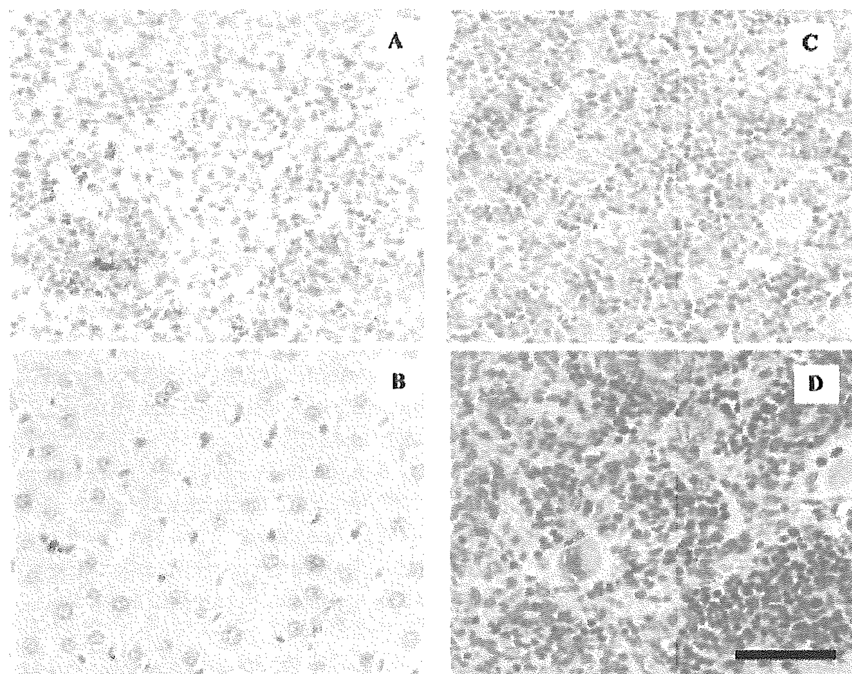

Fig. 5. Histology of rat spleen, liver, and marrow 14 days after exchange transfusion with HbV/rHSA or sRBC/rHSA. Spleen (A), liver (B), and marrow (C) of the HbV/rHSA group. The spleen and liver contained slight hemosiderin deposition, but not the marrow. The spleen of the sRBC/rHSA group (D) also contained slight hemosiderin deposition. Bar = 50 μm (Berlin blue method).

ful discussion of phagocytic and hematopoietic activities. The rHSA was obtained from Nipro Corp.

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トピックス

人工酸素運搬体の開発—現状と将来展望—

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検 査 と 技 術

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は信じられないほどである．同一遺伝子でも表現型(Phenotype)が違うのである．その表現型は環境によって左右されるのである．臨床検査データは一つの表現型であり，環境によって変化した結果も含んだデータである．遺伝子多型などの遺伝子情報と標準化された臨床検査データを蓄積し解析することで，個人が置かれた環境等の要因も考慮することができ，初めて個人の体質に合った医療(テーラーメード医療)が可能になると考えている．このような状況になると医療は劇的に変化するのではないだろうか．

今回JCCLSにできた臨床検査標準化基本検討委員会は，ここに述べたような手段で臨床検査ひいては医療の標準化の礎になることを目指している活動である．

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人工酸素運搬体の開発 ―現状と将来展望―

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歴史的背景

1900年にLandsteinerが血液型を発見，血液型をあわせ，交差試験を行うことにより，輸血の副作用は著しく減少した．20世紀，輸血は出血に対するなくてはならない治療法としてその安全性が追求され続け，その結果多くの血液型の理解が進み，輸血感染症が発見され，スクリーニング方法も確立した．また，移植片対宿主疾患(graft versus host disease, GVHD)などの免疫学的異常反応の解析と治療，輸血後急性肺傷害(transfusion related acute lung injury, TRALI)など，多くの問題に対して研究が行われ，その治療法が開発されて，輸血治療は安全・確実なものとなってきた．しかし，未知のウイルス感染症やプリオン病の問題など輸血治療の安全性にかかわる新たな問題が最近クローズアップされてきている．

いつでも，どこでも安全に投与できる人工血液の開発を目指し，厚生省(当時)は1997年に人工赤血球，人工血小板，人工抗体の創製と開発について科学研究班を組織し，精力的な研究が開始され，現在に至っている．

人工赤血球とは

輸血に用いる保存血は冷所で保存し，保存期間が21日間と短いこと，輸血を行う直前に交差試験を必要とすることなどの煩雑な点がある．交差試験が必要なく，長期保存のできる人工赤血球の開発は第二次世界大戦以前より始まっていた．戦後，パーフルオロ化合物の合成技術，高分子合成・分離技術，蛋白質精製技術などの成熟とあいまって1960年ごろより人工赤血球として人工酸素運搬体の開発が行われるようになった．日本でも人工赤血球の開発は早くから始められ，旧ミドリ十字社がパーフルオロ化合物の乳剤であるフルオゾールを開発し，一定の成績を収めてFDA (Food and Drug Administration，米国食品医薬品局)より承認を受けたが，特殊な適応症のみの承認であったために広く使用されるには至らなかった．一方，ヘモグロビン(hemoglobin，Hb)を精製・修飾して酸素運搬体として利用するタイプの人工赤血球は北米で多くの企業が開発を行ってきた．Baxter社が開発したDCL-Hbは第3相試験まで研究が進んだが開発が中断された．一方，Biopure社の開発している重合ウシヘモグロビンを用いた人工赤血球は，現在北米を中心に第3相試験を行っており，南アフリカでは臨床応用がなされている．

以上のように開発が開始されて久しいが，投与後の血管抵抗の上昇や，組織機能，酸素運搬などの点で解決しなければならない問題点が多く，真の意味での臨床に用いられている物質はない．

