Chapter 1

Drug-Induced Cutaneous Toxicity

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Additional information is available at the end of the chapter

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Abstract

The skin is the largest organ in the body and is continually exposed to external stimuli, such as chemical and environmental substances. Cutaneous toxicity can be broadly classified according to the mechanism of onset, namely: contact dermatitis, i.e., damage resulting from contact with a substance (irritant dermatitis, allergic contact dermatitis, chemical burns); photosensitivity, i.e., caused by combined effects of a substance and ultraviolet light (phototoxic dermatitis, photoallergic contact dermatitis); contact urticaria; chemical-induced acne; pigmentary disturbance; drug rash; hair disturbance; nail disturbance; or tumor-induced. This review outlines the function and structure of the skin, outlining characteristics of these types of cutaneous toxicity. In recent years, advances have been made in the development of pharmaceutical products targeting specific molecules or genes and nanotechnology-based pharmaceutical products, raising concerns about the onset of toxicity by novel mechanisms involving new pharmaceutical products. Therefore, it is important to understand the basic toxicity-related changes described herein.

Keywords: cutaneous toxicity, drugs, chemicals, toxicity studies

1. Introduction

Cutaneous adverse drug or chemical reactions in patients are not common. Among hospitalized patients, the incidence of adverse drug reactions concerning the skin ranges from 1% to 3%; however, the actual prevalence is much higher, as many mild forms of cutaneous adverse reactions are not reported [1]. We are constantly exposed to external stimuli, such as chemical and environmental substances, resulting in various skin symptoms. This article focuses on (1)

the function and structure of the skin and (2) characteristics of cutaneous toxicity of pharmaceutical products and chemical substances in humans and animals.

2. Function and structure of the skin

Skin is the largest organ of the body, covering the surface and accounting for approximately 15–20% of body mass. In addition to its constant barrier role, protecting the living body against external stimuli, skin is important for maintaining the body health (e.g., through regulation of body temperature, storage of fluids and electrolytes, and the synthesis of vitamin D) and also acts as an important component of the immune system. As a sensory organ, the skin can sense pain, touch, pressure, and temperature [2]. Histologically, the skin consists of the epidermis, dermis, and subcutaneous tissue. The epidermis is formed by keratinized squamous epithelia, stratified from the surface into the cornified layer, clear layer (only on the palms and soles), granular layer, spinous layer, and basal layer [3]. The epidermis also contains antigen-presenting Langerhans cells (mainly in the spinous layer); melanocytes (mainly in the basal layer), which produce melanin to protect epidermal cells against damage induced by ultraviolet light; and Merkel cells (mainly on the palms and soles), which are neuroendocrine cells. The dermis is composed of fibrous connective tissue with elastic and reticular fibers intermingled with collagen bundles, containing mast cells that are involved in allergic reactions, sweat glands, sebaceous glands, hair follicles, blood vessels, lymphatic vessels, and nerve fibers. Subcutaneous tissue is composed of loose connective tissue and subcutaneous adipose tissue. Adipose tissue is especially prominent in the footpads where it functions as a "shock absorber" and as an insulating layer [4]. Skin appendages are skin-associated structures that serve a particular function, including sensation, contractility, lubrication, and heat loss. They contain hairs (sensation, heat loss, filter for breathing, protection), sebaceous glands (secrete sebum onto hair follicles, which oils the hair), sweat glands (can produce sweat secreted with strong odor (apocrine) or with a faint odor (eccrine), and nails (protection). Hair growth occurs in three stages: anagen (growth phase), catagen (involution period), and telogen (resting phase during which hair shedding occurs) [5, 6]. The rate of hair growth and duration of the growth cycle vary in different areas of the body and are influenced by sex hormones and growth factors. Sebaceous glands are most often associated with hair follicles and produce sebum by holocrine secretion. Zymbal's gland is a specialized sebaceous gland in rodents located at the base of the external ear canal; the gland cells contain cytochrome P450 isoenzyme and peroxidases and are capable of chemical metabolism [7, 8]. Apocrine glands are distributed throughout the skin of most laboratory animals, whereas in humans they are located in axillary, pubic, and perianal areas, while they are only present in the plantar areas in rodents. There are a number of specialized apocrine glands, such as the anal sac gland of dogs, the ceruminous glands of the external ear canal, and the glands of Moll in the eyelids. Eccrine glands are found throughout the body in humans; however, these glands are limited to the footpads of carnivores and rodents.

In preclinical studies, cutaneous toxicity is rarely encountered, except in cutaneous application, intradermal administration, and subcutaneous administration. Cutaneous toxicity primarily

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involves either a direct local inflammatory reaction to the drug without involvement of an immunological mechanism or an indirect inflammatory reaction associated with a systemic manifestation [6]. In cutaneous application studies, both epidermis and skin appendages are important factors in transdermal drug absorption [9]. Experimental animals such as guinea pigs, monkeys, and swine exhibit similar absorption characteristics to humans [5]. Of all laboratory animals, swine skin is most structurally comparable to human skin [4]. Swine and humans have comparable stratum corneum, epidermal thickness, and hair follicle density, as well as similar chemical composition of the stratum corneum. Rodents have much thinner skin (especially the epidermis) with greater permeation compared to humans [4]. In general, skin is thicker over the dorsal and lateral surfaces and thinnest on the ventral and medial surfaces. Areas of skin that contact the ground, such as footpad and heels, have the thickest epidermis (**Figure 1**). The extent of transdermal drug absorption differs according to skin location. Sites in order of favorable absorption, due to the skin thickness, are the abdomen, forehead, palms, and soles of feet [5]. It should be noted that skin thickness varies considerably during the hair cycle (**Figure 2**). Skin thickness during the anagen stage is thickest and is thinnest during the catagen stage in rodents and rabbits. If the skin is damaged, the biological protective barrier function decreases, leading to a significant increase in drug absorption, which results in intensified systemic toxicity [10]. Microsomal enzymes in keratinocytes are capable of metabolizing topically applied chemicals, thus rendering them inactive or active. Dimethylbenz(a)anthracene (DMBA) becomes a potent skin carcinogen after metabolic activation by keratinocytes [11].

