थोरियम के प्रति जैविक प्रतिक्रियाओं में अंतर्दृष्टि

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मानव स्वास्थ्य एवं पर्यावरण के लिए थोरियम

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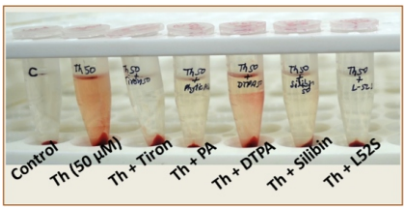

थोरियम सजावट के लिए तर्कसंगत रूप से चयनित एजेंटों की एक्स-विवो स्क्रीनिंगः मानव आरबीसी लाइसिस परख।

सारांश

थोरियम (Th-232) विकसित भारत की ऊर्जा मांग को पूरा करने के लिए एक अति आवश्यक नाभिकीय ईंधन है। नाभिकीय ऊर्जा के अलावा, थोरियम के अन्य समस्थानिक कैंसर के उपचार और अन्य पर्यावरणीय अनुप्रयोगों हेतु सहायक हैं। विभिन्न सामाजिक अनुप्रयोगों के लिए थोरियम की पूरी क्षमता को यथार्थ करने के लिए, हमारा रेडियोजीवविज्ञान अनुसंधान मानव कोशिकाओं (RBCs, यकृत और फेफड़ों की कोशिकाओं) और पशु मॉडल में थोरियम अंतःक्रिया, परिवहन और जैविक प्रतिक्रियाओं के तंत्र के बारे में महत्वपूर्ण अंतर्दृष्टि प्रदान करता है। विषाक्तता तंत्र और थोरियम के जैव-समन्वय से संभावित संकेतों ने थोरियम के लिए लिगैंड के तर्कसंगत अभिकल्पन और विकास का मार्ग प्रशस्त किया है, जिसमें कैंसर के उपचार और नाभिकीय विघटन कार्यनीतियों के विकास के लिए संभावित अनुप्रयोग हैं।

Insight into Biological Responses to Thorium

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Thorium for Human Health and Environment

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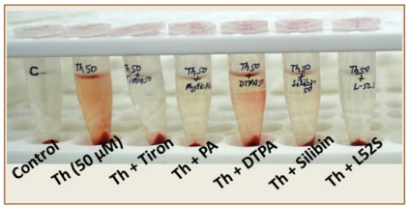

Ex-vivo screening of rationally-selected agents for Thorium decorporation: Human RBC lysis assay

ABSTRACT

Thorium (Th-232) is an indispensable nuclear fuel for meeting the energy demands of *Viksit Bharat* (developed India). In addition to nuclear energy, other Th isotopes hold promise for cancer treatment and other environmental applications. This article presents a comprehensive synthesis of our research findings on Thorium Biology accumulated over the past two decades as part of the Nuclear Radiobiology Research Program. To realize the full potential of Th for various societal applications, our radiobiology research provides significant insight about the mechanism of Th interaction, transport and biological responses in human cells (RBCs, liver and lung cells) and animal models. The potential clues from toxicity mechanism and bio-coordination of Th have paved the way for rational design and development of ligands for Th, having potential applications for cancer treatment and development of nuclear decorporation strategies.

KEYWORDS: *Thorium, Radiobiological Research, Cancer Therapy, Actinide Decorporation*

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Introduction

The nuclear energy is indispensable with little-to-zero carbon foot print. Importantly, Uranium has been utilized as a nuclear fuel for clean energy generation since last several decades. However due to its limited abundance, alternative nuclear fuels viz. Plutonium and Thorium are gaining significant importance. Plutonium availability is again dependent on natural Uranium. Therefore, Thorium owing to i) its 4-5 times more natural abundance (compared to U), ii) potential of its conversion into fissile fuel, iii) thermo-physical characteristics, iv) lesser long-lived radionuclide generation has been considered as a potential fuel for sustainable energy generation through advanced nuclear technologies, in particular molten salt reactor, high temperature reactor and accelerator driven sub-critical systems (Fig.1).

