Review

Mechanisms of Mycotoxin-induced Dermal Toxicity and Tumorigenesis Through Oxidative Stress-related Pathways

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Abstract: Among the many mycotoxins, T-2 toxin, citrinin (CTN), patulin (PAT), aflatoxin B1 (AFB1) and ochratoxin A (OTA) are known to have the potential to induce dermal toxicity and/or tumorigenesis in rodent models. T-2 toxin, CTN, PAT and OTA induce apoptosis in mouse or rat skin. PAT, AFB1 and OTA have tumor initiating properties, and OTA is also a tumor promoter in mouse skin. This paper reviews the molecular mechanisms of dermal toxicity and tumorigenesis induced in rodent models by these mycotoxins especially from the viewpoint of oxidative stress-mediated pathways. (DOI: 10.1293/tox.2013-0062; J Toxicol Pathol 2014; 27: 1–10)

Key words: dermal toxicity, dermal tumorigenesis, T-2 toxin, citrinin, patulin, aflatoxin B1, ochratoxin A

Introduction

Mycotoxins are fungal metabolites known to be harmful to human and animal health. To date, there are many reports of disorders caused by mycotoxins in the digestive, urinary, immune and reproduction systems[1], and the importance of oxidative stress through lipid peroxidation has been stressed as a trigger of mycotoxin-induced toxicity in these systems[2–6]. Recently, Doi and Uetsuka[7] reviewed the molecular mechanisms of neurotoxicity induced in rodent models by four kinds of mycotoxins, T-2 toxin, macrocyclic trichothecenes, fumonisin B1 (FB1) and ochratoxin A (OTA), from the viewpoint of oxidative stress-associated pathways.

The FAO[8] and WHO[9] have highlighted the need for toxicological evaluation of mycotoxins through dermal exposure. This is important because the skin is the major interface between the body and surrounding environment, and there is a chance that the skin of grain handling workers as well as of domestic animals is exposed to mycotoxins[10–12]. Concerning this point, it has been shown that such mycotoxins as aflatoxin B1 (AFB1)[13–15] and T-2 toxin[14, 16, 17] readily penetrate through human and animal skin and cause systemic toxic effects in their respective organs and also in the brain[18]. Recently, Boonen *et al.* examined the transdermal kinetics of seven kinds of mycotoxins, AFB1, OTA, FB1,

citrinin (CTN), zearalenone (ZEN) and T-2 toxin, using human skin in an *in vitro* Franz diffusion cell setup[19], and they reported that except for FB1, all mycotoxins penetrate through the skin and that OTA shows the highest penetration[19]. However, there have been few reports of toxic effects of mycotoxins on human skin.

Except for skin lesions induced by T-2 toxin[20–25], only limited information on mycotoxin-induced dermal toxicity has been available even in animal models. However, during the last decade, several researchers have added more information on dermal toxicity and/or tumorigenesis induced in mice by topical application of AFB1[15], patulin (PAT)[26, 27], CTN[12] and OTA[28, 29]. This paper reviews the molecular mechanisms of dermal toxicity and tumorigenesis experimentally induced in mice or rats by T-2 toxin, CTN, PAT, AFB1 and OTA especially from the viewpoint of oxidative stress-related pathways.

Mycotoxin-induced Dermal Toxicity and Tumorigenesis

T-2 toxin

T-2 toxin is a cytotoxic secondary fungal metabolite that belongs to the trichothecene mycotoxin family. It is produced by various species of *Fusarium* (*F. sporotrichioides, F. poae, F. equiseti* and *F. acuminatum*), which can infect corn, wheat, barley and rice crops in the field or during storage[30, 31]. T-2 toxin is a well-known inhibitor of protein synthesis through its high binding affinity to peptidyl transferase[32]. Subsequent inhibition of the peptidyl transferase reaction can trigger a ribotoxic stress response that activates c-Jun N-terminal kinase (JNK)/p38 mitogen-activated pro-

tein kinases (MAPKs)[33]. In addition, T-2 toxin inhibits the synthesis of DNA and RNA probably secondary to inhibition of protein synthesis[32, 34], interferes with the metabolism of membrane phospholipids and increases liver lipid peroxides (LPOs)[35, 36]. Moreover, oxidative stress is thought to be the main factor underlying the T-2 toxin-induced toxicity[3–5, 7].