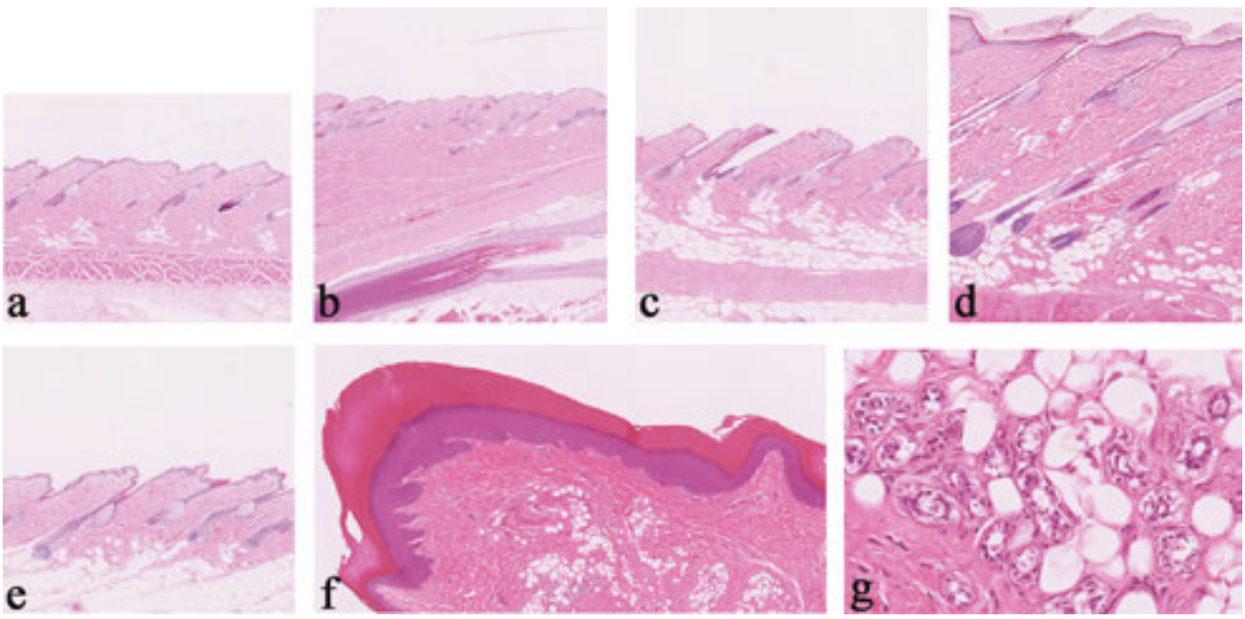

Figure 1. Comparative histology of different skin locations in rats. (a) Scalp region at the vertex. (b) Nose region. (c) Inguinal region. (d) Back region. (e) Abdominal region. (f) Footpad region. (g) Eccrine sweat gland in the footpad. Note that scalp, inguinal, and abdominal skin are thin. In contrast, back and footpad skin are thicker. Footpad skin is the thickest, especially the stratum corneum and epidermis. Eccrine sweat glands are located only in the footpad of rodents; however, these glands are found throughout the human body.

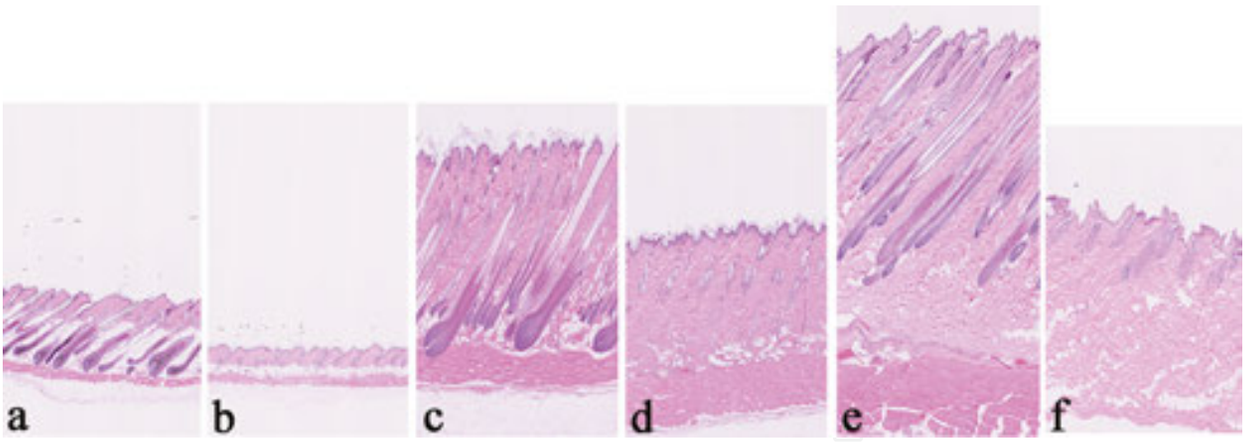

Figure 2. Comparative histology of back skin during different hair cycle stages. (a) Anagen stage in the mouse. (b) Catagen stage in the mouse. (c) Anagen stage in the rat. (d) Catagen stage in the rat. (e) Anagen stage in the rabbit. (f) Catagen stage in the rabbit. Note that skin thickness is thickest at the anagen stage and thinnest at the catagen stage in rodents.

3. Types of cutaneous toxicity (Table 1)

Cutaneous toxicity can be classified according to the mechanism of onset into the following: (1) contact dermatitis, i.e., damage resulting from contact of the skin with a drug (irritant dermatitis, allergic contact dermatitis, and chemical burns); (2) photosensitivity, caused by the combined effect of a chemical substance and ultraviolet light (phototoxic dermatitis and photoallergic contact dermatitis); (3) contact urticaria; (4) chemical-induced acne; (5) pigmentary disturbance; (6) drug rash; (7) hair disturbance; (8) nail disturbance; and (9) tumor-induced. Cutaneous toxicity can also be classified according to the route of exposure, i.e., either due to systemic effects or local irritation of the skin (local toxicity) [5, 6].

3.1. Contact dermatitis

Contact dermatitis is skin inflammation occurring as a result of direct contact of the skin with a drug that can be classified into the following three types, according to the mechanism of onset.

3.1.1. Irritant dermatitis

Irritant dermatitis is an inflammatory change caused by direct irritation of the skin that can be either acute or cumulative. Activation of mast cells, complement or prostaglandin synthesis results in reversible damage to the skin, observed as irritation within 4 hours following topical application of the chemicals. Irritant dermatitis is characterized by inflammatory cell infiltration, acanthosis, epidermal hyperkeratosis, and hyperplasia associated with other epidermal changes such as erosion/ulcer, necrosis, or vesicle formation [11]. Irritant dermatitis depends on the severity of the irritants and duration of their exposures [5, 6] (**Figure 3**). If the damage to the skin is irreversible, the lesion is clinically referred to as corrosion, which is characterized by full thickness necrosis of the epidermis penetrating into the underlying dermis [11]. In preclinical

studies of topical application agents, skin irritation testing is conducted using rabbits or guinea pigs to evaluate drug-induced irritation using the Draize method (with a 5-grade score based on macroscopic assessment of the severity of erythema, crusting, and edema) (**Figure 4** and **Table 2**). The Draize test consists of application of the chemical to the test site on shaved dorsal skin. The test sites undergo gross evaluation at 6, 24, and 72 postapplication.

