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TiO2 Nanoparticles as a Common Component of Sunscreens: An Experimental Study of Dermal/Ocular Safety Assessment

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Abstract:

BACKGROUND: The safety evaluations of sunscreens containing Titanium Dioxide-Nanoparticles (TiO2-NPs) were done by dermal exposure, acute dermal and eye irritation/corrosion, and skin sensitization according to the guideline for Organization for Economic Co-operation and Development (OECD).

OBJECTIVES: The aim of our study was the evaluation of safety and toxicity of TiO2-NPs following acute sunscreen exposure.

METHODS: TiO2 and TiO2-NPs (20-40 nm and 98% purity) were purchased in the anatase crystal phase, and five types of concentration for sunscreens were made which were carried out in five different treatment groups in mice and rabbits.

RESULTS: In acute eye irritation using rabbits, the only irritation effect was observed in the conjunctivae area within one hour after administration both in TiO2-NPs group and TiO2-Ps. In acute dermal irritation using rabbits did not show a significant difference among groups in different concentrations and durations. Similarly, in a skin sensitization test using mice, contact hypersensitivity (CHS) did not show a significant difference ($P<0.05$) among groups in 15% concentration of TiO2 in the different durations after application.

CONCLUSIONS: Our finding demonstrates that TiO2-NPs and TiO2-Ps in sunscreens are relatively safe and did not induce statistically significant eye and dermal irritation and skin hypersensitivity.

Keywords:

Dermal irritation, Eye irritation, OECD, Skin sensitization, TiO2-NPs

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Introduction

Nanotechnology is a growing technology that is widely used in a variety of fields. The various applications of these types of products will create a health challenge for populations, such as workers and ultimately, consumers. For solving this problem and to ensure the safety of products, companies and regulators must implement the systematic risk assessment process for risk identification and characterization of ultrafine particles-types. For the primary risk assessment a minimum base set of toxicity screening tests is needed which is usually designed by OECD (Organization for Economic Co-operation and Development). These protocols are used for the safety investigation of high production volume (Warheit et al., 2007; Wu et al., 2009).

TiO2 (Titanium Dioxide) is an inert biological substance that has been previously classified as a very safe substance (Lademann et al., 2006). It is often used in the cosmetic, pharmaceutical, paint and paper industries. In the cosmetic industry, since about 1990 titanium dioxide has been used in sunscreens because of its ability to filter UV-A and UV-B radiation. The white color skin is one disadvantage of TiO2 in the use of sunscreens. This disadvantage can be solved by the small particle of nanoparticles of TiO2 while retaining the sunscreen feature. Due to changes of dimension, TiO2 nanoparticles may show different biological, chemical, optical, magnetic and structural properties and may induce differential toxicity (Beer, Dokkedahl, Wang, Sutherland, & Nyengaard, 2015; Lademann et al., 2006).

Despite numerous in vivo and in vitro studies, there is contradictory information about inflammation, DNA damages, cytotoxicity, allergy, cancer following dermal exposure of TiO2-NPs (Titanium Dioxide-Nanoparticles). The possible biological and safety effects of TiO2-NPs dermal exposure and absorption have not been well studied and more investigations on the potential health hazards of the TiO2-NPs are needed, especially the viewpoint of physicochemical properties such as concentration in products like sunscreens (Lademann et al., 2006; McSweeney, 2016; Smijs & Pavel, 2011).

Unlike the similar studies, our study is a type of regulatory scientific literature which is mostly in line with the work of administrations such as Therapeutic Good Administration (TGA). The aim of our study was the primary evaluation of safety and toxicity of TiO2-NPs following short-term sunscreen dermal exposure to clarify possible risk and impact of TiO2-NPs in two animal models of mice and rabbit in a variety of TiO2-NPs concentrations. The toxicity screening tests include dermal and eye irritation/corrosion and skin sensitization.

