＜皮膚科＞

①atypical fibroxanthomaとその鑑別

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④癌診療指針のための病理診断プラクティス 皮膚腫瘍 （総編集 青笹克之, 専門編集 清水道生、新井栄一、中山書店）

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症例5 atypical fibroxanthoma とその鑑別

70代，男性

■現病歴

近距離被爆後の急性原爆症，喉頭癌の既往あり．

半年間の経過で増大した上肢の腫瘤を主訴に受診した．初診時，左上腕の外側にドーム状に隆起する20×19mm大の紅色腫瘤があり，軽度の落屑を伴っていた図1．下床との癒着はなかった．臨床的には隆起性皮膚線維肉腫（dermatofibrosarcoma protuberans：DFSP），Merkel細胞癌（Merkel cell carcinoma），皮膚付属器腫瘍などを疑われ切除された．

病理所見

表皮直下～真皮内で増殖する腫瘍性病変である．ドーム状に外方向性に隆起し，辺縁にはわずかに表皮のcollaretteを伴う．表皮は一部菲薄化し，ごく限局性にびらんを生じていた．内方向性にも腫瘍は増殖するが，脂肪織内への浸潤はなく，境界は比較的明瞭である図2a．腫瘍内に壊死はなかった．表皮直下より異型細胞は存在したが，表皮との明らかな連続性はなかった図2b．腫瘍は一部で疎な束状配列をみるほか，特定の配列を示さず増殖する多角形の細胞で構成されていた図2c．多核巨細胞や大型で奇怪な核をもつ異型細胞も散在していた．核分裂像も多く認められた図2d．また，本症例では間質のmyxoidな変化を伴っており，alcian blue染色で粘液の沈着が確認された．

免疫組織化学的にはCK34βE12，AE1/AE3，EMA，CAM5.2，S-100蛋白，CD31，CD34，SMA，desminは陰性，p63，CD68はごく一部の細胞のみ陽性，CD10，CD99が陽性だった図3．

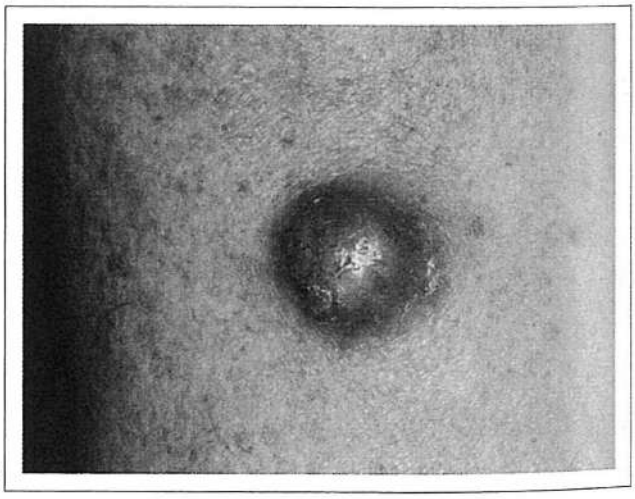

図1 左上腕に生じた紅色腫瘤

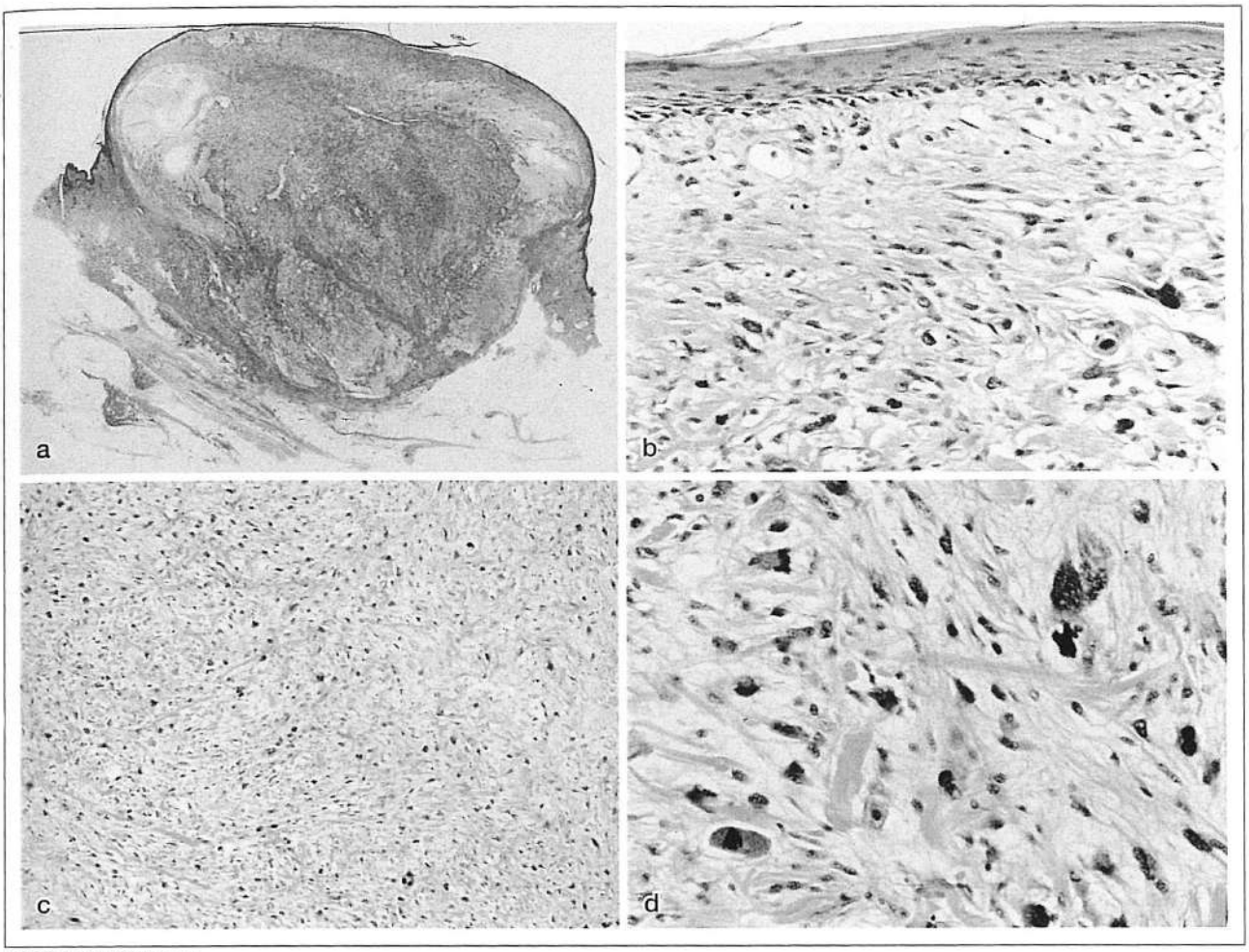

図2 組織学的所見
a：真皮に結節状態に増殖する腫瘍　　b：表皮との連続性はない.
c：疎な束状配列を示す.　　d：奇怪な核をもつ細胞や，多核巨細胞．核分裂像も散見される.

鑑別診断

atypical fibroxanthoma は高齢者露光部皮膚に生じる腫瘍である．白人男性に多く，紫外線が誘因といわれる．臨床的には，比較的急速に発育するドーム状腫瘤で，しばしば潰瘍化する.

病理組織学的には異型の強い紡錘形細胞，類上皮様細胞，奇怪な核をもつ巨細胞や多核細胞が増殖する．表皮はしばしば潰瘍化し，腫瘍辺縁には collarette を伴う．spindle cell variant，clear cell change，granular cell change，ヘモジデリン沈着による pigmented variant，with osteoclast-like giant cells，myxoid change，chondroid formation，osteoid formation など 多数の亜型が知られており，さまざまな病理組織像を呈することから鑑別は多岐にわたる．また，疾患に特異的な免疫組織化学的マーカーはなく診断は除外診断となるため，しばしば診断は非常に困難である.

