Radioprotective effects of kojic acid against mortality induced by gamma irradiation in mice

Seyed J. Hosseinimehr, Pharm D, PhD, *Saeed Emami,* Pharm D, PhD, *Vali Zakaryaee,* MSc, *Amirhossein Ahmadi,* Pharm D Student, *Daryush Moslemi,* MD.

ABSTRACT

الأهداف: لتقييم الآثار المولدة لحمض كوجيك على معدل الوفيات المحرض بواسطة أشعة غاما في الفئران. تمت مقارنة الكفاءة مع عقار اميفوستين كمرجع مولد للأشعة.

الطريقة: أجريت دراسة تجريبية في كلية الصيدلة بجامعة مازانداران للعلوم الطبية – مستشفى ساري وبابلسار للعلاج الإشعاعي – بابلسار – إيران، خلال الفترة ما بين أكتوبر 2006م وحتى يناير 2008م. تم إعطاء حمض كوجيك تحت الجلط كجرعات مفردة كالتالي: (142–175–232–350mg/kg)، قبل ساعة واحدة من جرعة أشعة غاما (8Gy). تم حقن عقار اميفوستين تحت الجلد بجرعة مقدارها (200mg/kg) بجرعات إشعاعية متشابهة. انخفض معدل الوفيات عند 30 يوماً بعد الإشعاع. تم تقييم مضاد السمية لحمض كوجيك بواسطة طريقة 1، 1-ديفينيل 2-بيسريلهيدرازيل الخالي من الإشعاع (DPPH).

النتائج: تم تقسيم 120 فأراً من نوع (NMRI) إلى 6 مجموعات بمعدل 20 فأر في كل مجموعة. بعد 30 يوماً من العلاج، بلغ معدل النجاة لكل مجموعة كالتالي: مجموعة التحكم 5%; 142 mg/kg, 5%; 175 mg/kg, 0%; 232 mg/kg, 30%; 350 mg/kg
وعقار اميفوستين 40%، و40% عند ساعة واحدة من العلاج قبل التعرض لأشعة غاما، على التوالي. ازداد معدل النجاة بشكل إحصائي لدى الحيوانات التي تلقت العلاج بحمض كوجيك (350mg/kg)، قبل ساعة واحدة من الإشعاع، بالمقارنة مع مجموعة التحكم. يثبط حمض كوجيك نشاط بقايا التركيز الذاتي على نشاط مضادات الأكسدة القوي بعملية (DPPH).

خاتمة: يبدو أن حمض كوجيك مع نشاط مضادات الأكسدة يخفض من معدل الوفاة المحرض بواسطة أشعة غاما.

Objectives: To evaluate the protective effects of kojic acid on mortality induced by gamma irradiation in mice. The efficacy was compared with amifostine as a reference radioprotector.

Methods: This experimental study was conducted in the Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari and Babolsar Radiotherapy Hospital, Babolsar, Iran, between October 2006 and January 2008. Kojic acid was administrated subcutaneously as single doses of 142, 175, 232, and 350 mg/kg, one hour prior to a lethal dose of gamma irradiation (8 Gy). Amifostine was injected subcutaneously at a dose of 200 mg/kg at a similar irradiation dose. The mortality was recorded 30 days after irradiation. The antioxidant activity of the kojic acid was assessed using the 1, 1-diphenyl-2-picrylhydrazyl free stable radical (DPPH) method.

Results: One hundred and twenty NMRI mice were divided into 6 groups with 20 mice in each group. At 30 days after treatment, the percentage of survival in each group was: control, 5%; 142 mg/kg, 5%; 175 mg/kg, 0%; 232 mg/kg, 30%; 350 mg/kg, 40%; and amifostine, 40% one hour treatment prior gamma irradiation. The survival rate was statistically increased in animals treated with kojic acid (350 mg/kg), one hour prior irradiation, as compared with the irradiated control group. Kojic acid exhibited concentration-dependent scavenging activity on DPPH possessing strong antioxidant activity.

Conclusion: Kojic acid with antioxidant activity reduced the mortality induced by gamma irradiation.