Material and Methods

Chemical: In our study, TiO2 and TiO2-NPs (20-40 nm and 98% purity examined by transmission electron microscope) in the anatase crystal phase purchased from International Fanavaran Araz Tajhiz Company.

Preparation of sunscreen: The five types of sunscreen were made to study the effects of sunscreen containing TiO2 particles.

1- Sunscreen containing about 10% TiO2-NPs (TiO2 10%, Glycerin 10%, emulsifier 13.5%, conservator 0.45%, liquid paraffin 3%, butylated hydroxytoluene

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0.05% and water 63%)

2- Sunscreen containing about 15% TiO2-NPs (TiO2 15%, Glycerin 10%, emulsifier 11.5%, conservator 0.45%, liquid paraffin 7.5%, butylated hydroxytoluene 0.05% and water 63%)

3- Sunscreen containing about 10% TiO2 (TiO2 10%, Glycerin 10%, emulsifier 13.5%, conservator 0.45%, liquid paraffin 3%, butylated hydroxytoluene 0.05% and water 63%)

4- Sunscreen containing about 15% TiO2 (TiO2 15%, Glycerin 10%, emulsifier 11.5%, conservator 0.45%, liquid paraffin 7.5%, butylated hydroxytoluene 0.05% and water 63%)

5- Vehicle Sunscreen (Glycerin 10%, emulsifier 13.5%, conservator 0.45%, liquid paraffin 10%, butylated hydroxytoluene 0.05% and water 66%)

Study condition: Our procedures on laboratory animals (rabbits and mice) were performed after approval by the Ethics Committee of the Faculty of the University of Tehran and conducted in accordance with the guidelines of Center for the Use and Care of Experimental Animals. The weight rabbits and mice in the study was 1.8±2 kg and 25±4 gr respectively. The temperature of the animal rooms was 20 °C (± 3 °C) for rabbits and mice. The relative humidity was 50-60%. Lighting program was 12:12. The conventional laboratory diets and drinking water were freely provided

Screening tests based on OECD protocols that included acute eye irritation (No, 2012) and acute dermal irritation/corrosion (Cooperation & Development, 2002) and other tests such as contact hypersensitivity (Yuen & Halliday, 1997).

Acute eye irritation/corrosion (OECD 405 guideline): Healthy young adult rabbits with a weight of 1.5±2 were used. Buprenorphine 0.01 mg/kg was administered subcutaneously for reducing possible pain and distress, sixty minutes before testing. 0.1 ml of the sunscreens containing TiO2-NPs (10% and 15%), TiO2 (10% and 15%) and control were separately applied in a single dose to one of the eyes of the rabbit, and the untreated eye served as the control. To identify reversible effects, the rabbits were observed for 21 days after administration of the TiO2-NPs and TiO2. The eyes were evaluated 1, 24, 48 and 72 h after treatment. The degree of eye irritation/corrosion was evaluated by scoring lesions of conjunctiva, cornea, and iris.

Acute dermal irritation/corrosion (OECD 404 guideline): Healthy young adult rabbits with a weight of 1.5±2 were used. Approximately 24 h before the study, fur was removed from the dorsal area of rabbits in five zones with an area of 6 cm2. 0.5 ml of the sunscreens containing TiO2-NPs (10% and 15%), TiO2 (10% and 15%) and control was separately applied in a single dose to some of the shaved areas, and the untreated shaved areas served as the control. At the termination of the test (usually 4 h after exposure), sunscreen was removed and areas were cleaned. To identify reversible effects, the rabbits were observed for one-day after administration of the TiO2-NPs and TiO2. The skin was evaluated 60 min, 24, 48 and 72 h after treatment and was evaluated by the scoring of oedema, erythema, and eschar formation.

