ORIGINAL ARTICLE

Systematic Review of Toxicity Profiles on Nano-TiO_2 for Cancer Therapy

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ABSTRACT

Introduction: With the increasing clinical use of titanium dioxide nanoparticles (nano-TiO_2), a better understanding of their safety in the human use is critical. The present study aims to review the potential application of nano-TiO_2 as targeted cancer therapy based on their toxicity risk which highly dependent on their physio-chemical properties. **Methods:** This review was performed based on PRISMA-P protocol that begin with literature searching on the selected databases; PubMed, Springer Link, Science Direct and general search engine; Google Scholar from 2013 to 2018. Studies retrieved by the pre-determined keywords (titanium dioxide nanoparticles, toxicity, genotoxicity, cytotoxicity, targeted cancer therapy) that assessed toxicity risk of nano-TiO_2 in cancer therapeutics were included. **Results:** The search retrieved 252 articles. Assessment of eligibility by application of inclusion criteria yielded 14 articles. Nano-TiO_2 induced cytotoxicity and genotoxicity in dose and time-dependent manner killing the cancerous cells. All studies used primary particles size < 100 nm with mean of 39.38 and standard deviation of 30.47 which is lower than the mean denoting diameter distribution from selected studies are concentrated from the mean. **Conclusion:** This review suggest that TiO_2 nanoparticles can be considered as an ideal candidate for drug-delivery vehicle for targeted cancer therapy by specifically tailored their physio-chemical properties of this nanoparticles according to desired target site and functions to ensure its optimal efficacy.

Keywords: Titanium dioxide nanoparticles, Targeted cancer therapy, Physio-chemical properties, Toxicity risk, Nanotherapeutics

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INTRODUCTION

Contemporary cancer treatment including surgery, chemotherapy and radiotherapy have been the main modality to treat cancer to date worldwide (1, 2). However, few studies show that there are limitations in their applicability and efficiency. Surgical procedure can only be done in solid tumours. Even though removal of a large bulk of tumour can relieve mass-effect which alleviate symptoms instantly, it is unable to completely kills the microscopic remnants around tumour margin consequently lead to recurrence (3-5). Chemotherapy has become a fundamental component of cancer treatment for most cancers. Despite years of effort on oncology research and discovery, conventional chemotherapeutic regimes still exhibit poor specificity by working non-selectively collaterally destroying the healthy normal cells. Besides, it is poor accessibility to tumour site making indispensable usage of higher dose drugs, narrow therapeutic window, and high intolerable toxicity (6, 7).

Nanotechnology has emerged in biotechnology and medical fields offering enormous potential in research and innovation. Recently, new advances have been developed and utilized in detection, diagnosis, imaging, monitoring and management of diseases (8). During the last decade, there is a strong focus on application of nanotechnology for cancer therapy. Cancer nanotechnology has brought about a significant breakthrough in cancer management (9, 10). It goes beyond just target-specific drug therapy by providing early diagnosis (*in vitro* and *in vivo*) of the cancer (11) accurate disease prognosis (12) along with a substantial

increase in the number of highly effective therapeutic and diagnostic agents.

The use of nano-biomaterials also promises the development of tailor-made drug-delivery devices which capable of carrying large doses of chemotherapeutic drugs or therapeutic genes directly into cancerous cells while sparing normal healthy cells (13, 14). These nanomaterials would greatly minimize or even eliminate the adverse side effects that often accompany conventional cancer therapies.

Till date, plenty of nanoparticles have been approved as effective transporters in drug-delivery system due to their compelling features. These include their ability to improve solubility of hydrophobic drugs, prolonging bioavailability, preventing inevitable adverse side effects, allowing for target specific and able to cross biological barriers which is limited in conventional drugs (15-19).

TiO_2 nanoparticles (nano-TiO_2) have demonstrated to be the most appropriate materials for targeted cancer therapy due to their splendid physiochemical properties; low toxicity level which is safe for human use, high chemical and physical stability, excellent photo catalyst liberating free-oxygen radicals and potent anti-microbial effects (20-23).