主な鑑別疾患は，悪性黒色腫（malignant melanoma），扁平上皮癌（squamous cell carcinoma：SCC），血管肉腫（angiosarcoma），平滑筋肉腫（leiomyosarcoma），undifferentiated pleomorphic sarcoma などである 図4．本症例のように myxoid な間質を有する際は myxofibrosarcoma なども否定する必要がある.

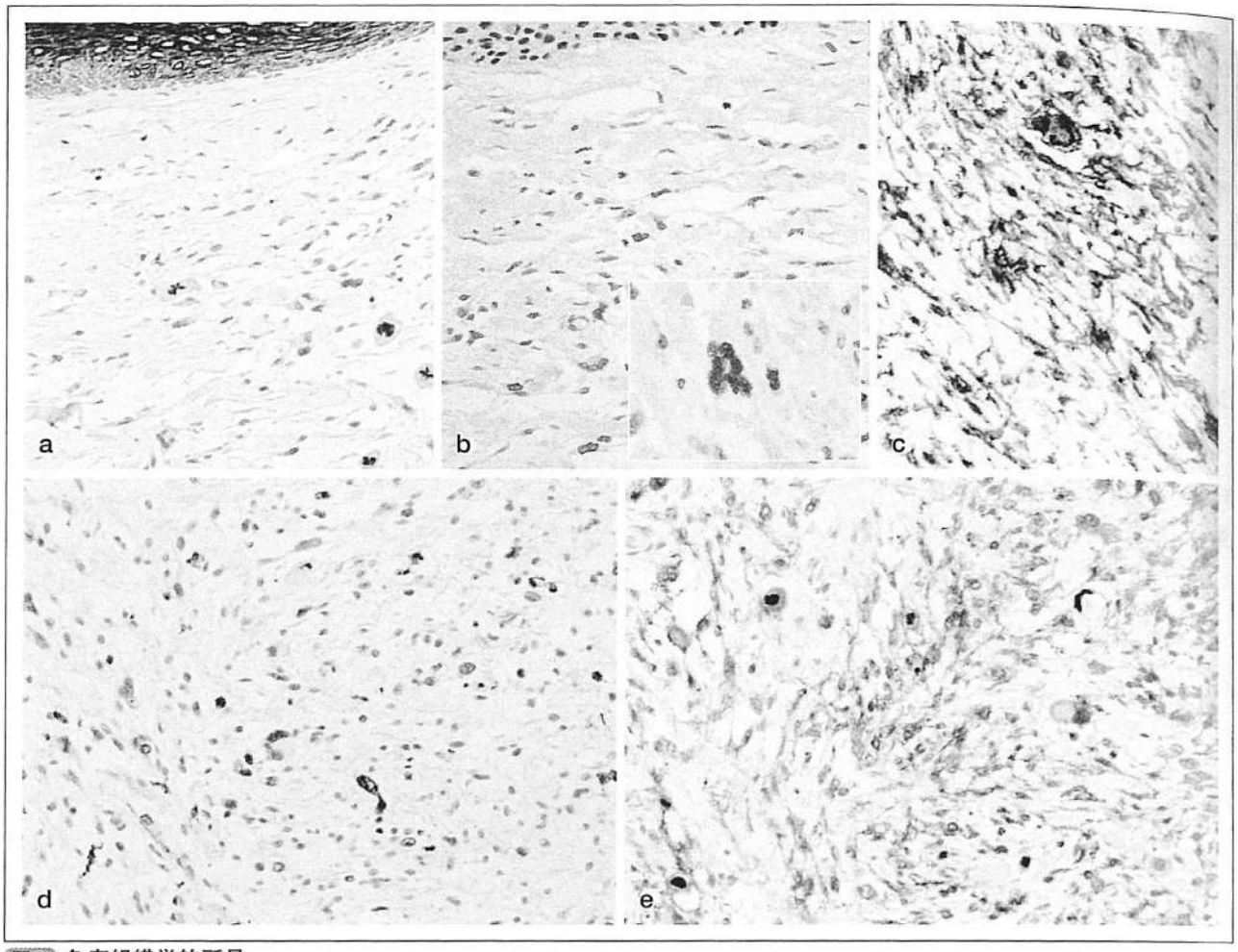

図3 免疫組織学的所見
a：CK34βE12　b：p63（挿入図：陽性細胞）　c：CD10　d：CD68　e：CD99

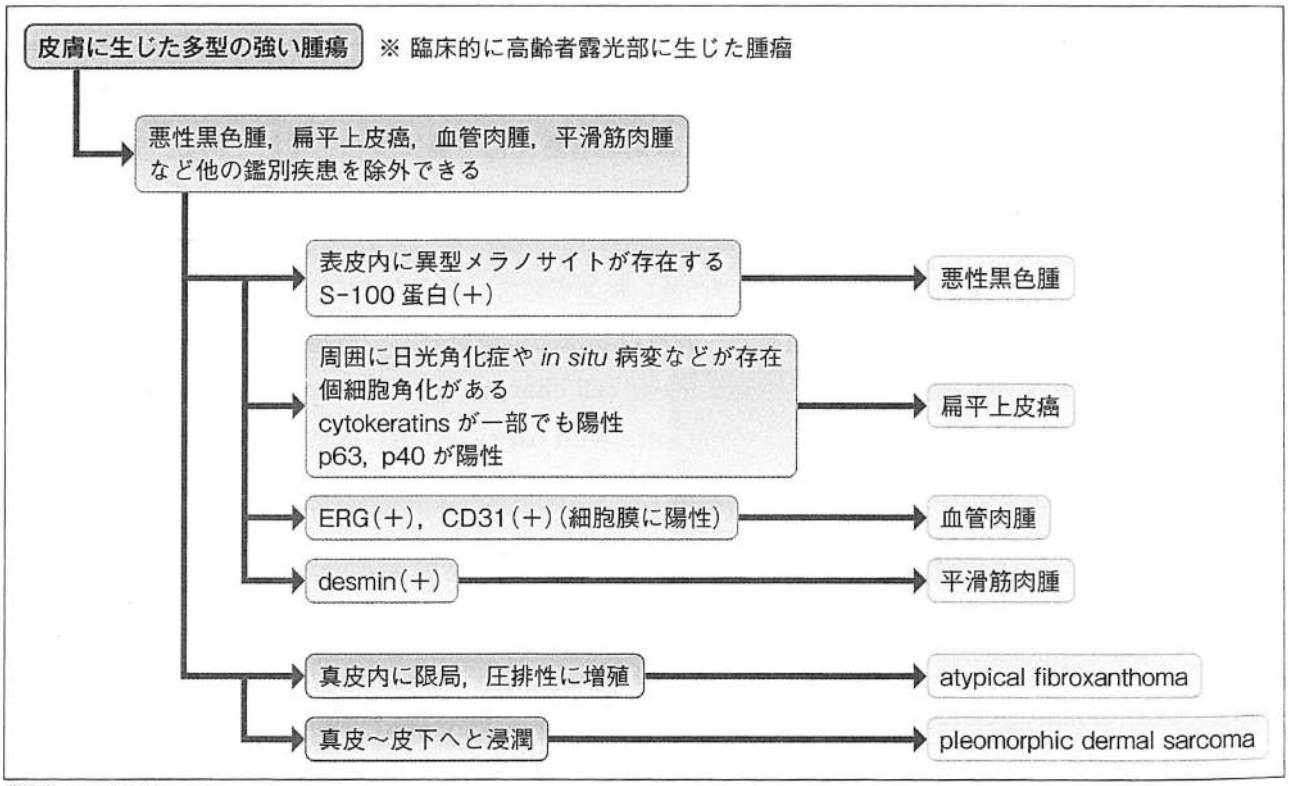

図4 鑑別診断のフローチャート

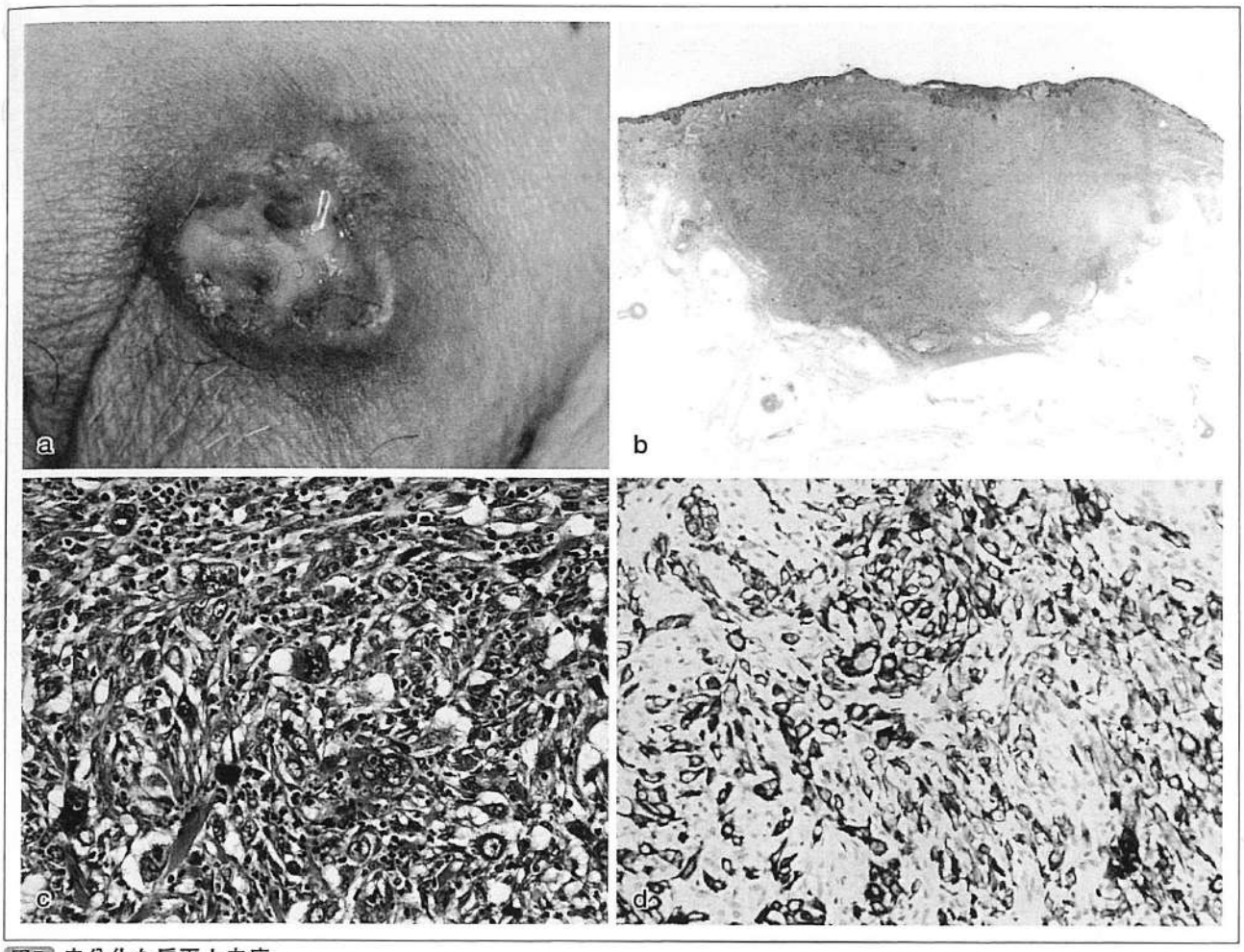

図5 未分化な扁平上皮癌
a：臨床像．恥丘部に生じた潰瘍を伴う腫瘍性病変
b：真皮より皮下脂肪織へと圧排性に増殖する．
c：明らかな角化傾向はなく，大型で奇怪な核を有する細胞や多核細胞が増殖する．
d：AE1/AE3 免疫染色では一部陽性である．
（b, c：木下麻衣子ほか．6 恥骨部腫瘍の1例．日本皮膚病理組織学会会誌 2006；22：22-5.）

鑑別の際に最も苦慮するのは扁平上皮癌および皮膚に生じた undifferentiated pleomorphic sarcoma であろう．

未分化な扁平上皮癌，あるいは spindle cell variant の扁平上皮癌は臨床的，病理組織学的に atypical fibroxanthoma と類似する 図5．atypical fibroxanthoma と上皮との関連性を示唆する報告も散見され，両者が同一スペクトラム上の疾患なのか異なる疾患であるのか，明確な結論はでていない．現在は，atypical fibroxanthoma はサイトケラチンは陰性でなければならない，と定義する病理学者が多い．しかし未分化な扁平上皮癌でもサイトケラチンの染色性が失われることがある．この際，鑑別には AE1/AE3 よりも CK34βE12 や CK5/6 など高分子サイトケラチンがより有用とされる．加えて p63 や，p63 のアイソフォームである p40 の陽性所見も扁平上皮癌を支持する結果となるが，p63 は atypical fibroxanthoma でも部分的に陽性となることがある．

真皮を主座として生じた undifferentiated pleomorphic sarcoma は，以前は atypical fibroxanthoma も併せて浅在型の悪性線維性組織球腫（malignant fibrous histiocytoma：MFH）と呼ばれた．しかし，atypical fibroxanthoma の予

表1 atypical fibroxanthoma と pleomorphic dermal sarcoma の鑑別

	atypical fibroxanthoma	pleomorphic dermal sarcoma