Contact Hypersensitivity (CHS): Animals were randomly divided into five groups and housed in cages for at least seven days before sunscreen usage. The mice abdominal hair was shaved in all of the groups, 24 h before sunscreen usage. Mice were sensi-

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tized topically with 100 µl of a %5 DNCB (2, 4-Dinitrochlorobenzene) in olive oil: acetone solution (1:4) on the ventral shaved abdomen for five days. On the sixth day, 10 µl of a %1 DNCB olive oil, acetone solution was each applied to both sides of the right ear. The left ear was exposed to a vehicle alone. For CHS assay, sunscreens containing TiO2-NPs (10% and 15%), TiO2 (10% and15%w) and control groups were used for sensitization and challenge on the right ear, and the left ear was as a control. Measurement of ear thickness to determine the cutaneous manifestation of CHS was performed according to the method of Gad et al. Also, ear thickness was measured at 24, 48 and 72 h.

Data analysis: For ordinal data, we used nonparametric test (Kruskal Wallis and Mann-Whitney test for comparison of groups and the Wilcoxon test for inter-group comparison), and numerical data were analyzed parametrically (t-test) using software SPSS version 21 with significant level of $P<0.05$.

Results

Acute eye irritation/corrosion: After application of TiO2-NPs and TiO2-Ps sunscreen for evaluation of acute eye irritation effects in the rabbit eyes in different concentrations and durations, the only acute eye irritation effect was observed in the conjunctivae area within one hour after administration both in TiO2-NPs group and TiO2-Ps and different concentrations. Also, significant difference ($P<0.05$) was shown among the different concentrations of TiO2 sunscreen with the control group in the conjunctivae area within one hour after administration (Table 1). The primary irritation index (PII) for the test sunscreens was 0.0625 that showed very little eye irritation during the experiment period. Furthermore, the reversibility of effects was not observed up to 21 days after administration of the TiO2-NPs and TiO2.

Table 1. Acute eye iiritation/corrosion tset of sunscreens containig TiO2-NPs and TiO2-Ps in rabbits. [a]Scoring according to OECD test guideline 405 (2012). Mean score of eye responses=(total score of cornea + total score of iris + total score conjunctivae + total score chemosis)/4; bPrimary irritation index (PII) = (mean score at 1 h + mean score at 24 h + mean score at 48 + mean score at 72 h)/4.

	TiO2-NPs	TiO2-Ps
Dermal response (mean score)a	0.25	0.25
1 hr after application of sunscreens	0.25	0.25
24 hr after application of sunscreens	0	0
48 hr after application of sunscreens	0	0
72 hr after application of sunscreens	0	0
Primary irritation index (PII)b	0.0625	0.0625

Table 2. Acute dermal iiritation/corrosion tset of sunscreens containig TiO2-NPs and TiO2-Ps in rabbits. [a]Scoring according to OECD test guideline 404 (2002). Mean score of eye responses=(total score of erythema and eschar formation + total score of edema formation)/4; bPrimary irritation index (PII) = (mean score at 1 h + mean score at 24 h + mean score at 48 + mean score at 72 h)/4.

	TiO2-NPs	TiO2-Ps
Dermal response (mean score)a	0	0
1 hr after application of sunscreens	0	0
24 hr after application of sunscreens	0	0
48 hr after application of sunscreens	0	0
72 hr after application of sunscreens	0	0
Primary irritation index (PII)b	0	0

Acute dermal irritation/corrosion: In the evaluation of acute dermal irritation effects, the number of scores of oedema, erythema, and eschar formation was zero, and no significant difference was observed among groups in different concentrations and times ($P<0.05$). Besides, the primary irritation index (PII) for the test sunscreens was 0.