Saudi Med J 2009; Vol. 30 (4): 490-493

From the Department of Medicinal Chemistry (Hosseinimehr, Emami, Zakaryaee), Pharmaceutical Research Center (Hosseinimehr, Emami), Student Research Committee (Ahmadi), Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran, and the Faculty of Medicine (Moslemi), Babol University of Medical Sciences, Babol, Iran.

Received 20th November 2008. Accepted 22nd March 2009.

Address correspondence and reprint request to: Dr. Seyed J. Hosseinimehr, Department of Medicinal Chemistry, Faculty of Pharmacy, Pharmaceutical Research Center, Mazandaran University of Medical Sciences, Sari, Iran. Tel / Fax. +98 (151) 3261244. E-mail: sjhosseinim@yahoo.com, sjhosseinim@mazums.ac.ir

Ionizing radiation has several potential applications in medicine such as radiotherapy, radiology, and nuclear medicine. Ionizing radiation passing through living tissues generates reactive free radicals. These free radicals can interact with critical macromolecules such as DNA, proteins, and membranes resulting in cell damage, and leading to cell biological dysfunction, and death.[1,2] With regards to side effects of ionizing radiation, it is important to protect humans from radiation-induced genotoxicity or lethality. Thiol compounds are the first class of radioprotective agents that are used as radioprotective agents, and cannot reduce mortality induced by lethal dose of gamma irradiation.[2] Amifostine is a powerful radioprotective agent belong to this class of compounds.[2,3] This drug is approved by the Federal Drug Administration (FDA) as a radioprotective agent for prevention of xerostomia induced by gamma irradiation in patients under radiotherapy. Amifostine, and other thiol compounds have several side effects including hypotension, nausea, vomiting, and allergy.[3,4] The search for an effective and less toxic radioprotectors, has spurred interest in the development of different compounds. One of the main approaches in this field is to explore the natural compounds as radioprotectors. Kojic acid [5-hydroxy-2-(hydroxymethyl)-4-pyrone] is a fungal metabolite produced by some species of *Aspergillus*, *Penicillium*, and *Acetobacter*. Kojic acid is widely used as a food additive for preventing enzymatic browning of raw crabs, and shrimps. This natural compound is widely used as a cosmetic agent for skin whitening due to inhibitory effects on polyphenol oxidase, or tyrosinase enzymes.[5-7] Kojic acid can stimulate lymphocyte proliferation, and enhances neutrophil functions, and scavenging of reactive oxygen species, which is increased by white blood cells in the blood.[8,9] We showed recently that administration of kojic acid 24 hours prior to gamma radiation can reduce mortality in mice.[9] With respect to the useful biological properties of kojic acid, safe food usage, and radioprotective effects in the recent study, the present study evaluates the radioprotective effects of kojic acid treatment one hour before lethal dose of gamma irradiation in mice, and is compared with amifostine as a radioprotective drug as well as antioxidant activity.

Methods. ***Chemicals.*** The kojic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were purchased from Merck Co. (Darmstadt, Germany) and Fluka Chemical Co. (Buchs, Switzerland). All other chemicals were obtained from Merck. The experiments were conducted in the Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, and Babolsar Radiotherapy Hospital, Babolsar, Iran, Tehran, Iran, between October 2006 and January 2008.

Animals. Eight-week old male NMRI mice (Pasteur Institute, North Branch, Amol, Iran) weighing 28 ± 3 g were used. Mice were kept in a good condition at the university's animal section and given standard mouse pellet, and water *ad Libitum*, and cared according to guide for laboratory animals in the university.[10] All animals were kept under controlled lighting conditions (light: dark, 12:12 h) and temperature (22±1°C). Their use was approved by the Research Committee of Mazandaran University of Medical Sciences.

