Research Article

Yuefen Zhang#, Pengfei Lu#, Hongzhi Qi, Ge Wu, Rui Mao*, Yongxing Bao*

Radiotherapy for the treatment of pulmonary hydatidosis in sheep

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Abstract: Hydatidosis is an endemic disease causing a severe threat to public health. Drugs and surgery have been utilized for treatment, but their efficiency is not adequate. Therefore, new methods are required for treating such diseases. In this study, we attempt to evaluate the efficiency of radiotherapy for hydatidosis in sheep. The sheep naturally infected with pulmonary hydatid were randomly divided into four groups, including the control group subjected to no irradiation and the other three groups subjected to 30, 45, and 60 Gy irradiation, respectively. On this basis, we speculated that 45 Gy might be a safe and effective dose of 45 and 60 Gy, respectively. Gene expression of caspase-3 and gadd45a and protein expression of BCL-2 and BAX in the lung tissues were evaluated after treatment. Our data showed that the irradiation with a dose of 30, 45, and 60 Gy significantly induced the expression of caspase-3 and gadd45a. Immunohistochemical staining showed that the BCL-2 protein was downregulated after exposure to 45 Gy of irradiation, whereas the BAX expression was downregulated after irradiation at a dose of 45 and 60 Gy, respectively. On this basis, we speculated that 45 Gy might be a safe and effective dose for treating pulmonary hydatidosis in sheep, which induced a downregulation of caspase-3 and gadd45a in the cyst and a downregulation of BCL-2 and BAX in the adjacent lung tissues.

Keywords: radiotherapy, pulmonary hydatidosis, sheep, apoptosis, irradiation

1 Introduction

Hydatidosis is an endemic disease in many countries, including South America, New Zealand, Canada, as well as Western China. At the adult stage, the Echinococcus granulosus lives in the intestine of carnivores, and the eggs are discarded in their feces. The eggs can survive for at least 1 year and could be infectious upon ingesting by an intermediate host such as sheep, goats, horses, or pigs, which finally grow into larvae within the bodies of these animals [1].

Lung is one of the most frequently involved organs by E. granulosus with an incidence of 10–40%, followed by the liver [2]. Patients newly infected by E. granulosus usually show small cysts and generally have no obvious symptoms. They are diagnosed occasionally during physical examination or chest X-ray scans. With the disease progression, some patients may present cough, sputum, chest pain, and hemoptysis combined with the enlargement of the cyst, causing compression, or inflammation [3].

The diagnosis of hydatidosis is mainly based on ultrasonography, X-rays, magnetic resonance imaging, and computed tomography (CT), as well as immunodiagnostic tests [4]. For the treatment of hydatidosis, patients are recommended to undergo surgery, chemotherapy, or observation. In a multicenter clinical study coordinated by the World Health Organization, benzimidazoles (BZD) such as albendazole and mebendazole are considered to be effective for treating hydatidosis [5]. To date, BZD and praziquantel are the major agents for antihydatid therapy. However, the response rates after administration of these agents are still not good due to a low blood drug concentration. In addition, there might be a high possibility of pulmonary hydatid rupture after chemotherapy [6]. It has been well acknowledged that surgery is still the main therapeutic approach for treating hydatidosis [7,8]. Nonetheless, many patients present recurrence after treatment.
Recently, our team has focused on the treatment of pulmonary hydatid infection using radiotherapy [9]. Our earlier studies indicated that radiotherapy contributed to the improvement of symptoms in those infected by pulmonary hydatid [10–13]. To date, there is still a lack of studies focusing on the molecular mechanisms of improvement of hydatidosis conditions after radiotherapy. In this study, we determined the gene expression of caspase-3 and gadd45a that were tightly associated with apoptosis and cell death. Also, we determined the expression of BCL-2 and BAX serving as two important proteins involved in cell death and necrosis [14].