Contact Hypersensitivity (CHS): The measurement of ear thickness to determine the cutaneous manifestations of CHS did

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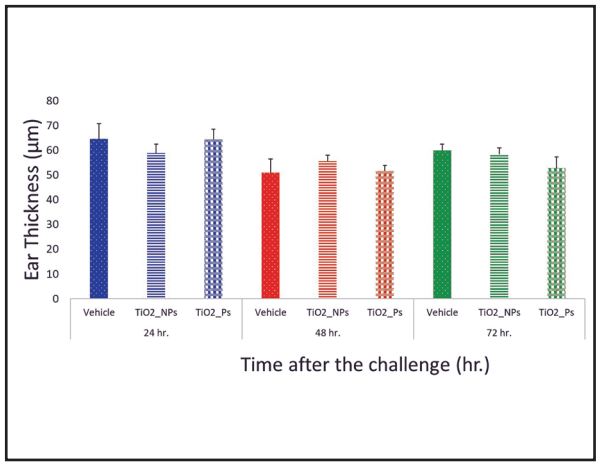

Figure 1. Contact hypersensitivity in mice ear. Sunscreen containing TiO2-NPs of 15%, Sunscreen containing TiO2-Ps of 15%, vehicle Sunscreen without TiO2-NPs and TiO2-Ps.

not show a significant difference (P<0.05) among groups in 15% concentration of Tio2 at 24, 48 and 72 h after application (Fig 1).

Discussion

Nowadays, the use of TiO2-nanoparticles in products like sunscreens is rising due to their ability to filter Ultraviolet-A and B and keep skin colorless. However, a significant concern of cosmetic products containing nanoparticles is their effects on health (Park et al., 2011; Smijs & Pavel, 2011).

Our study examined the safety concerns about sunscreen containing titanium dioxide (TiO2) nanoparticles (NPs). Three safety assays considered in this study for health risk assessment in animal models that include acute eye and dermal irritation and contact hypersensitivity.

Our results in different concentrations of TiO2-NPs and TiO2-Ps did not show a significant difference in the specific timetable of each test. The only significant difference was demonstrated in acute eye irritation test related to the conjunctivae evaluation within one hour after sunscreen application containing TiO2-NPs and TiO2-Ps in a concentration of 10% and 15% in comparison to control groups.

Several studies have been annually conducted to assess the safety risks associated with the use of sunscreens containing TiO2-NPs in vitro and in vivo models. The Australian Therapeutic Goods Administration (TGA) published two reviews on these issues for the identification of unacceptable and possible risks of harm/toxicity to consumers. In these reviews TiO2-NPs were reported as ingredients in sunscreens that were unlikely to cause harm/toxicity. In fact, for induction of toxicity dermal absorption is required following systemic absorption (McSweeney, 2016).

Xiangliang Yang et al. showed nano titanium dioxide in physiological pH causes the formation of protein tyrosine nitration in mouse skin homogenate. However, the authors pointed out further studies are needed to evaluate the relationship between photocatalytic protein tyrosine nitration and chronic cutaneous diseases and more attention should be given in the production and utilization process of products containing TiO2-NPs such as sunscreens (Lu et al., 2008).

In the study of DeLouise et al., allergic contact dermatitis test was used to survey the interaction of nanomaterials and allergic responses with mouse skin. Their finding for the first time suggested that nanoparticles modulate the elicitation phase of the allergic responses in the skin (Jatana, Palmer, Phelan, & DeLouise, 2017).

In the study of Lucian et al., exposure to TiO2 nanoparticles with pre-existent skin barrier dysfunction/defect could exacerbate atopic dermatitis symptoms and show initiation of skin pathologies by histamine discharge (Mocan et al., 2016).

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To address immunosuppressive effects of TiO2-NPs, following dermal exposure by contact hypersensitivity test in mice, Wimolnut Auttachoat et al. showed that TiO2-NPs dermal exposure caused irritancy and potential hypersensitivity responses (Auttachoat, McLoughlin, White Jr, & Smith, 2014).

In a study of safety evaluation of zinc oxide nanoparticles with dermal irritation and corrosion, and skin sensitization test, according to the guideline for OECD, Sung-Hwan Kim et al. reported that nano-sized ZnO used in their study was relatively safe (Kim et al., 2016). Also, in a review article of toxicology data from TiO2-NPs by Jinshun Zhao et al., in most dermal exposure studies, whether in vivo or in vitro, TiO2 NPs do not penetrate the stratum corneum (SC) and cannot cause skin harm/toxicity (Shi, Magaye, Castranova, & Zhao, 2013).