As mentioned above, it has been reported that topical exposure to T-2 toxin could induce histopathological changes in the skin of several animal species, and Yarom et al. suggested that T-2 toxin-induced epidermal degeneration might be secondary to ischemia brought about by microvessel degeneration in the dermis[22]. In 1999, Albarenque et al. started a series of studies to clarify the mechanisms of T-2 toxin-induced dermal toxicity using Wistar-derived hypotrichotic WBN/ILA-Ht rats[37, 38] focusing on the expression of apoptosis-related oncogenes and cytokines[10]. In their first study, they clarified that after topical application of T-2 toxin, depression of proliferating activity starts at 3 h and that apoptosis of basal cells starts soon after and becomes prominent at 12 h in the epidermis, while capillary and small vessel endothelial degeneration develops at 6 h in the dermis[10]; this suggests the direct toxic effect of T-2 toxin on the epidermis[10]. This is the first report of mycotoxin-induced apoptosis in the skin[10].

Thereafter, using the same experimental system, Albarenque et al. showed that the expression of oncogenes (c-jun and c-fos) as well as cytokines (TNF-α and IL-1β) mRNAs is significantly elevated prior to the peak time of apoptosis in keratinocytes after topical exposure to T-2 toxin[39, 40]. They also reported that the level of TGF-β1 mRNA of the whole skin tissue shows a slight elevation from 6 to 12 h and reaches a significantly higher level at 24 h and that the increase in signals of TGF-β1 mRNA detected by the in situ hybridization method starts at 3 h in the epidermis and progresses thereafter both in the epidermis and dermis[41]. Later, using rat keratinocyte primary cultures, they also showed that c-fos and c-jun and TNF-α and IL-1β play an important role in the development of T-2 toxin-induced apoptosis in keratinocytes[42].

C-fos is a type of immediate-early response gene, and its activation with other factors such as c-jun occurs as an early response to cell injury, resulting in an increase in the sensitivity of keratinocytes to apoptosis[43], and the expression of c-fos is said to precede the initiation of apoptosis or to be concomitant with apoptosis in many systems[43–45].

Keratinocytes can release pro-inflammatory cytokines such as TNF-α and IL-1β when they have been injured[46, 47]. There are many reports suggesting the possible role of TNF-α as an apoptosis-inducer in different kinds of cells including keratinocytes[48, 49]. TNF-α can interact with its receptors[50], and signals from the receptors are related to the induction of some genes and proteins such as c-myc, c-fos and caspase, resulting in the induction of apoptosis[51]. TGF-β1 is a multifunctional cytokine and is known as a negative growth regulator of normal epithelial cells[52], and human keratinocytes can undergo apoptosis after initial

growth arrest under the effect of TGF-β1[53]. TGF-β1 may have a relation to the early depression of epidermal basal cell proliferating activity in rat skin following topical application of T-2 toxin[41].

As mentioned above, trichothecenes mycotoxins trigger a ribotoxic stress response that activates JNK/p38[33], and JNK/p38 stimulates immediate-early genes, c-fos and c-jun, both of which encode components of transcription factor activator protein-1 (AP-1)[54]. In this regard, the c-fos gene plays an important role in the early phase of T-2 toxin-induced apoptosis in the lymphoid and hematopoietic tissues in mice and rats[55], and T-2 toxin increases expression of both oxidative stress-related genes and apoptosis-related genes (c-fos and c-jun), resulting in the induction of hepatocyte apoptosis in mice[4]. Moreover, T-2 toxin is also reported to cause oxidative stress and subsequent activation of MAPK pathways in pregnant and fetal rat tissues, resulting in the induction of apoptosis in these tissues[56].