2 Materials and methods

2.1 Animals and study design

Twenty female sheep (3–5 years; body weight, 45 ± 10 kg) naturally infected with pulmonary hydatid confirmed by ultrasonography obtained from pastoral areas of Xinjiang autonomous region were reared in the experimental animal center of our hospital. The animals were fed in a temperature of 18–22°C and relative humidity of 40–60%. After CT confirmation, the animals were kept in the animal room for 1 week. All animals used in this study were euthanized using pentobarbital (85.8 mg/kg) and phenytoin (11 mg/kg) through intravenous injection. Death was declared with the presence of apnea and absence of audible heartbeat or corneal reflex as previously described [15].

Twenty sheep were randomly divided into the following groups: (i–iii) irradiation groups, subject to irradiation of 30 Gy (n = 5), 45 Gy (n = 5), and 60 Gy (n = 5), respectively, and (iv) control group received the same treatment except for irradiation.

Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. All study protocols were performed in line with the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval No. IACUC-2014021002).

2.2 CT scan

After anesthesia, the sheep were fixed on an operating table using a thermoplastic membrane. Then CT scan was given using Big Bore Helical CT scanner (general electric). An experienced radiologist was responsible for depicting the irradiation area, and then the radiotherapy was performed by a professional radiotherapist. The radiotherapy was divided into three fractions with an interval of 2 days, and the predetermined dose was reached within 7 days. After radiotherapy, the cysts and the lung tissues adjacent to the endocyst were collected by biopsy for further detection.

2.3 Reverse-transcription quantitative PCR

Total RNA from endocyst was isolated using TRIzol (Takara Biotech, Dalian, China). The mRNA was reverse transcribed into cDNA by using a reverse transcriptase kit (Vazyme, China) according to the manufacturer’s protocols. Reverse transcript quantitative PCR (RT-qPCR) was performed on an Eppendorf MasterCycler platform (Wesseling-Berzdorf, Germany) using the SYBR Green system (Vazyme, China), and the gene transcription was normalized by β-actin. The relative expression level of each gene was calculated according to \(2^{-\Delta\Delta Ct}\) method. The primers are listed in Table 1.

2.4 Immunohistochemical analysis of lung tissue

The slices were deparaffinized with xylene and hydrated through a graded ethanol series (95, 90, 80, 70%, and pure water). After blocking with endogenous peroxidase, antigen retrieval was performed with sodium citrate. The sections were incubated with primary anti-Bax (1:50, Category No.: A7626, Bioss, China) and anti-BCL-2 antibody (1:400, Category No.: MG53719-CH, Bioss, China) at 4°C for overnight. Then the sections were incubated with an horseradish peroxidases-conjugated secondary antibody for 20 min at room temperature. After washing with phosphate buffer saline, the sections were incubated

**Table 1: Primers for RT-qPCR**

| Name         | Sequence (5’–3’)                          |
|--------------|-------------------------------------------|
| GADD45A F    | TCGTACATGAGATCGTTGGG                      |
| GADD45A R    | GTTGAACCTACATCCGCCCCCT                    |
| caspase3 F   | ATCCACGGCTCCCCTCCTT                       |
| caspase3 R   | AGCACCGTGTTAGCAC                         |
| β-actin–F    | CGCAAGATCCGCTGGAT                       |
| β-actin–R    | TAACGCAGCTAAGCTCCGC                     |
with 3,3’-diaminobenzidine, counterstained with hematoxylin, and promoted blue with blue liquid, followed by dehydration. Finally, the sections were cover-slipped with neutral plastic and observed under a microscope.

2.5 Statistical analysis

Data analysis was performed by one-way analysis of variance followed by Tukey’s multiple comparison tests using a GraphPad Prism 7 software (GraphPad Corporation). A P value of <0.05 was considered to be statistically significant.

3 Result

3.1 Conditions of hydatid cyst

The hydatid cysts in the lung tissues of sheep were multiple cysts of various sizes. The majority of lung tissues showed the presence of protoscoleces in the cysts (Figure 1). After radiation, the size showed a decline to some extent.