The opinion of European Union Scientific Committee on consumer safety (SCCS) for Titanium Dioxide (nano form) as UV-filter in dermally applied cosmetic product is as follows: In the dermal irritation/corrosion, it appears that the TiO2 nanomaterials are either mild or non-irritant to skin; In the mucous membrane irritation/eye irritation, eye irritation potential of nano-TiO2 appears to be low; In the skin sensitization, TiO2 nanomaterials appear to be a weak or non-sensitizer for skin applications, our findings are in accordance with their reporting(Henkler et al., 2012).

The finding of Kwangsik Park et al. provided evidence that sunscreen nanoparticles of TiO2 did not show corrosive and irritant effects in a rabbit model (Choi et al., 2014).

In conclusion, to evaluate the adverse effects of TiO2NPs- sunscreens, a minimum base set of toxicity/safety screening tests, including dermal and eye irritation/corrosion and, skin sensitization were performed in accordance with OECD test guidelines. The dermal toxicity/safety information about this study can assist in determining the risk of TiO2NPs sunscreens to consumers, and these findings indicate that there were no significant adverse effects of TiO2 nanoparticles sunscreens through toxicity/safety screening tests of OECD and contact hypersensitivity in animal models. Finally, it should be noted that the safety data in this study does not include permeability and any other organ risk assessment.

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Conflicts of interest

The author declared no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۷، دوره ۱۳، شماره ۱، ۸۶-۷۹

نانوذرات تیتانیوم دی اکسید به عنوان جزء رایج کرم‌های ضدآفتاب: مطالعه تجربی ارزیابی ایمنی/سمیت

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(دریافت مقاله: ۲۹ مرداد ماه ۱۳۹۷، پذیرش نهایی: ۵ آذر ماه ۱۳۹۷)

چکیده

زمینه مطالعه: برای ارزیابی ایمنی کرم‌های ضد آفتاب حاوی NP-TiO۲ به دنبال مواجهه کوتاه مدت پوست با کرم‌های ضد آفتاب، آزمون‌های تحریک حاد پوستی و چشم و حساسیت پوست بر اساس دستورالعمل‌های سازمان همکاری و توسعه اقتصادی (OECD) انجام شد.

هدف: هدف از این مطالعه ارزیابی ایمنی و سمیت NP-TiO۲ها پس از مواجهه کوتاه مدت پوستی و مخاطی بود.

روش کار: TiO۲ و NPs-TiO۲ (با درجه خلوص ۹۸٪ و اندازه ۲۰-۴۰ nm) در فاز بلور آناتاز خریداری شدند و به دنبال آن پنج نوع کرم ضد آفتاب ساخته شد که در آزمون‌های مختلف بر روی حیوانات انجام شد.

نتایج: در تحریک حاد چشم با استفاده از خرگوش، تنها اثر تحریک در ناحیه ملتحمه یک ساعت پس از تجویز در هر دو کرم حاوی NPs-TiO۲ و Ps-TiO۲ مشاهده شد. در تحریک پوستی حاد با استفاده از خرگوش، اختلاف معنی‌داری بین گروه‌ها در غلظت و زمان‌های متفاوت وجود نداشت. به طور مشابه، در آزمایش حساسیت پوستی با استفاده از موش، آزمون حساسیت تماسی (CHS) تفاوت معنی‌داری ($P>۰/۰۵$) در گروه‌ها در غلظت ۱۵٪ Tio۲ در زمان‌های مختلف پس از استفاده نداشت.

نتیجه گیری نهایی: یافته‌های ما نشان می‌دهد که NP-TiO۲ها و Ps-TiO۲ در کرم‌های ضد آفتاب نسبتاً ایمن هستند و باعث التهاب چشم و پوست و حساسیت پوستی نمی شوند.

واژه‌های کلیدی:
تحریک پوست، تحریک چشمی، OECD، حساسیت پوستی، NPs-TiO۲

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