臨床像	高齢者男性の露光部（頭部）	
	＜2cm まで	＜6～7cm まで
病理組織像	真皮に限局，圧排性の増殖 壊死巣（－） リンパ管・脈管侵襲像（－） 神経周囲への浸潤像（－）	皮下脂肪織～以深に浸潤性に増殖 50% で壊死巣（＋） リンパ管・脈管侵襲像（＋）のことあり 神経周囲への浸潤像（＋）のことあり
病理組織学的亜型	spindle cell clear cell pigmented granular cell change with osteoclast-like giant cells myxoid change with keloidal change in the collagen	spindle cell myxoid desmoplastic stromal change pseudoangiosarcomatous keloidal stromal change with osteoclast-like giant cell
免疫組織化学的所見	陰性：S-100 蛋白，cytokeratins, desmin, CD34 陽性になることあり：SMA, EMA, CD31（細胞質に陽性），p63, HMB45 しばしば陽性：CD10, CD99, CD68	陰性：S-100 蛋白，CD34, desmin, cytokeratins, ERG 陽性になることあり：SMA, CD31, EMA, Melan A, FLI1 しばしば陽性：CD10, CD68
予後	良好．まれに局所再発	low-grade malignant potential 局所再発，転移．まれに死亡例の報告がある

後は良好であることから，近年は皮膚に生じた undifferentiated pleomorphic sarcoma に対しては“pleomorfic dermal sarcoma”や，“undifferentiated pleomorphic sarcoma of skin”などの呼称が提唱されている．pleomorphic dermal sarcoma も臨床像は atypical fibroxanthoma と同様で，典型的には高齢男性露光部に生じる．病理組織学的にも高度な異型を有する紡錘形，多角形，類上皮様の細胞が真皮より皮下脂肪織，さらに深部へと浸潤性に増殖する．多彩な亜型を有する点も同様である．特異的な免疫組織学的マーカーはない 表1．現状では除外診断として atypical fibroxanthoma あるいは pleomoprhic dermal sarcoma の診断となった際に，さらに明らかな深部への浸潤傾向を有する，など atypical fibroxanthoma の定義を満たさないものを pleomorphic dermal sarcoma と診断することとなる．

atypical fibroxanthoma は臨床的に 2cm 大まで，病理組織学的にも深部皮下脂肪織へと浸潤することはなく圧排性の増殖形態を示し，また腫瘍内壊死，脈管侵襲像，神経周囲浸潤像はない．これらの所見に合致していることから本症例を atypical fibroxanthoma と診断した．