Irradiation. These experiments were conducted in the Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, and Babolsar Radiotherapy Hospital, Babolsar, Iran, between October 2006 and January 2008. Whole-body irradiation was performed with a cobalt-60 γ-radiation source (Theratron 780, AECL, Ontario, Canada) in Babolsar Radiotherapy Hospital, Iran. Mice were placed in a well-ventilated perspex box and irradiated in groups[11] of 20 mice simultaneously. The source-to-skin distance was 84 cm with a dose rate of 1.76 Gy/min at room temperature (23 ± 2°C).[9] One hundred and twenty mice were divided into 6 groups with 20 mice in each group. For radioprotective studies, groups of 20 mice were injected subcutaneously (s.c) one hour prior to γ-radiation. Mice were irradiated with a lethal dose of irradiation (8 Gy). Kojic acid was dissolved in distilled water. Mice were treated with kojic acid at doses of 142, 175, 232, and 350 mg/kg body weight (b.w) prior to a lethal dose of gamma irradiation. Amifostine was dissolved in the distilled water, and injected at a dose 200 mg/kg with an irradiation dose of 8 Gy. The treated animals were kept for 30 days, and lethality was recorded each day. The control group received an equal volume of sterile distilled water in the same manner. Survival was recorded on a daily basis.[12]

Measurement of free radical scavenging activity. The free radical-scavenging capacity of kojic acid was determined as bleaching of the stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH).[13] Different concentrations of kojic acid (0.04 to 1.6 mg/ml) were added, at an equal volume, to a methanolic solution of DPPH (100 µm). After 15 minutes at room temperature, the absorbance was recorded at 517 nm. The experiment was performed in triplicate. Butylated hydroxy toluene (BHT) was used as an antioxidant standard.[14]

Statistical analysis. Daily survival graph was taken using the Kaplan-Meier equation. The percentage of survival of various doses was compared using two-sample test for proportions.[12] GraphPad Prism 4 software was used.

Disclosure. This work was supported financially by the Iran National Science Foundation (INSF), grant NO. 85012.1.

Results. For evaluation of radioprotective activity, 142, 175, 232, and 350 mg/kg b.w doses of kojic acid were used. These doses were administrated one hour prior to 8 Gy irradiation. The survival results of radioprotection studies are summarized in **Table 1**, and **Figure 1**. Exposure of 8 Gy γ-irradiation induced mortality[9] and 95% of animals in the control+ 8 Gy irradiation group died within 30 days. As is evident from the data, kojic acid at doses of 232 and 350 mg/kg reduced mortality induced by radiation. Survival increased significantly in groups treated with kojic acid at doses of 232 and 350 mg/kg. Kojic acid did not show any enhancement of survival at doses of 142 and 175 mg/kg. In this study, administration of mice with kojic acid at the dose of 350 mg/kg, one hour prior irradiation had a more effective response compared to other doses. The DPPH free radical scavenging method can be used to evaluate the antioxidant activity of specific compound. An excellent scavenging effect was observed with kojic acid. The scavenging effect of kojic acid was enhanced with increasing concentration. The maximum inhibitory effects were obtained in 75% at a concentration of 1.6 mg/ml for kojic acid (**Table 2**).

Table 1 - Radioprotective effects of kojic acid and amifostine.

Compound and dose (mg/kg)	30-day survival*		30-day death	
	n	(%)	n	(%)
Control	1	(5)	19	(95)
Kojic acid				
142	1	(5)	19	(95)
175	0	(0)	20	(100)
232	26	(30)	14	(70)
350	8	(40)	12	(60)
Amifostine 200†	8	(40)	12	(60)

*Male NMRI mice were injected with a solution of compounds one hour before cobalt-60 γ-radiation at dose 8 Gy, 30-day survival was used
†Amifostine was dissolved in sterile distilled water and injected one hour prior irradiation

