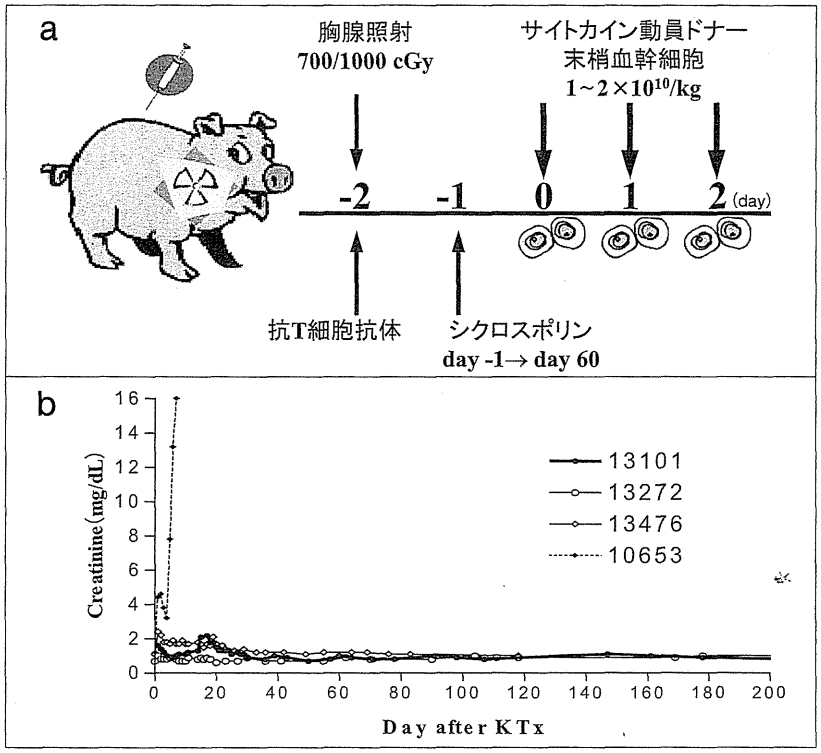

図2 ブタ MHC ミスマッチ間での非骨髄抑制性前処置による mixed chimerism の誘導と免疫寛容

a：非骨髄抑制性前処置のレジメン

b：13101，13272，13476：mixed chimerism が成立後，血液幹細胞のドナーと SLA が一致した腎臓を免疫抑制剤非投与下に移植したのちの血清クレアチニンの変動．10653：ナイーブのミニブタに one-haplotype ミスマッチの腎臓を免疫抑制剤非投与下に移植したのちの血清クレアチニンの変動

(Fuchimoto Y et al.：J Clin Invest 105：1779-1789, 2000[6]より)

をもとにして，WBI の回避を試みた．多量(1×10^{10}/kg)血液幹細胞移植を用いた非骨髄抑制性前処置のレジメンを示す．

基本的には SLA マッチ群と SLA one-haplotype ミスマッチ群で pCD3-CRM9 を使用した T 細胞除去と胸腺照射，1×10^{10}/kg の PBSC 移植，移植後の 60 日間のシクロスポリン(CyA)投与である(図 2a)．このレジメンでは WBI を受けた動物に比較して非常に健康で，感染はまったく認められなかった．4 匹中 4 匹のブタで安定した長期 MC の成立に成功した．一過性の GVHD を認めたブタもいたが，全例において 1 年以上安定した MC が維持された．また，このレジメンを受けたブタはいずれも骨髄移植後数カ月でドナーの腎移植を受けた．腎移植前に mixed lymphocyte reaction (MLR)，cell-mediated lympholysis (CML)の T 細胞機能解析においてドナーに対して低反応を示したが，3rd party には正常な反応を示すことより，ドナー特異的免疫寛容が証明された．全動物で免疫抑制剤をまったく使用せずにドナー腎は長期生着した(図 2b)[6]．

このレシピエントブタの胸腺の生検組織では皮質-髄質結合部にドナー細胞を認め，二重免疫染色ではドナー細胞は Class Ⅱ陽性で，形態学的に

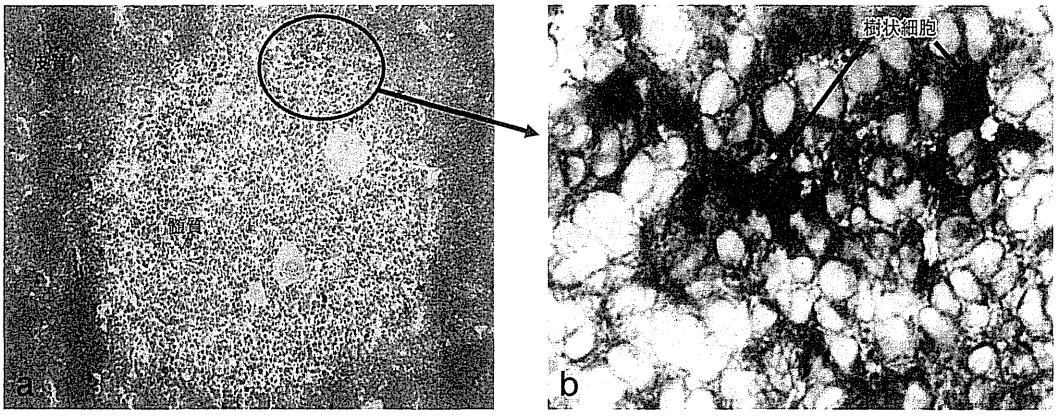

図3　安定した mixed chimerism のブタモデルでの
胸腺内ドナー細胞の存在

MHC ミスマッチ間の安定した MC を示すブタモデルのレシピエント胸腺内では，皮質-髄質結合部にドナー細胞を認めた(a：ドナー Class Ⅰ抗体)．この部分の二重免疫染色ではドナー細胞は Class Ⅱ陽性で，形態学的に樹状細胞と思われた(b)．また，FACS にても胸腺内に未熟な T 細胞(CD1)と成熟 T 細胞(CD3)が混在することが確認できた．以上より，このモデルにおける免疫寛容は胸腺内 clonal deletion を主とすることが強く推察された．

(Fuchimoto Y et al.：J Clin Invest　105：1779-1789, 2000[6]より)

樹状細胞と思われた．また FACS においても胸腺内にドナー骨髄由来の未熟 T 細胞(CD1 陽性)ならびに成熟 T 細胞(CD3 陽性)を認めた(図 3)．

以上のことより，このモデルにおける *in vitro* ならびに *in vivo* でのドナー特異的免疫寛容の機序は胸腺内における clonal deletion が強く示唆された[6]．ただ，このレジメンではブタのγヘルペスウイルス関連の移植後リンパ増殖性疾患(PTLD)を 25％程度認めたこと[7,8]から，1 Gy の WBI を追加することにより PTLD の発生も抑えられ，より安定した MC の確立が得られた[9]．

イヌにおける前臨床研究

ワシントン大学(シアトル)グループの Storb らは，血液系疾患の治療や免疫寛容誘導に MC を応用するために，イヌの MHC である dog leukocyte antigen(DLA)が一致したマイナー抗原のみの異なるイヌのモデルを使用して低侵襲での MC の確立の研究を行っている[10,11]．前処置としては基本的に 1.0～4.5 Gy の WBI と移植後に CyA，ミコフェノール酸モフェチル(MMF)，ラパマイシンなどの免疫抑制剤を併用して MC を確立したのちに，腎移植または肺移植によって免疫寛容を確認している[12～14]．

このイヌの研究でも MC は非常に長期に持続しており，免疫寛容の機序としてはブタと同様の機序が最も考えられた．

サルにおける前臨床研究

MGH のグループはマウス，ブタ，サルの研究成果から MC の臨床応用を開始した．

Kawai らは MHC 不適合間のサルにおいて低侵襲プロトコールにより，混合キメラと同種腎移植の免疫寛容誘導について精力的に研究を行ってきた．基本的プロトコールは，① 3 Gy の WBI，② 7 Gy の胸腺照射，③ 抗 T 細胞抗体，④ 脾摘，⑤ 移植後 1 カ月間の CyA 投与である[15～19](図 4)．このうち長期生存しているサルのなかには 10 年以上免疫抑制剤を中止した状態で正常な腎組織を示すものも存在する．脾摘は副刺激阻害である抗 CD154 抗体によってとってかわることが報告された[18]．

ただこのプロトコールによるサルのキメラは数

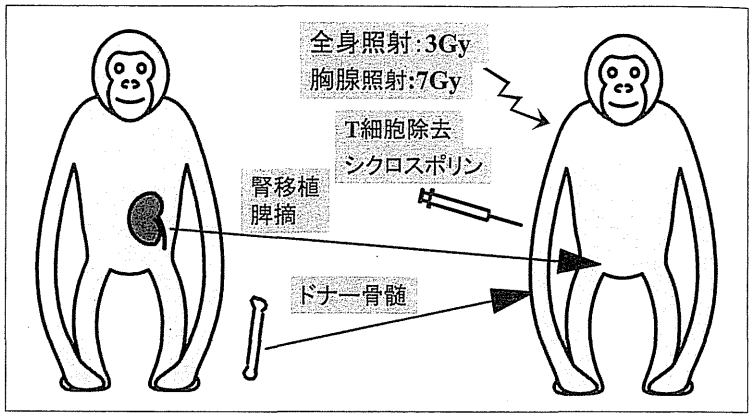

図4 サルにおけるMHCミスマッチ間での非骨髄破壊性前処置による mixed chimerism の誘導
レジメンは基本的に，① 3 Gy の全身照射，② 7 Gy の胸腺照射，③ 抗T細胞抗体，④ 脾摘，⑤ 移植後1カ月間のシクロスポリン投与である．
（河合達郎先生にご提供いただいた図より改変）