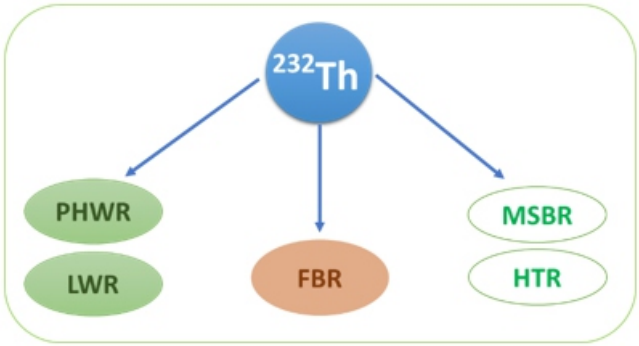

Fig.1: Scheme illustrates the recent development for possible utilization of naturally occurring Thorium (Th-232) in various reactor technologies. PHWR: Pressurised Heavy Water Reactor; LWR: Light Water Reactor; FBR: Fast Breeder Reactor; MSBR: Molten Salt Breeder Reactor; HTR: High Temperature Reactor.

Recently, Thorium-based fuel such as ANNEL (Advanced Nuclear Energy for Enriched Life) has also been developed for existing PHWR or CANDU reactors (Fig. 1). Additionally, the feasibility of Thorium utilization has been explored in Light Water Reactors [1]. Thorium-232 would be used as a blanket in Fast Breeder Reactors (FBR) to convert it into fissile U-233 for third stage reactors of DAE. Notably, the commencement of "Core Loading" at India's first indigenous PFBR (500MWe) at Kalpakkam, Tamil Nadu would pave the way for efficient utilization of India's vast reserve of Thorium. This review provides a comprehensive overview of our research findings on Thorium Biology, gathered over the past two decades through the Nuclear Radiobiology (Bio-Actinide) Research Program.

Thorium and Human Health

In view of Thorium as a future nuclear fuel, the fundamental research aimed towards understanding the radiobiological effects of Thorium in human cells and experimental animals is one of the most relevant areas of Radiation Biology Program of several research areas of nations. Having the largest reserve of Thorium and being equipped with advanced nuclear reactor technologies for Thorium utilization, this research program is even more relevant for India [2]. Following three sub-sections describe potential implications of Thorium biology research in i) targeted alpha cancer therapy, ii) fundamental radiobiology and iii) development of novel decorporation strategies for management of internal nuclear contamination.

Thorium for cancer therapy

One of the isotopes of Thorium is Th-227, which emits five high-energy alpha particles ($t_{1/2}$ =18.7d, average 5.9 MeV) and two beta disintegrations during its decay. These alpha particles are highly favourable for targeted therapy of cancer mainly due to the following reasons: i) short-path length resulting minimal damage to surrounding normal tissue, ii) high linear energy transfer leading to un-repairable and clustered DNA double strand breaks, and iii) cytotoxic effects of alpha radiation independent of oxygen status in tumour. Due to half-lives, large alpha particle energies and rapid decay chains, ^{225}Ac and ^{227}Th have immense potential for targeted alpha therapy of cancer. With regard to yield as compared to ^{225}Ac, ^{227}Th can be obtained from the beta decay of ^{227}Ac, which is a decay product of naturally-occurring ^{235}U. Alternatively, ^{227}Th can be produced by thermal neutron irradiation of ^{226}Ra followed by beta decay of ^{227}Ra. To achieve the full anticancer potential of Th-227 conjugates, a significant scope of research exists for development of constructs with tumor-targeting moiety and a specific bifunctional chelator. Recent reports have shown promising results for antitumor activity of ^{227}Th conjugates in preclinical tumor models and in phase-I clinical trial [3]. In summary, ^{227}Th-based targeted alpha therapy represents a viable cancer treatment modality in future.