Even though the usage of nano-TiO_2 as targeted cancer therapy seems promising, to the best of our knowledge, their toxicity risk profile are still scarce. Thus, the purpose of this study is to provide a better understanding of nano-TiO_2 toxicity risk in human which eventually suggest its actual potential on application for targeted cancer therapeutics.

MATERIALS AND METHODS

Search Strategy

Our systematic review was obliged to the PRISMA-P guidelines (24). We included manuscripts between 2013 till 2018 that were retrieved in the following databases: PubMed, Springer Link, Science Direct and general search engine Google Scholar. A Boolean strategy was applied. The following pre-determined keywords (titanium dioxide nanoparticles, toxicity, genotoxicity, cytotoxicity, targeted cancer therapy) were used and combined interchangeably with the Boolean operator 'AND' or 'OR' to broaden the searching outcomes.
Titles and abstracts of the potential articles were screnned independently by the author to retrieve relevant articles from the mentioned full-text electronic journal databases. In addition, the bibliographies of the selected articles were systematically screened by non-automated manual search to obtain other potentially relevant articles. Every steps taken in this process were meticulously documented to ensure transparency, replicability and feasibility to reanalyse. To avoid duplication of works, Endnote software version X7 was used as a reference manager to merge results of all extracted studies. Full text of each identified study was retrieved by author.

Study selection

We included all pre-clinical and clinical studies, original and in-press articles published within the past 6 years (2013 till 2018) to narrow down the review on the toxicity profile of nano-TiO_2 in recent studies. Studies involving usage of nanoparticles in other diseases than cancer, organic nanoparticles, and those in predatory or blacklisted journals were excluded.

Data extraction

The articles were thoroughly reviewed to verify the eligibility based on the inclusion criteria and assessed the quality of the articles. The author independently extracted the data on the relationship of physio-chemical properties of nano-TiO_2 and the toxicity outcome on the target cells (*in vitro*) and organism (*in vivo*). Data included are primary particles size, zeta-potential, surface area, exposure time and dosage. All the extracted data were sorted in tables using Microsoft Excel and the reference citations were exported to the EndNote Version X7 reference manager software.

Data analysis

The data collected from the studies were sorted, concluded respectively and compared to ascertain the strength of the study. Results from toxicity risk of nano-TiO_2 were pooled, sorted, extrapolated and descriptively analysed using statistical Minitab version 6.0 software to determine the relationship of particles diameter and toxicity effects. The quality of evidence for outcomes were determined using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (25).

RESULTS

In total, 252 articles were identified by the indexed in PubMed, Springer Link, Science Direct and general search engine Google Scholar. After application of inclusion and exclusion criteria, we obtained 14 articles discussed on the *in vivo* and *in vitro* toxicity of nano-TiO_2 in cancer therapeutics (Figure 1).

Present studies on *in vitro* and *in vivo* toxicological profiles have described on the Nano-TiO_2 physiochemical properties with the desired functions (Table I, Table II). Nano-TiO_2 induced cytotoxicity and genotoxicity in dose and time-dependent manner killing the cancerous cells but reverted after 24 H. Nano-TiO_2 with size < 100 nm were not translocated and deposited in organs and were clearly excreted by the kidney. All studies in the review used primary particles size < 100 nm with mean of 39.38 representing the centre of this diameter distribution data. While the standard