To summarize, T-2 toxin-induced dermal toxicity is considered to occur as follows. T-2 toxin causes oxidative stress, which induces a ribotoxic stress response and subsequent activation of MAPK pathways. Then, this stimulates expression of c-fos and c-jun, resulting in the induction of keratinocyte apoptosis. In addition, the keratinocytes affected primarily by ribotoxic stress release TNF-α and IL-1β, and these cytokines are also thought to be involved in the mechanisms of T-2 toxin-induced keratinocyte apoptosis.

To date, there have been no reports of T-2 toxin-induced skin tumorigenesis. In this regard, Lambert et al. reported that deoxynivalenol, one of the trichothecene mycotoxins, induces a mild diffuse squamous hyperplasia in the epidermis but shows no potential for initiation or promotion when topically applied as part of a two-stage skin tumorigenesis treatment regimen in Sencar mice[57].

Citrinin (CTN)

CTN is a secondary metabolite of several fungal species belonging to the genera *Penicillium, Aspergillus* and *Monascus*. It contaminates various commodities of plant origin, cereals in particular, and is usually found together with another nephrotoxic mycotoxin, OTA[58, 59]. CTN triggers nephropathy and hepatotoxicity as well as renal adenoma formation in various cellular and animal models[60–63]. To date, the mechanisms of CTN-induced *in vivo* toxicity have not fully been understood[58], although several studies have shown the involvement of reactive oxygen species (ROS) in CTN-mediated toxicity characterized by apoptosis in certain *in vitro* models[64–68].

Kumar et al. were the first to investigate molecular mechanisms of CTN-induced dermal toxicity from the viewpoints of oxidative stress, DNA damage, cell cycle arrest, and apoptosis in mouse skin[12]. They showed that CTN under *in vivo* condition has the ability to cause oxidative stress, which is indicated by significant depletion of glutathione (GSH) as well as inhibition of glutathione peroxidase (GPx) and catalase activities, along with an increase in LPOs and protein carbonyl content, and subsequent ROS-

mediated DNA damage as evaluated by the comet assay in mouse skin upon topical exposure to CTN (25–100 µg/mouse for 12–72 h)[12], as reported in the above-mentioned *in vitro* studies[64–68].

ROS-mediated DNA damage in mouse skin leads to enhanced expression of p53 and p21^{waf1} that causes cell cycle arrest at the G0/G1 and G2/M phases[12] as reported by Abbas and Dutta in *in vitro* models[69], to enhanced Bax/Bcl2 ratio and cytochrome c release, and to activated caspase 9 and 3 but not caspase 8, which result in apoptosis through the mitochondria-mediated pathway[12]. The p53 protein plays a key role in the DNA damage response pathway by transmitting a variety of stress signals associated with antiproliferative cellular responses that lead to apoptosis[70], and the lack of enhancement in caspase 8 activity indicates that the extrinsic or death receptor pathway of apoptosis is not activated by CTN in mouse skin. Moreover, Kumar *et al.* clarified that topical treatment of bio-antioxidants such as butylated hydroxyanisole, quercetin and α-tocopherol abolishes CTN-induced oxidative stress, cell cycle arrest and apoptosis, confirming the direct involvement of ROS in CTN-induced toxicological manifestations in mouse skin[12].

To summarize, CTN under *in vivo* conditions has the ability to cause oxidative stress and ROS-mediated DNA damage in mouse skin upon topical exposure, leading to enhanced expression of p53, p21^{waf1} and BAX proteins that causes cell cycle arrest at the G0/G1 and G2/M phases and apoptosis in mouse keratinocytes through the mitochondria-mediated pathway.

