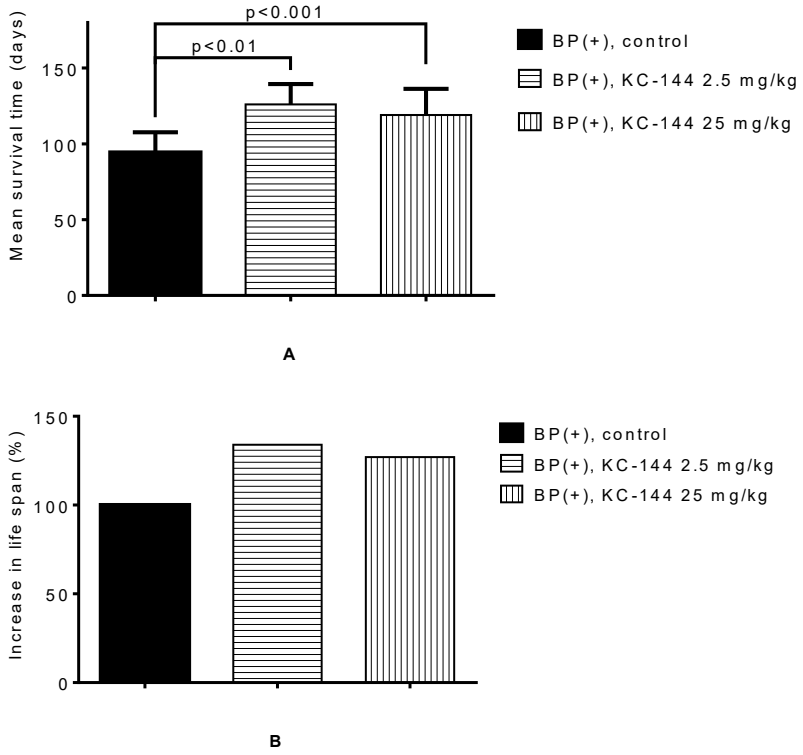

Fig. 2. KC-144 increased the survival after BP injected. The effect of KC-144 on mean survival time (A) and increase in life span (B) of mice. The significant differences according to one-way ANOVA, followed by post hoc Holm-Sidak's multiple comparisons test. (n=10).

The application of KC-144 in both minimal and maximal doses resulted in an extended lifespan of animals compared to the positive control. The antineoplastic effect of iodine is well documented [12, 13], with studies showing the antineoplastic activity of povidone-iodine on various mesothelioma cell lines and the involvement of molecular iodine and its derivatives, like 6-iodolactone, in triggering apoptotic effects in cells. 6-iodolactone, a product of molecular iodine transformation, is known to activate apoptosis [14]. Another mechanism for iodine's antitumor activity may involve its effect on activating the immune response via the Th1 pathway [15]. Additionally, iodine solutions used in cancer radiotherapy have anti-inflammatory effects on mucous membranes [16]. Previous research has demonstrated that KC-144 can induce the production of IFN-gamma *in vitro* when treating peripheral blood mononuclear cells (PBMCs) [17]. Iodine is also recognized for inhibiting the harmful effects of sulfur mustard on the skin of mice and guinea pigs by activating the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in skin cells [18, 19].

This stage of the research underscores KC-144's low toxicity and its preventive action against benzo(a)pyrene-induced tumor in mice, aligning with the broader search for new anticancer drugs that exhibit both direct and mediated cytotoxic effects against cancer cells,

with a particular emphasis on mechanisms such as apoptosis activation and immune response modulation.

Therefore, it can be concluded that the new iodine complex KC-144 is characterized by low toxicity and exhibits a preventive effect in the model of benzo(a)pyrene-induced tumor in mice.