人工赤血球開発の現状

われわれは厚生労働省科学研究の一環として1985年より早稲田大学理工学部と共同で，人工酸素運搬体の開発と評価を行ってきた．現在二種類の人工酸素運搬体について研究を進めている．一つ目は期限切れの輸血用血液よりヘモグロビンを分離精製し，ウイルス除去，不活化を行ってからリン脂質小胞体(リポソーム)内に封入し，粒径を250 nmに制御したヘモグロビン内包型リポソームであるヘモグロビン小胞体(Hb小胞体)と，二つ目がアルブミンに人工合成のヘムを包接させたアルブミンヘムとである(図)．これらの開発現状について紹介し，臨床検査法の開発についても言及する．

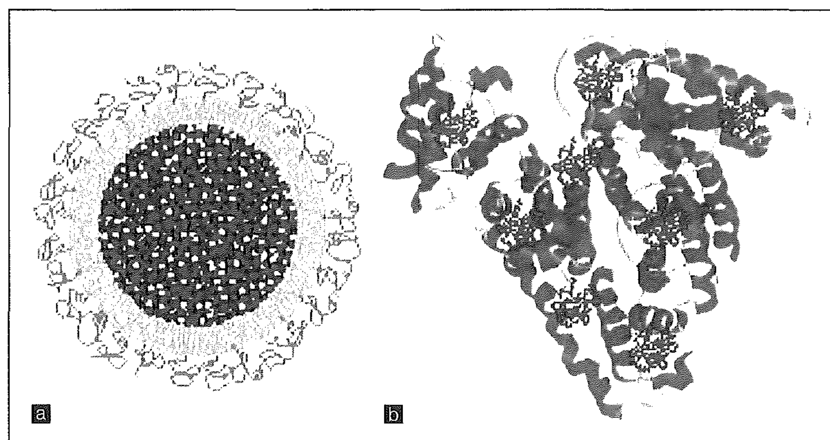

図　ヘモグロビン小胞体(a)とアルブミンヘム(b)の模式図
a：精製したヘモグロビンをリン脂質二重膜に封入している．直径250 nm．b：人工合成のヘムをアルブミン分子に包接させた．

1．ヘモグロビン小胞体(Hb小胞体)

Hb小胞体は精製ヘモグロビンをヒトの赤血球のように脂質二重膜で被覆して粒径を制御し，酸素運搬能を持たせた物質である[1]．通常リポソームは自己粒子間の凝集や内皮細胞への付着，血小板の活性化などが問題となる．互いの凝集を防止し，血管内皮との相互作用を防止するためにポリエチレングリコール(polyethylene glycol，PEG)で膜表面を修飾し，血小板の活性化を抑制する負電荷脂質を用いてリポソームを形成している．また，長期保存を可能とするためには小胞体内のヘモグロビンが酸化されない環境を保持することが重要で，現在では窒素雰囲気下でHb小胞体を脱酸素化したデオキシ体として保存することによって1年以上の長期保存を可能としている[2]．

生体内での酸素運搬能，循環保持能力については，交換輸注試験[3]や出血性ショックの蘇生試験を中心に検討が行われている．ラット，およびウサギ，ビーグル犬において種々の検討が行われ，十分な酸素運搬能を有し，ショック蘇生に有効であることが解明されている．生体内での半減期は35時間(ラット)近くあることが報告された[4]．

投与後の生体に与える変化であるが，成長阻害もなく，実験動物の体重の増加も順調であった．血中より消失したHb小胞体がどのように代謝されるかを病理組織を用いて検討したところ，3時間後より脾臓の赤脾髄に集積し始め，7日後をピークとして脾臓が腫大，重量も最大となり，その後正常域に復することが明らかとなってきた．腫大は赤脾髄のマクロファージがHb小胞体を貪食することによって起こり，脾臓内のマクロファージはいったん，貪食胞が飽和に達したと思えるほどHb小胞体を貪食した後，経時的に正常像に回復し，7日後には正常の組織構築を呈することをSakaiらが報告している[5]．このような脾臓の変化はラットの保存血液を用いて行った同様の試験でも認められるので，Hb小胞体に特有の現象ではないことも明らかとなった．

通常の薬品を開発する際には，LD_{50}から，最大投与量を決定し，通常使用量を決定してゆく．しかし人工酸素運搬体に関しては，最大投与量をどのように決定すべきなのかについて明らかなコンセンサスは得られておらず，今後検討すべき課題であると考えられる．

長期生存に与える影響，免疫系に与える影響についても研究が進んでおり反復投与でも成長，血液生化学的検査などでの明らかな異常は認められていない[6]．

リポソーム製剤を血管内に投与する場合，投与後に採血した血液内，特に血漿層にリポソームが分散し，血液・生化学検査が正しく評価できるかについて検討する必要がある．特に比色や比濁法で定量する臨床検査法では，Hb小胞体の干渉作用のため検査結果に影響が出ることが予想された．この点に関しては採血後に血清を超遠心分離操作にかけてHb小胞体を沈殿除去することにより結果に影響を及ぼさないことが確認されている[7]．

2．アルブミンヘム

アルブミンは血中で最も多い蛋白質であり，膠質浸透圧，粘度を維持し，体内のホメオスタシスを保持する重要な蛋白質である．アルブミンヘムはこの蛋白質に人工合成のヘムを包接という方法で導入し，酸素運搬を可能とした物質である．体内で酸素運搬能を有

し，出血性ショックの治療薬として有効である可能性が示唆されており[8,9]，現在研究が続行中である．血中にアルブミンヘムが投与された場合の臨床検査法についても，今後開発を進めてゆく必要がある．

まとめ

人工赤血球は通常の薬剤と異なり，血中で酸素を受け渡しすることで機能を発揮し，血中にある程度の期間とどまることが要求されている．出血に対する治療薬としての用途のほか，虚血領域への酸素運搬を治療法としたOxygen Therapeutics(酸素治療)についても研究が進んでいる[10]．臨床応用が始まれば，臨床検査現場での対応も必要となるため，機器の開発，応用に関しても研究の展開が必要と考えられている．