週間しか持続せず，MCは一過性であり，その免疫寛容機序としてはブタとは異なることが推察された．

大動物研究から臨床応用へ

これまでMCを臨床応用して一定の成果を得ている施設として，Harvard大学(MGHプロトコール)，Stanford大学(Stanfordプロトコール)，Louisville大学(Northwesternプロトコール)がある．

MGHプロトコールでは，シクロホスファミド，抗CD2抗体，リツキシマブの前処置後にHLA haplo-identicalなドナーの骨髄と腎臓を同時に移植した[20]．現在まで10人に施行し，7人で免疫抑制剤を中止できている(図5)．最長では8年以上，免疫寛容の状態が持続している患者も存在している．

Stanfordプロトコールにおいては，HLAマッチであるが腎移植後に抗胸腺細胞抗体，全身リンパ腺照射(TLI)，骨髄移植を行って長期MCを得て，免疫寛容の報告を行っている[21]．HLAミスマッチ間では成功例はないが，HLAマッチ間では長期のMCが確認されている点から注目に値する報告である．ただ末梢血のMCを認めていても臓器を拒絶する症例もあり，末梢血のMCは必ずしも免疫寛容を保証するものではないことはブタの研究でも報告があり，興味深い[9,22,23]．現在までに16人に同プロトコールを行い，8人に6カ月以上のMCを獲得し，そのうち4人で免疫抑制剤を中止できたと報告している[24,25]．

最近報告されたNorthwesternプロトコールでは，HLAミスマッチ間にてフルダラビン，シクロホスファミドと2 GyのWBIの前処置後に腎移植とfacilitating cellの注入にて，GVHDもengraftment syndrome(骨髄生着時のサイトカインストームによるvascular leak syndrome)も起さず，8人中5人に非常に安定したMCと免疫抑制剤の中止が可能であった[26]．HLAミスマッチで安定した高レベルのMCを得て，腎移植の免疫寛容を誘導した点で非常に画期的な報告であるが，facilitating cellのcharacterization，GVHDがなぜ起きないかの理由など不明な点も多く，今後のさらなる検討が必要である[27,28]．

ブタ・イヌとサル・ヒトの免疫寛容誘導機序の考察

興味深いことに，ブタ・イヌの場合にMCは半

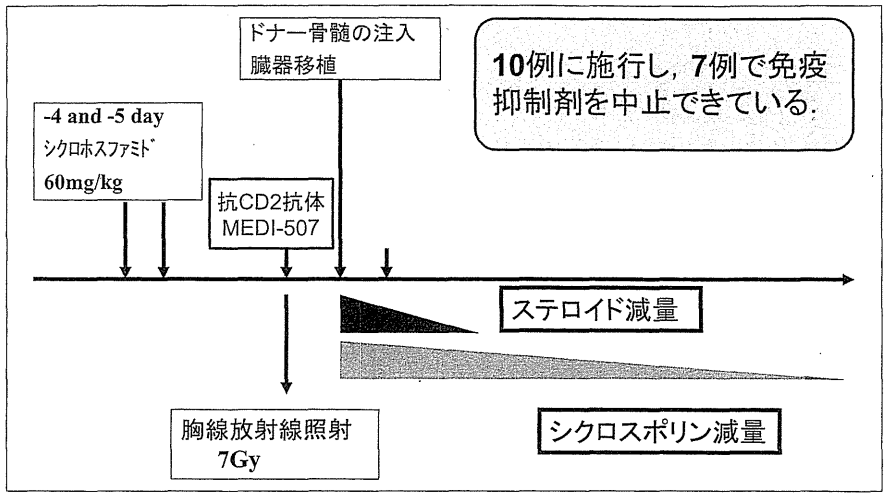

図5　MGH プロトコールによる臨床免疫寛容誘導
移植前 5,4 日にシクロホスファミド 60 mg/kg を投与．胸腺照射を行ったのちに抗 CD2 抗体の投与，その後，骨髄移植と腎移植を同日に行う．3 例目に液性拒絶がみられたため，これにリツキシマブの投与を行っている．移植後はステロイドならびにシクロスポリンを数カ月で漸減してゆく．
（河合達郎先生にご提供いただいた図より改変）

永久的に持続するのに対して，サル・ヒトでは少なくとも MHC ミスマッチ間において MC は一過性でその後には消失してしまう．この一過性の MC は GVHD にとっては都合がよいのであるが，両者で臓器の免疫寛容の機序が異なる可能性がある．

ブタならびにイヌの場合は安定した MC を作製し，数カ月後にドナーの腎臓あるいは肺を移植して免疫寛容を確認している．臓器移植後に免疫抑制剤は使用していない．この免疫寛容誘導機序は移植臓器が関与しない中枢性免疫寛容と思われる．ブタによる解析では末梢血のドナー細胞キメラは必ずしも免疫寛容を保証するものではなく，骨髄，胸腺，末梢血におけるドナー細胞の生着こそがドナー臓器の免疫寛容を保証するとの報告がある[9]．

これに対して，サル・ヒトの場合は臓器移植と骨髄移植は同時に行われ，臓器自体が免疫寛容に深く関わっている可能性が強く示唆される．実際，臨床症例の末梢血ならびに移植臓器のリンパ球の解析結果から免疫寛容に末梢性の調節性機序が報告されている[20,29]．また，最近ではサルの研究から T 細胞除去を行っても memory CD8 T 細胞の残存により MC の誘導が阻害されることがわかり，この memory CD8 T 細胞を除去すること，ならびに骨髄移植のタイミングを調節すること（いわゆる“delayed tolerance”），にて MC を高率に誘導できることが示された[30,31]．これには移植した臓器が同じ抗原を持った血液幹細胞の生着を助け，また血液幹細胞の生着が移植臓器の免疫寛容を強化している可能性がある．

臓器移植における免疫寛容の将来

MC 確立後に臓器移植を行うことが理想的だと思われる．しかし，骨髄移植を先行する方法は，生体移植では可能であるが，脳死移植では不可能である．そのため，これからは調節性 T 細胞または facilitating cell の輸注を併用した安定した MC の誘導にさらなる研究が進められることになるであろう．それには今後，ブタ・サルでの大動物における研究に戻り，確認を行いながら臨床応用し

て，最適なプロトコールを模索してゆくことが重要と思われる．

本稿で紹介したサルの研究ならびに臨床でのMGHプロトコールの内容について情報をご提供いただきました，マサチューセッツ総合病院外科の河合達郎教授に深謝いたします．

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CASE REPORT Open Access

Li-Fraumeni syndrome with simultaneous osteosarcoma and liver cancer: Increased expression of a CD44 variant isoform after chemotherapy

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Abstract

Background: Li-Fraumeni syndrome (LFS) is a hereditary cancer predisposition syndrome that is commonly associated with a germline mutation in the tumor suppressor gene *p53*. Loss of p53 results in increased expression of CD44, a cancer stem cell (CSC) marker, which is involved in the scavenging of reactive oxygen species (ROS). Here, we report a change in the expression of a CD44 variant isoform (CD44v8-10) in an 8-year-old female LFS patient with osteosarcoma and atypical liver cancer after chemotherapy.

Case presentation: The patient visited a clinic with a chief complaint of chronic pain in a bruise on her right knee. Magnetic resonance imaging (MRI) raised the possibility of a bone malignancy. Biochemical testing also revealed significantly elevated levels of AFP, which strongly suggested the existence of a primary malignancy in the liver. MRI imaging showed the simultaneous development of osteosarcoma and liver cancer, both of which were confirmed upon biopsy. Combined therapy with surgical resection after chemotherapy was successful in this patient. Regardless of the absence of a familial history of hereditary cancer, a germline mutation in *p53* was identified (a missense mutation defined as c.722 C>T, p.Ser241Phe). To better understand the cancer progression and response to treatment, immunohistochemical (IHC) analysis of biopsy specimens obtained before and after chemotherapy was performed using a specific antibody against CD44v8-10.

Conclusion: This case demonstrates the ectopic up-regulation of CD44v8-10 in a biopsy sample obtained after cytotoxic chemotherapy, which confers high levels of oxidative stress on cancer cells. Because the alternative splicing of CD44 is tightly regulated epigenetically, it is possible that micro-environmental stress resulting from chemotherapy caused the ectopic induction of CD44v8-10 *in vivo*.

Keywords: Li-Fraumeni syndrome (LFS), cancer stem cells (CSCs), CD44 variant isoforms

Background

Li-Fraumeni syndrome (LFS) is a familial cancer predisposition syndrome, which is inherited in an autosomal dominant manner. This syndrome is most frequently associated with soft tissue sarcoma, osteosarcoma, premenopausal breast cancer, brain cancer, and adrenocortical carcinoma, but it can also result in other types of tumors. LFS is classified into two major subgroups: classic LFS and Li-Fraumeni-like (LFL) syndrome, which shares some, but not all, of the features of LFS [1-3]. Juvenile development of simultaneous and multiple cancers raises the possibility of LFS.