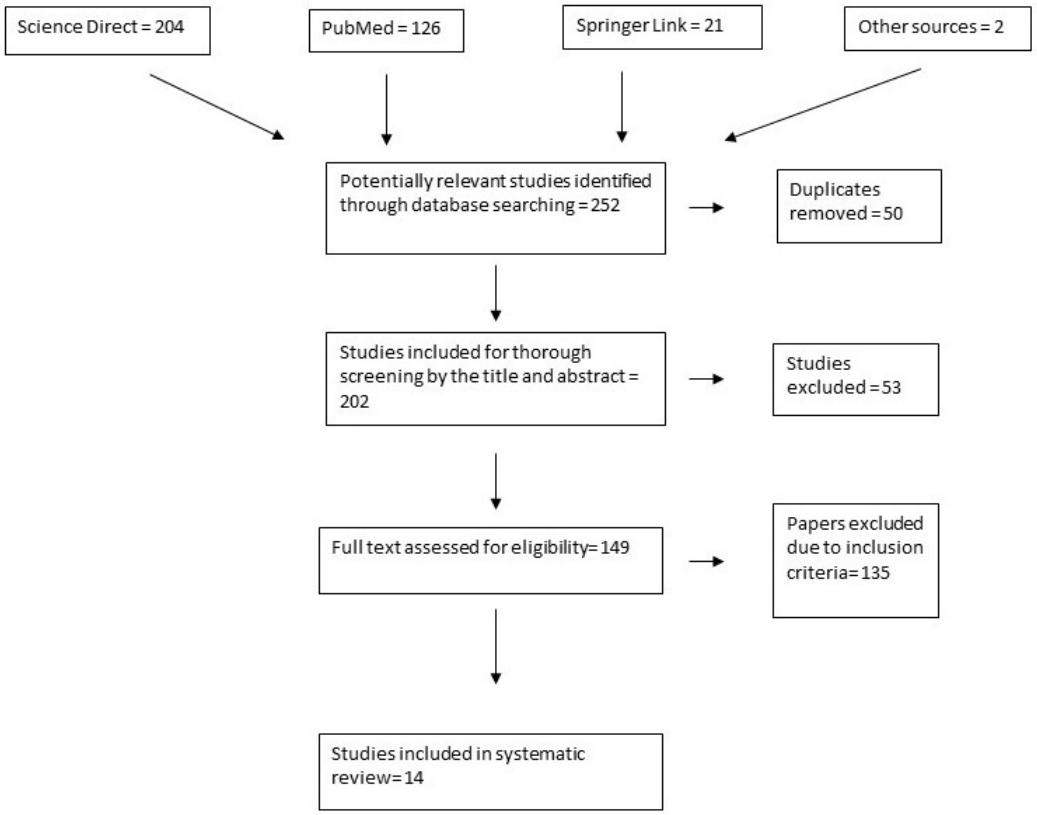

Figure 1: Flow chart of literature searching from selected databases. The flow chart above described the results from the searching process up till screening of eligible studies included in the reviewin all mentioned electronic databases on toxiciy risk nano-TiO2 as targeted cancer therapy.

deviation is 30.47 which is lower than mean denoting the diameter distribution from selected studies are concentrated from the mean (Figure 2, Table III). On the other hand, the modified diameter has primary size of > 100 nm with mean of 294.4 and standard deviation of 295.8 suggested slightly un-concentrated form the mean (Figure 3, Table 4). Despite the modified sizes are larger than 100 nm, note that the nano-TiO_2 enhanced its delivery at the targeted site.

DISCUSSION

Toxicity of nano-TiO_2 are greatly distributed by liberation of free radical including radical oxygen species (ROS). Increased oxidative stress (OS) is a common cellular response proved by upregulation of OS-related biomarkers collected from production workers in workplace that suggested nano-TiO_2 can induce OS in humans (26). However, most conclusions about the role of OS in the nano-TiO_2 toxicity of were obtained from *in vivo* and *in vitro* studies.

In vivo studies disclosed that nano-TiO_2 contents in main organs increased after animals received nano-TiO_2 through various administration route, which, in turn, induced OS and dysfunctional organs. Periera et al., found there is depletion of endogenous anti-oxidant system and swelling of the mitochondria of Male Wistar rats upon administration of nano-TiO_2 through oral gavage (27). However, study by Dobrzynka et al., shown no significant cytotoxicity or genotoxicity induced upon intravenous exposure of nano-TiO_2 (28).

In vitro studies conducted by De Angelis et al., and Zijno et al., showed that exposure of nano-TiO_2 in human epithelial colorectal adenocarcinoma cells (Caco-2 cell line) lead to elevation of oxidative stress after 6 H but reverted after 24 H. However, studies by Vales et al., Filippi et al. Lopes et al. and Valdiglesias et al., shown no significant increase in oxidative stress induced (29 - 32). Besides, study by Dubey et al., showed a dose dependent increase in DNA damage, lipid peroxidation and protein carbonyl content with a significant decrease in activity of superoxide dismutase, catalase, total glutathione levels and total antioxidant capacity indicated that the cells were under oxidative stress (33).