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①先天性巨大色素性母斑から発生し、悪性黒色腫との鑑別を要した proliferative noduleの1例

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④日本小児皮膚科学会雑誌

⑤第36巻1号、p61-64

先天性巨大色素性母斑から発生し，悪性黒色腫との鑑別を要した proliferative nodule の1例

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Key words：悪性黒色腫，先天性巨大色素性母斑，Ki-67，proliferative nodule

要　　旨

先天性巨大色素性母斑は悪性黒色腫の発生母地となることが知られているが，先天性色素性母斑からは，他に良性の経過をとる proliferative nodule を生じることがあり，悪性黒色腫との鑑別が問題となる。本症例では，先天性巨大色素性母斑を有する患児が1歳時に結節を切除した際，病理組織像で核分裂像が多く散見され，また Ki-67 の高い陽性率（30％）を認めたことから，悪性黒色腫との鑑別に苦慮した。全体的な腫瘍の組織構築は左右対称性であり，深部への浸潤傾向なく，異常核分裂像や壊死巣は認めなかったことから，注意深く経過観察した。母斑の切除のみ継続し現在5歳6か月であるが，明らかな再発および転移はなく，経過も含めて proliferative nodule と診断した。このような症例では慎重な経過観察が必要と考えられた。

はじめに

先天性巨大色素性母斑は出生2万人に1人の稀な疾患である。Viana らの報告によると巨大色素性母斑の5～10％に悪性黒色腫を発症すると推定されており[1]，早期に母斑を除去することが望ましい。一方，proliferative nodule は，新生児～乳幼児期に，巨大色素性母斑の病巣内に生じる結節性病変であり，悪性黒色腫との鑑別が問題になる。今回，病理組織像から悪性黒色腫との鑑別に苦慮した症例を経験したため，若干の考察を加えて報告する。

症　　例

症例：日齢15，女児。

既往歴・家族歴：特記すべきことなし。

現病歴：出生時より全身に黒色斑が多発しており，当科を紹介され受診した。

現症：右胸腹部～背部にかけて広範な黒色斑を認め，その他側頭部，下顎，頬部，体幹，下肢に大小の黒色斑が散在していた（図1a，b）。

経過：先天性巨大色素性母斑と診断し，他院で戻し表皮移植や色素斑の部分切除術が繰り返し行われていた。1歳6か月頃より腹部の結節の増大に両親が気づき，1歳9か月時に腹部の8×6mm大結節（図2a，b）を3cm離して切除した。

病理組織学的所見：病変は真皮内で結節状に増生し，メラノサイトで構成される腫瘍であった。線維性被膜に被包され結節状に増生する腫瘍成分と被膜外に浸潤する領域がみられた（図3）。いずれの領域でもメラノサイトは核異型を有してお

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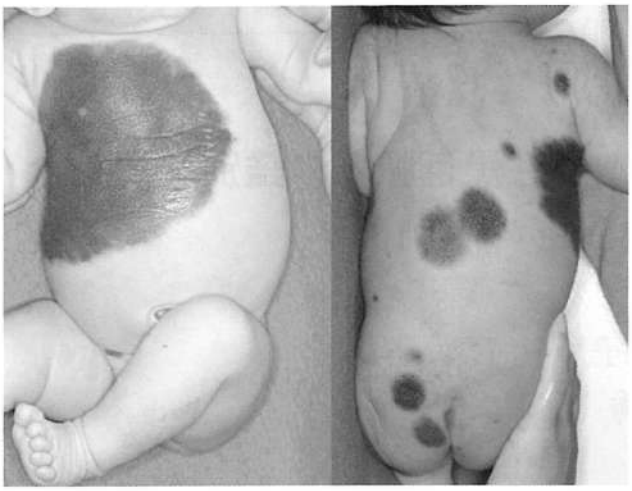

図 1a　　図 1b

図 1a, b　右胸腹部～背部に広範な黒色斑を認め，その他体幹などに大小の黒色斑が散在している．

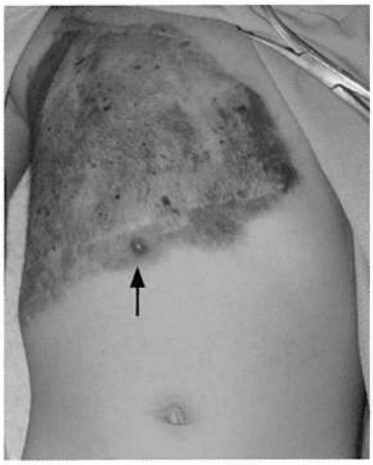

図 2a

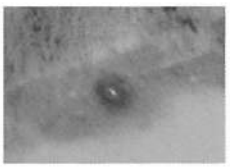

図 2b

図 2　切除時臨床像　腹部の結節（↑）(a) とその拡大像 (b)

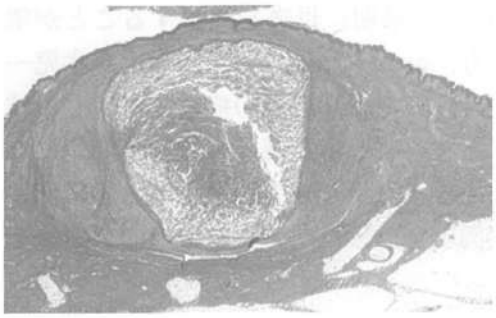

図 3　病理組織像（ルーペ像）　線維性被膜に被包され結節状に増生する腫瘍成分と被膜外に浸潤する領域がみられた．

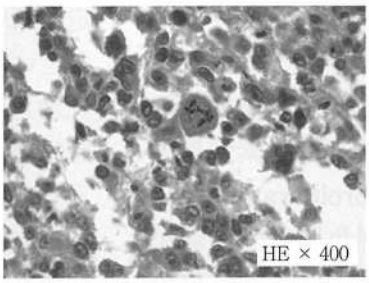

図 4a　病理組織像（被膜内部×400）　変性した細胞がみられた．

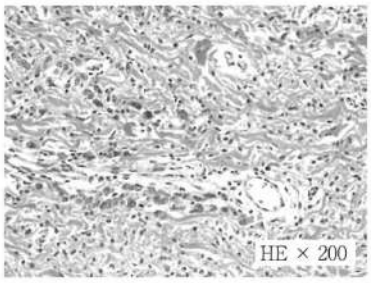

図 4b　病理組織像（被膜外部×200）　周囲に浸潤する細胞が存在した．

り，変性した細胞（図 4a）や，一部には周囲に浸潤する細胞（図 4b）も存在した。また，腫瘍細胞は多型が強く，核分裂像が多く散見された（図 4c）が，異常核分裂像や壊死巣は認めなかった。全体的な腫瘍病変の構築は左右対称性であり（図 3），深部への浸潤傾向はみられなかった。

免疫組織学的所見：被膜内の腫瘍細胞に Ki-67 や cyclin D1 の陽性細胞は乏しかったが，被膜外浸潤を示す腫瘍細胞には多数の Ki-67 や cyclin D1 陽性細胞がみられた（図 4d-1, 2）。

切除後経過：全体的な腫瘍の組織構築は左右対称性であり，深部への浸潤傾向なく，異常核分裂像や壊死巣は認めないことから注意深い経過観察とした。母斑の切除のみ継続し現在 5 歳 6 か月であるが，明らかな再発および転移はなく，経過も