Radiobiological research

Under this topic, we summarize the key findings from our learning experience on the basic mechanisms of Thorium interaction with human cells and proteins and its consequences. A more than decade-long research activities revealed crucial answers for i) the mechanism of effects of

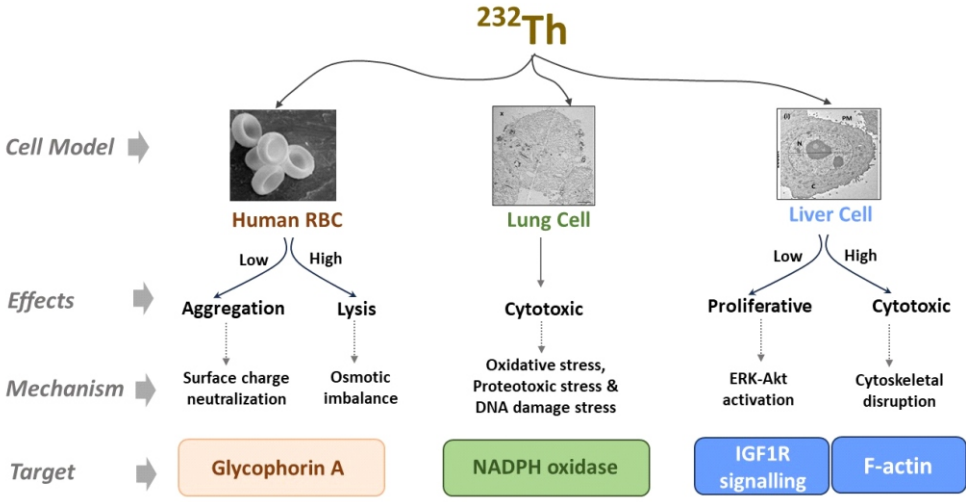

Fig.2: Scheme illustrates the effect of Thorium, possible mechanisms and potential target of toxicity in the indicated human cell models under in vitro conditions. Disclaimer: These effects were observed under experimental conditions and may not occur in real exposure scenario.

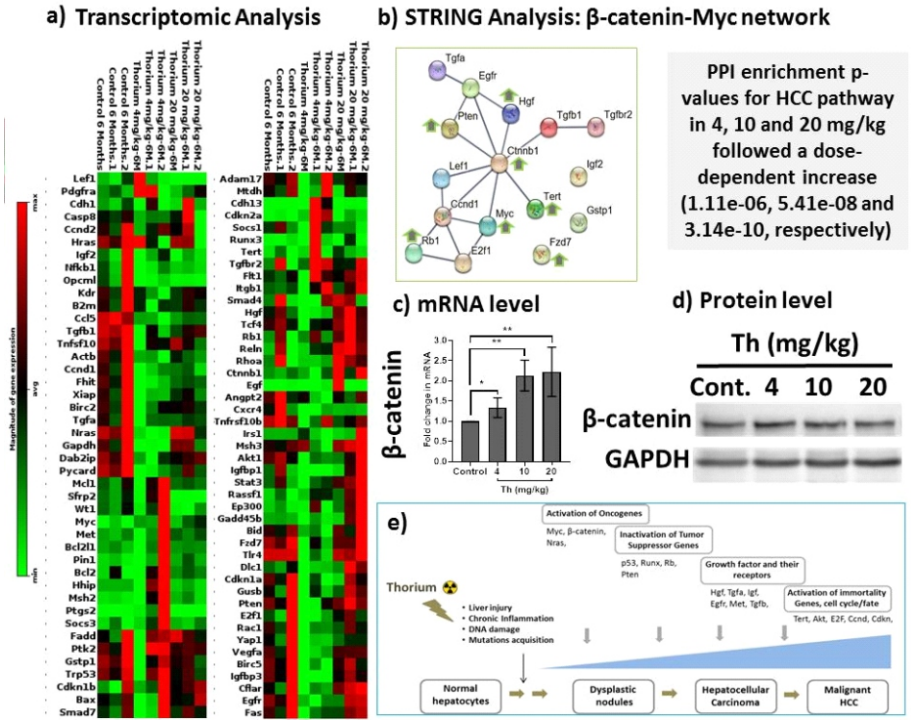

Fig.3: (a) Transcriptomic profile of liver tissues of mice following 6 or 12 months of Th administration (4, 10 and 20 mg/kg). (b) Protein-protein interaction analysis by STRING revealed statistically significant functional interaction among the proteins of hepatocellular carcinoma network, which identified β-catenin as the most significant signalling nodes. (c & d) Validation of role of β-catenin at mRNA and protein level. (e) Scheme illustrates the possible role of Th-induced genes in the sequential process of liver carcinogenesis. Reprinted from "Mechanistic Insights into Thorium-232 induced Liver Carcinogenesis: The Driving Role of Wnt/β-Catenin Signaling Pathway by R. Yadav, S. K. Das, M. Ali, N. G. Shetake, B. N. Pandey and A. Kumar, Science of Total Environment 907 (2024) 168065 with permission from Elsevier.