Classification	Type	Definition and characteristics
A. Classification according to the route of exposure to the drug	Cutaneous toxicity due to systemic effect Local irritation of skin (local toxicity)	
B. Classification according to the mechanism of onset		
1. Contact dermatitis	Irritant dermatitis	Skin inflammation occurring as a result of direct contact of the skin with a drug, without involvement of an immune mechanism
	Allergic dermatitis	Skin inflammation upon re-exposure to a drug that had been previously administered and bound as a hapten to a protein in the skin to become immunogenic (type IV allergic reaction)
2. Photosensitivity	Phototoxic dermatitis	A condition caused by a drug with covalent binding as a result of a photochemical reaction with ultraviolet light
	Photoallergic dermatitis	Skin inflammation upon re-exposure to a previously administered drug that absorbed ultraviolet light and was transformed to act as a hapten to bind with a protein in the skin to become immunogenic (type IV allergic reaction)
3. Contact urticaria		Acute erythema with involvement of histamine release from mast cells (increased vascular permeability), occurring soon after contact with the drug
4. Chemical acne		Inflammation of hair follicles due to excessive keratin and sebum in hair follicles
5. Pigmentary disturbance	Hyperpigmentation	A condition occurring in association with increased melanin production due to activation of melanocytes, hemosiderin deposition due to hemorrhage, or deposition of the drug itself
	Hypopigmentation	A condition occurring in association with loss of melanin or selective damage to melanocytes
6. Drug rash (cutaneous reaction)	Toxic epidermal necrolysis, oculomucocutaneous syndrome	The mechanism remains unknown, although an allergic reaction has been speculated. Reported for greater than 1100 drugs, including sulfa drugs
7. Hair disturbance	Alopecia	A condition due to drugs with an androgenic effect acting on hair follicles to shorten the hair cycle, or drugs with an antimitotic effect inducing atrophy of hair follicles and prolongation of the resting phase of the hair cycle
	Hypertrichosis	A condition due to prolongation of the anagen phase of hair follicles induced by certain immunosuppressants, antihypertensives (minoxidil), or drugs for benign prostatic hyperplasia (finasteride)
8. Nail disturbance	Nail transverse ridges, onycholysis, discoloration	A condition arising from damage to the nail matrix cells due to drugs with an antimitotic effect or deposition of the drugs themselves
9. Tumors		

This table has been modified from [5].

Table 1. Classification of drug-induced cutaneous toxicity.

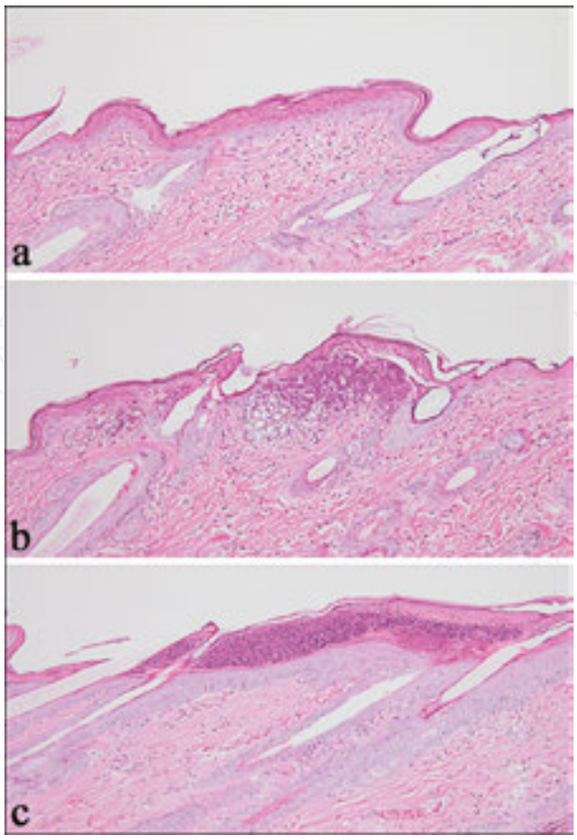

Figure 3. Sequential stages of inflammatory changes in irritant contact dermatitis following a single exposure to sodium lauryl sulfate (SLS) in the guinea pig. (a) Epidermal necrosis and slight infiltration of neutrophils in dermis are observed 24 hours after exposure. (b) Epidermal necrosis and severe infiltration of neutrophils in epidermis and dermis are observed 24 hours after exposure. (c) Epidermal abscess (pustule) and acanthosis (epidermal regeneration) are observed 48 hours after exposure. Owing to its emulsifying properties, SLS is an anionic surfactant used in many hygienic and cleaning products, including shampoos, toothpastes, and shaving foams.

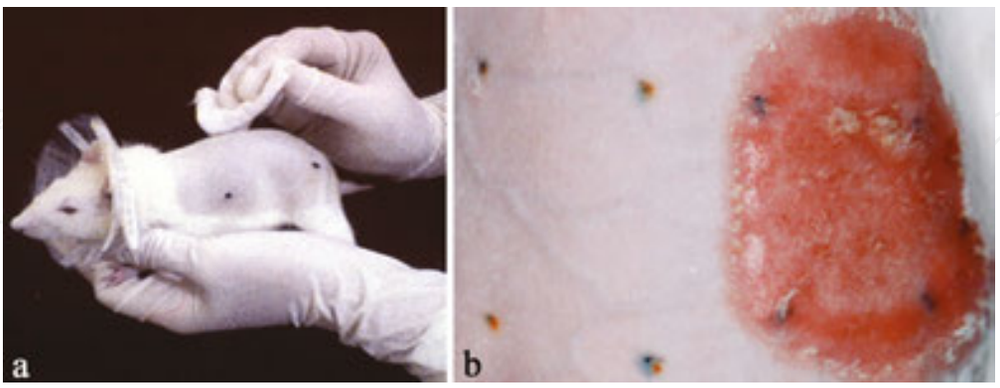

Figure 4. Macroscopic photos from a cumulative dermal irritation study in animals. (a) After the hair on the back of the rat is shaved, the drug is continually applied to the same area. The rat wears the Elizabethan collar to prevent the animal from biting or licking the exposure site. (b) Cumulative dermal irritation study in a rabbit (left: vehicle application, right: drug application). The site of drug application is observed with erythema, redness, swelling, and moistness. The change spreads beyond the site of application, indicating a strong irritant property of the drug.

Skin reaction	Grading value
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema to slight eschar formation	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (raised edges of area well defined)	2
Moderate edema (raised more than 1 mm)	3
Severe edema (raised more than a mm and extending beyond the area of exposure)	4

Eschar is scab or crust formation.
This table has been modified from [4].

Table 2. Skin irritation test (Draize scale).

3.1.2. Allergic contact dermatitis

Allergic contact dermatitis is a condition caused by a delayed (type IV) allergic reaction. A low molecular weight drug binds as hapten to a protein in the body to act as a complete antigen. Characteristically, inflammation is induced approximately 12 hours following recontact of a sensitized animal with the drug. Known sensitizing substances include preservatives contained in topical application agents, nickel sulfate, potassium dichromate, neomycin, aroma chemicals, formaldehyde, rubber/latex medical supplies, and plants (e.g., rhus lacquer).

3.1.3. Chemical burns

Chemical burns are an injury caused by a chemical substance that is extremely corrosive or irritating (e.g., strong acid or strong alkali), often involving itching and/or ulceration due to local coagulative necrosis (**Figure 5**). No currently available pharmaceutical products cause this type of injury. Accidental exposure to skin or oral ingestion of these chemicals represents a pediatric emergency problem and these chemicals have a history of being common agents used for suicide [12, 13]. Cement burn is well known in the developed world. The majority of patients are either workers in the construction industry or do-it-yourself enthusiasts, commonly kneeling or standing in cement. The mechanism of injury is a combination of the effects of cement alkalinity and mechanical abrasion. Besides denaturing protein, alkalis saponify fat-producing liquefactive necrosis [14].