4 Conclusion

The antitumor activity of iodine is of significant interest for the development of new drugs due to the issues of drug resistance in cancer diseases. It is known that iodine exerts both direct and mediated cytotoxic effects on cancer cells. One of the mechanisms of action is the activation of cell apoptosis. Iodine also affects the polarization of Th1/Th2 immune cells, also be used in combination with other anticancer drugs. Highly toxic substances are used in cancer chemotherapy. Therefore, the search for new anticancer drugs with low toxicity is relevant. To reduce the local irritating action of inorganic iodine solutions in the form of potassium or sodium triiodides, complexation with various bioorganic ligands from amino acids to polymeric molecules is used. As a result, medicinal substances suitable for the development of pharmaceutical forms are obtained. The new iodine complex KC-144 includes peptides and dextrin as complexing agents, while lithium cations enhance the polarization of triiodide. The study of acute toxicity in mice showed that the LD_{50} of KC-144 exceeds 2500 mg/kg. Based on these results, the new iodine complex KC-144 can be classified as a category 5 toxicity (low-toxicity substances). Various experimental models are used to study the antitumor activity of developed drugs. We selected the model of benzo(a)pyrene-induced cancer. The substance KC-144 was used in a prophylactic scheme. As a result, it was shown that intraperitoneal administration of BaP over two weeks at a dose of 100 mg/kg causes tumor development in mice. Oral administration of KC-144 for a week and throughout the entire treatment period of BaP-treated mice increases lifespan.

Given the promising results observed in this study, further investigation into the molecular mechanisms of KC-144's protective activity, its efficacy in other cancer models, and its potential synergistic effects with existing cancer therapies is warranted. The development of KC-144 represents a novel avenue for cancer treatment, emphasizing the need for additional research to fully understand its therapeutic potential and applicability in clinical settings.

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References

1. P. García-Solís, Y. Alfaro, B. Anguiano, G. Delgado, R.C. Guzman, S. Nandi, M. Díaz-Muñoz, O. Vázquez-Martínez, and C. Aceves, Inhibition of N-methyl-N-nitrosourea-induced mammary carcinogenesis by molecular iodine (I_2) but not by iodide (I^-) treatment. Molecular and Cellular Endocrinology, 236(1-2), 49-57 (2005). https://doi.org/10.1016/j.mce.2005.03.001
2. O. Soriano, G. Delgado, B. Anguiano, P. Petrosyan, E.D. Molina-Servín, M.E. Gonsebatt, and C. Aceves, Antineoplastic effect of iodine and iodide in dimethylbenz[a]anthracene-induced mammary tumors: association between