輸血は20世紀の医療を大きく変えた治療法の1つであり，今日の日本での輸血は限りなく安全になっている．しかし，貯蔵，使用時の注意点を考えると人工血液があることによって輸血を補完できるシステムを構築できる可能性がある．この点で人工赤血球の開発は21世紀の医療の進歩に貢献できると考えられる[11]．

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新規腫瘍マーカーとしての尿中ジアセチルスペルミン

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はじめに

尿中ジアセチルスペルミンはポリアミンの尿中代謝産物の一つである．ポリアミンは活発に増殖する組織に多量に含まれており，細胞増殖に重要な役割を果たしていると考えられるが，アセチル化され，尿へ排泄されるが，その大部分はモノアセチル体である．ジアセチルスペルミンはポリアミンの1種であるスペルミンがジアセチル化されたもので，尿中ポリアミンの1%以下にすぎない．しかし，最近，癌患者に特異的に尿中排泄が増加することが知られるようになってきた．検体が尿であるため，採血の苦痛や針刺し事故の危険がなく，癌マーカーとしての機序より考えると臓器特異性がないことが推察され，癌検診に有用な“汎用性癌マーカー”として期待されている．

本稿では尿中ジアセチルスペルミンの研究の現況について概説した．

1．尿中ポリアミン

複数のアミノ基を持つアルキルアミンをポリアミンと総称する．ヒトの体内には4種類のポリアミンと，

3．細径気管支鏡

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key words　ultrathin bronchoscope, CT guided bronchoscope, transbronchial biopsy, virtual bronchoscopy

動　向

気管支鏡の発達は，多彩な処置のできる処置用内視鏡の開発と，末梢領域まで到達でき，細かい観察と検査が可能な気管支鏡の開発との2つの方向で進んできた．最近ファイバーの改良が進み，解像力に優れ，また，暗かった画面をCCD装置を組み込むハイブリッド構造とすることによって日常の気管・気管支の観察と評価に苦痛を感じない細径気管支鏡が出現した．機器の改良が進む細径気管支鏡が臨床現場で果たす役割について述べる．

A．細径気管支鏡の用途（表1）

気管支鏡は気管・気管支の内腔観察，透視や超音波を併用した病変部位の検体採取といった診断目的に使用される場合と，気管・気管支内病変の切除，異物の除去，喀痰の吸引，薬剤の注入などの治療・処置の目的で使用される場合がある．

細径気管支鏡でも同様に診断目的で使用される場合と処置を目的として使用される場合がある．診断で用いられる場合には通常の気管支鏡では到達が困難な5次気管支より末梢の気管支に到達して観察あるいは検体の採取を行うことが主体となる．その他の診断用途としては気道狭窄症例での狭窄部以遠の観察を行うこと，空洞性病変内の内

表1　細径気管支鏡の用途

	利用法
診断目的	亜区域枝以遠の気管支内腔の観察
	気道狭窄症例における狭窄部以遠の観察
	小児，新生児の気道内の観察
	末梢の空洞性病変の内腔観察
	末梢の肺病変の生検，ブラシによる細胞診，細菌検査
処置目的	麻酔時における一側肺換気用チューブの挿入におけるガイド
	空洞性病変内への薬剤の直接注入
	小児における気管，気管支内分泌物の除去
	小児例での気管内挿管のガイド

腔観察と検体採取などがある[1,2]．細径気管支鏡を診断に用いる場合，観察所見について種々の報告がなされており所見と疾患との関係についての解析も報告があり，今後の検討課題である[1]．

処置目的としては呼吸器外科手術や食道手術の際に一側肺換気用気管挿管チューブ留置のためのガイドとして用いることが多い．空洞性病変を有する疾患において空洞に到達して空洞内を観察し，直接薬剤を注入して診断と治療に有用であったとの報告が散見されるようになり[2-4]，今後このような使用法も増えてくると思われる．

当然のことながら，新生児や，小児においては気管内腔が狭いため，細径の気管支鏡は診断，処置のために必須の機器である．

B. 細径気管支鏡の使用領域

細径気管支鏡を用いた際の観察範囲は患者の体格と機器の外径によって決まるが，現在細径気管支鏡として汎用されるようになった外径2.8mmの気管支鏡では通常の電子内視鏡で観察可能な気管支より最大で7次末梢の気管支まで観察が可能である（たとえば右上葉B3であれば電子内視鏡では4次気管支すなわち亜々区域枝B3biαまでの観察が可能であるが，細径気管支鏡を用いると8次気管支B3biαxxyまで観察可能である．最大で12次気管支にまで挿入することができると報告されている[5]．

細径気管支鏡では光源を導くライトガイドも細いため，太い気道では画像が暗くなり診断の役に立たない．このため，太い気道では通常の気管支鏡あるいは気管支ビデオスコープを使用する必要があった．しかし，観察用ファイバーの性能向上と細いファイバーの使用，実装技術の改良により画像の解像度も向上したため，スクリーニングとしての検査を行う場合には支障がない程度となっている．さらにはCCDをあらかじめ気管支鏡本体に組み込んだハイブリッド気管支鏡では自動露光装置を備え，光量に応じて画像の明るさを調節するため，観察に光量の必要な気管や主気管支でもある程度の観察は可能となり，細径気管支鏡の守備範囲は気管から末梢領域までをカバーできると考えられている．

C. 診断に使用できる処置具について

診断のために検体を採取する場合，検体採取に用いる処置具には機器のチャンネル径により制約がある．現在，1.2mmチャンネルを有する細径気管支鏡ではブラシと生検鉗子が使用できる．生検された標本は病理検体としては非常に小さい．病理検査部門とよく連絡を取り合って数回の生検を行っておくことが推奨されている[5]．ブラシも使用できるが，採取できる細胞の量は多くはない．洗浄液の吸引は時間をかければある程度可能であるが，末梢領域にウェッジしての肺洗浄では吸引圧が強いと気道が虚脱し洗浄液の回収が困難なことが多い．