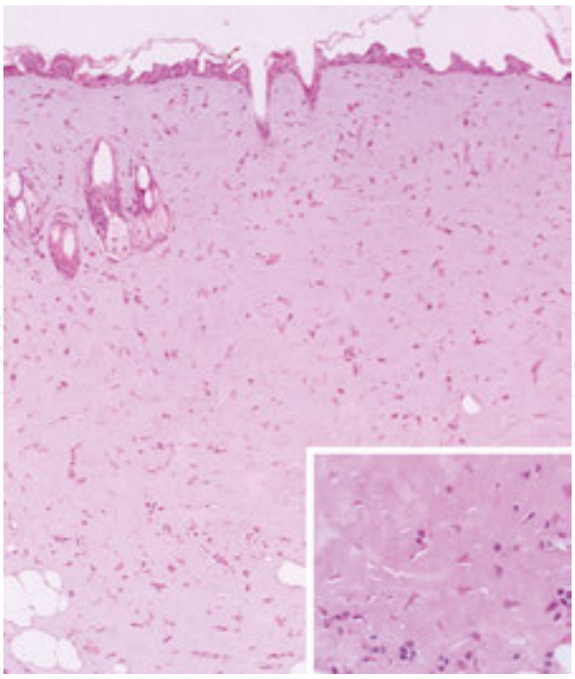

Figure 5. Sodium hydroxide-induced burn in the back skin of a rat. Severe coagulative necrosis is observed in all cutaneous layers. Insert is a higher magnification image of the same photo.

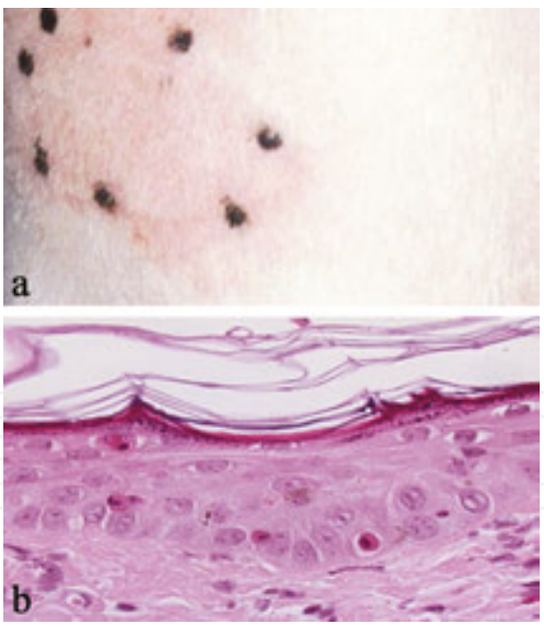

Figure 6. Macroscopic photo from a phototoxic study of 8-methoxypsoralen in guinea pigs. After the hair on the back of the guinea pig was shaved, the drug was applied to the same area and irradiated with ultraviolet light, and the reaction was subsequently evaluated. (a) The site on the left was irradiated with ultraviolet light (UVA) after drug application, while the site on the right was not irradiation after drug application. Erythema is observed at the site with ultraviolet light irradiation. This reaction to 8-methoxypsoralen with ultraviolet light has been utilized in ultraviolet light therapy (PUVA) for psoriasis in humans. (b) Apoptotic epidermal cells (sunburn cells) are observed at the site with ultraviolet light irradiation.

3.2. Photosensitive dermatitis

3.2.1. Photosensitivity

Photosensitive dermatitis (photosensitivity) is a general term that refers to skin inflammation caused by the combined effect of a drug and light. It can be classified into two types, either with or without involvement of an immunological mechanism. Numerous systemic and topical drugs, aroma chemicals, plants, and cosmetics have been reported to induce this condition. Some examples of photosensitizing drugs are phenothiazine, tetracyclines, sulfonamides, chlorpromazine, nalidixic acid, and fluorocoumarins (psoralens).

3.2.2. Phototoxic dermatitis

Phototoxic dermatitis is skin damage caused by a drug that is sensitive to light (ultraviolet light), not by the drug alone, but after absorption of photon energy, without involvement of an immune mechanism. Free radicals and peroxidative injuries have been reported to be involved in this reaction. In preclinical studies of topical application agents, phototoxicity testing is conducted using guinea pigs for evaluation of drug phototoxicity (**Figure 6**).

3.2.3. Photoallergic contact dermatitis

Photoallergic contact dermatitis is a condition caused by a delayed (type IV) allergic reaction. A drug sensitive to light (ultraviolet light) absorbs photon energy and is transformed into a substance (i.e., hapten) that combines with a protein in the body to act as a complete antigen. Characteristically, inflammation is induced approximately 12 hours following recontact of a sensitized animal with the drug.

3.3. Contact urticaria

Contact urticaria is acute redness or rash that occurs within several minutes to one hour following exposure to a drug. It can be caused by a direct effect of the drug on vascular walls, by an indirect effect on vascular walls via histamine release from mast cells (without involvement of an immune mechanism), or by an IgE-mediated immediate (type I) allergic reaction with involvement of an immune mechanism. For immune contact urticaria, known conditions include systemic reactions to penicillin or food, as well as urticaria due to natural rubber products (latex allergy), but it is generally difficult to reproduce such conditions in preclinical studies using experimental animals.

3.4. Chemical-induced acne

Chemical-induced acne is a disease of hair follicles caused by a chemical substance and is characterized by keratin plugs in hair follicles due to excessive proliferation of keratinocytes in hair follicles (comedo), sebum retention and inflammation [11]. Known examples of chemical-induced acne include occupational skin disorders of oil acne, caused by frequent exposure of the skin to cutting oils, as well as chloracne, induced by dioxins such as TCDD and PCB [15]. Clinically, the lesions are located around the eyes, ears, back, and genitalia; and

other symptoms include hyperpigmentation, conjunctivitis, and ocular discharge. A notorious event occurred when Ukraine President Viktor Yushchenko was stricken with facial chloracne resulting from deliberate poisoning with TCDD during his presidential campaign [16].

3.5. Pigmentary disturbance

Pigmentary disturbance is only observed in animals with scarce hair or animals that have been shaved, thus is difficult to detect in preclinical studies. Altered pigmentation is a condition that sometimes follows skin inflammation and is characterized histopathologically by an increase or decrease in the number of melanocytes as well as melanin production. Hyperpigmentation can occur in association with increased melanin production due to drug-induced activation of melanocytes, hemosiderin deposition due to hemorrhage, or deposition of a heavy metal or drug itself (**Figures 7** and **8**). Melanin production is increased by busulfan, cyclophosphamide, long-term high-dose ACTH, and inorganic arsenic. In addition, chlorpromazine or minocycline can form a complex with melanin or hemosiderin with deposition in the skin, leading to blue-gray discoloration of the skin. In contrast, hypopigmentation results from loss of melanin due to damage to melanocytes. Depigmenting agents such as phenols, catechols, and hydroquinone have a similar structure to tyrosine, thus can inhibit melanin synthesis and induce hypopigmentation (**Figure 8**). Recently, an unexpected outbreak of patients with leukoderma occurred in Japan with use of brightening/lightening cosmetics containing rhododendrol, which is a competitive tyrosinase inhibitor, thereby inhibiting melanin synthesis [17]. This type of leukoderma is induced by not only apoptosis of melanocytes but also subsequent immune reactions with CD8-positive T cell infiltration toward melanocytes [18, 19].