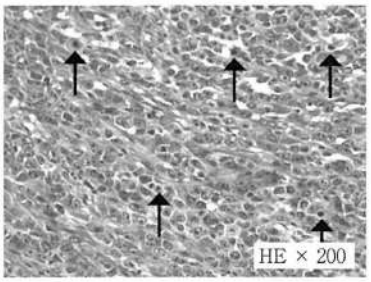

図 4c　病理組織像（被膜外部×200）　腫瘍細胞は多型が強く，核分裂像が多く散見された（↑）．

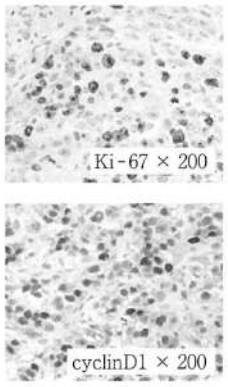

図 4d-1, 2　病理組織像（被膜外部×200）　被膜外浸潤を示す腫瘍細胞には多数の Ki-67，cyclin D1 陽性細胞を認めた．

含めてproliferative noduleと診断した。

考　察

先天性の色素性母斑はごく小型のものから巨大なものまであり，新生児の1～2％に見いだされる[2]。Kopfらは最大径が20cm以上のものを巨大色素性母斑と定義しており[3]，巨大色素性母斑に生じる悪性黒色腫は5歳までに約50％[4]，約70％が思春期までに発症していると報告されている[5]。また，わが国の小児悪性黒色腫の1/3が先天性巨大色素性母斑を発症母地とし，その発症年齢の平均は3.6歳であったとの報告もあり[6]，早期に母斑を除去すべきと考えられている。

一方，新生児～乳幼児期に先天性巨大色素性母斑の病巣内に良性の結節性病変を生じることがあり，proliferative noduleと称される。結節の大きさは径5mm程度までのことが多いが，さらに大型のこともあり，ときに多発する。病理組織学的に悪性黒色腫との鑑別が問題になることがあるが，予後は良好である。組織学的鑑別点として，増殖する細胞が多角形状～類円形状の比較的小型の細胞であること，異型性や核分裂像が目立たないこと，壊死や破壊性増殖像は認められないこと，結節辺縁部で周囲の母斑細胞への移行像がうかがわれることが挙げられている[7]。本症例では全体的な腫瘍構築は左右対称性であり，深部への浸潤傾向や壊死巣はみられないものの，構成する腫瘍細胞は多型性に富み核分裂像も多く，Ki-67陽性率も30％と高かった。このため悪性黒色腫との鑑別に苦慮した。Bastianらは先天性色素性母斑に随伴した増殖病変を病理組織学的にGroup Ⅰ～Ⅵに分類し[8]，Group Ⅳを「先天性母斑上に生じたnodular melanomaに類似する病変で，細胞充実度が高く，核異型があり増殖率も著しいもの」と定義した。本症例はBastianのGroup Ⅳに類似するが，Bastianらの報告でGroup Ⅳに分類された9症例中，経過の得られた5例はいずれも悪性黒色腫への進展はなかった。また，Group Ⅳの9例中7例（78％）でComparative genomic hybridization（CGH）で染色体異常がみられたが，その内容は主に1本の染色体すべてが欠損・増幅するというもので，悪性黒色腫で多くみられるような染色体の部分的な欠損・増幅が多岐にわたるパターンとは異なっていた[8]。Bastianらは，この染色体異常の差からGroup Ⅳは良好な経過をたどる可能性があると推測している[8]。また，本症例のKi-67陽性率は30％であったが，Nguyenらの報告した症例は，先天性巨大色素性母斑上に生じた結節性病変で非常に多くの核分裂像（27 per mm^2）を持ち，Ki-67標識率も60％と高かった。Fluorescence in situ hybridization（FISH）を用いてproliferative noduleと診断し，活発な分裂像を認めても，それだけで悪性黒色腫と診断すべきではないと結論づけている[9]。van Hautenらは，proliferative noduleの99例をまとめ，proliferative noduleは臨床的にも病理学的にも非常に多様性があり，核分裂像が多いこともあるが，異常核分裂像は認めなかったことを報告している[10]。本症例の病理組織像でも異常核分裂像はみられず，proliferative noduleの診断を支持する所見であった。本症例のように診断に苦慮する場合は，FISHやCGHのような分子学的検索も診断の一助となる可能性がある。症例の蓄積と今後の診断技術の向上に期待したい。

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日本小児皮膚科学会の定める利益相反に関する開示事項はありません。

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A proliferative nodule arising in a giant congenital nevus and closely resembling malignant melanoma

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Proliferative nodules arising in congenital melanocytic nevi (CMN) can be difficult to distinguish from malignant melanoma, which may also develop within giant CMN. We treated a one-year-old girl who presented with a melanocytic nodule measuring 8 × 6 mm within a CMN on the abdomen. After the nodule was excised, histopathological examination revealed a significant number of mitoses and a Ki-67 labeling index of 30%, which made it difficult to distinguish from malignant melanoma. However, the overall histopathological structure was symmetrical, with no deep invasion, atypical mitosis, or necrosis. We carefully monitored the patient for three years and nine months, and confirmed no recurrence or metastasis. On the basis of the histopathological features and clinical course, we finally diagnosed a proliferative nodule. Careful monitoring appears to be necessary to definitively diagnose nodules developing in CMN.

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Key words : giant congenital melanocytic nevus, Ki-67, melanoma, proliferative nodule

＜皮膚科＞

①Injury due to extravasation of thiopental and propofol: Risks/ effects of local cooling/warming in rats

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Injury due to extravasation of thiopental and propofol: Risks/effects of local cooling/warming in rats

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ABSTRACT

Inadvertent leakage of medications with vesicant properties can cause severe necrosis in tissue, which can have devastating long-term consequences. The aim of this study was to evaluate the extent of extravasation injury induced by thiopental and propofol, and the effects of cooling or warming of local tissue on extravasation injury at macroscopic and histopathologic levels. Rats were administered intradermally thiopental (2.5 mg/100 μL) or propofol (1.0 mg/100 μL). Rats were assigned randomly to three groups: control (no treatment), cooling and warming. Local cooling (18–20 °C) or warming (40–42 °C) was applied for 3 h immediately after agent injection. Lesion sizes (erythema, induration, ulceration, necrosis) were monitored after agent injection. Histopathology was evaluated in skin biopsies taken 24 h after agent injection. Thiopental injection induced severe skin injury with necrosis. Peak lesions developed within 24 h and healed gradually 18–27 days after extravasation. Propofol induced inflammation but no ulceration, and lesions healed within 1–2 days. Local cooling reduced thiopental- and propofol-induced extravasation injuries but warming strongly exacerbated the skin lesions (e.g., degeneration, necrosis) induced by extravasation of thiopental and propofol. Thiopental can be classified as a "vesicant" that causes tissue necrosis and propofol can be classified as an "irritant". Local cooling protects (at least in part) against skin disorders induced by thiopental and propofol, whereas warming is harmful.

1. Introduction

Accidental leakage of certain medications into the body from an intravenous drip is not uncommon. Inadvertent leakage of medications with vesicant properties can cause severe necrosis in tissue, which can have devastating long-term consequences. Most instances of extravasation are attributed to cytotoxic agents, and the prevalence has been reported to be 0.1–6% [1,2]. In addition, several non-cytotoxic agents have been reported to possess vesicant properties due to their high osmolarity, extremely acidic or basic pH, and vasoconstrictive activity [3]. Several guidelines for overall management of extravasation have suggested that recognition of potential risks for each agent is important so that clinicians can manage extravasation depending on the severity of such risks [4,5]. Intravenous cytotoxic agents can be classified into three categories according to the extent of damage from extravasation: vesicants, irritants, and non-tissue-damaging agents [1,2,4]. Vesicants can cause tissue necrosis even at small volumes of extravasation because they are inherently toxic to cells. Irritants can cause an inflammatory reaction (but not necrosis) at the extravasation site. Non-tissue-damaging agents do not damage tissue at all.