Sequence analysis of the entire *p53* coding region (exons 2–11) detects about 95% of *p53* mutations, most of which are missense mutations. A functional assay may be useful for determining the clinical significance of novel missense mutations [4]. It has been indicated that approximately 70% of LFS patients and 8–22% of

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patients with LFL syndrome have detectable *p53* mutations [5]. Comprehensive analyses of genotype-phenotype correlations have led to a better understanding of tumors that are associated with germline *p53* mutations [6].

CD44 is an adhesion molecule for extracellular matrix components such as hyaluronic acid and osteopontin [7], and plays an important role not only in wound healing and cell migration, but also in tumor invasion and metastasis. CD44 has numerous isoforms generated through alternative mRNA splicing. For instance, CD44v6 interacts with c-Met, the receptor of hepatocyte growth factor (HGF), thereby increasing the survival and proliferative ability of tumor cells That is one of the reasons why the expression of the CD44 splice variant CD44v6 is correlated with the metastasis of colon cancer to the liver and a poor clinical prognosis [8]. CD44 has been recently identified as one of the cellular surface markers associated with cancer stem cells (CSCs) in several types of tumors. Notably, CD44 variants (CD44v) are exclusively expressed in epithelial-type cells, whereas the CD44 standard isoform (CD44s) is expressed in both epithelial and mesenchymal cells [9].

Loss-of-functional mutations in the *p53* gene promote tumor development. CD44 expression is generally suppressed by *p53* binding to the *CD44* promoter, so that increased expression of CD44 is detected in tumor cells with mutant *p53* [10].

CSCs are defined as the small population of cancer cells with multi-lineage differentiation potential. These self-sustaining cells have the exclusive ability to self-renew and maintain the tumor tissue [11]. CSCs often fail to respond to chemotherapy, thereby causing distant metastasis and latent relapse. For this reason, we focused on the change in tumor expression of CD44 as a result of chemotherapy. Minimal residual disease (MRD) after chemotherapy is expected to be enriched in CSCs compared with the pre-chemotherapy tumor specimen.

Alternative splicing is mainly responsible for the diversity of CD44 isoforms. Among these variants, CD44v8-10 enhances reduced glutathione (GSH) synthesis by stabilizing the xCT transport system for cysteine, the precursor of GSH. Hence, CSCs acquire an enhanced reactive oxygen species (ROS) defense system [12]. Cytotoxic drugs such as Adriamycin induce apoptosis by causing oxidative stress, which damages DNA. To better understand the influence of oxidative stress on the tumor microenvironment, changes in the expression of CD44v8-10 were evaluated in response to chemotherapy. We hypothesized that oxidative stress due to the administration of cytotoxic chemotherapy would affect the expression of CD44v8-10 in *p53*-mutated cancer cells in this patient.

Case presentation

An 8-year-old female slipped on the stairs and had a bruise on her right knee. However, since the pain persisted, the patient visited a nearby orthopedic clinic. Magnetic resonance imaging (MRI) revealed the possibility of a bone malignancy (Figure 1). The patient was then admitted to the hospital for further examination. Biochemical testing revealed significantly elevated levels of both serum AFP (AFP: 79016 ng/ml, L3: 3555 ng/ml (4.5%), PIVKA-II: 128 IU/l), which suggested possible malignancy of the liver. A serum AFP level as high as 79,000 ng/ml was also indicative of a liver tumor. Antibodies against hepatitis virus B and C were not detected. The development of hepatoblastoma was atypical in terms of the age of onset, given that hepatoblastomas

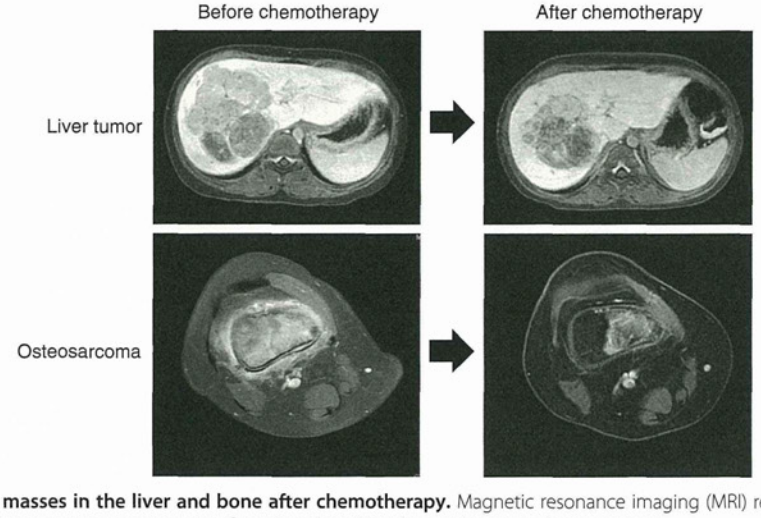

Figure 1 Reduced tumor masses in the liver and bone after chemotherapy. Magnetic resonance imaging (MRI) revealed that the sizes of both the liver tumor and the osteosarcoma were significantly reduced by the combination chemotherapy.

typically arise in patients under 3 years of age. Pathological diagnosis was transitional liver cell tumor (TLCT). A subset of liver cell tumors in older children and adolescents may develop as an intermediate between blastomatous tumors and adult-type tumors. This type of tumor, referred to as TLCT, is highly dependent on Wnt signaling than other categories of liver tumors [13]. MRI imaging showed the simultaneous development of osteosarcoma and liver cancer (Figure 1), both of which were confirmed by biopsy. After two courses of standard neoadjuvant chemotherapy for hepatoblastoma (a combination of cisplatin (CDDP) and tetrahydropyranyl-adriamycin (THP-ADR)), the residual liver tumor was removed completely by surgical extended lobectomy. After treatment with the standard neoadjuvant chemotherapy protocol for osteosarcoma (a combination of CDDP, ifosfamide (IFO), and methotrexate (MTX)), the diminished osteosarcoma was totally resected and an artificial joint was placed. The levels of transitional serum biomarkers for the liver tumor and osteosarcoma are shown (Figure 2), in which the blue arrows indicate repeated neoadjuvant chemotherapy. To date, there has been no sign of relapse or latent metastasis for about two years after last surgery. Combined therapy with surgical resection after chemotherapy was, therefore, successful in this patient. Given that we found the simultaneous development of a liver tumor and osteosarcoma, we performed genetic testing to evaluate the patient's susceptibility to malignancy. Despite the absence of a familial history of hereditary cancer, a germline mutation in *p53* (c.722 C>T, p.Ser241Phe) was identified. Notably, the frequency of *de novo* mutations in LFS is between 7% and 20% [14]; it is, therefore, conceivable that the *p53* mutation occurred *de novo* during embryonic development.

Immunohistochemical (IHC) analysis of the patient's tumors prior to and following chemotherapy was performed using an antibody against the human CD44 variant isoform. CD44v8-10 was not expressed in both the liver tumor and the osteosarcoma before chemotherapy, but was ectopically expressed after chemotherapy (Figure 3).

Discussion and conclusion

CD44 exists in as many as 16 different isoforms, which are generated through alternative mRNA splicing. CD44v8-10 is generated by epithelial splicing regulatory protein 1 (ESRP1), an RNA-binding protein [15]. Whereas the standard CD44 isoform (CD44s), which contains exons 1–5 and 16–20, is expressed predominantly in hematopoietic cells and normal epithelial cell subsets, CD44 variant isoforms with insertions in the membrane-proximal extracellular region are abundant in epithelial-type cancers, including liver tumors. Recently, CD44v8-10 was reported to have a novel function; it inhibits oxidative stress in tumor cells by promoting the GSH synthesis [12]. However, exactly transcriptional factors or epigenetic mechanisms controlling the induction of ESRP1, the master regulator of CD44v8-10, remain unclear [9]. We have already performed IHC using the specific antibody against human ESRP1 and ESRP2. Unfortunately, there was no antibody which was suitable for IHC due to reactions to the non-specific antigens, so

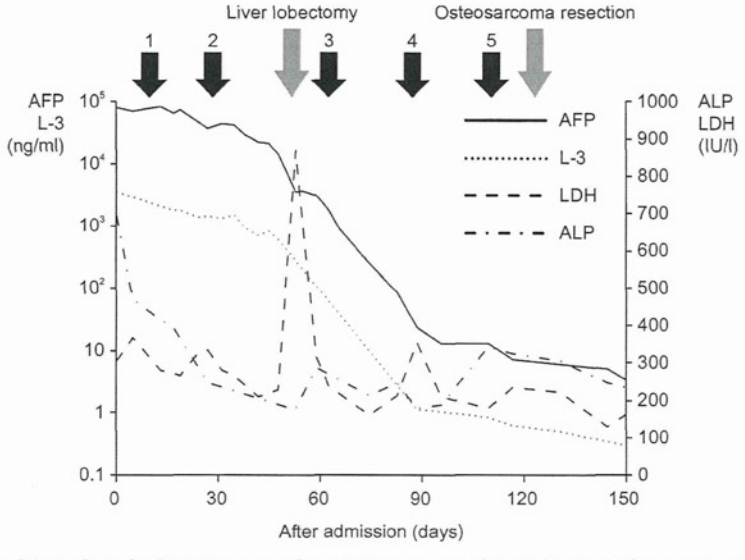

Figure 2 Transitional serum biomarkers for liver cancer and osteosarcoma. Combined therapy with surgical resection after chemotherapy resulted in a significant decrease in serum tumor markers for liver cancer and osteosarcoma. AFP and L-3 are serum markers for the hepatic tumor, whereas LDH and ALP are serum markers for the osteosarcoma.