To date, there have been no reports of CTN-induced skin tumorigenesis. However, as mentioned above, CTN treatment causes prominent DNA damage, suggesting its genotoxic and mutagenic potential in the skin[12]. Moreover, although the cell cycle arrest by CTN may permit DNA repair, if it is faulty, it may allow proliferation of mutated cells, which is generally observed in cases of tumorigenesis[71].

Patulin (PAT)

PAT is a toxic chemical concomitantly produced by several species of mold, especially within *Aspergillus, Penicillium* and *Byssochlamys*. It is the most common mycotoxin found in apples and apple-derived products such as juice, cider, compotes and other food intended for young children. Exposure to this mycotoxin is associated with immunological, neurological and gastrointestinal outcome[72], and PAT has been classified as a group-3 carcinogen[73]. To date, studies on the mechanisms of PAT-induced toxicity have been done using various cell lines, focusing on the immunotoxic and genotoxic effects of the toxin[72], and it has been reported that PAT has the potential for inducing formation of ROS[74, 75], DNA damage[64, 76, 77], rapid activation of extracellular signal-regulated kinase (ERK)1/2[78] or of p38 and JNK[79], and effects on cell cycle distribution responsible for overexpression of a functional p53 protein[80].

Saxena *et al.* were the first to study the mechanisms of PAT-induced dermal toxicity in mice[26], and they reported that dermal exposure of PAT in mice for 4 h results in a dose-dependent (40–160 µg/animal) and time-dependent (up to 6 h) enhancement of ornithine decarboxylase (ODC) activity and increase in biosynthesis of polyamines[26]. Polyamines, cell proliferation, and apoptosis are tightly connected in a quite complex interplay[81], and polyamine levels within cells are regulated and modulated by the key enzymes that control polyamine biosynthesis, particularly ODC[82, 83]. Elevated ODC activity and increased biosynthesis of polyamines serve as a novel stimulus to induce the ataxia-telangiectasia mutated (ATM)-DNA damage signaling pathway and cell death in normal keratinocytes[84]. Wei *et al.* revealed the correlation of elevated ODC activity with apoptotic cell death in normal keratinocytes via the induced generation of reactive aldehydes and H_2O_2, followed by subsequent activation of the ATM-DNA damage response pathway[84]. Saxena *et al.* also reported that topical application of PAT (160 µg/mouse) for 24–72 h causes DNA damage depicted by alkaline comet assay and significant G1- and S-phase arrest along with induction of apoptosis in skin cells[26]. Moreover, they showed that PAT leads to overexpression of p21^{waf1}, Bax and p53 proteins and that PAT-induced apoptosis is mediated through the mitochondrial intrinsic pathway as revealed through the release of cytochrome c protein in cytosol, leading to enhancement of caspase 3 activity in mouse skin cells[26]. Thus, the PAT (160 µg/mouse)-induced cascade of events in mouse skin is considered to occur as follows. Induction of ODC activity generates polyamines and H_2O_2, which cause DNA damage, resulting in enhancement of p53 expression and subsequent cell cycle arrest at the G0/G1 and S phases through enhanced p21 expression along with induction of apoptosis through enhanced BAX expression and caspase 3 activity.

After that, Saxena *et al.* also reported that a single topical application of PAT (400 nmol/mouse) resulted in enhanced cell proliferation as evaluated by ^{3}H-thymidine uptake along with increased generation of ROS and activation of ERK, p38 and JNK MAPKs, in mouse skin[27]. PAT exposure also results in activation of downstream target proteins, c-fos, c-jun and NF-κB transcription factors. They thought that the observed early activation of JNK and NF-κB appears to be a direct response to PAT, while later activation of ERK, p38, c-jun, c-fos and NF-κB may be due to enhanced generation of ROS as reported by Benhar *et al.*[85]. This suggests that PAT-induced ROS acts as second messengers in intracellular signaling cascades and may mediate cell proliferation by activating ERK and p38 along with activation of downstream targets, c-jun, c-fos and NF-κB[85]. Moreover, Saxena *et al.* reported that specific inhibitors of MAPK, especially p38 and JNK, pathways are able to significantly suppress ^{3}H-thymidine uptake by keratinocytes in mouse skin following a single topical application of PAT (400 nmol/mouse), suggesting that p38 and JNK pathways may be involved in PAT-induced cell proliferation[27]. In addition, as mentioned above, PAT enhances the activity of ODC in mouse skin following a single application[26]. It is well known that ODC plays an important role in the regulation of cell proliferation, and it is stressed that ODC-related