3.2 Irradiation induced the expression of caspase-3 and gadd45a in the hydatid cyst

To investigate the effects of irradiation on hydatid cysts, the expression levels of two key genes (caspase-3 and gadd45a) involved in apoptosis and cell death were evaluated using RT-qPCR. The relative expression of gadd45a mRNA in the 30 Gy group was about 2.4-fold higher than in the control group. In addition, the expression of gadd45a mRNA in the 45 and 60 Gy groups was about 2.1–2.2 fold higher than that of the control group (Figure 2). For the expression of Caspase-3 mRNA, its expression in the 30 Gy group was nearly 3.0-fold compared with that of the control, whereas the expression of Caspase-3 mRNA in 45 and 60 Gy groups was 2.0-fold and 2.2-fold compared with that of the control group (Figure 2). All these indicated that irradiation caused apoptosis and necrosis in cystic cells.

3.3 Irradiation reduced the expression of BCL-2 and BAX in lung tissue involved by the cyst

With the continuous growth of *E. granulosus*, the surrounding organs and tissues were compressed, resulting...

**Figure 1**: Morphology of cyst in the lung tissues in (a) control group and (b) radiation group.
Figure 2: Effect of irradiation on the expression of caspase-3 and gadd45a. The mRNA level of gadd45a and caspase-3 was evaluated by using RT-qPCR (n = 5 in each group). The presented values are the means ± standard error mean. *P < 0.05, compared with control group.

Figure 3: Effects of irradiation on the expression of BCL-2. The protein level of BCL-2 was detected by using immunohistochemistry under a magnification of 40×, 100×, 200×, and 400×, respectively.
in cell death and tissue atrophy or necrosis, as well as final dysfunction [16]. In the present study, we detected the expression levels of BCL-2 and BAX in the lung tissue using immunohistochemistry, which showed no significant difference in the expression of BCL-2 after exposure to irradiation with a dose of 30 and 60 Gy, respectively. In contrast, the expression level of BCL-2 was significantly downregulated after exposure to a dose of 45 Gy (Figure 3). This indicated recovery from cell death induced by the cyst. BAX expression was significantly downregulated in the cyst after exposure to a dose of 45 and 60 Gy, rather than that of 30 Gy (Figure 4), indicating 45 and 60 Gy of irradiation reversed the cell death induced by the cyst.

4 Discussion

Hydatid disease is a serious zoonosis caused by *Echinococcus* hydatid parasitism in animal hosts, with a strong infectious potency to human individuals [17]. Hydatid disease has been considered to be an epidemic in the world. In recent years, extensive studies have been conducted to investigate the ecology and biological features of hydatid disease, and a comprehensive prevention system has been established [18–20]. This, to some extent, greatly promotes clinical diagnosis and treatment [21].

The progression of hydatid disease is prolonged, and some patients are asymptomatic for several years. The larva of hydatid acts as tumors that are slowly growing and gradually invading the organ. The severity of the disease may be closely related to the marked fibrosis of the tissue around the cysts. Due to hematogenous spread, it will gradually extend to the adjacent tissues and distant organs [22]. To date, the treatment of hydatid disease is still mainly reliant on the surgery and administration of agents (e.g., BZD). Recently, few studies have been available to focus on utilizing irradiation for treating such disease. In an earlier study, Zhao et al. indicated that heavy-ion radiation could induce the extinction of hydatid cysts in vitro [23]. However, rarely studies have been focused on the roles of radiation in hydatid disease under in vivo conditions [24].

![Figure 4: Effects of irradiation on the expression of BAX. The protein level of BAX was detected by using immunohistochemistry under a magnification of 40×, 100×, 200×, and 400×, respectively.](image-url)
The caspase family is a key element in the process of cell apoptosis. Its activation and overexpression could regulate cell apoptosis through interacting with multiple protein factors [25]. Caspase-3 is the main effector in the process of apoptosis [26]. At present, a large number of patients may present recurrence after surgery, administration of drugs, and other methods. To our best knowledge, irradiation can effectively kill the hydatid, causing relatively low injuries to normal tissues, which may serve as a safe and effective option for treating hydatidosis. In this study, we investigated the efficacy of radiation therapy for hydatidosis through detecting the apoptosis and cell death-related genes and proteins in the cyst and the adjacent lung tissue, respectively. Our data showed that irradiation could significantly induce the expression of caspase-3 and gadd45a, serving as two important genes involved in apoptosis and cell death. This indicated that the irradiation could effectively kill the hydatid in the lung tissues of sheep.