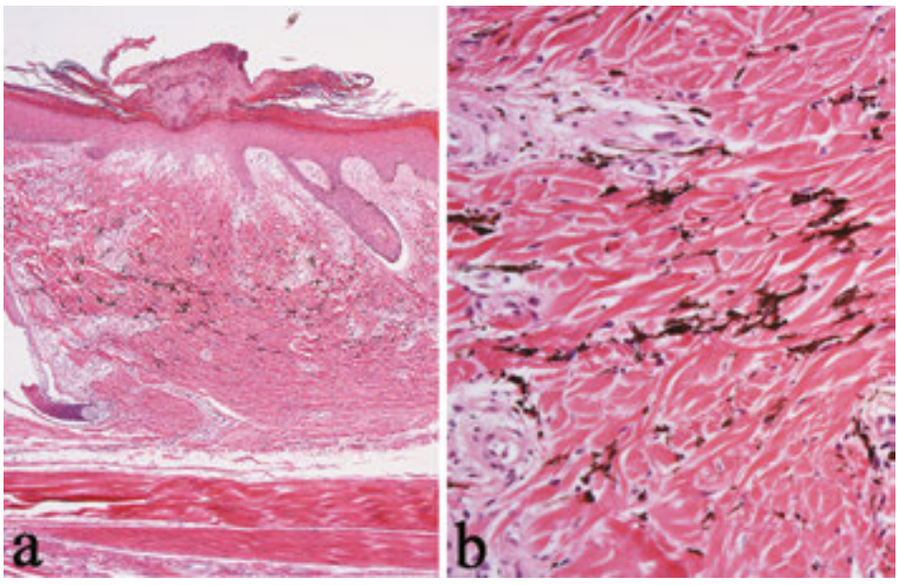

Figure 7. Hyperpigmentation due to melanin deposition in dermis at a drug injection site in the monkey. (a) Crust and epidermal hyperplasia are observed in the epidermis, and black pigment is observed in the dermis. (b) High power field of (a). Note that the black pigment is scattered throughout the dermis.

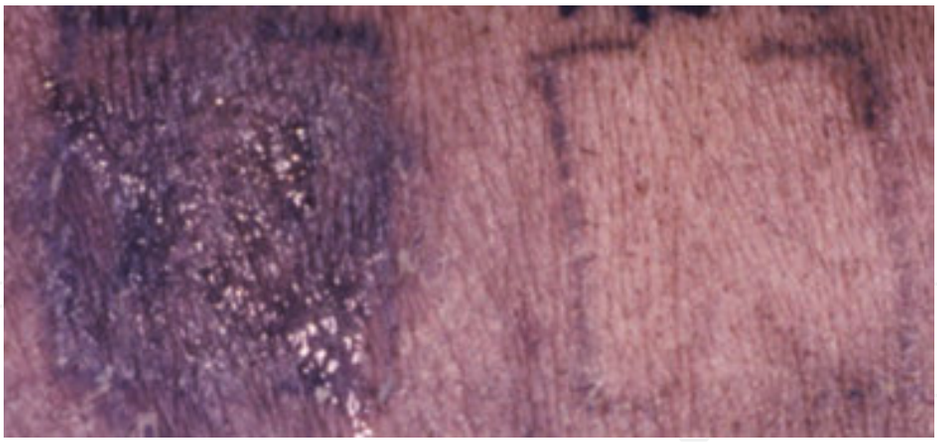

Figure 8. Macroscopic photo from a dermal application study of two drugs in mini-pigs (dark Yucatan pigs). The normal skin of Yucatan pigs appears black, because the skin contains a large amount of melanin pigment. With one drug applied to the left regions of the image, darkening of the skin is shown compared to the normal portion of skin, indicating excessive pigmentation due to drug application. With another drug applied to the right side of the image, lightening of the skin is shown with decreased pigmentation.

3.6. Drug rash (cutaneous reaction)

Drug rash (cutaneous reaction) is the most common adverse drug reaction reported to occur with antibiotics. The most serious forms of drug rash are toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome, which are known to occur with the use of various drugs, including penicillin derivative or cephem derivative antibiotics, antipyretic analgesics (particularly NSAIDs), allopurinol, amine antiepileptic drugs (phenytoin and carbamazepine), and sulfa drugs. The Ministry of Health, Labour and Welfare of Japan announced that of 110,023 cases of adverse drug reactions reported from 2005 to 2009, approximately 2.2% of the cases (2370) were toxic epidermal necrolysis or Steven-Johnson syndrome [20]. Although the mechanism of onset remains unknown in many instances, a type III allergic reaction is often speculated.

Many new antitumor drugs with specific molecular targets have been approved in recent years (the so-called "targeted therapies"), and their adverse effects are highly specific with respect to the skin. Cutaneous reactions to these therapies are among the most frequently observed and, when severe or protracted, can result in significant morbidity, requiring dose modification or drug discontinuation [21, 22]. Hyperplastic changes of the epidermis can be attributed to numerous causes, including response to stimulation from growth factors, such as epidermal growth factor (EGF). The repeated administration of EGF to cynomolgus monkeys results in cutaneous desquamation and epidermal hyperplasia [23]. Epidermal growth factor receptor (EGFR), multikinase, c-Kit, BRAF, or MEK inhibitors induce papulopustular rash, maculopapular rash, and hand-foot syndrome in humans [24, 25]. EGFR inhibitor-induced lesions are associated with the inhibition of EGFR in undifferentiated, proliferating keratinocytes in the basal and suprabasal layers of the epidermis [26]. Other inhibitor-related rashes appear to be associated with the inhibition of vascular endothelial growth factor (VEGF) receptors in the skin [24].

3.7. Hair disturbance

Many drugs induce hair disorders, such as hair loss, stimulated hair growth, or, more rarely, changes in the hair shape and color [27]. Hair loss (hypotrichosis or alopecia) is a common problem that affects approximately 60 million men, women, and children in the United States, with a total cost for medical consultation and treatment of US$1.3 billion per year [28]. The onset of alopecia (toxic alopecia) often depends on the hair cycle at the time of drug administration. Drugs with an androgenic effect can cause alopecia by acting on the resting phase of the hair cycle to shorten the cycle. In addition, drugs with an antimitotic effect (e.g., anticancer drugs) or irradiation can cause alopecia by inducing apoptosis of hair follicles during the anagen phase of the hair follicle, thereby causing atrophy of hair follicles and prolongation of the resting phase of the hair cycle (chemotherapy or radiation-induced follicular dystrophy) [29, 30] (**Figure 9**). Hypertrichosis refers to drug-induced promotion of hair growth or induction of the anagen phase [31] **Figure 10**, and has been reported in organ transplant recipients and animal models treated with cyclosporine [32], as well as an antihypertensive (minoxidil) and a drug for treating benign prostatic hyperplasia (finasteride) [33]. Minoxidil and finasteride have been approved for clinical use as drugs to stimulate hair growth [34]. Hypertrichosis is observed as an increase in the length, thickness, and number of eyelashes in glaucoma patients treated with prostaglandin F2α agonists [35]. Bimatoprost has been used as a therapy for eyelash insufficiency or as eyelash restorer. As in the cases of the pigmentary disturbances described above, these changes can only be observed in animals with scarce hair or animals with shaved hair and are thus difficult to detect in typical preclinical studies.