hyperproliferation and altered differentiation in skin keratinocytes have been linked with deregulation of MAPK signaling pathways[86, 87].

To summarize, a single topical application of PAT (160 μg/mouse in the former case and 400 nmol/mouse in the latter case) to mouse skin brings about different events in mouse keratinocytes. Namely, in the former case, induction of ODC activity generates ROS, which causes DNA damage in keratinocytes, resulting in p53-mediated cell cycle arrest along with apoptosis through the intrinsic mitochondrial pathway. In the latter case, ROS generation activates MAPKs signaling pathway leading to transcriptional activation of downstream target proteins c-fos, c-jun and transcription factor NFκB, resulting in keratinocyte proliferation.

Regarding PAT-related skin tumorigenesis, Saxena *et al.* have shown that a single topical application of PAT (400 nmol/mouse) followed by twice weekly application of 12-tetradecanoyl phorbol myristate acetate (TPA) results in tumor formation (squamouse cell carcinoma) after 14 weeks[27]. In this PAT/TPA group, a significant increase in LPO activity and significant decreases in free sulfhydryls, catalase, superoxide dismutase (SOD) and glutathione reductase (GR) activities are observed. The DNA damaging ability of PAT in skin cells is in agreement with other *in vitro* findings in which PAT was shown to cause oxidative DNA damage in a few mammalian cells[26, 64, 77, 88]. On the other hand, Saxena *et al.* described that no tumors were observed when PAT was used either as a complete carcinogen (80 nmol) or as a tumor promoter (20 nmol) (single dose of 7,12-dimethylbenz[a]anthracene (DMBA) followed by twice weekly application of PAT) for 25 weeks[27]. However, it may be possible that prolonged exposure to PAT at a high dose may induce tumor promotion and cause further toxicological manifestations in the skin, since earlier reports have revealed that long-term exposure to PAT is tumorigenic in Wistar rats, leading to sarcoma at the injection site on subcutaneous administration[89], and causes benign tumors of the forestomach and glandular stomach in Sprague-Dawley rats after gavage treatment[90].

To summarize, PAT (400 nmol/mouse) clearly has a tumor-initiating ability in mouse skin through skin cell proliferation mediated by ROS-induced MAPKs signaling pathway leading to transcriptional activation of downstream target proteins, c-fos and c-jun and transcription factor NFκB. Moreover, PAT may probably have skin tumorigenic potential as a promoter and/or a complete carcinogen in mouse skin after long-term and higher-dose application.

Aflatoxin B1 (AFB1)

Aflatoxins are secondary metabolites of the molds *Aspergillus flavus*, *A. parasiticus*, *A. tamari* and *A. nominus*[91]. AFB1 is by far the most potent teratogen, mutagen and hepatocarcinogen of all aflatoxins[92]. The carcinogenic potential of AFB1 following oral administration has been shown in several animal species[93], while limited knowledge is available regarding the epidermal carcinogenesis

of AFB1, and therefore, the WHO has clearly highlighted the need for toxicological evaluation of aflatoxins through dermal exposure[94]. *In vitro* and *in vivo* studies have shown that glutathione-S-transferase (GST) plays a crucial role in modulation of AFB1-DNA adduct formation[95], and AFB1 is said to mediate oxidative damage through generation of ROS including the hydroxyl ion[96]. In addition, *in vitro* studies have also shown that AFB1 can stimulate the release of free radicals, which leads to chromosomal damage[97].