The immunohistochemical staining showed that 30 Gy irradiation triggered no reduction in the expression of BCL-2 and BAX. However, a dose of 45 Gy significantly suppressed the expression of BCL-2 and BAX in the lung tissues involved by the cyst, suggesting beneficial effects on the host. Interestingly, 60 Gy irradiation did not reduce the protein level of BCL-2. This may be related to the fact that excessive irradiation causes injuries to the lung tissue, which indicates that the irradiation dose should be within the tolerance of normal tissues. Irradiation significantly enhanced the protein expression of BAX and BCL-2. This indicated that the killing effects of radiation on the hydatid disease were associated with the activation of the apoptosis pathway. Similarly, in an earlier study, irradiation could induce cell death by facilitating apoptosis [27].

There are limited reports about the treatment of hydatidosis based on irradiation, as most of the studies were carried out under in vitro conditions [28]. This is the first study to report the treatment of pulmonary hydatidosis in sheep by using irradiation, and we hope this may provide some insight for the clinical treatment. However, there are some limitations to this study. We only investigated the efficiency of radiation on treating hydatidosis through determining the expression of apoptosis-related markers. Little is known about the potential mechanism in it. Besides, the sample size is not large due to difficulty in sampling.

Three irradiation doses were used in this experiment, and the results showed that 45 Gy might be safe and effective for treating pulmonary hydatidosis in sheep. This was supported by an induction of caspase-3 and gadd45a expression in the cyst and a downregulation of BCL-2 and BAX in the adjacent lung tissues.

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Data availability statements: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Santivanez S, Garcia HH. Pulmonary cystic echinococcosis. Curr Opin Pulm Med. 2010;16(3):257–61.
[2] Lashkarizadeh MR, Hooshmand N, Nasibi S, Mohammadi MA, Shamsaddini S, Kamyabi H, et al. Genetic profile of hydatid cysts in patients with multi-organ involvement: mixed infections by different strains. Vector Borne Zoonotic Dis. 2019;19(10):724–30.
[3] Butt A, Khan JA. Cystic echinococcosis: a 10-year experience from a middle-income country. Trop Doct. 2020;50(2):117–21.
[4] Turgut AT, Altinok T, Topçu S, Koşar U. Local complications of hydatid disease involving thoracic cavity: imaging findings. Eur J Radiol. 2009;70(1):49–56.
[5] Bartels M, Schmidt T, Lübbert C. Successful surgical management of hepatic alveolar echinococcosis by inductive therapy with albendazole – a case report. Z Gastroenterol. 2020;58(1):63–7.
[6] Hasdiraz L, Onal O, Oguzkaya F. Bilateral staged thoracotomy for multiple lung hydatidosis. J Cardiothorac Surg. 2013;8:121.
[7] Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 2010;114(1):1–16.
[8] Tatar D, Senol G, Gunes E, Unsal S, Perim G. Diagnosis and treatment of pulmonary cystic hydatidosis. Indian J Pediatr. 2008;75(10):1003–7.
[9] Rui M, Ge W, Hui W, Lu P, Zhang W. Effects of X-ray on the metacestodes of Echinococcus granulosus in vitro. BMC Infect Dis. 2017;17(1):636.
[10] Deng C, Li J, Li L, Sun F, Xie J. Effects of hypoxia ischemia on caspase-3 expression and neuronal apoptosis in the brain of neonatal mice. Exp Ther Med. 2019;17(6):4517–21.
[11] Liu PF, Hu YC, Kang BH, Tseng YK, Wu PC, Liang CC, et al. Expression levels of cleaved caspase-3 and caspase-3 in tumorigenesis and prognosis of oral tongue squamous cell carcinoma. PLoS One. 2017;12(7):e0180620.
[12] Liu J, Jiang G, Mao P, Zhang J, Zhang L, Liu L, et al. Down-regulation of GADD45A enhances chemosensitivity in melanoma. Sci Rep. 2018;8(1):4111.