今後，処置具の改良は必要であるが，機器の形から限界もある．鉗子あるいはブラシをどれだけ正確に病巣に近づけることができるかに診断の成否がかかってきている．

D. 診断の再現性について

末梢発生の肺癌では腫瘍径が小さくなるほど関与する気管支は少なくなる．そこで検査時あるいは再検査時に特定の気管支に到達するためには気管支分岐を正確に同定する必要がある．

気管支の命名は，1950年に日本気管支分岐命名委員会が制定した第5次気管支までの定義を用いていたが，1999年に気管支学会（現在の日本呼吸器内視鏡学会）において5次気管支より末梢の気管支分岐の命名法を決定するためのワーキン

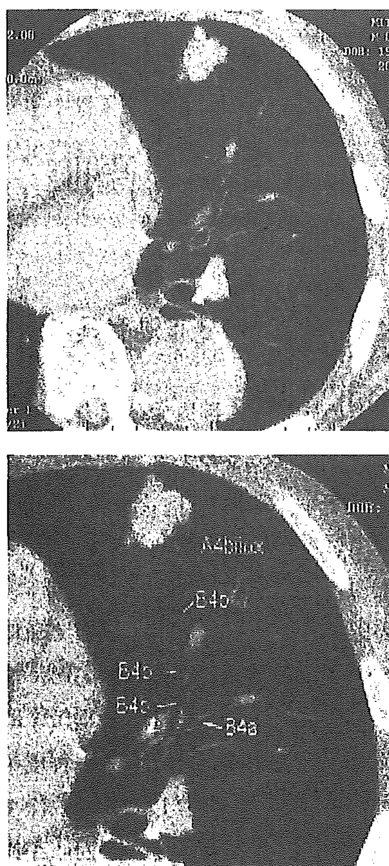

図1　細径気管支鏡の到達範囲

腫瘍へはB4bii αが関与していることが気管支およびその気管支に伴走する肺動脈の分岐様式より判定できる．細径気管支鏡にて同気管支を同定し，生検を行う．

ググループが組織され，2000年に6次以降の命名法が定義された[6]．この規定によって細径気管支鏡の到達部位を簡潔に記載することが可能となり，再現性をもって検査が遂行できるようになった．

図1にあげた症例は左S4bii αの関与が疑われる直径2.5cmの結節であるが，通常の気管支鏡では生検鉗子が腫瘍の前内側方にずれ，キュレットも同様に内側方の気管支（S4bii α y）に挿入されてしまうため，正確に結節に到達することができず，気管支鏡検体からの診断はできなかった．そこで，細径気管支鏡を用いて観察を行ったところ，B4bii αの次の分岐において硬い喀痰による閉塞があることが認められた．この閉塞を直視下生検にて解除した後，S4bii α xxに生検鉗子を挿入，透視において腫瘍に一致することを確認の上，生検を行い，中分化扁平上皮癌の診断を得た．

HRCT像では第5次分岐以降の気管支分岐も正確に追うことができ，この情報を上手に活用することによって細径気管支鏡検査の診断精度と診断率を向上させることが可能であると考えられている．また，これにCT透視を組み合わせて診断精度を高める検査法を行う施設もある．

細径気管支鏡は回転軸が術者が考えている回転軸と必ずしも一致しない（たとえば上葉肺尖部をねらうときには気管支鏡の切り欠きの部分が前を向いているのか外側を向いているのかがわからない）という欠点がある．これは気管支鏡が細く，径に比較して軟性部の長さが長いために挿入経路が長くなると壁に当たることによってねじれが生じ，手元の操作がダイレクトに気管支鏡先端に伝わらないためである．このような際には透視を用いて現在の位置を確認し，気管支分岐の正確な評価を行う必要がある．分岐次数が高次になるにしたがって，病巣に到達するために気管支分岐を正確に判別する必要が生まれてくる．この操作を簡略にそして正確に検査に反映させるために virtual bronchoscopy とよばれるHRCTあるいはMDCTによって得られた画像を再構成することによって病巣へ向かう気管支を内腔側から同定できるようにしたシステムの開発が進んでいる[7]．こうしたシステムの使用と単純な透視ではなくデジタル透視台の使用やCT併用の気管支鏡検査が行われるようになれば[8]，病巣への到達率が格段に向上し，正確な cytopathological diagnosis を得ることが可能となると思われる．

E. 今後の細径気管支鏡の位置づけ

肺癌を代表とする肺疾患の多くは初期には病巣も小さく，進展範囲も限られていることが多い．このような状態で正確な診断を行って治療を行うことが治療成績の向上につながることも明らかである．しかるに現状では電子内視鏡の開発によって解像度は向上したが，末梢への到達性はまだ充分とはいえなかった．細径気管支鏡の改良により，解像度，到達性に優れた機器が手に入るようになり，末梢小型病変に対する気管支鏡診断の向上が期待できる．小結節に対する診断ではCTガイドの針生検あるいは針吸引細胞診の診断率が高いという報告が多い[9,10]．しかし，針生検は手技自体が気胸や癌細胞の播種，喀血や空気塞栓などの合併症の頻度が高い．細径気管支鏡が普及することによって正確なcytopathologic diagnosisが得られることとなれば，安全性，反復してできるという面から細径気管支鏡が今後見直されるようになると考えられる．

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Neurotoxicity of Intrathecally Administered Bupivacaine Involves the Posterior Roots/Posterior White Matter and Is Milder Than Lidocaine in Rats

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Background and Objectives: *Clinical and laboratory studies suggest that lidocaine is more neurotoxic than bupivacaine. However, histological evidence of their comparative neurotoxicity is sparse. We thus pathologically and functionally compared the intrathecal neurotoxicity of these agents.*

Methods: Rats received 0.12 μL/g body weight lidocaine (0%, 2%, 10%, or 20%) or bupivacaine (0%, 0.5%, 2.5%, or 5%) in distilled water via an intrathecal catheter. The influence of high osmolarity was also examined using 5% bupivacaine in 20% glucose solution (5% BG) and a control 25% glucose solution. The L3 spinal cord, the posterior and anterior roots, and the cauda equina were examined by light and electron microscopy. Walking behavior and sensory threshold were investigated as neurofunctional tests.