Rastogi *et al.* were the first to study the skin tumorigenic potential of AFB1 using a two-stage mouse skin tumor protocol[15]. In their study, skin topical application of AFB1 (80 nmol) as a tumor initiator, followed by twice weekly application of TPA (4 nmol) for up to 24 weeks, resulted in tumor formation (squamous cell carcinoma) after 13 weeks, but no skin tumorigenic potential was observed when AFB1 was used either as a complete carcinogen (16 nmol) or as a tumor promoter (4 nmol)[15]. They also showed that weekly topical application of AFB1 causes significant induction of cutaneous CYP1A monooxygenases without any effect on hepatic levels, while GST activity, which detoxifies a number of LPO products, is induced more in the liver than skin; they further showed that topical application of AFB1 also results in increased hepatic and cutaneous LPO with concomitant depletion of GSH content, indicating the induction of oxidative damage[15].

Later, Rastogi *et al.*[98] reported the protective effect of an alcoholic extract of the leaves of *Ocimum sanctum* on AFB1- and AFB1/TPA-induced skin tumorigenicity using the same experimental system used in their previous study[15]. *O. sanctum* is a well-known medical plant widely distributed throughout India[99], and the aqueous and alcoholic extracts from the leaves of this plant have been shown to possess antioxidant, anticarcinogenic, hepatoprotective, and radioprotective properties[100–102]. The skin of AFB1/TPA-treated animals demonstrated papillomatous growth comprising of proliferation of squamous cells, hyperkeratinization and keratin pearl formation while the skin of animals topically pretreated with *O. sanctum* leaf extract showed small papillomatous growth lacking pearl formation. In addition, pretreatment with *O. sanctum* leaf extract significantly decreased the number of skin tumors induced by AFB1/TPA.

The expression of cutaneous γ-glutamyl transferase (GGT) and glutathione-S-transferase-P (GST-P) protein increased after AFB1 or AFB1/TPA treatment, but pretreatment with *O. sanctum* leaf extract led to a reduction in the expression of these proteins[98]. GGT is considered to be a late marker of tumor progression, and its overexpression in hepatic and skin tumors has been well documented[103]; GST-P expression is also said to increase in chemically induced hepatic tumors[104]. Pretreatment with *O. sanctum* leaf extract led to the reduction of cutaneous phase I enzymes that had been elevated by AFB1 or AFB1/TPA treatment and to the elevation of cutaneous phase II enzymes, suggesting the possibility of impairment in reactive metabolites formation resulting in a reduction of skin carcinogenicity[98]. Moreover, pretreatment with *O. sanctum* leaf extract increased

the cutaneous GSH level and reduced cutaneous LPO levels that had been elevated by AFB1 or AFB1/TPA treatment[98]. Enhanced levels of GSH resulting from treatment with *O. sanctum* leaf extract may reduce the rate of LPO as well as decrease the expression of heat shock protein (HSP) 70 protein, which has been reported to be altered during carcinogenesis[105]. Since HSP70 is also involved in oxidative stress[106], it is quite likely that this protein may have a role in cancer, which is also associated with oxidative stress and inflammation[107, 108]. Thus, Rastogi *et al.* concluded that leaf extract of *O. sanctum* provides protection against AFB1/TPA-induced skin carcinogenesis by acting as an antioxidant, by modulating phase I and II enzymes and/or by exhibiting antiproliferative activity[98].

To summarize, it is considered that AFB1 acts as a skin tumor initiator through reactive metabolite formation, LPO-mediated oxidative stress, and GST-mediated AFB1-DNA adduct formation. Like in the case of PAT, AFB1 may also have skin tumorigenic potential as a promoter and/or a complete carcinogen in mouse skin after long-term and higher-dose application.