Studies also shown release of pro-inflammatory mediator (IL-8) upon exposure to nano-TiO_2 (34, 35). Studies by Schneider et al., Ghosh et al., and Stoccoro et al., showed a significant damage of the DNA (36-38). Elevation of OS due to generation of intracellular ROS, increased hydrogen peroxide levels, decreased glutathione peroxidase and reduced glutathione level decreasing the mitochondrial membrane potential. The genes responsible with DNA-fragments break were then affected consequently followed release of cytochrome C into cytosol and activating caspase 3 to induce cancer cell apoptosis. In addition, Thai at al., showed expression of different gene responsible for translation initiation

Table I: *In vitro* toxicological profiles of Nano-TiO2 and the physiochemical properties used in the present study

TARGET	PHYSIOCHEMICAL PROPERTIES	PRIMARY SIZE (nm)	MODIFIED SIZE (nm)	EXPOSURE TIME	DOSE	OUTCOME	REFERENCE
Murine Balb/3T3 cell	• Surface area (g/m^2) Uncoated= 154 Citrated= 156 Silicate= 86 • Zeta potential(mV)= Uncoated= 41.2±0.5 Citrated= 57.5±2.6 Silicated= 32.2 ±4.1	83.5±10.4	• Citrated= 57.5±2.6 • Silicated= 115.6 ± 22.1	48h	1.25-80 $\mu g/cm^2$	Cytotoxicity: Induced cytotoxicity Genotoxicity: -Citrated and the lowest P25- statistically significant genotixicity -2h and 24h significant increase of primary DNA damage Neoplastic transformation (cell transformation assay): Citrate and the P25 induced type-III foci	Stoccoro et al. 2016
Human neuroblastoma SHSY5Y	• Surface area= TiO_2-S= 200-220m^2/g TiO_2-D= 35-45m^2/g • Zeta potential(mV)= -10.7	25	TiO_2-S= 447.9 TiO_2-D= 160.5	3/ 6/ 24h	0–150 g/ml	Cytotoxicity: -No decrease viability -No morphological alterations Genotoxicity: Micronuclei (dose dependent) Oxidative damage: No damage at any concentration and time	Valdiglesias et al. 2013
Caco-2 cells	• Surface area= NA • Zeta potential(mV)= -14.7± 0.4	<25	284±43	6h/ 24h	5, 25 and 100 mg/mL	Cytotoxicity: No decrease viability Oxidative damage: Increase at 6hr Proinflammatory mediator release (IL-8): Slight release after 24 h	De Angelis et al. 2013
Human erythrocyte lymphocyte	• Surface area= $14.0m^2/g$ • Zeta potential= NA	35-56	48	3h	0-100 µg/mL	Cytotoxicity: Significant cytotoxicity Significant reduction in mitochondrial dehydrogenase activity Genotoxicity: Significant increase DNA damage followed by gradual decrease Haemolytic effect: significant haemolysis Morphological alterations: spherocytosis and echinocytosis	Ghosh et al. 2013