Thorium in human cells; ii) the mechanism of cellular internalization of Thorium; iii) fundamental aspects of the binding of Thorium to protein and their functional consequences; and iv) major target organs of Thorium and their early and late chronic effects. Understanding these aspects of Thorium toxicology at organ, cell and molecular levels using biophysical, biochemical, microscopic, spectroscopic and computational approaches, we have designed and developed the rational antidotes for removal of Thorium and mitigation of its associated radiological and chemical effects. In conclusion, the Bio-Thorium Research Program at BARC would have significant implications for developing India's capability for efficient utilization of Thorium with adequate human health and environmental protection.

With regard to one of our research objectives, 'the mechanism of effects of Thorium in human cells', we have envisaged to investigate the effect of Th-232 on various human cells viz. red blood cells [4], lung cells [5], liver cells [6-8], and bone [9], which represent the target organ/sites of Thorium accumulation/toxicity in human/animal models (Fig.2). Our experimental data suggest that radiobiological response to Thorium depends on the cell type, chemical forms, concentration and exposure time of Thorium. Briefly, in human lung cells, Th-dioxide (colloidal) was found to be more toxic than the Th-nitrate at equivalent metallic concentration of Thorium. This was found to be due to higher uptake of Th in cells exposed to its dioxide form as compared to the Thorium uptake from its nitrate form of exposure. Moreover, transmission electron microscopy in combination with confocal microscopy further revealed the mechanism of Thorium internalization, which was found to be a clathrin/caveolin-mediated endocytosis following Th-dioxide exposure as compared to membrane perforation in cells exposed to Th-nitrate. Following internalization, Thorium induces oxidative stress, DNA damage response and proteotoxic stress, which play major roles in determining cytotoxicity [5]. Following transmigration through air-blood barrier in lung, Th gets circulated via blood and finally accumulates in liver and skeleton. Our studies on human liver cells have identified the role of cytoplasmic calcium in Thorium uptake, suggesting the possible application of calcium modulators for minimizing Th internalization. Using ultrasensitive analytical techniques, we have identified cytoskeleton as the major intracellular target of Thorium [7]. Interestingly, effect of Thorium on human red blood cells (RBCs) was found to be determined by Th:RBCs ratio. Lower Th-to-RBCs ratio caused aggregation due to neutralization of surface negative charge. However, at higher Th-to-RBCs ratio, RBCs undergoes cell lysis (hemolysis) through colloid-osmotic mechanism [4]. These mechanisms of Th-induced cytotoxicity have significant implications to develop rational approaches for mitigation of Th-toxicity.

During circulation in blood, Thorium ions can also interact (in addition to RBCs) with soluble proteins such as albumin/globulin in blood plasma as well as haemoglobin localized on RBC's membrane. Using biophysical tools, we have determined the binding sites of Thorium ions in human serum albumin [10] and haemoglobin proteins [11]. Interestingly, Th ions were found to perturb the structural and functional integrity of Fe-containing heme of haemoglobin. This was due to the similar charge-to-ionic-radii ratio of Th with Iron. This further explains the binding of Th at Fe-binding sites in iron transport/storage proteins (e.g. transferrin, ferritin, catalase, etc.). Thorium interaction with haemoglobin was also observed in the environmentally-important aquatic midge, *Chironomus*, which may serve as bioindicator of Th contamination in aquatic bodies [11]. Importantly, our spectroscopic data determined the ability of actinide ions including Thorium to unfold the proteins with significant alteration in their functionally important conformations. In this direction, further research in our laboratory using computational modelling and biochemical