Ochratoxin A (OTA)

OTA is a fungal metabolite produced by *Aspergillus ochraceus* and *Penicillium verrucosum*. OTA is found in a variety of plant food products such as cereals. Because of its long half-life, it accumulates in the food chain[109, 110] and is frequently detected in the human plasma at nanomolar concentrations[111]. The main target organ for OTA toxicity is the kidney[112, 113], and OTA also has immunotoxic[114], teratogenic[115], genotoxic[116] and neurotoxic effects[117]. In addition, there is sufficient evidence in experimental animals for the carcinogenicity of OTA[112], although there is still insufficient evidence in humans.

Kumar *et al.* were the first to investigate the OTA-induced toxicity and tumorigenesis in mouse skin[29]. In their study, after a single topical application of OTA (20–80 μg/mouse for 12–72 h), significant DNA damage as assessed by alkaline comet assay along with an elevated γ-H2AX level, a sensitive marker of DNA damage, was detected in mouse skin[29]. In addition, the level of nuclear factor erythroid 2-related factor (Nrf2), the master regulator for maintaining the balance of ROS[118], in the nucleus decreased after 24 h of OTA exposure, indicating an inhibitory effect of OTA on Nrf2 signaling. OTA-induced Nrf2 suppression may cause significant depletion of GSH content as well as inhibition of the activities of catalase, GST and GR along with enhanced production of LPOs and protein carbonyls dose- and time-dependently, and this indicates increased generation of ROS and subsequently enhanced oxidative stress in mouse skin.

Kumar *et al.* also reported that OTA activates ERK1/2 in the early phase and then p38 and JNK in the later phase in mouse skin after topical exposure[29], and they suggested that the early activation of ERK1/2 is the result of a direct response to OTA but that later activation of p38 and JNK may be the result of OTA-induced ROS, which acts as secondary messengers in the intracellular signaling cascade in mouse

skin[85]. Moreover, they reported that exposure to OTA results in a significant increase in the proportion of cells in the G0/G1 phase with a concomitant decrease in S phase cells, followed by an increase in apoptosis through elevated expression of p53 and p21[waf1], enhancement of the Bax/Bcl-2 ratio and cytochrome c level, and increased activities of caspase 9 and 3 in mouse skin[29]. On the other hand, Kumar *et al.* reported that a single topical application of OTA (100 nmol/mouse) causes significant enhancement of short-term markers of skin tumor promotion such as ODC activity, DNA synthesis and hyperplasia as well as expression of cyclin-G1 and cyclooxygenase-2 (COX-2) in mouse skin[28]. The enhancement in ODC activity has been reported to occur in response to growth factors as well as promoters such as TPA[119], and the overexpression of cyclin-D1 and COX-2 proteins is said to play a role in cell proliferation and tumor promotion of various tissues including skin[120, 121].

To summarize, a single topical exposure of OTA at the dose level of 20–80 μg/mouse activates ERK1/2 directly and p38 and JNK through OTA-induced ROS, resulting in the induction of apoptosis through the mitochondrial pathway in mouse skin. On the other hand, a single topical exposure of OTA at a dose level of 100 nmol/mouse causes significant enhancement of short-term markers of skin tumor promotion in mouse skin.

In a two-stage mouse skin tumorigenesis protocol, Kumar *et al.* reported that a a single topical application of OTA (80 μg/mouse) followed by twice weekly application of TPA for 24 weeks leads to tumor formation (squamous carcinoma with proliferation of epidermal layers)[29]. They suggested that some cells damaged by a single topical application of OTA may pass though a p53-mediated cell cycle checkpoint by faulty repair, which may introduce mutations in OTA-induced animals, and subsequent application of TPA, a tumor promoter, fixes the mutations and confers a selective advantage in those cells, which leads to tumorigenesis[29]. They concluded that OTA has skin tumor-initiating properties under *in vivo* conditions, which may be related to oxidative stress, MAPK signaling and DNA damage in mouse skin[29].