Thiopental and propofol are used for the induction and maintenance of anesthesia. Extravasation of these agents is common because anaesthetized patients cannot indicate pain during injection [6]. Risk of extravasation injury is increased because propofol is administered forcefully using automated syringe drivers. Several case reports from 1961 through to 2014 have highlighted extravasation by thiopental or propofol [3]. It is well known that thiopental can act as a vesicant [7] and propofol can act as an irritant [8–11]. However, few reports have shown the vesicant effects of propofol [12–14]. Thus, the risk of skin lesions induced by propofol extravasation is understood incompletely because data

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are limited to a few case reports. Clinicians manage extravasation injuries according to the potential risks of agents. Hence, classification of non-cytotoxic agents into the three categories described above (as well as cytotoxic agents) depending on their toxicity would be useful.

Prompt interdisciplinary management of tissue damage induced by extravasated agents is important for successful therapy. Several reports have shown that stopping the drug infusion as well as surgical excision, thermal application and/or pharmacologic interventions aid management of injury due to extravasation of agents [4,5]. Cooling or warming of local tissue are major supportive measures to reduce skin lesions induced by certain agents [1,3,4,15]. According to one overview of extravasation management [16], hospitals should ensure the availability of "extravasation kits containing cold–hot packs" at the treatment unit. However, there is little scientific basis for the effects of poultices on skin lesions. With regard to management of thiopental extravasation, warming is recommended by the manufacturer, though there is no evidence to indicate therapeutic effects. With respect to management of propofol extravasation, only one report has shown that cooling reduces pain [17]. Thus, the usefulness of cooling or warming of local tissue to manage injuries induced by extravasation of thiopental or propofol is not known.

In the present study, we first evaluated the extent of extravasation injuries induced by thiopental or propofol and classified these agents (as well as cytotoxic agents) on the basis of macroscopic and histopathologic evaluations of skin damage. Next, the effects of local cooling or warming on extravasation injury were evaluated to provide a comprehensive view of management strategies for extravasation of thiopental and propofol.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the *Guide for Animal Experimentation from the Committee of Research Facilities for Laboratory Animal Sciences of Hiroshima University* (permit number: A15-31).

Thirty-three male Wistar albino rats (8 weeks; body weight, 250–270 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan). Rats were housed in individual cages in a temperature-controlled room at 23 °C on a 12-h light–dark cycle. They were fed a standard laboratory diet (MF, Oriental Yeast Company, Tokyo, Japan) and water *ad libitum* for >1 week before experimentation.

2.2. Extravasation models

Rats were anesthetized with pentobarbital (40 mg/kg, i.p.). According to a previous report [18], the hair on the back of rats was shaved with an electrical clipper (Thrive 2100; Daito Electric Machine Industry Co. Ltd., Osaka, Japan). Sodium thiopental (Ravonal®; Tanabe–Mitsubishi, Osaka, Japan) was dissolved at 2.5 mg/100 μL in water for injection according to manufacturer instructions. Twenty-four hours after hair removal, rats with no wounds were injected intradermally (i.d.) with a solution of thiopental or propofol (1.0 mg/100 μL of Diprivan®; AstraZeneca, Osaka, Japan) at 100 μL (the minimum volume at which lesions can be observed macroscopically). As a negative control group, physiologic (0.9%) saline was injected (i.d.) at 100 μL as well as thiopental and propofol. Intradermal injections were undertaken after grabbing dorsal skin using a 26-G needle at the center of a hair-free site 7 cm from the ear. Two injections were made on the axisymmetric dorsal side of each rat. Right-side lesions were monitored until the injury healed completely. Left-side lesions were punch-biopsied (using a dermal punch) 24 h after intradermal injection for histopathologic evaluation under anesthesia with pentobarbital.

2.3. Cooling and warming of local tissue

Rats that had undergone intradermal injection of thiopental or propofol were assigned randomly to three experimental groups of 5 rats each: no treatment (control); cooling (treatment with a cold pack); warming (treatment with a hot pack). Cooling and warming of local tissue were done for 3 h immediately after intradermal injection using cold or hot packs (3M HealthCare, Tokyo, Japan), respectively [19]. Three rats were assigned to a group in which saline was injected via the intradermal route without treatment. Skin temperature was monitored every 10 min using electronic thermocouple probes (BTM-4208SD; Sato-Tech, Kanagawa, Japan), and was maintained at 18–20 °C or 40–42 °C for cooling and warming, respectively.

2.4. Macroscopic evaluation

Extravasation injury to skin was evaluated macroscopically according to a method described previously [18]. Briefly, the widest perpendicular diameters of skin lesions were measured using a caliper by an investigator blinded to group allocation. Each lesion site was inspected every day during the first week after intradermal injection, then every 5 days from day-7. Four parameters of lesions (erythema, induration, ulceration, necrosis) were assessed. The area of lesion sites was calculated in cm^2 as the product of diameters [18]. The area under the lesion–time curve (AUC) was calculated in cm^2 days using the trapezoidal method [18]. The AUC, peak area of the lesion, and damage duration were analyzed until the injury healed completely.

2.5. Histopathologic evaluation

Lesion sites were biopsied (using a dermal punch) with a diameter of 4 mm at 24 h after intradermal injection according to the peak time of lesions using phenytoin (which is thought to possess strong alkaline properties similar to those of thiopental) [20]. Tissue samples were suspended in 10% formaldehyde for fixation before dehydration, Sections (5 μm) from the paraffin-embedded tissue blocks were stained with hematoxylin and eosin, in addition to standard histopathologic evaluation under a light microscope (BX51; Olympus, Tokyo, Japan). Each sample was analyzed by independent pathologists blinded to the experimental procedure.

2.6. Statistical analyses

Data are the mean ± standard error of the mean (SEM). Differences among each treatment group were analyzed using Kruskal–Wallis test followed by the Student Newman–Keuls multiple-comparison *post hoc* test. $P<0.05$ was considered significant.

3. Results

3.1. Macroscopic findings

For saline-injected rats, no change was observed at any injection site. In thiopental-treated rats, skin lesions developed immediately and reached maximal intensity within 24 h after injection (Fig. 1a). At 2–3 days after thiopental injection, the epidermis had regenerated and exhibited eschar formation, granulation and excoriation of necrotic sites. Typical shape of these skin lesions was ovoid, and induration, erythema, and ulceration (in descending order of size) were observed. Epidermal integrity was

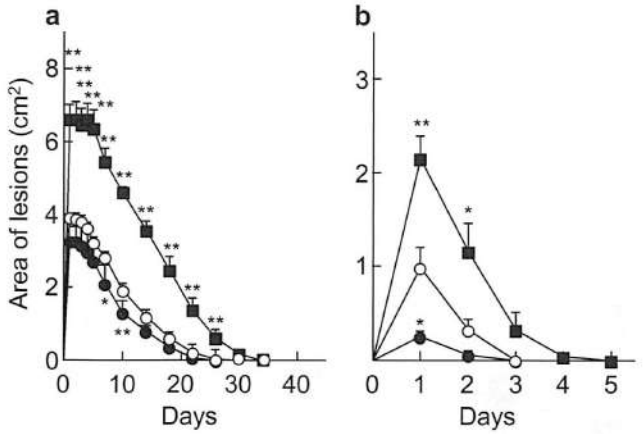

Fig. 1. Effects of local cooling or warming on macroscopic injury induced by thiopental (a) or propofol (b) extravasation in rats. Thiopental (2.5 mg) or propofol (1.0 mg) were administered intradermally (ID) at a volume of 100 μL. Local cooling (18–20 °C) and warming (40–42 °C) were performed for 3 h immediately after ID of thiopental or propofol. Remaining lesions were monitored until the injury was healed completely. Open circles, closed circles and closed squares represent lack of treatment, cooling and warming, respectively. Each value represents the mean ± SEM of results from five rats. $^{*}P < 0.05$, $^{**}P < 0.01$: significantly different from controls (non-treatment).

Table 1
Effects of local cooling or warming on thiopental- and propofol-induced skin lesion parameters in rats.