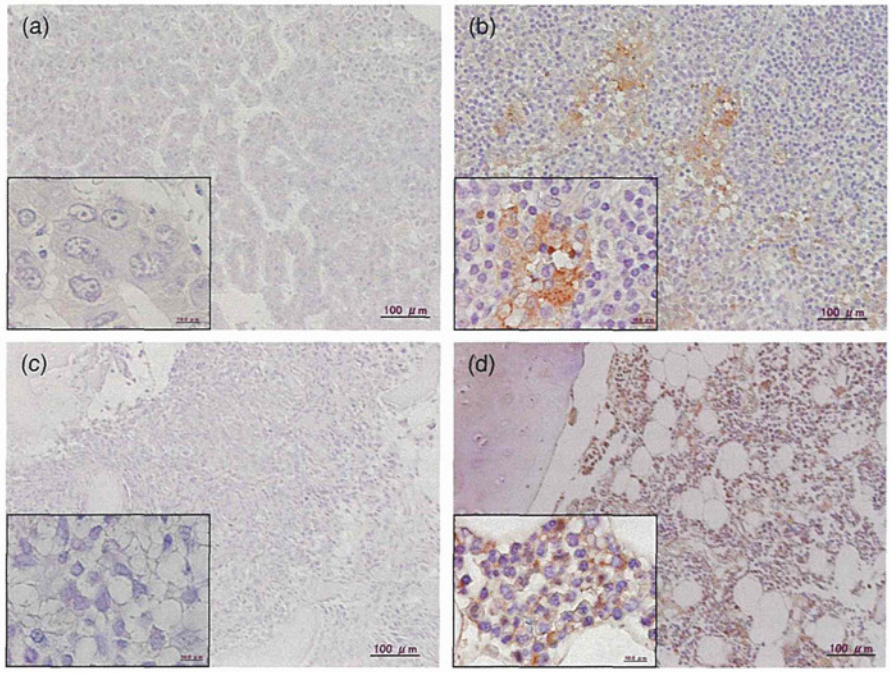

Figure 3 CD44v8-10 IHC of both tumors before and after chemotherapy. Immunohistochemical analysis of a transitional liver cell tumor (TLCT) prior to chemotherapy (**a**), a post-chemotherapy TLCT tissue (**b**), an osteosarcoma biopsy tissue sample obtained prior to chemotherapy (**c**), and a post-chemotherapy osteosarcoma tissue (**d**) stained with an antibody against CD44v8-10 (Inset: higher magnification; scale bar: 10 μm). (Inset: higher magnification; scale bar: 10 μm).

that we could not obtain data that shows ESRP1 or ESRP2 exclusively in the nucleus (data not shown).

Osteosarcoma originates from mesenchymal tissue, not epithelial tissue, meaning that the CD44 variant isoforms expressed in osteosarcoma may have a completely different function from those expressed in liver cancer. Whereas the function of CD44v8-10 in osteosarcoma is unknown, the only v6 expression is negatively correlated with 5-year metastasis-free survival. Therefore, overexpression of the variant isoform, CD44v6, is considered a poor prognostic marker in patients with osteosarcoma [15]. However, no specific data has been reported regarding the biological significance of CD44v8-10 in osteosarcoma. The cytotoxic drugs CDDP and THP-ADR increase the ROS level in cancer cells, thereby inducing DNA damage and apoptosis. It is possible that CD44v8-10 is induced following chemotherapy in osteosarcoma to counteract this oxidative stress.

Considering the lack of familial history of malignancy in this case, it is supposed that the de novo p53 germline mutation occurred during early development. The p53 protein suppresses expression of CD44 by binding to the promoter region of CD44 [10], and CD44v8-10 was not expressed in both tumors before chemotherapy. The absence of p53 likely enabled the upregulation of CD44v8-10 in response to chemotherapy. However, this finding does not rule out the possibility that other CD44 isoforms were fully expressed prior to chemotherapy. Although cancer cells may acquire resistance to oxidative damage by upregulating CD44v8-10, there has been no sign of relapse or metastasis so far in this patient. The fact that CD44v8-10 expression was not correlated with increased malignant potential in this case seems to be contradictory to our previous research [12]; however, the effect of CD44v8-10 expression may depend on the origin of the tumor. It is possible that CD44v8-10 may function differently in hepatic tumors and osteosarcomas with p53 mutations than in gastric and colorectal cancers.

Potential mechanisms for the ectopic induction of CD44v8-10 expression in osteosarcoma are shown (Figure 4):

a) Cancer cells expressing CD44v8-10 may have existed in the original tumor, but were too rare to be detected by IHC. These cells survived even under conditions of oxidative stress caused by cytotoxic chemotherapy.
b) Cancer cells expressing CD44v8-10 did not exist originally, but the oxidative stress caused by chemotherapy induced the expression of CD44v8-10 in the tumor.

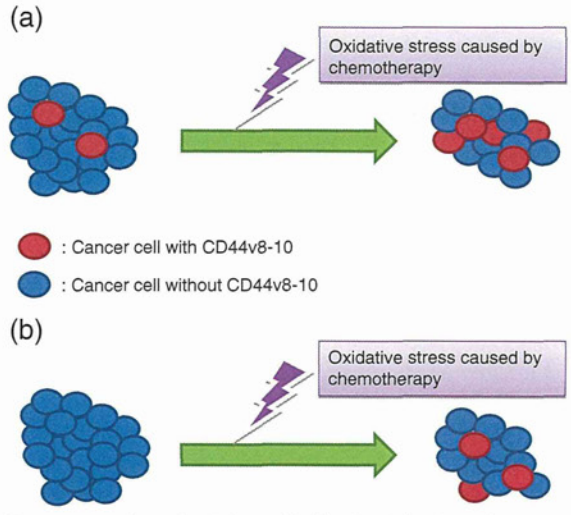

Figure 4 Two hypothetical models for the induction of CD44v8-10 after chemotherapy. Cancer cells expressing CD44v8-10 are present in the original tumor and clonally expand in response to anti-cancer drugs (**a**). Cancer cells with CD44v8-10 are absent from the original tumor, but oxidative stress caused by chemotherapy induces the ectopic expression of CD44v8-10 (**b**).

Differentiating between these possibilities will require further investigation into how the alternative splicing machinery for CD44 is affected by chemotherapy. There has been no sign of relapse or metastasis so far in this patient, but it will be crucial to gather additional data regarding CSC molecular dynamics during the course of chemotherapy. Therefore, a larger number of cancer cases need to be analyzed, and longer-term follow-up studies must be conducted, to confirm the results of this study.

Consent

Written informed consent was obtained from the patient for publication of this case report and the accompanying images. A copy of the written consent is available for review upon request.

Abbreviations

(LFS): Li-Fraumeni syndrome; (CSC): Cancer stem cell; (ROS): Reactive oxygen species; (MRI): Magnetic resonance imaging; (LFL): Li-Fraumeni-like; (GSH): Glutathione; (MRD): Minimal residual disease; (TLCT): Transitional liver cell tumor; (IHC): Immunohistochemical; (ESRP1): Epithelial splicing regulatory protein 1; (CDDP): Cisplatin; (THP-ADR): Tetrahydropyranyl-adriamycin.

Competing interests

The authors declare that they have no competing interests to disclose.

Authors' contribution

GJY: carried out the immunological staining analysis, and reviewed the literature, drafted and edited the manuscript, YF, TO, HS, SH and HM: were involved in the patient active management , drafted and edited the manuscript, MM, YM and MS: processed and provided pathology, TK: critically revised the manuscript. All authors read and approved the final manuscript.

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Cyclosporine A-Based Immunotherapy in Adult Living Donor Liver Transplantation: Accurate and Improved Therapeutic Drug Monitoring by 4-hr Intravenous Infusion

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Background. A paucity of data exists for evaluating therapeutic drug monitoring in association with clinical outcomes of cyclosporine A (CYA) treatment in living donor liver transplantation (LDLT).

Methods. A retrospective cohort analysis was conducted on 50 consecutive adult patients who underwent LDLT between 2001 and 2009 to investigate the feasibility and efficacy of 4-hr continuous intravenous infusion of CYA-based immunotherapy (4-hr CYA-IV, n=27) and compare the pharmacokinetic profile and short-term prognoses with an oral microemulsion formulation of CYA (CYA-ME, n=23).

Results. All patients in the 4-hr CYA-IV group reached target CYA peak by day 3 compared with only 22% in the CYA-ME group ($P<0.001$). Adjustability to achieve the target range was easier in the 4-hr CYA-IV group compared with the CYA-ME group ($P=0.017$). Acute cellular rejection rate was lower in the 4-hr CYA-IV group (0%) compared with the CYA-ME group (17%, $P=0.038$). A subset analysis of the CYA-ME group revealed that CYA exposure was affected by external bile output ($P=0.006$). Patients in the CYA-ME group showed increased risk of switch to tacrolimus (35%) compared with the 4-hr CYA-IV group (7%, $P=0.030$). Toxicities and mortality rates were equivalent. The optimal initial dose of oral CYA at conversion from the 4-hr CYA-IV was considered to be 3-fold greater than that of the intravenous dose.

Conclusions. In LDLT, our 4-hr CYA-IV immunosuppression protocol was superior to CYA-ME oral dosing and allowed accurate therapeutic drug monitoring with excellent patient compliance.