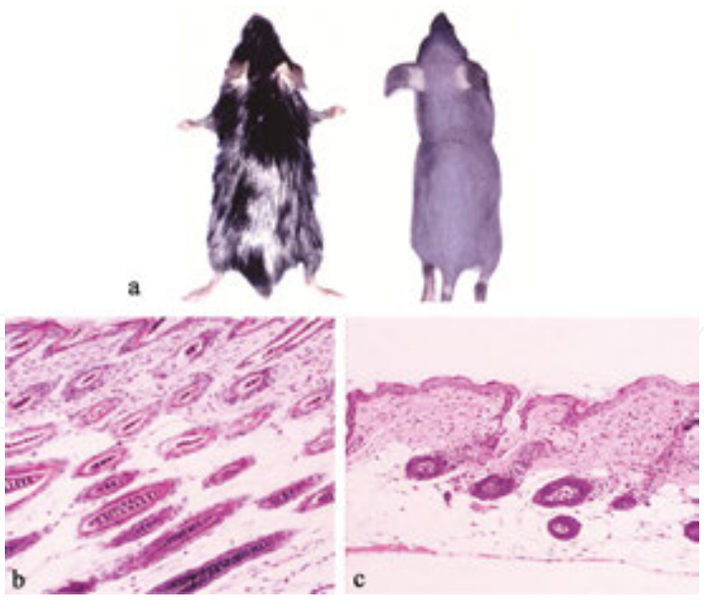

Figure 9. *N*-Methyl-*N*-nitrosourea (MNU)-induced follicular dystrophy in C57BL mice. (a) Systemic hair loss and whitening skin color are observed in the mouse treated with MNU (right side), compared to the control mouse with abundant hair (left side). MNU is an alkylating agent used as an antimitotic chemical, and induces apoptosis of hair follicles during the anagen phase of the hair follicle causing atrophy of hair follicles and prolongation of the resting phase of the hair cycle. (b) Anagen stage of hair cycle in a control mouse. (c) Catagen stage of hair cycle (follicular dystrophy) in a MNU-treated mouse. (b) and (c) are at the same magnification.

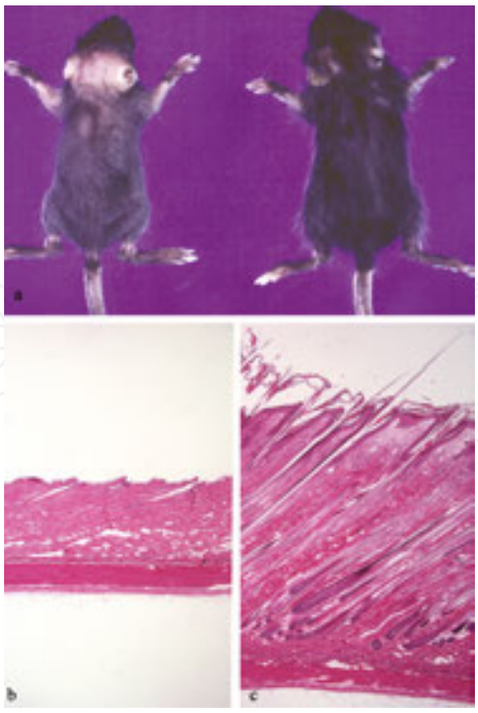

Figure 10. Immunosuppressant drug-induced hypertrichosis in a mouse follicular dystrophy model. (a) Compared to *N*-methyl-*N*-nitrosourea (MNU)-induced follicular dystrophy in the treated mouse (left side), the mouse treated with the immunosuppressant drug after MNU exposure has abundant body hair (right side) (b) MNU-induced follicular dystrophy is characterized by a prolonged catagen stage of hair cycle and thinness of the back skin. (b) and (c) are at the same magnification. (c) The hair cycle in the mouse treated with the drug after MNU exposure was found to be in the anagen stage with thickening of the back skin. (b) and (c) are at the same magnification.

3.8. Nail disturbance

Nail changes that reflect a previous general condition are a barometer of health that can be used to predict the presence or absence of an abnormality several weeks before its presentation [6]. Transverse ridges (Beau's line), washboard nail plates, and onycholysis are known to occur with use of metoprolol, retinoids, anticancer drugs, or irradiation [5]. Adverse effects of targeted molecular therapies, such as EGFR inhibitors, are also highly specific with respect to nails in human patients [22, 36]. In addition, yellow nail discoloration is known to occur with penicillamine, and black nail discoloration with Futraful (tegafur), anticancer drugs, or gold drugs. Administration of nucleoside analogs to dogs results in nail loss and footpad erosions with associated radiomimetic defects in the stratum germinativum [4]. In general, onycholysis can be induced by anticancer drugs or irradiation in experimental animals, while other changes are difficult to detect in preclinical studies.

3.9. Skin carcinogenesis

Some photoirritants, such as 8-methoxypsoralen, have been associated with UV-induced skin carcinogenesis. Treatment of psoriasis by photochemotherapy (PUVA) with oral methoxsalen, a psoralen, in conjunction with UVA radiation, is associated with an increased risk of irregular

pigmented skin lesions, squamous cell carcinoma, and malignant melanoma [37, 38]. Chemically induced skin tumors have been associated with numerous topically applied and systemically administered compounds in rodents; however, there appear to be few clinically used drugs that are suspected of being involved in skin carcinogenesis in humans. Rodent models of skin carcinogenesis are widely used for studies of carcinogenic mechanisms and the evaluation of carcinogenesis associated with chemical substances. Huff et al. performed a retrospective investigation of carcinogenicity tests on 379 chemical compounds conducted by the US National Toxicology Program (NTP) and reported that increased skin carcinogenesis was observed with 19 chemical compounds [39] (**Table 3**). Currently used methods used to determine skin carcinogenesis of drugs/chemical substances or methods to clarify carcinogenic mechanisms include: 2-year dermal application carcinogenicity studies (**Figure 11**); DMBA/TPA two-stage skin carcinogenesis models using 12-O-tetradecanoylphorbol-13-acetate (TPA) and 7,12-dimethylbenzanthracene (DMBA) (DMBA as an initiator, TPA as a promoter) [40]; studies in Tg.AC transgenic mice with expression of the v-Ha-ras gene in the epidermis [41]; and studies in SENCAR (SENsitivity to CARcinogen) mice [42]. Two-stage skin carcinogenesis models using metallothionein-I/II knockout mice have shown significant increases in skin carcinogenesis, thereby indicating an important role of metallothionein as an inhibitory factor of carcinogenesis in skin [43]. p53 is a protein that causes cell cycle arrest, apoptosis, or senescence that is crucial in the process of tumor suppression in several cell types [44]. In the