[13] Li FH, Han N, Wang Y, Xu Q. Gadd45a knockdown alleviates oxidative stress through suppressing the p38 MAPK signaling pathway in the pathogenesis of preeclampsia. Placenta. 2018;65:20–8.

[14] Dong D, Dong Y, Fu J, Lu S, Yuan C, Xia M, et al. Bcl2 inhibitor ABT737 reverses the Warburg effect via the Sirt3-HIF1α axis to promote oxidative stress-induced apoptosis in ovarian cancer cells. Life Sci. 2020;255:117846.

[15] Barletta M, Hofmeister EH, Peroni JF, Thoresen M, Scharf AM, Quandt JE. Influence of sedation on onset and quality of euthanasia in sheep. Res Vet Sci. 2018;117:57–9.

[16] Lamonaca V, Virga A, Minervini MI, Di Stefano R, Provenzani A, Tagliareni P, et al. Cystic echinococcosis of the liver and lung treated by radiofrequency thermal ablation: an ex vivo pilot experimental study in animal models. World J Gastroenterol. 2009;15(26):3232–9.

[17] Kotoulas S, Grapatsas K, Leivaditis V, Panagiotou I, Spiridakis E, Le UT, et al. Massive pulmonary embolism due to hydatid cysts: a rare postoperative complication of liver echinococcosis. Respir Med Case Rep. 2020;30:101054.

[18] Padayachy LC, Dattatraya M. Hydatid disease (Echinococcus) of the central nervous system. Childs Nerv Syst. 2018;34(10):1967–71.

[19] Khuroo MS. Hydatid disease. Indian J Gastroenterol. 2001;20(Suppl 1):C39–43.

[20] Araj GF, Mourad Y. Hydatid disease: the Lebanese contribution. J Med Liban. 2014;62(4):217–26.

[21] Falagas ME, Bliziotis IA. Albendazole for the treatment of human echinococcosis: a review of comparative clinical trials. Am J Med Sci. 2007;334(3):171–9.

[22] Bresson-Hadni S, Miguet JP, Mantion G, Giraudoux P, Vuitton DA. Alveolar echinococcosis: a disease comparable to a slow growing cancer. Bull Acad Natl Med. 2008;192(6):1131–8. Discussion 9.

[23] Zhao Y, Gui W, Zhang Y, Mo G, Li D, Chong S. Inhibitory effect of ionizing radiation on echinococcus granulosus hydatid cyst. Diseases. 2019;7(1):23.

[24] Ma C, Luo X, Mahan W, Tang Y, Xie Z. Outcomes of radiotherapy for osseous echinococcosis of Meriones meridianus. BioMed Res Int. 2020;2020(7):1–8.

[25] Aljuhani N, Ismail RS, El-Awady MS, Hassan MH. Modulatory effects of perindopril on cisplatin-induced nephrotoxicity in mice: Implication of inflammatory cytokines and caspase-3 mediated apoptosis. Acta Pharm. 2020;70(4):515–25.

[26] Fan R, Wang H, Zhang L, Ma T, Tian Y, Li H. Nanocrystallized oleanolic acid better inhibits proliferation, migration and invasion in intracranial glioma via Caspase-3 pathway. J Cancer. 2020;11(7):1949–58.

[27] Teraoka S, Kakei Y, Akashi M, Iwata E, Hasegawa T, Miyawaki D, et al. Gold nanoparticles enhance X-ray irradiation-induced apoptosis in head and neck squamous cell carcinoma in vitro. Biomed Rep. 2018;9(5):415–20.

[28] Zhang YF, Xie ZR, Ni YQ, Mao R, Qi HZ, Yang YG, et al. Curative effect of radiotherapy at various doses on subcutaneous alveolar echinococcosis in rats. Chin Med J (Engl). 2011;124(18):2848–8.