Keywords: Cyclosporine A, Immunosuppression, Living donor liver transplantation, Therapeutic drug monitoring, Rejection.

(*Transplantation* 2011;92: 100–105)

Despite development of a wide range of novel drugs, calcineurin inhibitors (CNIs) remain the major agents for immunosuppression in liver transplantation. Tacrolimus (Tac) has been widely accepted for immunotherapy, whereas for patients unable to tolerate Tac, cyclosporine A (CYA) has been described as a valuable rescue therapy (*1–3*). One meta-analysis demonstrated that as a primary immunosuppressive agent, Tac was superior to CYA (including both the original oil-based formulation and the newer microemulsion formulation) in terms of mortality, graft loss, and rejection at 1 year (*4*). Nonetheless, all but one study included in this meta-analysis measured CYA trough levels to attain adequate levels of exposure (*4*).

Recently, Levy et al. (*5*) reported a randomized, multicenter study indicating decreased overall incidence of and statistically less severe acute cellular rejection in liver transplant recipients on an oral microemulsion formulation of CYA (CYA-ME) when 2-hr postdose levels were monitored, as a surrogate marker of CYA peak, instead of conventional CYA trough level. More importantly, a subset of patients in the 2-hr postdose monitoring group who reached the minimum target CYA peak range by day 3 demonstrated a significantly lower incidence of acute cellular rejection compared with patients who only achieved the target peak level by days

The authors declare no conflicts of interest.

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7 and 10, suggesting that reaching target levels at an early stage after transplant is crucial when a 2-hr postdose monitoring strategy is to be implemented (*5*). Subsequently, several randomized trials including the LIS2T study have reported promising results in terms of efficacy, toxicity, and pharmacoeconomics with CYA-ME, showing equivalent results in patient groups receiving CYA-ME with 2-hr postdose monitoring or Tac for patient and graft survivals and the overall incidence of acute rejection (*6–8*).

Nevertheless, data collected for the previous studies were generally based on deceased donor liver transplantations, and these results cannot be simply applied to adult living donor liver transplantation (LDLT). The reduced size of graft livers (usually hemi-liver), prolonged intestinal paralysis because of lengthy operation, and posttransplant external bile diversion described in recent publications from high-volume LDLT centers mostly in Japan (*9–12*) are the distinctive features of adult LDLT, contributing to delayed graft functional recovery and poor enteral absorption, which in turn substantially interfere with achieving and maintaining the therapeutic CYA peak blood concentration mentioned earlier.

To overcome these problems, intravenous CYA infusion may become a promising option because of its ability to ensure sufficient CYA exposure to exert immunosuppressive effects regardless of enteral absorption and biliary drainage. Clinical evidence of the efficacy and safety of intravenous CYA in liver transplantation is scarce, and appropriate therapeutic drug monitoring remains to be elucidated (*13, 14*). Moreover, no study has compared the clinical outcomes of intravenous infusion of CYA (CYA-IV) with CYA-ME regimens in LDLT to date. In the era of individually tailored immunosuppression, establishing a standard intravenous CYA protocol in LDLT is paramount, as an alternative CNI-based immunotherapy with potential advantages over Tac with regard to posttransplant new-onset diabetes mellitus and in the treatment of transplant patients with hepatitis C virus (HCV) or primary biliary cirrhosis and as a salvage immunosuppressive regimen in cases of Tac-related side effects (*15–17*). In this study, we evaluated the feasibility and efficacy of 4-hr intravenous CYA immunotherapy for LDLT, focusing on its therapeutic drug monitoring in comparison with a CYA-ME regimen.

RESULTS

Patient Demographics

The CYA-ME (Neoral, Novartis Pharma K. K., Tokyo, Japan, n=23) group and the 4-hr continuous intravenous infusion of CYA (4-hr CYA-IV; Sandimmun, Novartis Pharma K. K., n=27) groups were comparable for age, indications, Child-Pugh grade, model for end-stage liver disease scores, preoperative conditions, Eastern Cooperative Oncology Group performance status, graft lobe, graft:recipient weight ratio, graft volume/recipient standard liver volume, donor age, and blood loss (Table 1). The number of males in the CYA-ME group was higher compared with that in the 4-hr CYA-IV group (P=0.035; Table 1). Regarding surgical factors, the proportion of patients who underwent duct-to-duct reconstructions was higher (P=0.044), and cold and warm ischemia times were longer (P=0.002 and P<0.001, respectively) in the 4-hr CYA-IV group compared with those in the CYA-ME group (Table 1).

TABLE 1. Patient demographics

Variables	CYA-ME	4-hr CYA-IV	*P*
Total	23 (100)	27 (100)	
Age (yr)	47±9	50±12	0.47
Gender			
Male	17 (74)	12 (44)	0.035
Female	6 (26)	15 (56)	
Indication for LDLT			
HCV	8 (35)	9 (33)	1.00
HBV	2 (9)	3 (11)	
FHF	2 (9)	3 (11)	
PBC	3 (13)	3 (11)	
PSC	1 (4)	1 (4)	
Alcohol	3 (13)	3 (11)	
Others	4 (17)	5 (19)	
Child–Pugh grade			
A	0 (0)	1 (4)	0.27
B	6 (26)	3 (11)	
C	17 (74)	23 (85)	
MELD score	19±6	17±6	0.49
Preoperative condition			
Outpatient	13 (57)	15 (56)	1.00
Hospitalized	7 (30)	8 (30)	
ICU	3 (13)	4 (15)	
ECOG performance status			
0–2	17 (74)	17 (63)	0.41
3, 4	6 (26)	10 (37)	
Graft lobe			
Left (±caudate lobe)	10 (43)	17 (63)	0.17
Right	13 (57)	10 (37)	
GRWR	0.90±0.27	0.90±0.21	0.92
GV/RSLV (%)	46±10	47±11	0.86
Biliary reconstruction			
Duct-to-duct	14 (61)	24 (89)	0.044
Roux-en-Y	9 (39)	3 (11)	
Donor age (yr)	41±15	38±12	0.35
Cold ischemia time (min)	53±22	80±32	0.002
Warm ischemia time (min)	45±10	63±14	<0.001
Blood loss (mL)	4863±4424	5041±5873	0.91

Data are presented as N (%) and mean±standard deviation.

CYA-ME, oral microemulsion formulation of cyclosporine A; 4-hr CYA-IV, 4-hr continuous intravenous infusion of cyclosporine A; LDLT, living donor liver transplantation; HCV, hepatitis C virus; HBV, hepatitis B virus; FHF, fulminant hepatic failure; PBC, primary biliary cirrhosis; PSC, primary sclerotic cholangitis; MELD, model for end-stage liver disease; ICU, intensive care unit; ECOG, Eastern Cooperative Oncology Group; GRWR, graft:recipient weight ratio; GV/RSLV, graft volume/recipient standard liver volume.

Pharmacokinetic Profiles of 4-hr CYA-IV and CYA-ME Groups

For the CYA-ME group, 9 of 15 patients (60%) who were not switched to other CNIs completed full pharmacokinetic evaluations on day 3. Of these nine patients, only two (22%) reached the target peak range of 700 to 1000 ng/mL (2-hr postdose CYA level 484±272 ng/mL; Fig. 1A). In con-

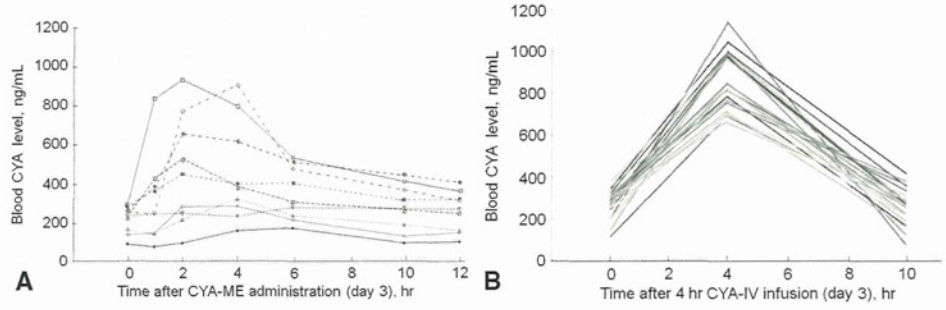

FIGURE 1. Comparison of pharmacokinetic profiles on posttransplant day 3. (A) The mean 2-hr postdose cyclosporine A (CYA) level in the CYA-ME group was 484±272 ng/mL, and only two of nine patients (22%) reached the target CYA peak range of 700 to 1000 ng/mL. (B) In contrast, the mean 4-hr postdose level in the 4-hr CYA-IV group was 856±129 ng/mL, allowing all 27 patients (100%) to achieve the target peak level. 4-hr CYA-IV, 4-hr continuous intravenous infusion of cyclosporine A; CYA-ME, oral microemulsion formulation of cyclosporine A.

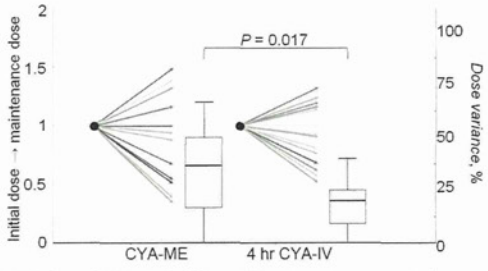

FIGURE 2. Adjustability of immunosuppressive agents. The *black dots* indicate the initial cyclosporine A (CYA) doses in both treatment groups normalized to 1, whereas the *radial arrows* correspond to the dose modifications relative to the initial dose that were required to maintain the target range for blood cyclosporine A concentrations in each case. The box-and-whisker diagrams depict dose variance in each group, calculated as the proportion of CYA dose difference required to achieve the target trough and peak levels over the initial dose. Dose variance was significantly smaller in the 4-hr CYA-IV group (18.3%±13.9%) compared with the CYA-ME group (35.3%±21.9%, P=0.017) group. CYA-ME, microemulsion oral formulation of cyclosporine A; 4-hr CYA-IV, 4-hr continuous intravenous infusion of cyclosporine A.