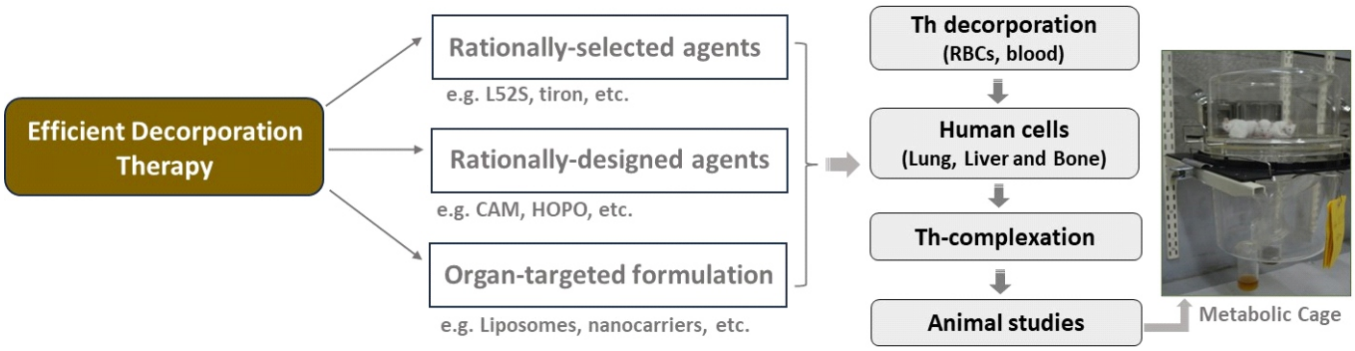

Fig.4:This scheme represents three different approaches for development of efficient decorporation therapy for Thorium and other relevant nuclear contaminants. The lead thorium chelator or its liposomal formulation are assessed for thorium removal efficacy from various human cells and blood. Thorium complexation is studied by a variety of spectroscopic and computational modelling. Finally, animal studies are performed to test thorium decorporation efficacy using in-house fabricated metabolic cages.

approaches, is in progress to characterize the bio-coordination of Thorium and other actinide ions with relevant proteins, which have been identified as a molecular target of toxicity.

We have carried out extensive studies in experimental mice/rat models to identify the major sites of Th accumulation and underlying mechanism of toxicity at cellular and molecular levels. Liver, skeleton and spleen were found to be the major target organs of Thorium following its administration [12-14]. We have determined the biodistribution, histological and functional changes as well as alterations in gene expression in organs/tissues after 6 to 12 months of Thorium exposure. Our recent analysis of gene expression and system biology approaches [15] revealed the identification of β-catenin/Myc driven signalling pathways responsible for Thorium-induced oncogenesis in mice (Fig.3). Currently, mechanism of radiobiological effects of Thorium on lung using specially-designed nose-only inhalation exposure facility is an active area of investigation, which led to the identification of surfactant protein-D as a potential biomarker of Th exposure [16].

Thorium decorporation technologies

Design and development of effective mitigation approaches for internal contamination with Thorium and other radiometals in human and environment is the most important area of research in this field. Presently, there is no FDA-approved antidote for treatment of human subjects in case of Thorium contamination. DTPA (Ca/Zn salt of diethylenetriamminepentacetate) is the only available agent recommended for Plutonium, Americium and Curium contamination. However, our animal data suggest that DTPA is not effective to remove Thorium ions from liver and skeleton. Therefore, further research is required to design Th-specific rational antidote. Our experience on the mechanism of Th interaction at cell and protein levels has provided significant clues about the biological ligands (proteins/DNA, etc.) with which antidote (chelator) need to potentially compete for Th decorporation. In this direction, we adopted multidisciplinary three-pronged approaches viz.: i) rationally-selected existing molecules; ii) rationally-designed new agents and iii) improvement of organ distribution profile of chelators using drug delivery approach (Fig.4).