Results: The posterior root and posterior white matter showed axonal degeneration in rats treated with 10% and 20% lidocaine and 5% bupivacaine in distilled water (5% BDW) and in 5% BG, but not in rats treated with 2% lidocaine, 0.5% and 2.5% bupivacaine, distilled water, or 25% glucose solution. The histological damages were more severe in 20% lidocaine-treated rats than in 5% bupivacaine-treated rats. The damage of posterior white matter was observed only when the posterior root was severely injured. No significant difference of histological findings was observed between 5% BDW and 5% BG. Functional abnormalities were found only in rats treated with 20% lidocaine.

Conclusions: The neurotoxic lesions caused by bupivacaine and lidocaine were indistinguishable in the primary site and the extending pattern, such as axonal degeneration originating from the posterior roots and extending to the posterior white matter. The intrathecal neurotoxicity is greater in lidocaine than in bupivacaine. *Reg Anesth Pain Med 2005;30:464-472.*

Key Words: Local anesthetics, Neurotoxicity, Histopathology, Sensory impairment, Axonal degeneration.

Spinal anesthesia has been widely accepted as a safe technique with various advantages. Recently, however, concerns have risen regarding the neurotoxicity of intrathecal local anesthetics. Several reports have documented a variety of spinal neurologic disorders, such as transient neurologic symptoms and cauda equina syndrome after uneventful spinal anesthesia,[1-4] although the exact etiology of each syndrome is unknown. In most of these clinical reports, the incidence of neurologic sequelae is commonly higher after spinal anesthesia with lidocaine than that with bupivacaine.[3-7] Various *in vitro* studies of sciatic nerve,[8] sensory neuron,[9] and posterior root[10] also indicate that lidocaine causes more severe impairment of neuronal function compared with bupivacaine. Although these *in vitro* studies point to the pure effect of local anesthetics on cultured neuronal tissue, they do not identify the location of the primary lesion caused by

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local neurotoxicity after intrathecal drug injection. Furthermore, even *in vivo* studies do not clearly demonstrate the primary neurotoxic lesion and its characteristics. Previously, we reported on the primary location and pathologic feature of the lesions observed after intrathecal administration of lidocaine.[11] An experimental study of rats[12] and many clinical reports have described that pharmacologic potency of bupivacaine is 4 times higher than that of lidocaine.[13] Practical usage of these drugs is recommended to be 0.5% and 2% in bupivacaine and lidocaine, respectively. Thus, the comparative effect of bupivacaine with lidocaine is 4 times higher in concentration in this study. The purpose of this study is to examine the features of the neurotoxic lesion induced by intrathecally injected bupivacaine and to compare its neurotoxicity with lidocaine.

Materials and Methods

The study was approved by the Ethics Committee on Animal Research of Kitasato University School of Medicine. Studies were conducted on 55 male Wistar rats (12 weeks old; body weight 278 to 300 g). The animals were housed 3 per cage in the experimental facility for 1 week before the experiment and were maintained under a 12-12-hour light-dark cycle at a room temperature of 22°C. They were allowed free access to food and water.

Surgical Procedure for Intrathecal Catheterization

The rats were anesthetized with sodium pentobarbital (intraperitoneal 50 mg/kg), and the subarachnoid space was cannulated with a polyethylene tube (0.6 × 700 mm) through the atlanto-occipital membrane by application of the modified method of Yaksh and Rudy.[14] The tip of the catheter was advanced 7.5 cm caudal to the level of L2. We fixed the other end of the catheter in the subcutaneous tissue to avoid dislocation of the catheter. The rats were allowed to recover, and the test drug was injected 1 week later. Rats that showed symptoms of traumatic nerve damage were excluded from further experiments.

Intrathecal Administration of Drugs

On day 7 after intrathecal catheterization, the rats were divided into 9 groups. Rats received either 2%, 10%, or 20% lidocaine in distilled water, 0.5%, 2.5%, or 5% bupivacaine (Astra Zeneca, Tokyo, Japan) in distilled water, or 5% bupivacaine in 20% glucose. Distilled water and 25% glucose solutions were also administered by the same method in the control groups. Each solution was prepared aseptically by a pharmacist (S.M.) on the morning of the day of the injection. The total volume of the injection was 0.12 μL/g body weight, in addition to 6 μL for the dead space of the catheter. Cutting of the skin under inhalational ether anesthesia delivered through a face-snout mask to reduce distress exposed the catheter embedded subcutaneously, and, thereafter, each solution was administered for 15 seconds through the catheter. Immediately after drug injection, ether inhalation was stopped (total inhalation time was less than 5 minutes), and the wound was closed with the catheter left under the cutaneous tissue. The rats were allowed to breathe room air until recovery from anesthesia.

The osmolarity of each solution was measured by the method of freezing-point depression (Fiske One-10 osmometer, Norwood, MA).

Recovery Time to Ambulation

The behavior of each rat was evaluated by analysis of its ability to walk with or without limitation. Evaluation was performed at 0.25, 0.5, 1, 2, 3, and 4 hours after intrathecal injection on the day of injection (postinjection day [PID] 0) and every morning from the next day (PID 1) to PID 4. The recovery time to normal ambulation was recorded.

Paw Stimulation Test

A technician blinded to the animal groups performed the paw stimulation test. The latency of the hindlimb withdrawal response to radiant heat delivered on the plantar surface was measured before injection of the drug (prelatency) and on PID 4 (postlatency). Measurements were repeated 6 times on both the left and right paws in each rat. The data were converted to percent maximum possible effect (%MPE) calculated as ([postlatency − prelatency]/[cutoff time − prelatency] × 100). We fixed the cutoff (i.e., maximum exposure) time to 20 seconds to prevent thermal injury.