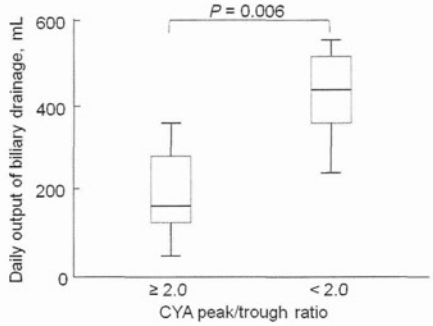

FIGURE 3. Effect of daily bile output on blood cyclosporine A (CYA) concentration in stable posttransplant patients taking oral CYA. A subset analysis of the CYA-ME group in stable patients tolerating regular diet around posttransplant weeks 2 to 3 revealed that patients who achieved adequate CYA exposure (CYA peak:trough ratios ≥2.0) had significantly lesser amounts of external biliary drainage (173±50 mL/day) compared with those who did not (403±46 mL/day, P=0.006). CYA-ME, oral microemulsion formulation of cyclosporine A; 4-hr CYA-IV, 4-hr continuous intravenous infusion of cyclosporine A.

trast, adequate and stable blood CYA trough/peak levels were successfully achieved at posttransplant day 3 in all 27 patients (100%) of the 4-hr CYA-IV group (4-hr postdose CYA level 856±129 ng/mL; Fig. 1B).

Adjustability of Immunosuppressive Agents

To evaluate the effort needed to adjust the immunosuppressive agents between the two groups, dose variance was calculated for each case as follows:

$$\text{Dose variance (\%)} = \frac{|\text{CYA dose required to achieve the target level} - \text{initial CYA dose}|}{\text{initial CYA dose}} \times 100$$

A Student's t test showed that patients in the 4-hr CYA-IV group had a significantly smaller dose variance (18.3%±13.9%) compared with those in the CYA-ME (35.3%±21.9%; P=0.017) group, indicating minor interpatient and intrapatient variability (Fig. 2).

Effect of External Bile Diversion on Oral CYA Absorption

In the CYA-ME group, daily bile output and blood CYA peak (2 hr after dose)/trough levels were measured in 14 of 15 patients (93%) at posttransplant weeks 2 to 3 when bowel functions were deemed to have returned to their baselines, and patients could tolerate regular diet. Patients with CYA peak:trough ratios more than or equal to 2.0, demonstrating adequate exposure to CYA, had a significantly lesser amount of biliary drainage (173±50 mL/day) compared with those with ratios less than 2.0 (403±46 mL/day, P=0.006; Fig. 3).

Short-Term Outcomes

No patients (0%) in the 4-hr CYA-IV group suffered acute cellular rejections, and this rate was significantly lower compared with that observed in the CYA-ME group (17%,

TABLE 2. Short-term outcomes

Variables	CYA-ME	4 hr CYA-IV	*P*
Total	23 (100)	27 (100)	
Acute cellular rejection	4 (17)	0 (0)	0.038
Adverse events			
Dialysis-dependent renal insufficiency	4 (17)	4 (15)	1.00
Neurotoxicity	2 (9)	2 (7)	1.00
Infection	8 (35)	9 (33)	1.00
CMV antigenemia	16 (70)	18 (67)	0.83
CNI switch	8 (35)	2 (7)	0.030
In-hospital mortality	2 (9)	4 (15)	0.67

Data are presented as N (%).

CYA-ME, oral microemulsion formulation of cyclosporine A; 4-hr CYA-IV, 4-hr continuous intravenous infusion of cyclosporine A; CMV, cytomegalovirus; CNI, calcineurin inhibitor.

P=0.038; Table 2). All four episodes of acute cellular rejection in the CYA-ME group occurred within 1 month posttransplant (range 7–21 days) while the patients were on oral CYA. The incidences of posttransplant comorbidities, including dialysis-dependent renal insufficiency, neurotoxicity, infection, and cytomegalovirus antigenemia, were similar between the two groups (Table 2). None of the patients in either group developed hypertension, severe electrolyte disturbance, or hyperlipidemia.

In the 4-hr CYA-IV group, 2 of 27 patients (7%) required a CNI switch to Tac because of seizures and severe antibody-mediated rejection. This rate was significantly lower than that observed in the CYA-ME group (8/23 patients, 35%, P=0.030; Table 2). The main reason for a CNI switch in the CYA-ME group was inability to achieve target trough levels, which occurred for five patients. The other causes included acute cellular rejection, seizures, and acute pancreatitis. In-hospital deaths occurred in 2 of 23 patients (9%) in the CYA-ME group (chronic rejection and multiple organ failure) and 4 of 27 patients (15%) in the CYA-IV group (multiple organ failures, posttransplant lymphoproliferative disease, and recurrent pneumonia). Mortality rates were comparable between the two groups (P=0.67; Table 2).

Optimal Initial Dose of Oral CYA After 4-hr CYA-IV

In the 4-hr CYA group, 4 cases of in-hospital mortalities were excluded, and the remaining 23 patients underwent trials of oral CYA conversion from 4-hr CYA-IV. Conversions were successful for 18 of 23 patients (78%) with a median posttransplant day of 27 (range 10–82 days). For the other 5 of 23 patients (22%), target trough/peak levels were not achieved, and their CNI was switched to Tac. The median doses of 4-hr CYA-IV before oral CYA conversion and initial oral CYA were 30 mg (range 10–65 mg) and 83 mg (range 25–200 mg), respectively. The median dose ratio of 4-hr CYA-IV before conversion to initial oral CYA was 1:3 (range 2.0–3.8). The median difference between the oral CYA dose at conversion and at discharge was 20% (range 0%–50%). From oral CYA conversion to time of discharge, none of the 18 patients experienced acute cellular rejections or adverse events, and adequate trough CYA levels were well maintained throughout the study period.

Long-Term Prognoses

During a median follow-up period of 52 months (range 5–108 months), the 5-year overall survival rates for patients in the CYA-ME and 4-hr CYA-IV groups were 78% and 81%, respectively (P=0.88).

DISCUSSION

This is the first series to demonstrate the feasibility and efficacy of 4-hr CYA-IV immunotherapy after LDLT in comparison with the CYA-ME regimen, a milestone in CNI-based immunotherapy. Our 4-hr CYA-IV protocol demonstrated excellent immunosuppressive potency (acute cellular rejection rate of 0%) with a similar toxicity profile and mortality rate to those of the CYA-ME regimen.

Our 4-hr CYA-IV regimen allowed effortless achievement of target CYA trough and peak levels in all patients (100%) with small interindividual and intraindividual dose variation by posttransplant day 3, which is considered to be the critical period for preventing acute cellular rejection (*5*). In LDLT, intravenous CYA infusion for 4 hr facilitates adequate and stable CYA exposure to reproduce the unique area under the concentration-time curve of CYA-ME exhibited in deceased donor liver transplantation recipients, characterized by a rapid increase in blood CYA concentrations usually within 2 hr after drug administration (*18*, *19*). This CYA peak level correlates well with area under the concentration-time curve and shows strong association with freedom from graft rejection (*19*). On the contrary, dose adjustment in the CYA-ME group was demanding in our series with only 22% of patients reaching the minimum target CYA peak level by day 3. Marked dose disparity among CYA-ME patients was also observed, along with a significantly higher incidence of acute cellular rejection and an increased risk for switch to Tac compared with the 4-hr CYA-IV group. Published evidence demonstrating successful adoption of CYA-ME with 2-hr postdose monitoring in deceased donor liver transplantation recipients cannot be extrapolated to LDLT patients, whose CYA exposure levels are unpredictable with inferior outcomes when oral administration is used. Thus, when CYA is used as the primary immunosuppressive agent in LDLT, our 4-hr CYA-IV protocol provides ideal therapeutic drug monitoring for optimization of CYA dosing and effect in the early stage after transplant.

In contrast to a recent report (*14*), we did not identify factors (including graft:recipient weight ratio) that significantly correlated with initial blood CYA trough levels in the 4-hr CYA-IV group. The timing of measurement and the dissimilarity in patient backgrounds may explain this difference. However, we did not extensively investigate this subject because reaching target peak CYA level within several days posttransplant is a matter of utmost importance (*5*). We successfully achieved an ideal concentration-time curve at posttransplant day 3 in all patients of the 4-hr CYA-IV group with no acute cellular rejection. This is one of the striking advantages of intravenous CYA infusion.