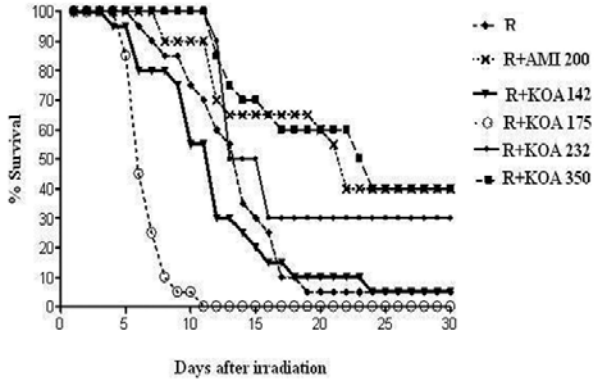

Figure 1 - Effect of different doses of kojic acid (KOA) on the survival of irradiated mice. The NMRI mice received KOA one hour before gamma irradiation with dose 8 Gy. N - 20 mice per group. R - radiation, AMI 200 - amifostine (200 mg/kg) R+KOA - irradiated + KOA treated groups, KOA - Kojic acid treated group.

Discussion. In this study, a single administration of natural compound kojic acid, before gamma irradiation reduced mortality induced by irradiation. The maximum protection was observed at a dose of 350 mg/kg of kojic acid one hour prior to irradiation, and it had a protection efficacy similar to amifostine at a dose of 200 mg/kg. We previously showed that synthetic compounds containing thiol protected mice against a lethal dose of γ-irradiation, and increased survival rate.[15,16] Difficulties were encountered when administrating aminothiols to humans leading to adverse toxic effects such as hypotension, nausea, vomiting, and allergy.[13,17] Some natural origin compounds can scavenge free radicals, such as hydroxyl radicals generated by γ-rays in cells. Previously, we showed that chlorogenic acid as a natural phytochemical origin compound reduces mortality induced by gamma irradiation in mice. Chlorogenic acid has excellent antioxidant activity.[18] Kojic acid is a fungal metabolite produced by some species of *Asperigillus*, and is used for topical application. Kojic acid can inhibit superoxide anions, and hydroxyl radicals.[19] We showed that kojic acid exhibits antioxidant activity using the DPPH method in vitro. Kojic acid significantly decreased the levels of reactive oxygen species (ROS) generated by neutrophils.[19] Ionizing radiation, by passing through living tissues, can generate free radicals. Interaction of

Table 2 - Antioxidant activity of kojic acid and butylated hydroxy toluene (BHT) against 1,1-diphenyl-2-picrylhydrazyl stable radical.

Compound	Concentration (mg/ml)	% Inhibition (mean ± SD)*
Kojic acid	0.4	29.23±1.57
	0.8	61.67±1.53
	1.2	67.80±0.61
	1.6	74.87±1.63
BHT	0.05	59.67±3.01
	0.1	72.40±1.35
	0.2	78.67±1.53
	0.4	93.60±0.36

*Each value in the table was obtained by calculating the average of 3 experiments ± standard deviation

free radicals with DNA can induce DNA damage. Since kojic acid has good free radicals scavenging effects, then it probably scavenged free radical induced by gamma irradiation in environmental cells. Therefore, it is possible that kojic acid, being an antioxidant, scavenged the radiolytically generated free radicals, and reduced mortality induced by gamma irradiation in mice. We previously showed that kojic acid, and its complexes have radioprotective effects when administrated 24 hours prior gamma irradiation in mice. We did not test the efficacy of kojic acid treatment at this time immediately before irradiation. Kojic acid showed a protection of 63% when injected s.c 24 hours prior to a lethal dose of gamma irradiation.[9] In this study, injection of kojic acid immediately at one hour before gamma irradiation protected mice against a lethal dose of γ-irradiation with a protection percentage of 40%.

In conclusion, we investigated the radioprotective effects of the naturally occurring kojic acid in mice. The results of in vivo radioprotection study showed that this agent exhibits radioprotection effects against a lethal dose of γ-irradiation in mice. The antioxidant activity is probably a mechanism of kojic acid, due to its radioprotection effect. Further investigations are required to determine the mechanisms of kojic acid as a radioprotective agent in biological system, such as its antioxidant and immunostimulant properties.