Kumar *et al.* also reported that a single topical application of DMBA (120 nmol/mouse) followed by twice weekly application of OTA (50 nmol/mouse) for 24 weeks leads to tumor formation in mouse skin (squamous carcinoma with proliferation of epidermal layers), indicating the skin tumor promoting activity of OTA[28]. Moreover, based on the results of *in vitro* study using primary murine keratinocytes exposed to a noncytotoxic dose of OTA (5.0 μM), they proposed that OTA-induced cell proliferation seems to be responsible for skin tumor promotion by activating epidermal growth factor receptor (EGFR), MAPKs and Akt signaling involving NF-κB, AP-1 transcription factors, cyclin-D1 and COX-2 genes[28]. EGFR signaling leads to enhancement of phosphorylation of MAPKs as well as the activity of AP-1 and transcription factors and utilizes MAPK pathways to mediate its growth and stimulative effects[122], and MAPKs are said to play a crucial role in skin tumorigenesis[123–125]. EGFR also acts through the Akt pathway, which plays a

role in tumor promotion and progression[126]. The transcription factor AP-1 mediates gene regulation in response to a variety of extracellular stimuli including growth factors, cytokines, oncogenes, tumor promoters and chemical carcinogens[127], and upon activation, both transcription factors NF-κB and AP-1 translocate to the nucleus, where they bind to promoter regions of various target genes including cyclin-D1 and COX-2[128].

To summarize, OTA has skin tumor-initiating properties that may be related to oxidative stress, MAPK signaling and DNA damage, and OTA also has skin tumor-promoting properties that involve EGFR-mediated MAPKs and Akt pathways along with NF-κB and AP-1 transcription factors. Cyclin-D1 and COX-2 are target genes responsible for the tumor-promoting activity of OTA.

Conclusions

This paper reviewed the mechanisms of dermal toxicity and/or tumorigenesis induced in rodents by T-2 toxin, CTN, PAT, AFB1 and OTA.
(1) The T-2 toxin-induced cascade of events in rat skin is considered as follows. T-2 toxin brings about oxidative stress, which induces a ribotoxic stress response and subsequent activation of MAPK pathways. Then, this stimulates expression of c-fos and c-jun, resulting in keratinocyte apoptosis. In addition, TNF-α and IL-1β, which are released from keratinocytes primarily affected by ribotoxic stress, are also involved in T-2 toxin-induced keratinocyte apoptosis.
(2) CTN has the ability to cause oxidative stress and ROS-mediated DNA damage in mouse skin upon topical exposure, leading to enhanced expression of p53, p21^{waf1} and Bax proteins that causes cell cycle arrest at the G0/G1 as well as G2/M phases and apoptosis in mouse keratinocytes through the mitochondria-mediated pathway.
(3) PAT (160 µg) has a potential to induce DNA damage leading to p53-mediated cell cycle arrest along with apoptosis through the mitochondria-mediated pathway in mouse skin that may also be correlated with enhanced polyamine production as shown by induction of ODC activity. On the other hand, topical application of PAT (400 nmol) to mice results in cell proliferation, which is mediated by ROS-induced MAPKs signaling pathway leading to transcriptional activation of downstream target proteins c-fos, c-jun and transcription factor NFκB, and this is related to the skin tumor-initiating ability of PAT.
(4) AFB1 acts as a skin tumor initiator through reactive metabolite formation, LPO-mediated oxidative stress, and GST-mediated AFB1-DNA adduct formation. AFB1 may also have skin tumorigenic potential as a promoter and/or a complete carcinogen in mouse skin after long-term and higher-dose application.
(5) OTA has skin tumor-initiating properties that may be related to oxidative stress, MAPKs signaling and DNA damage in mouse skin. OTA also has skin tumor-promoting properties that involve EGFR-mediated MAPKs and Akt pathways along with NF-κB and AP-1 transcription factors. Cyclin D1 and COX-2 are the target genes responsible for the tumor-promoting activity of OTA.

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