We also investigated the optimal initial dose and time for converting from 4-hr CYA-IV to oral CYA administra-

tion. Data are limited for determining the conversion dose ratio of CYA-IV:oral CYA, ranging from 1:2 to 1:9 (*14, 20, 21*). Our current policy, based on clinical data, is to administer initial oral CYA at a dose 3-fold greater than that of intravenous CYA. This is in accordance with previous reports that described the absolute bioavailability of oral CYA as 38%±10% in healthy volunteers and the use of 4-hr CYA-IV for LDLT (*14, 22*). Regarding the time, although we sought to convert CYA from intravenous to oral administration at posttransplant weeks 2 to 3, considerable interindividual discrepancies in patient and graft recoveries hindered prompt conversion, and only 6 of 23 patients (26%) were successfully changed to oral administration by day 21. Because the amount of external biliary drainage obviously affected CYA-ME absorption (Fig. 3), intermittent tube clamping in stable LDLT recipients with more than or equal to 300 mL/day bile output, or simply increasing the initial oral CYA dose at the time of oral CYA conversion, may be a reasonable strategy to provide sufficient trough and peak CYA levels. Our goal is to start oral CYA once the patient's condition is stabilized, ideally around posttransplant day 7 to 10, and a safe and rapid oral conversion protocol has yet to be determined.

The present series has several limitations. It was a retrospective analysis of data collected in a single center, and the number of patients was small. Era bias may exist. A significant number of patients in the CYA-ME group required CNI switching. The selection of antimetabolites and biliary reconstruction techniques were not standardized. A prospective study might be required to validate the proposed 1:3 ratio of intravenous CYA:oral CYA dose at the time of conversion.

In conclusion, our 4-hr CYA-IV protocol enables accurate therapeutic drug monitoring and provides safe and effective immunosuppression for LDLT. Excellent patient compliance is expected because of the minor interindividual variances. A 4-hr CYA-IV regimen is superior to CYA-ME and would be a potent alternative strategy for primary immunosuppression in LDLT. Conversion to oral CYA is affected by external biliary drainage and is occasionally demanding, in which case establishing optimal dose and time is warranted.

MATERIALS AND METHODS

Study Population

We undertook a single-center, retrospective cohort analysis of 50 adult patients (older than 18 years) who underwent primary ABO-compatible LDLTs between April 2001 and December 2009. Written informed consent was obtained from all patients. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was conducted under the approval of the Institutional Review Board of Keio University School of Medicine (2010-075).

Regardless of the type of biliary reconstruction (duct-to-duct vs. Roux-en-Y), external bile diversion was performed in all patients. Biliary drainage tubes were routinely left open during the first 2 weeks and then clamped according to the general condition of the patient. Study subjects were divided into the following two groups based on the primary CNI administered as an immunosuppressive agent: CYA-ME and 4-hr CYA-IV. We introduced CYA-based immunotherapy in 2001, and CYA-ME was employed as the primary immunosuppression until 2004. A transition was made in 2005 to the current protocol of 4-hr CYA-IV. Patient backgrounds, CNI dose adjustability, and clinical outcomes were compared between the two groups.

Immunosuppressive Regimens

Patients were principally treated with a standard triple regimen comprising CNI, corticosteroids, and an antimetabolite. The patients in the 4-hr CYA-IV group received a 4-hr continuous intravenous infusion of CYA at an initial dose of 0.8 mg/kg twice daily. In the CYA-ME group, oral administration of CYA was initiated at 2.5 mg/kg twice daily. In both groups, the trough CYA levels were measured twice daily. The peak CYA levels were measured frequently whenever the dose was modified, at the end of infusion in the 4-hr CYA-IV group and at 2 hr after oral administration in the CYA-ME group. For patients in the 4-hr CYA-IV group, a switch to oral CYA administration was attempted 2 to 3 weeks after transplant when the patients were considered clinically stable; the basic starting dose was 3-fold greater than that of intravenous CYA and was administered twice daily. The CYA doses were adjusted to maintain therapeutic levels according to the posttransplant period (target trough and peak ranges 300–400 and 700–1000 ng/mL, respectively, to month 1; 150–300 and 500–700 ng/mL, respectively, to month 3; and 80–150 and 300–500 ng/mL, respectively, thereafter). The decision to switch from CYA to Tac was made if a patient was unable to reach the target CYA range during the first 3 days after LDLT or whenever acute rejection or CYA toxicity occurred. Blood CYA concentrations were measured by fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL). Methylprednisolone was given intravenously to all patients at a dose of 10 mg/kg at the time of graft reperfusion, at 2 mg/kg/day for days 1 through 3, at 1 mg/kg/day for days 4 through 6, and at 0.5 mg/kg/day thereafter, and then tapered and terminated at approximately 6 months after LDLT. For stable patients, antimetabolites were added to supplement the immunosuppressive regimen at the discretion of the transplant team; mycophenolate mofetil (500–1500 mg/day) or mizoribine (2–3 mg/kg/day) was used in most patients. Because the antimetabolites were prescribed on an auxiliary basis, they were prone to switching to other agents or withdrawal if a patient suffered rejection, infection, or suspected drug-induced toxicities.

In 2004, for patients with liver failure because of HCV infection, basiliximab (antiinterleukin 2 receptor α chain monoclonal antibody) was introduced. They were maintained steroid-free throughout the posttransplant period, but the dosage of CNIs and antimetabolites was kept identical to that with non-HCV patients.

Pharmacokinetic Evaluation

For the 4-hr CYA-IV group, a complete pharmacokinetic profile was obtained on day 3 by measuring blood CYA concentrations at 0, 4, and 10 hr after the start of infusion. For the CYA-ME group, the pharmacokinetic profile on day 3 was obtained by measuring blood CYA concentrations at 0, 1, 2, 4, 6, 10, and 12 hr after oral administration.

Rejection and Adverse Events

An acute cellular rejection episode was defined as a biopsy-proven, histologic diagnosis of moderate to severe rejection according to the Banff Schema that required a corticosteroid increment, including steroid pulse therapy, with or without CNI switches (*23*). Patients who received dialysis after transplant and who were not already known to have renal failure were classified as suffering from dialysis-dependent renal insufficiency. Neurotoxicity included convulsions, altered mental status, and leukoencephalopathy. Infection was identified whenever antimicrobial therapy was initiated separately from the routine prophylactic antibiotics. Cytomegalovirus antigenemia was checked twice weekly until discharge.

Statistical Analysis

Demographic data were presented as means±standard deviations or medians (ranges). Categorical variables were compared using a chi-square test or a Fisher's exact test. Continuous variables were compared using a Student's *t* test. If variables were not normally distributed with unequal variances (Levene's test), a Wilcoxon Mann–Whitney *U* test was used, when appropriate. Overall survivals were determined by the Kaplan–Meier method and compared using a log-rank test. *P* less than 0.05 was considered statistically significant. All data analyses used SPSS version 17.0 (SPSS Inc., Chicago, IL).

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症例報告

総排泄腔奇形根治術後遠隔期に発症し，診断に苦慮した慢性腹痛・腹壁痛：ACNES（abdominal cutaneous nerve entrapment syndrome）の1例

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要 旨

小児において慢性腹痛はごく一般的な症状である．慢性腹痛のうち10～30%は腹壁の痛みであるとされているが，比較的認知度が低くしばしば見落とされている．慢性腹壁痛は非常に限局した痛みと体表の圧痛を特徴とし，その原因としてはabdominal cutaneous nerve entrapment syndrome（ACNES）が最も一般的であると考えられている．Carnett's test（腹壁筋の緊張により局所の圧痛が増強する）が診断に有用である．今回我々は，総排泄腔奇形術後遠隔期に発症したACNESの1例を経験した．結果として局所麻酔薬とステロイドの局所注射により疼痛は消失し，長期間再発を認めていない．しかしながら疾患概念の欠如のため診断に苦慮し多くの時間と医療コストを要した．慢性腹痛の診療においては，常に腹壁痛を念頭に置いておく必要があると考えられた．

索引用語：慢性腹痛，慢性腹壁痛，ACNES，Carnett's test，小児

I はじめに

慢性腹痛はごく一般的な症状であるが，そのうちの10～30%は腹壁に原因がある慢性腹壁痛であると考えられている[1]．男女比は1：1と女性に多く[2]，好発年齢は30～50歳であるが[1]，小児にも報告例がある[3)-6)]．慢性腹壁痛は通常，鋭い痛みで体動により増強し，再現性をもって疼痛部位を指で指し示せるという特徴を有している[1]．その原因としてはabdominal cutaneous nerve entrapment syndrome（ACNES）が最も多く，Carnett's testという簡単な臨床検査が診断に有用である[1)2)7)]．Carnett's testの具体的な方法は，患者を仰臥位として圧痛の最強点を同定し，胸の前で手を組んだまま半座位に上体を起こさせる．この際に圧痛が増強したものを，Carnett's test陽性という[8)9)]．疼痛が内臓由来の場合，腹壁の緊張はこれを保護して圧痛が軽減するはずなので，腹壁の緊張により増強する（Carnett's test陽性の）疼痛は腹壁由来であるという理論に基づいている．圧痛点の局所麻酔で疼痛が消失すればACNESの診断が確定する[1]．このように慢性腹壁痛，ACNESは比較的容易に診断可能であるが，疾患の認知度が低いために多くの無駄な検査や不必要な手術が行われ，多大なコストがかさんでいると警鐘されている[1)9)]．

今回我々は，総排泄腔奇形術後遠隔期に慢性腹痛を認め，診断に苦慮したが最終的にACNESと診断し治療が奏功した症例を経験したので報告する．

II 症 例

1．現 病 歴

7歳女児．日齢1に総排泄腔奇形のため横行結腸人工肛門造設，1歳時に根治術を施行し，2歳時に人工肛門を閉鎖した．その後排尿・排便機能は良好．1年前に慢

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性右下腹部痛を認めたが，機能性ディスペプシアの診断のもとプロトンポンプ阻害薬の内服で軽快した．1か月前に左側腹部痛のため救急外来を受診したが，原因は不明で経過観察となった．その後も疼痛が持続して激しい運動（剣道）が不能となり，精査加療目的に入院となった．