References

1. Joiner MC. Induced radioresistance: an overview and historical perspective. ***Int J Radiat Biol*** 1994; 65: 79-84.
2. Hosseinimehr SJ. Trends in the development of radioprotective agents. ***Drug Discov Today*** 2007; 12: 794-805.
3. Hosseinimehr SJ. Radioprotective agents in radiotherapy [monograph on the internet]. Elsevier: SciTopics; 2008 [cited 27 June 2008]. Available from: http://www.scitopics.com/Radioprotective_Agents_in_Radiotherapy.html
4. Koukourakis MI, Kyrias G, Kakolyris S, Kouroussis C, Frangiadaki C, Giatromanolaki A, et al. Subcutaneous administration of amifostine during fractionated radiotherapy: a randomized phase II study. ***J Clin Oncol*** 2000; 18: 2226-2233.
5. Burdock GA, Soni MG, Carabin IG. Evaluation of health aspects of kojic acid in food. ***Regul Toxicol Pharmacol*** 2001; 33: 80-101.
6. Perez-Bernal A, Munoz-Perez MA, Camacho F. Management of facial hyperpigmentation. ***Am J Clin Dermatol*** 2000; 1: 261-268.
7. Chen JS, Wei CI, Rolle RS, Otwell WS, Balaban MO, Marshall MR. Inhibitory effect of kojic acid on some plant and crustacean polyphenol oxidases. ***J Agr Food Chem*** 1991; 39: 1396-1401.
8. Niwa Y, Akamatsu H. Kojic acid scavenges free radicals while potentiating leukocyte function including free radical generation. ***Inflammation*** 1991; 15: 303-315.
9. Emami S, Hosseinimehr SJ, Taghdisi SM, Akhlaghpoor S. Kojic acid and its manganese and zinc complexes as potential radioprotective agents. ***Bioorg Med Cem Lett*** 2007; 17: 45-48.
10. Hosseinimehr SJ, Nemati A. Radioprotective effects of hesperidin against gamma irradiation in mouse bone marrow cells. ***Br J Radiology*** 2006; 79: 415-418.
11. Hosseinimehr SJ, Zakaryaee V, Froughizadeh M. Oral oxymetholone reduces mortality induced by gamma irradiation in mice through stimulation of hematopoietic cells. ***Mol Cell Biochem*** 2006; 287: 193-199.
12. Hosseinimehr SJ, Emami S, Taghdisi SM, Akhlaghpoor S. 5,7-Dihydroxychromone-2-carboxylic acid and its transition-metal (Mn and Zn) chelates as non-thiol radioprotective agents. ***Eur J Med Chem*** 2008; 43: 557-561.
13. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. ***Phytochem Anal*** 2002; 13: 8-17.
14. Hosseinimehr SJ, Azadbakht M, Jahanabadi A. Protective effect of hawthorn extract against genotoxicity induced by cyclophosphamide in mouse bone marrow cells. ***Environ Toxicol Phar*** 2008; 25: 51–56
15. Hosseinimehr SJ, Shafiee A, Mozdarani H, Akhlagpour S. Radioprotective effects of 2-iminothiazolidine derivatives against lethal doses of gamma radiation in mice. ***J Radiat Res*** 2001; 42: 401-408.
16. Hosseinimehr SJ, Shafiee A, Mozdarani H, Akhlagpour, S, Froughizadeh M. Radioprotective effects of 2-imino–3{(chromone-2-yl) carbonyl} thiazolidines against gamma irradiation in mice. ***J Radiat Res*** 2002; 43: 293-300.
17. Kligerma MM, Glover DJ, Thuris AT, Norflett AL, Yuhas JM, Coia LR, et al. Toxicity of WR-2721 administered in single and multiple doses. ***Inter J Radiat Oncol Biol Phy*** 1984; 10: 1773-1776.
18. Hosseinimehr SJ, Zakaryaee V, Ahmadi A, Akhlagpoor S. Radioprotective effects of chlorogenic acid against mortality induced by gamma irradiation in mice. ***Methods Find Exp Clin Pharmacol*** 2008; 30: 13-16.
19. Gomes AJ, Lunradi CN, Gonzalez S, Tedesco AC. The antioxidant action of polypodium leucotomos extract and kojic acid: reaction with reactive oxygen species. ***Braz J Med Biol Res*** 2001; 34: 1487-1494.