Caco-2	• Surface area= NA • Zeta potential(mV)= -9.2±0.5	20-60	220±68	2, 4, 6, 24h	1 and 2.5 lg/ cm^2	Genotoxicity: No significant genotoxicity Oxidative damage: Induced but revert within 24hr	Zijno et al. 2015
HepG2	• Surface area= A- 52.9 B- 22.2 C- 118 D- 49.8 H- 11.6 I- 6.99 • Zeta potential= NA	A-31 B-59 C-25 D-22 H-214 I-142	A- 402.8 B- 534.0 C- 331.2 D- 328.0 H- 379.0 I- 467.9	3d	0.3-1000 µg/ mL	Cytotoxicity: No significant cytotoxicity Differently expressed gene: • A, B, I, H- affected expression of genes translation initiation, EIF2, mTOR signalling and regulation of eIF4 and p70S6K • D and C- not	Thai et al. 2016
HaCaT	• Surface area= NA • Zeta potential(mV)= −5.59 ± 1.70	18	1369.0 ± 27.97	1, 24h	0.16-25 µg/ml	Cytotoxicity: No significant cytotoxicity Autophagic response (LC3 translocation): Significant increase eGFP-LC3 dots Oxidative damage: Not significant	Lopes et al. 2016
Human broncho-epithelial (BEAS-2B)	• Surface area= NA • Zeta potential(mV)= -19.5±0.5	21.7±0.6	575.9 ± 8	4w (long exposure)	1-20 µg/ mL	Cytotoxicity: No significant Proinflammatory mediator release (IL-8): No significant increase in expression Genotoxicity: Not significant Oxidative damage: Not significant Acquired phenotype (soft agar assay): Significant dose-dependent increase	Vales et al. 2015
WAG cell line	• Surface area= NA • Zeta potential= NA	- 35.21 ± 14.1	249.7	24h	1.56mg/l	Cytotoxicity: Not significant Genotoxicity: Not significant Oxidative damage: Dose dependent increase Lipid peroxidation: Dose-dependent increase Protein carbonyl content: Dose-dependent increase	Dubey et al. 2015
Balb/3T-3mouse fibroblasts	• Surface area= NA • Zeta potential(mV)= Anatase −5.64 ± 0.77 Rutile +0.04 ± 0.13	 11-18 10-35	 51.42 ± 0.35 134.40 ± 1.02	24, 72h	1-10 µg/ ml	Cytotoxicity: Anatase- Not significant Rutile- Significant cytotoxicity Genotoxicity: Not significant Neoplastic transformation: Rutile induced significant dose-dependent	Uboldi et al. 2016
Human colorectal HT29	• Surface area= NA • Zeta potential(mV)= −10.20 ± 3.20		27.38 ± 5.90	24h	0-10 µg/ ml	Cytotoxicity: Not significant Genotoxicity: Increased amount of DNA strand breaks and oxidized purine bases	Schneider et al.2017
C3A cells	Surface area= NA Zeta potential (mV)= −5.6±0.3	30.5±1.8	119±16	4h	0-10 µg/ ml	Hepatocyte glycogen metabolism: Very limited effect on glycogen breakdown, glucose, LP release, even at the highest doses tested Phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression: No significant increase Oxidative damage: Not significant	Filippi et al. 2015