Tissue Preparation

On PID 4, after evaluations of 2 functional tests, the animals were deeply anesthetized and then perfused transcardially with a fixative solution (2.5% cacodylate-buffered glutaraldehyde) for histologic examination. After fixation, the lumbar spinal cords with the anterior and posterior roots and cauda equina were removed *en bloc* and each was dissected into 4 samples (A to D): transverse section with both roots of L3 (A), posterior (B), or anterior (C) roots just proximal to the dorsal ganglion, and cauda equina nerves (D) for light and electron microscopic examination, as described in our previous study.[15] All specimens were embedded in epoxy

resin. The semithin sections (0.5 to 1.0 μm thick) were stained with polychrome dyes. Ultrathin sections (70 to 80 nm thick) were double-stained with uranyl acetate and lead citrate and examined under a JOEL FX2000 electron microscope (Nippon Denshi, Tokyo, Japan) at 100 kV. The histologic damages were estimated as follows. Grade of distribution score (D-score) of the lesion was divided into 3 scores (0 = no lesion, 1 = lesion limited to the posterior root, 2 = lesion observed both in the posterior root and posterior white matter). Severity score (S-score) of the lesion was divided into 3 scores (0 = mild, 1 = moderate, 2 = severe). D-score plus S-score was the total injury score.

Statistical Analysis

Values of %MPE were expressed as mean ± SD and analyzed by Dunnett's test for comparison of the mean control (distilled water) with each of the other group means. Differences in the incidence of pathologic lesions after bupivacaine and lidocaine injections were compared by the chi-squared test. Differences in injury score and time to walking behavior were analyzed by the Mann-Whitney *U*-test. All statistical procedures were performed by StatView software version 4.5 J (Abacus Concept, Inc., Berkeley, CA). A P value <.05 was considered to denote a significant difference.

Results

Four rats were excluded from the study because of hindlimb palsy caused by traumatic injury during catheterization. A total of 51 rats were analyzed. The number of rats in each group was as follows: control groups (distilled water, n = 6; 25% glucose, n = 5), lidocaine groups (2%, n = 6; 10%, n = 6; 20%, n = 6), and bupivacaine groups (0.5%, n = 6; 2.5%, n = 5; 5% in distilled water [5% BDW], n = 6; 5% in 20% glucose [5% BG], n = 5). The pH and osmolarity of each drug solution are listed in Table 1.

Table 1. Osmolarity and pH of Drug Solutions Injected Intrathecally

	Concentration	pH	Osmolarity (mOsm/kg H_2O)
Lidocaine	2%	5.6	176
	10%	6.0	895
	20%	6.3	2,038
Bupivacaine	0.5%	5.8	41
	2.5%	5.5	120
	5% BDW	5.4	241
	5% BG	4.8	1,890
Vehicle			
Distilled water		6.1	0
25% Glucose		5.5	20,100

Abbreviations: 5% BDW, 5% bupivacaine dissolved in distilled water; 5% BG, 5% bupivacaine dissolved in 20% glucose.

Table 2. Time of Recovery to Normal Ambulation After Intrathecal Drug Injection

Drug	n	Time (hour) Median	Range
Lidocaine			
2%	6	0.5	0.5–1*
10%	6	4	3–4*
20%	6	more than PID 4*†	
Bupivacaine			
0.5%	5	1	1–2
2.5%	5	2	1–2
5% BDW	6	2.5	2–4‡
5% BG	5	2	1–3
Vehicle			
DW	6	0.25	
25% G	5	0.25	

NOTE. Data are expressed as median and range (minimum–maximum).

Abbreviations: 5% BDW, 5% bupivacaine dissolved in distilled water; 5% BG, 5% bupivacaine dissolved in 20% glucose; DW, distilled water; 25% G, 25% glucose solution.

*P < .05 lidocaine *v* bupivacaine at concentrations of similar pharmacologic potency, 10% lidocaine *v* 2.5% bupivacaine, 20% lidocaine *v* 5% BDW or 5% BG.

†All rats injected with 20% lidocaine showed walking limitation even on PID4.

‡P < .05 5% BDW *v* 5% BG.

Neurofunctional Deficits

Recovery time to ambulation. Control animals showed complete recovery within 15 minutes after intrathecal injection of the drug (Table 2). Although rats treated with 2% lidocaine recovered more rapidly than those treated with 0.5% bupivacaine (P = .013), rats treated with ≥10% lidocaine showed longer recovery time than the ≥2.5% bupivacaine groups (P = .003). In the 20% lidocaine group, all rats exhibited walking limitation even on PID 4. In contrast, rats treated with 5% BDW and 5% BG started to walk normally within 4 hours on PID 0. Comparison between 5% BDW and 5% BG showed that the time to ambulation in the latter group was significantly faster than that of the former group (P = .045).

Paw Stimulation Test. The effects of both drugs on the sensory threshold expressed as %MPE are shown in Figure 1. In lidocaine-treated rats, the %MPE decreased in a concentration-dependent manner. A significant difference was seen in %MPE between rats treated with 20% lidocaine and those injected with distilled water alone (control) (P = .0001). In contrast, %MPE values of all rats treated with each of ≤10% lidocaine and bupivacaine was not significantly different from those of the control group.

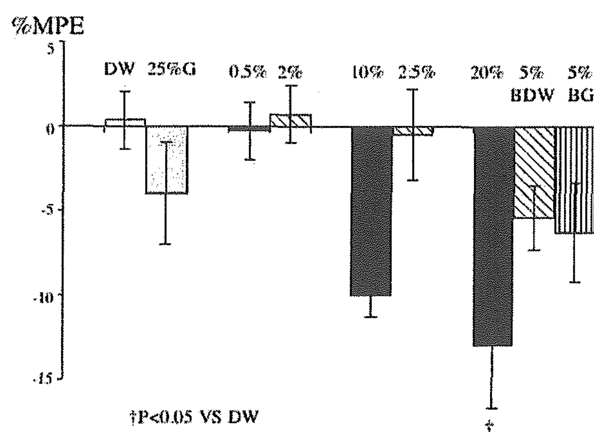

Fig 1. Changes in percent maximum possible effect. Open bar indicates distilled water group. Solid bar indicates lidocaine group. Diagonal bar indicates bupivacaine–distilled water group. Gray bar indicates 5% bupivacaine–20% glucose group. †$P < .05$ compared with distilled water. A significant decrease in %MPE was only noticed in 20% lidocaine compared with distilled water ($P = .0001$). Other groups did not show any significant changes in sensory threshold.