lactoperoxidase and estrogen-adduct production. Endocrine-Related Cancer, **18(4),** 529-539 (2011). https://doi.org/10.1530/ERC-11-0065
3. B. Kerdelhué, C. Forest, and X. Coumoul, Dimethyl-benz(a)anthracene: A mammary carcinogen and a neuroendocrine disruptor. Biochimie Open, **3**, 49-55 (2016). https://doi.org/10.1016/j.biopen.2016.09.003
4. B. Moorthy, C. Chu, and D.J. Carlin, Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. Toxicological Sciences, **145(1)**, 5-15 (2015). https://doi.org/10.1093/toxsci/kfv040
5. B.A.K. da Silva, R.D. Aydos, I.S. Silva, D.M. Pereira, T.C. de Carvalho, D.M. Dourado, F.A. dos Reis, R.S. and Nacer, Lung apoptosis after intra-pulmonary instillation of Benzo(a)pyrene in Wistar rats. Acta Cirurgica Brasileira, **25(1)**, 117-120 (2010). https://doi.org/10.1590/s0102-86502010000100023
6. S.S. Hecht, Tobacco Smoke Carcinogens and Lung Cancer. JNCI Journal of the National Cancer Institute, **91(14)**, 1194–1210 (1999). https://doi.org/10.1093/jnci/91.14.1194
7. Patent No. 35927 (2022). Abekova, A.O., Islamov, R.A., Yuldashova, G.Ab-R., & Ilin, A.I. Coordination compound of dilithium (I) (1→4)-L-D-poliglucopyran triiodide chloride, polypeptide, possessing antitumor activity. JSC SCAID RK. [Republic of Kazakhstan Patent]
8. J.S. Kim, T.Y. Jeong, C. Kwan Cho, Y.W. Lee, and H.S. Yoo, Antitumor effect of skin of venenum bufonis in a NCI-H460 tumor regression model. Journal of Acupuncture and Meridian Studies, 3(3), 181-187 (2010). https://doi.org/10.1016/s2005-2901(10)60034-9
9. D. Sani, P.A. Abdu, M. Mamman, K.O. Jolayemi, P.O. Yusuf, and A.D. Andamin, Research Note: Evaluation of acute oral toxicity of povidone-iodine in cockerels using the up-and-down procedure. Poultry Science, **100(2)**, 631–634 (2021). https://doi.org/10.1016/j.psj.2020.11.002
10. A.P. Southern, and S. Jwayyed, Iodine Toxicity. PubMed. (2023). https://pubmed.ncbi.nlm.nih.gov/32809605
11. A. Ilin, and A. Nersesyan, Toxicology of iodine: A mini review. Archive of Oncology, **21(2)**, 65-71 (2013). https://doiserbia.nb.rs/Article.aspx?id=0354-73101302065I
12. A. Fiorelli, F. Pentimalli, V. D'Urso, D. Di Marzo, I.M. Forte, G. A., M. Di Domenico, M. Accardo, U. Di Serio, and M. Santini, Antineoplastic activity of povidone-iodine on different mesothelioma cell lines: results of in vitro study. European Journal of Cardio-Thoracic Surgery, **45(6)**, 993-1000 (2014). https://doi.org/10.1093/ejcts/ezt534
13. E. Rojas Arroyo-Helguera, G. Delgado, and C. Aceves, Signaling pathways involved in the antiproliferative effect of molecular iodine in normal and tumoral breast cells: evidence that 6-iodolactone mediates apoptotic effects, Endocr Relat Cancer., **15**, 1003-1011 (2008). https://doi.org/10.1677/ERC-08-0125
14. M. Nava-Villalba, and C. Aceves, 6-Iodolactone, key mediator of antitumoral properties of iodine. Prostaglandins & Other Lipid Mediators, **112**, 27-33 (2014)**.** https://doi.org/10.1016/j.prostaglandins.2014.07.001
15. O. Cuenca-Micó, E. Delgado-González, B. Anguiano, F. Vaca-Paniagua, A. Medina-Rivera, M. Rodríguez-Dorantes, and C. Aceves, Effects of molecular iodine/chemotherapy in the immune component of breast cancer tumoral microenvironment, Biomolecules. **11(10)**, 1501-1501 (2021). https://doi.org/10.3390/biom11101501
16. R. Rahn, I.A. Adamietz, H.D. Boettcher, V. Schaefer, K. Reimer, and W. Fleischer, Povidone-iodine to prevent mucositis in patients during antineoplastic radiochemotherapy. Dermatology, **195(2)**, 57-61 (1997). https://doi.org/10.1159/000246032

17. R.T. Kenzhebekova, A.O. Abekova, K.D. Raziyeva, Z.S. Abramova, R.A. Islamov, A.K. Nersesyan, and A.I. Ilin, Investigation of the impact of iodine coordination compound on production of interleukin-4 and interferon-γ in vitro and primary evaluation of local irritation in vivo. International Journal of Biology and Chemistry, **11(2)**, 4-10 (2018). https://doi.org/10.26577/ijbch-2018-2-339
18. Wormser U., Sintov A., Brodsky B., Casillas R. P., & Nyska A. Protective effect of topical iodine containing anti-inflammatory drugs against sulfur mustard-induced skin lesions. Archives of Toxicology, **78(3)**, 156-166 (2004). https://doi.org/10.1007/s00204-003-0523-2
19. M. Ben-Yehuda Greenwald, M. Frušić-Zlotkin, Y. Soroka, S. Ben-Sasson, H. Bianco-Peled, and R. Kohen, A novel role of topical iodine in skin: Activation of the Nrf2 pathway. Free Radical Biology & Medicine, **104**, 238-248 (2017). https://doi.org/10.1016/j.freeradbiomed.2017.01.011