Agent/Treatment	Peak area (cm^2)	AUC (cm^2 days)	Damage duration (days)
Thiopental			
None	3.9 ± 0.2	42.0 ± 2.3	20.9 ± 0.9
Cooling	3.3 ± 0.2	31.5 ± 2.1	17.6 ± 1.1*
Warming	6.6 ± 0.5**	98.4 ± 6.2**	26.8 ± 1.3**
Propofol			
None	1.0 ± 0.2	1.3 ± 0.3	1.5 ± 0.2
Cooling	0.3 ± 0.1*	0.3 ± 0.1	1.1 ± 0.2
Warming	2.2 ± 0.2**	3.7 ± 0.7**	2.2 ± 0.3

AUC, area under the lesion-time curve. Thiopental (2.5 mg) and propofol (1.0 mg) were administered intradermally (ID) at a volume of 100 μL. Local cooling (18–20 °C) and warming (40–42 °C) were performed for 3 h immediately after ID of thiopental or propofol. Remaining lesions were monitored until the injury was completely healed. Each value represents the mean ± SEM of results from five rats.
* $P < 0.05$.
** $P < 0.01$: significantly different from controls (non-treatment).

returned completely over 18–27 days. Local cooling slightly improved skin lesions as represented by the peak area of lesions, AUC, and damage duration ($p < 0.05$). Warming significantly increased the peak area and AUC by ≈ 1.7-fold ($p < 0.01$) and 2.3-fold ($p < 0.01$) compared with those in the control group, respectively (Table 1). Damage duration was also extended by ≈ 6 days in warming-treated rats compared with that in control rats ($p < 0.01$). Propofol injection also induced maximal skin lesions within 24 h (as shown in thiopental-treated rats). Lesions in propofol-treated rats had subtle erythema but no ulceration (unlike those in thiopental-treated rats). Propofol-induced lesions healed within 2 days compared with the 18–27 days needed by thiopental-treated rats (Fig. 1b and Table 1). Thus, the extent of propofol-induced lesions was much milder compared with that induced by thiopental. Local cooling reduced the peak area ($p < 0.05$), AUC and damage duration, whereas warming increased the peak area ($p < 0.01$), AUC ($p < 0.01$) and damage duration (Table 1).

3.2. Histopathologic findings

In saline-treated control rats, skin tissue showed cells with intact architecture as well as regular morphology of skin tissue (Fig. 2a, b). At 24 h after thiopental injection, degeneration, edema, necrosis and infiltration of inflammatory cells were observed in epidermal, dermal and subcutaneous tissues (Fig. 2c, d). In good agreement with macroscopic findings, local cooling suppressed the edema and degeneration in muscle seen in thiopental-treated rats, suggesting that cooling also reduced the skin disorders caused by thiopental injection at the morphologic level (Fig. 2e, f). In contrast, warming promoted more severe degeneration and necrosis in epidermal, dermal, subcutaneous tissues, blood-vessel walls and muscle compared with those in untreated rats (Fig. 2g, h). Some nuclear debris was observed in the deep dermis of these warmed rats. These results clearly suggested that skin lesions induced by thiopental injection in warmed rats was the most severe among the three groups. Propofol injection did not result in necrosis, but led to more inflammatory cells infiltrating into dermal and subcutaneous tissues and muscle in untreated controls compared with those in thiopental-treated rats. Furthermore, cooling and warming did not affect the lesions of propofol-injected rats at the histopathologic level (Fig. 3).

4. Discussion

Recognition of the potential risks for extravasation of agents is very important. However, the risks of extravasation injury induced by thiopental and propofol are understood incompletely because extravasation injuries caused by these agents has not been evaluated at macroscopic and histopathologic levels. We examined the intrinsic risks of extravasation injury by thiopental and propofol and classified them into three categories according to macroscopic and histopathologic findings of skin damage using a rat model. Effects of local cooling or warming of tissue on extravasation injury were also evaluated.

Thiopental injection induced skin lesions such as erythema, induration, ulceration and tissue necrosis within 24 h after its extravasation (Fig. 1a). In good agreement with macroscopic findings, histopathologic evaluation of skin lesions showed degeneration, edema, and necrosis in the epidermal, dermal and subcutaneous tissues of thiopental-treated rats within 24 h (Fig. 2). These results suggest that extravasation injury due to thiopental is severe, and we classified thiopental as a "vesicant". With respect to the mechanism of tissue damage from extravasation of non-cytotoxic agents, the high osmolarity, acidic or alkaline pH and/or vasoconstrictive activity have been reported [3]. According to manufacturer instructions, thiopental injection leads to an osmotic pressure ratio of 0.8 and alkaline pH of 10.2–11.2. Thus, the ulceration and necrosis induced by thiopental could be attributed to its alkalinity rather than its osmolarity. Propofol caused an inflammatory reaction with erythema, but ulceration was not observed macroscopically in the extravasation site. Histopathologic evaluation showed that propofol exerted no vesicant effects (including induction of necrosis though the increased number of inflammatory cells infiltrating into the dermis and subcutaneous tissue) (Fig. 3). Thus, we categorized propofol as an "irritant", in agreement with previous reports [8–11]. Propofol has been recognized as a less invasive drug because of its chemical properties, isotonicity, and neutral pH (7.0–8.5) [8]. However, even a less invasive agent such as propofol can induce tissue injury if large volumes of solution are extravasated because high hydrostatic pressures lead to tissue ischemia [21,22]. Propofol can leak readily because it is injected forcefully using automated syringe drivers. Thus, tissue compression by large volumes of extravasated

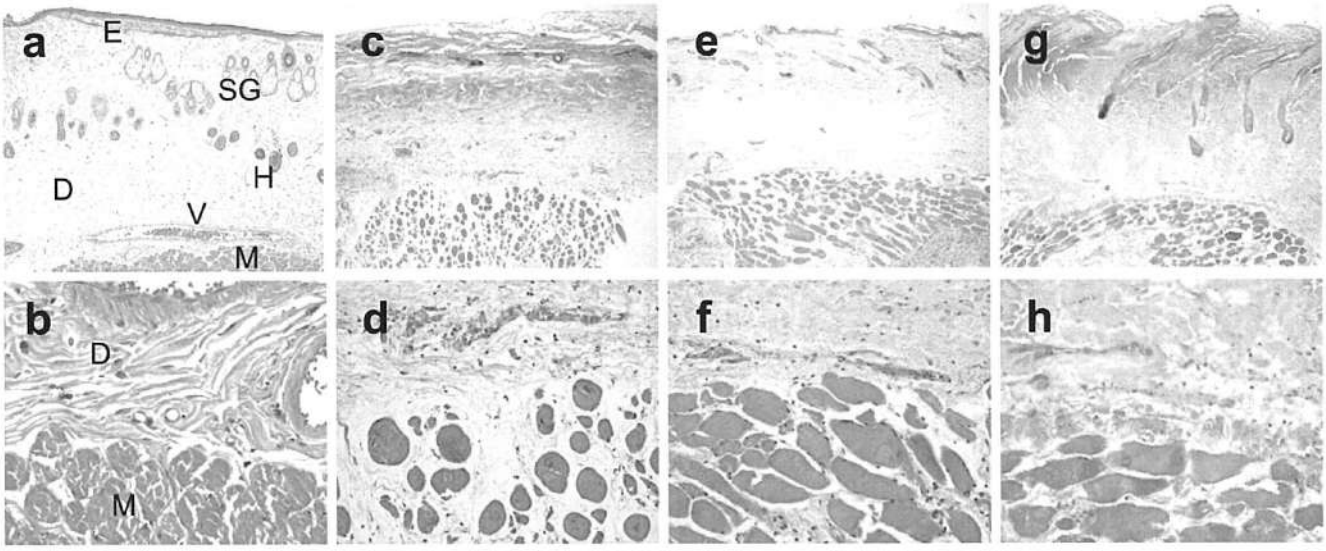

Fig. 2. Effects of local cooling or warming on histopathological skin lesions induced by thiopental extravasation in rats. Normal skin (a, b) after intradermal injection (ID) of saline shows complete architectural construction of dermis, skin appendixes, epithelium (E), dermis (D), muscle (M), hair follicle (H), sebaceous glands (SG), vessel (V). Control (c, d) is a group without any treatment after ID of thiopental (2.5 mg). Cooling treated with cold pack (18–20 °C) for 3 h immediately after thiopental ID, reduced edema (e, f). Warming treated with hot pack (40–42 °C) for 3 h immediately after thiopental ID, caused degeneration and necrosis in the deep dermis and muscle (g, h). Hematoxylin and eosin stain: (a, c, e, g) magnification × 4; (b, d, f, h) magnification × 20.