Histopathologic Assessment of Neurotoxicity

Histologic abnormalities were evident in 10% and 20% lidocaine groups, in the 5% BDW group, and in the 5% BG group but not in the other groups (2% lidocaine, 0.5% bupivacaine, 2.5% bupivacaine, distilled water, and 25% glucose). The histologic abnormality was defined as a lesion with infiltration of macrophages and destruction of myelin sheaths and axons. These histopathologic changes were mainly observed in the proximal portion of the posterior root at its entry to the spinal cord (within sample A) (Table 3). In addition to the posterior root lesion, the posterior white matter was damaged, especially in cases with severe posterior root lesions (Table 4 and Fig 2). No histological abnormality was detected in any other areas (samples B, C, and D). Relationship between catheter locations and lesions is also depicted in Figure 3.

Electron microscopic examination showed widespread degeneration of both the myelin sheaths and axons in severely injured areas such as the proximal portion of the posterior root in rats treated with 20% lidocaine. Axonal degeneration with the almost-intact myelin sheath in mildly injured areas was mainly observed in rats treated with less than 10% lidocaine and 5% bupivacaine (Fig 4B). Thus, the neurotoxic lesions caused by lidocaine and bupivacaine seemed to commonly spread from the posterior root just at the entry, into the spinal cord, and to the nearby posterior white matter. These histologic changes were likely caused by axonal degeneration.[11,15]

Histopathologic characters of neurotoxicity caused by lidocaine and bupivacaine were virtually identical. Thus, we scored the histologic damage in each group (Table 4). Histologically determined injury scores of rats treated with 10% and 20% lidocaine were much higher than those of rats treated with bupivacaine at the equipotent concentrations.

Table 3. Incidence of Lesions Induced by Lidocaine, Bupivacaine, and Control Groups

	Posterior Root		Posterior White Matter			
Drug	Proximal	Distal	FC	FG	DH	AH
Lidocaine						
2% (n = 6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
10% (n = 6)	6 (100)	0 (0)	6 (100)	0 (0)	0 (0)	0 (0)
20% (n = 6)	6 (100)	0 (0)	6 (100)	0 (0)	0 (0)	0 (0)
Bupivacaine						
0.5% (n = 6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2.5% (n = 5)	0 (0)‡	0 (0)	0 (0)*	0 (0)	0 (0)	0 (0)
5% BDW (n = 6)	3 (50)†	0 (0)	2 (33)*	0 (0)	0 (0)	0 (0)
5% BG (n = 5)	2 (40)†	0 (0)	1 (20)**	0 (0)	0 (0)	0 (0)
Vehicle						
DW (n = 6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
25% G (n = 5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

NOTE. Proximal indicates posterior root at entry into the spinal cord. Distal indicates peripheral portion of the posterior root just above the dorsal ganglion.

Abbreviations: FC, fasciculus cuneatus (lateral area of the posterior column); FG, fasciculus gracilis (median area of the posterior column); AH, anterior horn; 5% BDW, 5% bupivacaine dissolved in distilled water; 5% BG, 5% bupivacaine dissolved in 20% glucose; DH, dorsal horn.

Significant differences in the incidence of posterior root lesions† or FC lesions* between 10% lidocaine *v* 2.5% bupivacaine, 20% lidocaine *v* 5% BDW; or 5% BG (chi-squared test, †, *$P < .05$, **$P < .01$).

Table 4. Histological Injury Score

Drug	n	Total Score					
		0	1	2	3	4	5
2% Lidocaine	6	6	0	0	0	0	0
10% Lidocaine	6	0*	0	0	0	2	4
20% Lidocaine	6	0*	0	0	0	0	6
0.5% Bupivacaine	6	6	0	0	0	0	0
2.5% Bupivacaine	5	5	0	0	0	0	0
5% BDW	6	3	0	1	0	2	0
5% BG	5	3	0	1	0	1	0
Distilled water	6	6	0	0	0	0	0
25% Glucose	5	5	0	0	0	0	0

NOTE. Distribution Score (D-score): 1 = lesion limited to the posterior root; 2 = lesion extending to the posterior white matter from the posterior root.

Severity of lesion score (S-score), most severe lesion in the posterior root or posterior white matter: 0 = no lesion; 1 = mild lesion, focal disruption of myelin sheath and axon; 2 = moderate lesion, middle grade between 1 and 3; 3 = severe lesion, diffuse disruption of myelin sheath and axon. Injury score was calculated as the total score of D-score and S-score. Data are number of rats with each total score (0 to 5).

Abbreviations: 5% BDW, 5% bupivacaine dissolved in distilled water; 5% BG, 5% bupivacaine dissolved in 20% glucose.

*$P < .05$ lidocaine *v* bupivacaine at concentrations of similar pharmacologic potency, 10% lidocaine *v* 2.5% bupivacaine, 20% lidocaine *v* 5% bupivacaine.

Discussion

The present study shows that intrathecally injected lidocaine and bupivacaine cause indistinguishable neurotoxic lesions with regard to the primary site of the lesion. Lesions caused by both drugs spread from the posterior roots (entry zone) just at the entry into the spinal cord, to the posterior white matter. Our histologic study indicates that these lesions are likely caused by axonal degeneration and that the histologic damage caused by lidocaine and bupivacaine appears to involve the sensory system but not the motor system. However, the incidence and severity of the lesions are quite different between lidocaine and bupivacaine groups; lesions induced by lidocaine were more widely distributed and more severe than those induced by bupivacaine at concentrations of equivalent potency. Moreover, at higher concentrations, lidocaine produced sensory impairment and persistent hindlimb limitation, whereas bupivacaine did not. Considered together, intrathecal lidocaine likely induces more severe neurotoxicity than does bupivacaine in rats.