2．入院時現症

右季肋部に手術瘢痕を認めた．左側腹部に自発痛，圧痛を認めたが，反跳痛，筋性防御は認めなかった．痛みは face scale で運動時 3～4/5，安静時 2～3/5 と運動時にやや増強する疼痛であり，食事との関連は明らかでなかった．

3．検査所見

血液検査，上部消化管内視鏡，腹部超音波，MRI，cine MRI では明らかな異常所見を認めなかった．小腸造影で圧痛部口側の空腸がやや拡張しており癒着の可能性も考えられたが，明らかな原因解明には至らなかった．

4．経　過

検査で有意な所見が出なかったことから機能性消化管障害と考え，様々な内服治療を試みた．1年前に著効したプロトンポンプ阻害薬をはじめ，消化管運動改善薬，抗アレルギー薬，漢方などはいずれも効果を認めなかった．アセトアミノフェンは唯一若干の効果を認めたが，満足されるレベルではなかった．3環系抗うつ薬も検討したが家族の同意が得られず投与しなかった．

診断，治療に難渋したため入院 21 日目に身体所見を取り直した．圧痛点は腹直筋外縁に位置し指で示せるほど非常に限局していた（図1）．前屈のような圧痛点の腹壁筋を弛緩する運動は疼痛を引き起こさずに可能であったが，後屈や右への側屈，背筋運動のような圧痛点の腹壁筋を伸展する運動は疼痛のために不可能であった．

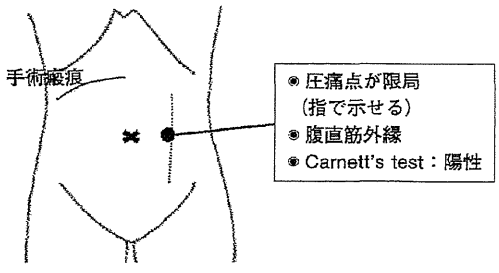

図1　身体所見

右季肋部に手術瘢痕を認めた．圧痛は左側腹部の腹直筋外縁に指で示せるほど限局しており，Carnett's test 陽性で，腹壁痛の診断基準を満たしていた．

腹筋運動は疼痛があるものの何とか可能であった．また，嘔気や顔面蒼白などの自律神経反射を伴わない疼痛であった．これらの所見から腹壁を起源とした体性痛を疑い，圧痛点皮下に局所麻酔（1%プロカイン 7 ml）を行ったところ，数時間疼痛の消失を認め診断に確証を得た．Carnett's test を施行したところ陽性であり，リドカイン貼付剤も有効であった．長期間の疼痛消失を得るために，局所麻酔薬（0.375%ロピバカイン 1.5 ml）とステロイド（ベタメタゾン 1 mg）の局所注射を行ったところ著効を示し，入院 54 日目に退院した．入院費用は 135 万円を要した．以後外来経過観察中であるが，1年6か月間再燃を認めていない．

III 考　察

Carnett は 1926 年に慢性腹壁痛の原因を肋間神経痛であると記載しており[8]，1972 年には Applegate が肋間神経の前皮枝が腹直筋鞘を貫通する部位で絞扼されることが原因であるとして（図2），これを abdominal cutaneous nerve entrapment syndrome（ACNES）と名付けた[10)11)]．慢性腹壁痛と ACNES は同義に使用されている場合もあるが，ACNES は慢性腹壁痛の最も一般的な原因の一つにすぎないと記載されている場合もある[7)11)]．慢性腹壁痛の他の原因としては帯状疱疹，腫瘍，外傷などによる神経痛や，血腫，Spigelian ヘルニアなど多岐にわたるが，ACNES よりも頻度が低いと言われている[1)7)]．Th8～12 の肋間神経前皮枝は腹直筋外縁の約 1 cm 正中側で腹直筋を貫いており，患者はここを疼痛部位としてはっきりと指し示せることが多い[1)]．

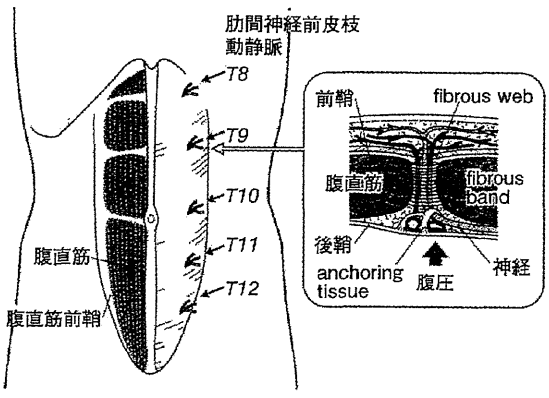

図2　Abdominal cutaneous nerve entrapment syndrome（ACNES）の解剖

肋間神経前皮枝の分布と腹壁における走行を示す．腹直筋前鞘で神経が絞扼されて疼痛を引き起こす．

ACNESの誘因としては，外傷や手術瘢痕，急激な運動や腹壁筋の酷使，腹壁筋の筋力低下・肥満・腹水・妊娠などによる腹壁の緊張などが挙げられる[1]．経口避妊薬や妊娠による腹壁痛の報告からは，エストロゲン，プロゲステロンによる組織の浮腫が腹壁痛の誘因となり得ることも推察される[3)12)]．本症例においては，疼痛部位とは離れていたが上腹部の手術歴があったことや，急激な運動（剣道）を行っていたことがACNESの誘因の一つとなったと考えられた．また，臨床的には問題となっていなかったが，総排泄腔奇形根治術後のため排便時にかかる腹圧が通常よりも高かった可能性もあると思われた．

表1 腹壁痛の診断基準

非常に限局した疼痛
or
圧痛点が固定している
AND
体表の圧痛（腹直筋の深さ）
or
圧痛点の径 ≦ 2.5 cm
or
腹壁筋の緊張により圧痛が増強する
(Carnett's test 陽性)

Srinivasan R, et al, 2002[7] より引用一部改変.

Carnett's testは簡便で腹壁痛の診断に有用な検査であるが，精神的な原因による慢性腹痛でも陽性になるとの報告もあり[13]，単独での診断感度は78%，特異度は88%と報告されている[7]．表1に示した腹壁痛の診断基準を用いると感度85%，特異度97%となる[7]．本症例の腹痛も後からみれば腹直筋外縁に位置して指で示せるほど非常に限局しており，腹壁筋の伸展・緊張で腹痛が増強すること，Carnett's testが陽性であったことなど，この診断基準を満たしていた．しかし我々には当初，腹壁痛という疾患概念が欠如していた．更に，上腹部の手術歴や機能性ディスペプシアの既往から腹部内臓の器質的疾患や機能的疾患を第一に考えてしまったことが診断に苦慮した理由として挙げられる．

一方，腹壁痛の存在は腹部内臓の疾患の否定にはならないこともまた事実である[7]．Greenbaumら[11]は慢性腹壁痛と仮診断した72名のうち4名（6%）に後から器質的疾患が判明したと報告しており，その内訳は総胆管狭窄，逆流性食道炎，大腸憩室周囲炎，糖尿病性神経症であった．Thomsonら[14]は腹壁の圧痛を認めた24名中1名（4%）に，Grayら[15]は24名中5名（21%）に虫垂炎を認めたと報告している．このため，腹壁痛の診断後も最低3か月は経過観察して器質的疾患の除外をする必要がある[7)9)]．

表2 慢性腹壁痛の小児報告例

報告者	年	年齢	性別	部位	診断	治療	転帰
Peleg[3]	1999	15	F	右下腹部	ACNES	局所麻酔薬＋ベタメタゾン3mg 局所注射	治癒
Skinner[4]	2007	16	F	右下腹部手術瘢痕	末梢神経損傷	局所麻酔薬＋トリアムシノロン10mg RSB 3回	治癒
		11	F	右下腹部	ACNES	局所麻酔薬＋メチルプレドニゾロン40mg RSB 3回	治癒
		13	F	右下腹部	ACNES	局所麻酔薬＋トリアムシノロン20mg RSB	不明
		15	M	右下腹部手術瘢痕	末梢神経損傷	局所麻酔薬＋トリアムシノロン40mg RSB	疼痛軽減
		13	F	右下腹部手術瘢痕	末梢神経損傷	局所麻酔薬＋トリアムシノロン40mg RSB	疼痛軽減
		15	F	臍上部	ACNES?	局所麻酔薬＋トリアムシノロン40mg RSB 2回	6か月，2か月で再燃
		13	F	臍周囲	ACNES?	局所麻酔薬＋トリアムシノロン10mg RSB	9時間後再燃
Ivens[5]	2008	11	F	右季肋部	ACNES	局所麻酔薬＋ベタメタゾン1.5mg 局所注射	治癒
Simpson[6]	2011	15	F	上腹部正中	ACNES	局所麻酔薬 TAP block 局所麻酔薬＋トリアムシノロン40mg TAP block	疼痛軽減
自験例	2011	7	F	左側腹部	ACNES	局所麻酔薬＋ベタメタゾン1mg 局所注射	治癒

ACNES: abdominal cutaneous nerve entrapment syndrome, RSB: rectus sheath block, TAP: transversus abdominis plane.