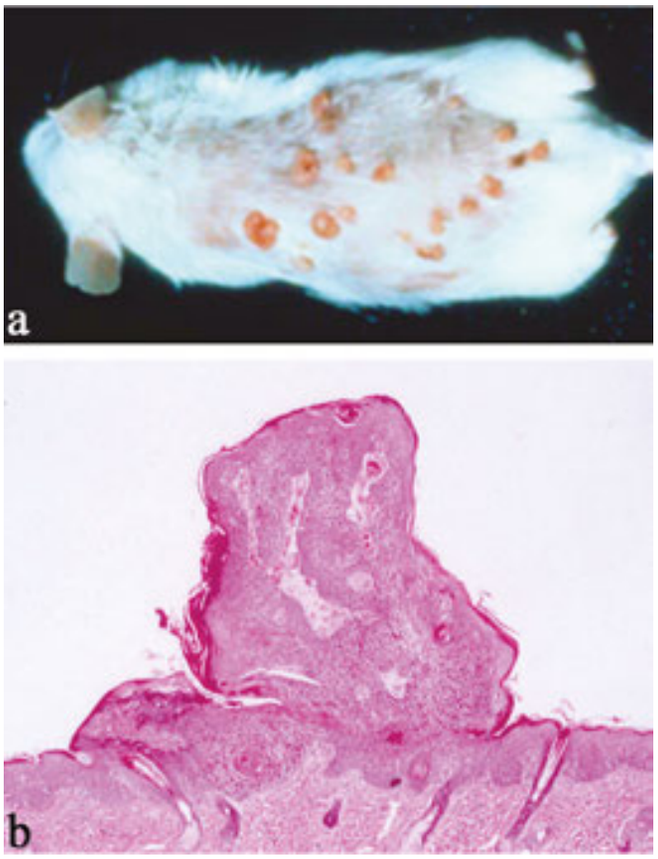

Figure 11. A 2-year dermal application study of a drug in CD1 mice. (a) Macroscopically, multiple reddish skin tumors are observed on the back. (b) Histological findings are consistent with squamous cell papilloma.

DMBA/TPA two-stage skin carcinogenesis model, the absence of p53 in stratified epithelia leads to the appearance of a higher number of tumors that grow faster and become malignant more frequently than tumors arising in mice with the wild type p53 genotype [45]. The carcinogenic risk of a chemical after topical application is traditionally investigated in rats; however, in recent years, Tg.AC mice have become a popular alternative. The skin of Tg.AC mice is genetically initiated, thus the induction of epidermal papilloma in response to dermal or oral exposure to a chemical agent acts as a reporter phenotype for the carcinogenicity of the test chemical [11, 46]. The SENCAR mouse is an outbred strain (not genetically engineered) that was selected specifically for increased skin tumor multiplicity and decreased tumor latency in response to known dermal carcinogens [41]. A recent report described a possible animal model for human keratoacanthoma involving a single intraperitoneal injection of 50 or 75 mg/kg *N*-methyl-*N*-nitrosourea in male Sprague-Dawley rats at 4 weeks of age [47] (**Figure 12**). Keratoacanthoma is a benign tumor believed to arise from the epithelium of hair follicles [48]. Peroxisome proliferator-activated receptor agonists have been associated with the development of hemangiosarcomas in mice and hamsters and liposarcomas and fibrosarcomas in rats [49].

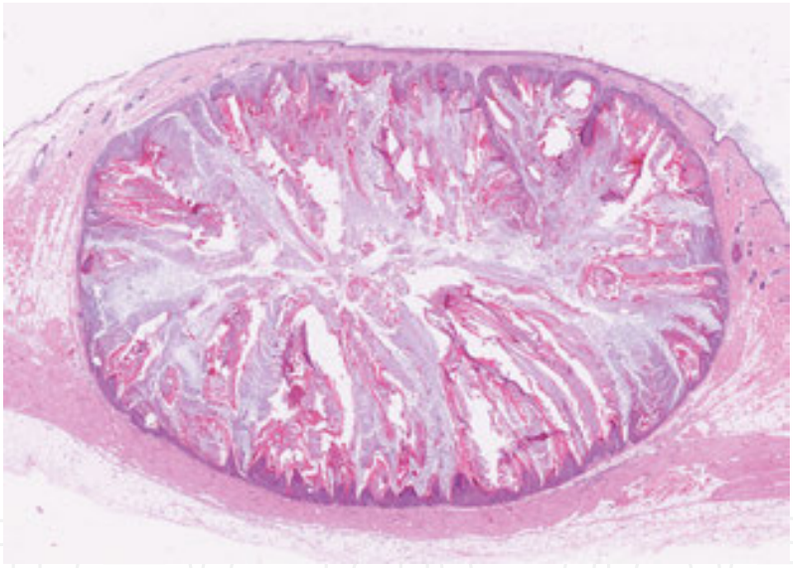

Figure 12. Animal model for human keratoacanthoma following a single intraperitoneal injection of *N*-methyl-*N*-nitrosourea in a male rat.

3.10. Other cutaneous toxicity due to systemic toxicity

3.10.1. Acne formation due to anti-inflammatory analgesics

This is a common clinical adverse reaction to NSAIDs or steroids and involves proliferation of acne bacteria leading to worsening of inflammation (steroid acne). In preclinical studies, spontaneous interdigit inflammation may worsen in beagle dogs following NSAID administration, eventually leading to skin ulcers in all extremities in severe cases (**Figure 13**).

Name of chemical compound	Route	Any skin carcinogenesis (2-year carcinogenesis study)				Mutagenicity (Ames)	TR No.
		F344 rats		B6C3F1 mice			
		Male	Female	Male	Female		
3-Amino-9-ethylcarbazole	Dietary	+	+	+	+	+	093
Benzene	Oral	CE	CE	CE	CE	–	289
Chloroethane	Inhalation	?	?	?	CE	+	346
C.I. acid red 114	Drinking water	CE	CE			+	405
C.I. basic red 9 monohydrochloride	Dietary	CE	CE	CE	CE	+	285
C.I. Direct Blue 15	Drinking water	CE	CE			–	397
2,4-Diaminoanisole sulfate	Dietary	+	+	+	+	+	084
3,3′-Dimethoxybenzidine dihydrochloride	Drinking water	CE	CE			+	372
3,3′-Dimethoxybenzidine-4,4′-diisocyanate	Dietary	+	+	–	–	+	128
3,3′-Dimethoxybenzidine dihydrochloride	Drinking water	CE	CE			+	390
2,4-Dinitrotoluene	Dietary	+	+	–	–	+	054
Fenthion	Dietary	–	–	?	–	+/–	103
Glycidol	Oral	CE	CE	CE	CE	+	374
Nithiazide	Dietary	–	+	+	?	+	146
5-Nitro-O-anisidine	Dietary	+	+	?	+	+	127
Nitrofurazone	Dietary	?	CE	–	CE	+	337
Rhodamine 6G	Dietary	?	?	–	–	–	364
Tris(aziridinyl)-phosphine sulfide	Subcutaneous	+	+	+	+	+	058
4-Vinyl-1-cyclohexene diepoxide	Dermal application	CE	CE	CE	CE	+	362

+: Positive, CE: apparent increase in incidence, ?: increased incidence but not significantly, −: negative, +/−: positive or negative, TR No.: National Toxicology Program (NTP) study number.
This table has been modified from [39].