Table II: *In vivo* toxicological profiles of Nano-TiO2 and the physiochemical properties used in the present study

TARGET	PHYSIOCHEMICAL PROPERTIES	SIZE (nm)	EXPO-SURE	EXPOSURE DOSE	OUTCOME (TUMOUR SIZE)	REFERENCE
Male Wistar rats (gavage)	• Surface area= 35-65 m^2/g • Zeta potential= -5.07 ±1.11mV	21	21 days	100 µg/kg/day	Mitochondrial effects: -Structurally swelling -Mitochondria bioenergetic not affected -Depleted endogenous anti-oxidant system -Induced oxidative stress	Pereira et al. 2018
Male Wistar rats (intravenous)	• Surface area= NA • Zeta potential= -33.7mV	21	Once	5mg/kg bw	No significant cytotoxicity and genotoxicity	Dobrzynska et al. 2014

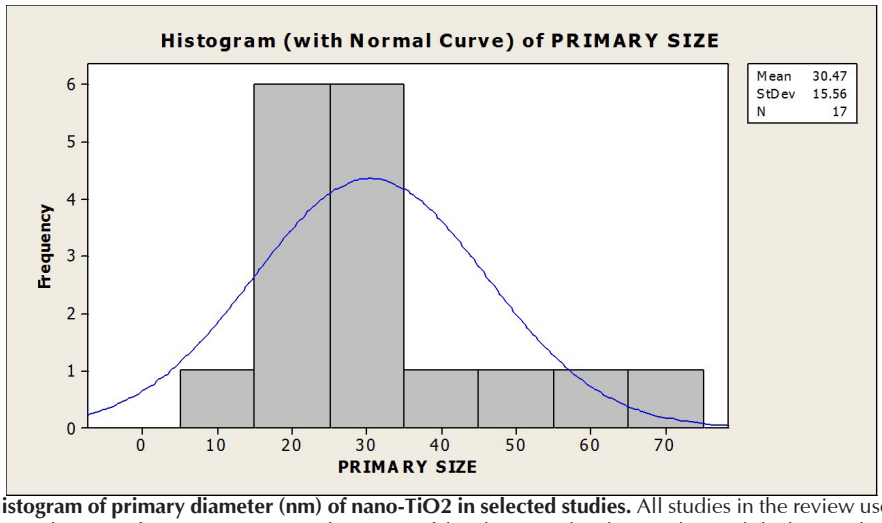

Figure 2: Histogram of primary diameter (nm) of nano-TiO2 in selected studies. All studies in the review used primary particles size < 100 nm with mean of 39.38 representing the centre of this diameter distribution data. While the standard deviation is 30.47 which is lower than mean denoting the diameter distribution from selected studies are concentrated from the mean.

Table III: Descriptive analysis on primary particles size of nano-TiO_2 in selected studies

Descriptive Statistics: PRIMARY SIZE

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
Primary Size	21	0	39.38	6.65	30.47	14.50	21.48	25.00	48.28

Variable	Maximum
Primary size	133.38

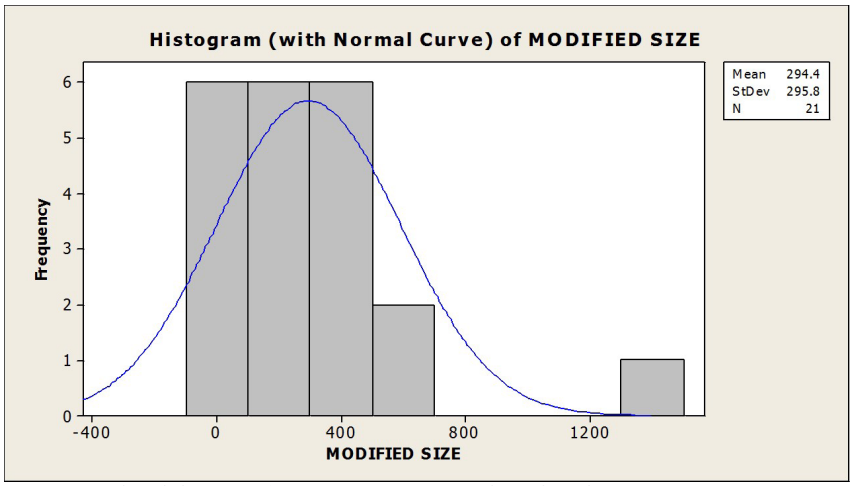

Figure 3: Histogram of modified diameter (nm) of nano-TiO2 in selected studies. The modified diameter has primary size of > 100 nm with mean of 294.4 and standard deviation of 295.8 suggested slightly un-concentrated form the mean.

Table IV: Descriptive analysis on nano-TiO_2 with modified particles size

Descriptive Statistics: MODIFIED SIZE									
Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
Modified Size	21	0	294.4	64.5	295.8	21.5	241.0	25.00	425.4
Variable	Maximum								
Modified Size	1341.0								

including EIF-2, mTOR signalling and regulation of eIF4 and p70S6K in human liver HepG2 cells upon exposed to nano-TiO_2 (39). Note that the mechanisms of nano-TiO2 toxicity are largely determined by their physio-chemical properties such as their size, shape, specific surface area, surface charge, catalytic activity, and the presence or absence of a shell and active surface groups.

CONCLUSION

This work suggests that nano-TiO_2 with primary size < 100 nm can be considered an ideal candidate for drug-delivery vehicle for targeted cancer therapy. This current study is limited to precisely determine the actual potential of nano-TiO_2 to be an effective and safe targeted cancer therapy agent as there is a need of more research described more comprehensive physio-chemical properties of nano-TiO_2. There is a need of meta-analysis in order to demonstrate statistically significant physio-chemical properties of nano-TiO_2 which could lead to comprehend development of this nanoparticles as cancer therapeutics.

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