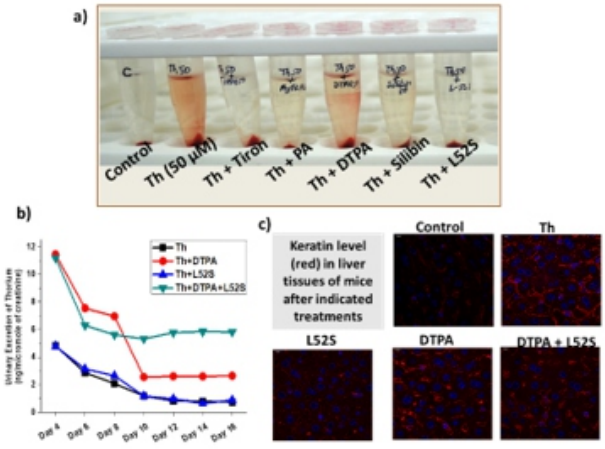

Fig.5: (a) Ex-vivo screening of rationally-selected agents for Thorium decorporation: Human RBC lysis assay; (b) The lead candidate L52S in combination with DTPA enhances Th-232 decorporation in mice and © potentially mitigates liver damage. Reprinted from "Thorium decorporation efficacy of rationally-selected biocompatible compounds with relevance to human application" by M. Ali, B. Sadhu, A. Boda, N. Tiwari, A. Das, S. K. Musharaf Ali, D. Bhattacharya, B. N. Pandey and A. Kumar, Journal of Hazardous Materials 365 (2019) 952-61 (License: 5924850861041) and "Enhanced thorium decorporation and mitigation of toxicity through combined use of Liv52® and diethylenetriamine pentaacetate" by M. Ali, S. K. Das, N. G. Shetake, B. N. Pandey, and A. Kumar, Journal of Hazardous Materials, 477 (2024) 135234 with permission from Elsevier (License 5924851197485).

Our efforts on screening of rationally-selected existing molecules have identified L52S (a hepatoprotective formulation) for its potential of Th decorporation [17]. L52S has been found to be significantly effective to enhance removal of Th from target organs in combination with DTPA (Fig. 5) [18]. Regarding other approaches, we have designed and tested liposomal-DTPA [19] and other ligands [20, 21] for Th decorporation. Further research is in plan to develop more efficient organ-targeted vehicle system to deliver chelating agent for organ-specific actinide decorporation. Recently, collaborative efforts are in progress for development of multidentate chelating agent for actinide decorporation.

Thorium for isotope hydrology

Recent research showed that the monitoring of Th concentrations in water can be used as a chemical signature for rock fracture events, which are integral to the hydrology and geochemistry of watersheds [22]. Moreover, ratiometric determination of Th isotopes (Th-228/230) was found to significantly predict the flood events, suggesting the novel application of Th measurements for environmental and climate change studies [23]. Since Th is sparingly soluble in water, its concentration can be monitored as a signature of water contamination from mining/milling activities also.

Future Research Perspectives

Further research is directed to gain deeper insights about the cellular and molecular mechanisms of radiobiological effects of Thorium and other heavy metal radionuclides in cells and tissues. These biological investigations would lead to the identification of sensitive and

specific biomarkers of Thorium exposure/effects. Our learning suggested the need for development of decorporation therapy according to the route of exposure, which can be applied in real practical scenario for effective management of internal contamination with actinides.

Conclusion

We have delineated the mechanism of cytotoxic effects of Th-232 in relevant human cells (blood cells, liver and lung cells). Cellular effects of Th were found to be dependent upon the cell type and its chemical form. Human RBCs were found to undergo aggregation or lysis upon Th exposure. Th uptake in liver cells was observed to be sensitive to cytoplasmic calcium level and its channels. In lung cells, Th followed either membrane perforation or endocytosis mechanism depending on nitrate or oxide form for internalization. At protein level, Th prefers to bind iron or calcium-binding sites, which is followed by interaction with carbonyl or amide groups. At organ level, omics data revealed the possible mechanism of Th-induced carcinogenesis in mice liver. Extensive efforts are underway to develop rational decorporation therapy for removal of internalized Th from lungs, surface wounds and other vital organs based on the understanding of mechanisms of Th toxicity and interaction.

Acknowledgment

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