Table 3. Chemical compounds reported to produce skin carcinogenesis from systemic exposure (US NTP study).

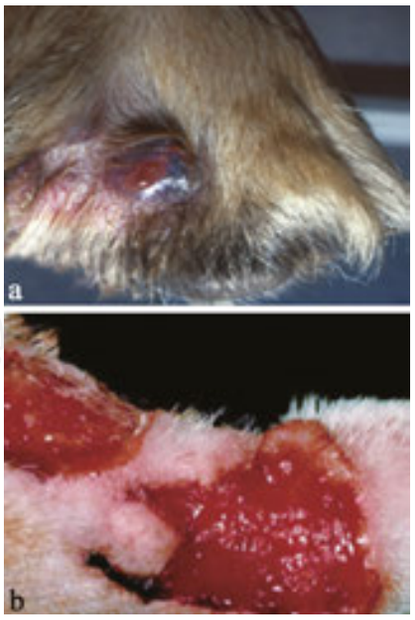

Figure 13. Nonsteroidal anti-inflammatory analgesic drug (NSAID)-induced skin lesion in a subacute toxicity study using beagle dogs. (a) Macroscopic observation of an interdigit lesion in the foot pad. Severe swelling and ulceration due to inflammation with local bacterial infection are observed. (b) In severe cases, the local lesions progress to skin ulcers on all extremities (subcutaneous phlegmon).

3.10.2. Drug-induced skin atrophy

Skin atrophy can be observed with long-term, repeated use of corticosteroids due to inhibitory effects on cell proliferation and/or fiber production, leading to decreases throughout the epidermis, skin appendages (hair follicles, sweat glands, and sebaceous glands) and subcutaneous adipose tissue [50] (**Figure 14**). Skin atrophy is also commonly observed with systemic exposure to anticancer drugs in preclinical studies.

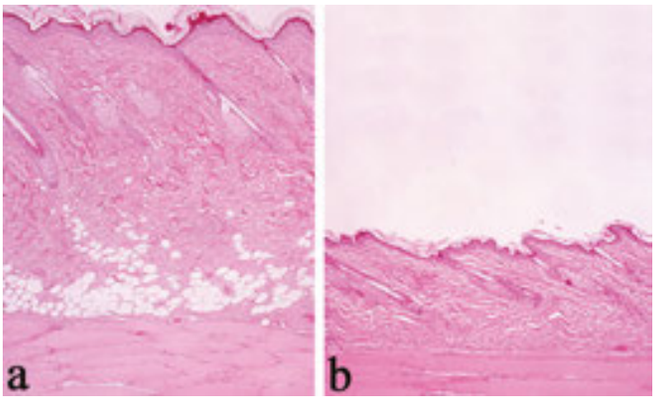

Figure 14. Corticosteroid-induced cutaneous atrophy in the rat. (a) In normal rat skin, the skin is thick with large hair shafts, sebaceous glands, and subcutaneous adipose tissue. (b) In the drug-exposed skin, the skin is thin with a severe decrease in all cutaneous layers, skin appendages (hair follicles and sebaceous glands), and subcutaneous adipose tissue.

3.10.3. Skin ulcer due to peripheral circulatory insufficiency

A lesion similar to cutaneous gangrene seen in diabetic patients can be induced in monkeys with certain drugs, and is speculated as a consequence of peripheral circulatory insufficiency due to the involvement of a vascular disorder [5].

3.10.4. Drug-induced granulomatous reaction

Hypodermic injections of certain drugs induce granulomatous inflammation located at the injection site, which is highly painful for the patients (**Figure 15**). Granulomas induced by luteinizing hormone-releasing hormone analogues have been reported in some patients for the treatment of prostatic cancers [51]. Histopathologically, epithelioid granulomatous inflammation with small vacuoles derived from the constituent ingredients of drug microcapsules has been observed [52]. In some patient cases, vaccinations induce granulomatous reactions at the injection site due to specific inflammation and irritation [53]. Recently, treatment with interferon has been associated with cutaneous granulomatous reactions and sarcoid reactions [54].

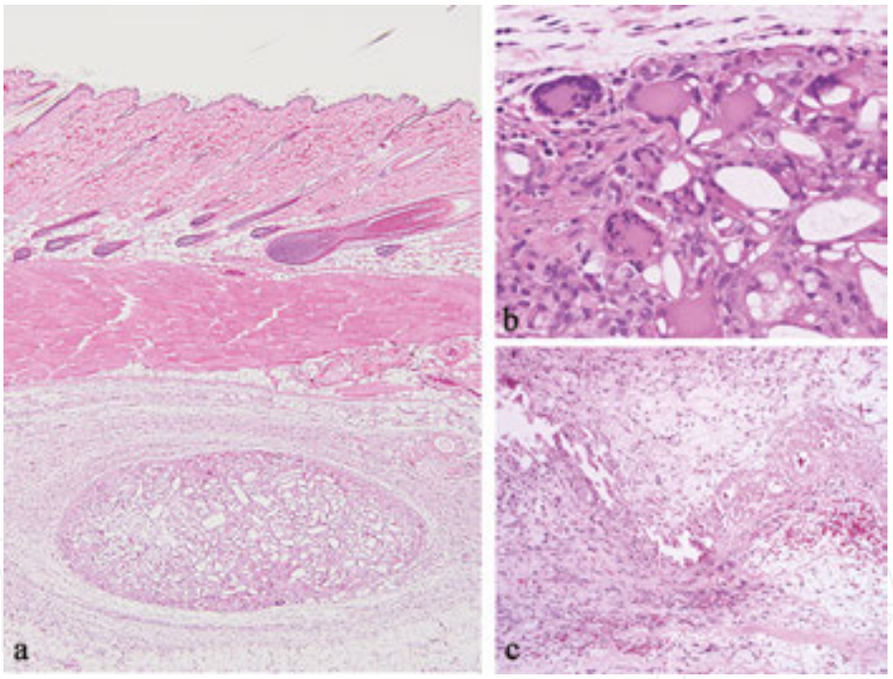

Figure 15. Drug-induced granuloma in subcutaneous tissue of the rat. (a) Granuloma is observed in the subcutaneous tissue. (b) Many foreign body giant cells that phagocytose lipoid materials are observed. Lipoid materials are derived from the contents of the drug. (c) Dystrophic calcification is observed in the obsolete lesions.

4. Closing remarks

This review has outlined the types and characteristics of drug-induced cutaneous toxicity, as well as providing descriptions of the methods of cutaneous toxicity testing required for safety

evaluation. It should be emphasized that cutaneous toxicity of drugs or chemical substances may appear in various forms. In recent years, advances have been made in the development of pharmaceutical products targeting specific molecules, genes, or nanotechnology-based pharmaceutical products. Due to the potential onset of cutaneous toxicity involving novel mechanisms with new pharmaceutical products, it will continue to be important to understand the basic toxic changes described here.

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