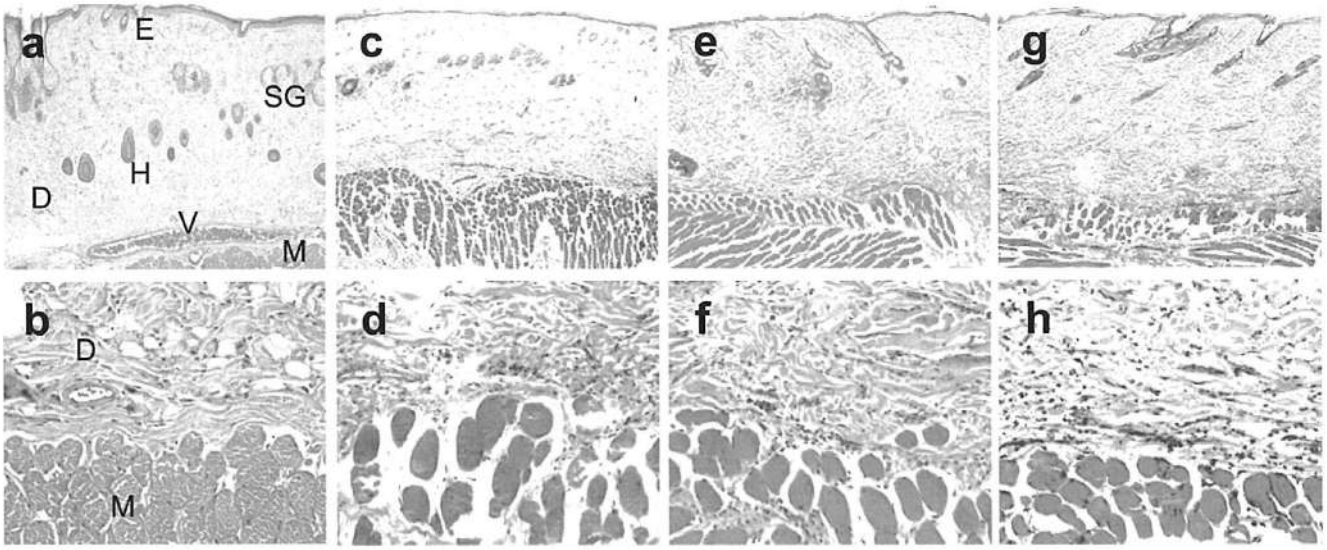

Fig. 3. Effects of local cooling or warming on histopathological skin lesions induced by propofol extravasation in rats. Normal skin (a, b) after intradermal injection (ID) of saline shows complete architectural construction of dermis, skin appendixes, epithelium (E), dermis (D), muscle (M), hair follicle (H), sebaceous glands (SG), vessel (V). Control (c, d) is a group without any treatment after ID of propofol (1.0 mg). Cooling (e, f) treated with cold pack (18–20 °C) for 3 h immediately after propofol ID and warming (g, h) treated with hot pack (40–42 °C) for 3 h immediately after propofol ID. Skin tissues were biopsied at 24 h after ID of saline or propofol. Hematoxylin and eosin stain: (a, c, e, g) magnification × 4; (b, d, f, h) magnification × 20.

solution may be involved in the development of skin toxicity by extravasated propofol. In addition, several reports have shown that skin necrosis by propofol is involved in malnutrition and predisposition to the bleeding caused by sepsis and diabetes mellitus [12,23,24]. Thus, further study is needed to confirm that propofol is an irritant by distinguishing the intrinsic risk properties of agents and other risk factors (e.g., physical destruction of tissue due to excess accumulation of injected fluid in connective tissues).

With regard to the management of extravasation injury induced by cytotoxic agents, application of cooling or warming and pharmacologic agents (e.g., dexrazoxane for anthracycline and hyaluronidase) has been reported [25]. We examined the effects of cooling or warming on extravasation injury induced by thiopental and propofol because these supportive measures are conservative. Cooling reduced the severity of thiopental-induced lesions, including edema and necrosis in dermal, subcutaneous and intramuscular tissue. The mechanism of injury reduction by cooling may be due to the induction of vasoconstriction, which results in reduced dispersion of these agents [25]. Dorr et al. reported that warming can reduce the extravasation injury caused by vinca alkaloids though mechanisms that are understood incompletely [26]. In contrast, in our study, warming exacerbated rather than improved the extravasation injury caused by thiopental at macroscopic and histopathologic levels. Warming also exacerbated the severity of macroscopic skin lesions in propofol-treated rats, but exerted no histopathologic changes in propofol-treated rats

(Fig. 3). The reason for this difference in the effect of warming on propofol-induced injury at macroscopic and histopathologic levels is not clear. Dispersion of these agents away from the extravasation site, and promotion of an inflammatory reaction and/or edema resulting from the increased blood circulation by warming, could be considered as mechanisms of exacerbation [1,3,15]. Conversely, local warming in combination with an acidic agent (e.g., lidocaine) could improve the extravasation injury induced by alkaline agents because warming enhances the neutralization of alkaline agents by lidocaine resulting from promotion of their dispersion [3].

Studies have shown that dimethyl sulfoxide (DMSO) improves anthracycline-induced tissue injuries by scavenging free radicals [27,28]. Extravasation of alkaline agents, as well as anthracycline agents, generates free radicals such as the hydroxide radical [29]. Hence, topical administration of DMSO could enable scavenging of the free radicals generated by extravasated alkaline agents. DMSO also promotes redistribution of poorly water-soluble extravasated agents from lesions into the systemic blood circulation by increasing their solubility [25]. Thiopental and propofol are poorly soluble in water but have high solubility in DMSO. Thus, a combination of topical DMSO and cooling applied after extravasation could improve the skin lesions induced by these agents by scavenging free radicals and/or promoting removal of these agents from tissues to a greater extent compared with cooling alone.

Systematic methods of cooling and warming, such as the compression time and temperature for extravasation injury, have not been determined [22]. Multiple cooling or warming poultices have been applied to reduce extravasation injury (e.g., four-times daily for 20-min each for 1–2 days) [29]. Protection, rest, ice, compression, and elevation have been used for ankle-joint sprains [30] and muscle bruises, as has cryotherapy [31]. Thus, multiple cooling may be effective for treatment of the skin lesions induced by thiopental and propofol. Oyama et al. reported that a single 3-h cooling session caused a greater reduction in counts of inflammatory cells expressing C5a receptor 1 and interleukin-8 receptor, and a peripheral nerve fiber bundle expressing transient receptor potential vanilloid type 1 than four-times daily cooling for 20 min in doxorubicin-injected mice [32], suggesting that using a poultice for 20 min may not be sufficient to treat the extravasation injury cause by vesicants. However, so far, there are no data demonstrating whether multiple cooling or single cooling is more relevant to treat skin lesions induced by extravasated thiopental and propofol. In our study, single cooling at 18–20 °C for 3 h reduced the inflammation and ulceration caused by thiopental and propofol. Thus, we recommended use of a cool poultice for the 3 h to treat the extravasation injury by thiopental or propofol. Warm compresses must be avoided to treat the extravasation injury caused by these agents. This information could provide a reference for identification of the risk of extravasation of propofol or thiopental, and management of such extravasation.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.09.005.

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