Previous studies indicated that hyperosmolarity of 5% lidocaine solution potentially produced neurotoxic effects.[16] However, several other study groups subsequently reported that the addition of glucose to a local anesthetic solution did not increase the local neurotoxicity.[17-19] Similarly, the present study demonstrated the lack of obvious histologic abnormality in rats treated with 25% glucose alone, the osmolarity of which exceeds that of 20% lidocaine. Our results may also exclude the possibility that the excessively high concentration of glucose increases neurotoxicity when it is mixed with the anesthetics, because the observed neurotoxic effects of 5% BDW were similar to those of 5% BG, whose osmolarity was 7 times higher than that of 5% BDW and close to that of 20% lidocaine. Thus, the extent of histologic damage is exclusively caused by the drug itself rather than the osmolarity of the solution. In our previous report on the neurotoxicity of tetracaine,[15] rats treated with 20% tetracaine elicited severe histologic damages and walking disturbance, whereas rats treated with 0.5% tetracaine did not elicit any histologic or neurofunctional abnormalities, although the osmolarity of both solutions was similar (650 mosm/kgH_2O for 0.5% tetracaine *v* 750 mosm/kgH_2O for 20% tetracaine). This result also supports the observation that the histologic or functional abnormality is not caused by excessively high osmolarity.

The present histologic findings suggest that the neurofunctional impairment is sensory dominant because the lesions are always limited to the sensory system. In contrast, the motor system was morphologically intact, even in groups with high concentrations of the anesthetic agents. Although the intact histology of the motor system does not prove the lack of a local anesthetic effect on motor neurons, our histologic findings suggest that the motor neuron is less susceptible to local neurotoxicity than are sensory neurons. Therefore, we presume that the walking disturbance observed in the 20% lidocaine group is most likely caused by a decrease in the sensory threshold that results from severe lesions in the posterior root and posterior white matter and not from impairment of motor

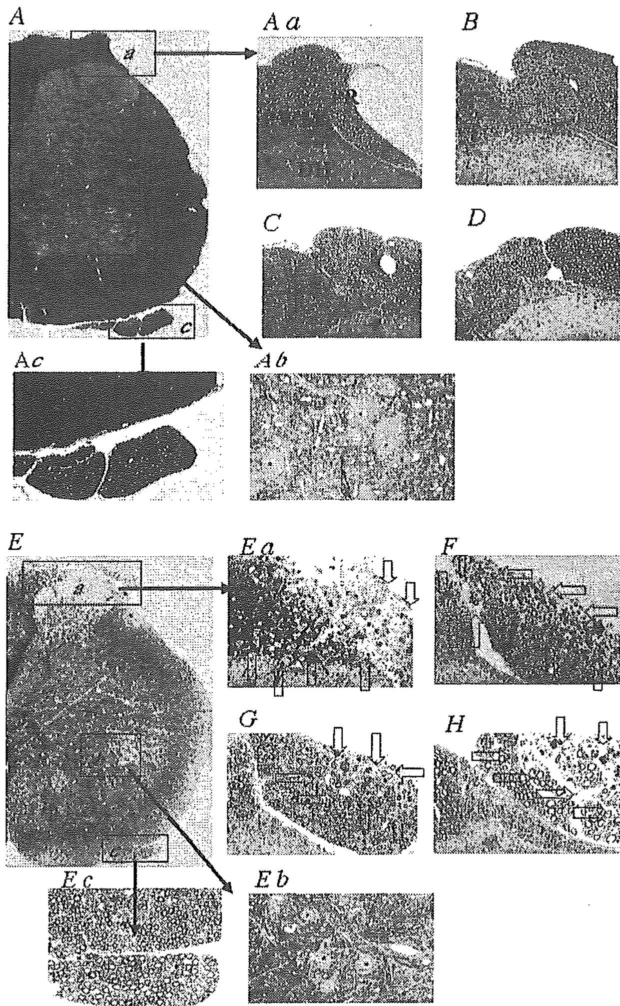

Fig 2. Light microscopic findings in the posterior root and posterior white matter according to treatment. (*A*) 25% glucose, (*B*) distilled water, (*C*) 2% lidocaine, (*D*) 2.5% bupivacaine, (*E*) 20% lidocaine, (*F*) 10% lidocaine, (*G*) 5% bupivacaine in distilled water (BDW), (*H*) 5% bupivacaine in 20% glucose (BG). Magnification ×200. PR: posterior root, PWM: posterior white matter, DH: dorsal horn. *A a* and *E c* show posterior root and posterior white matter magnified in each spinal cord. *A b* and *E b* indicate anterior cell, and *A c* and *E c* indicate anterior root treated with each drug ×200. Open arrows show histologic lesions. Histologic abnormalities are observed only in posterior root and posterior white matter of 10% and 20% lidocaine and both 5% BDW and 5% BG (*E–H*). The posterior root and posterior white matter lesions in 20% lidocaine group are the most severe among the groups, and the extent and severity of the lesions are the mildest in both 5% BDW and 5% BG. No obvious difference is seen in histologic findings between 5% BDW and 5% BG. Anterior horn and anterior root are always intact, even at 20% lidocaine.

neurons themselves. On the other hand, abnormalities of both walking behavior and sensory threshold were not detected in 10% lidocaine–treated and 5% bupivacaine–treated rats, although histologic damage of the sensory system was evident in most of these animals. The reason a discrepancy between histologic and neurofunctional results is noted in cases with mild and focal histologic damage is believed to be a functional compensation of the remaining intact nerve fibers in loci.

In the behavior tests, rats in the 2% lidocaine group exhibited significantly faster recovery than did the 0.5% bupivacaine group, which confirmed the well-accepted fact that lidocaine is a shorter-acting anesthetic than is bupivacaine. However, at more than 10% lidocaine concentrations, the recovery time after injection of lidocaine significantly increased over that observed after bupivacaine injection (Table 2). This prolongation is explained by the histologic damage and associated neurotoxic effect. In contrast, the faster recovery in the 5% BG

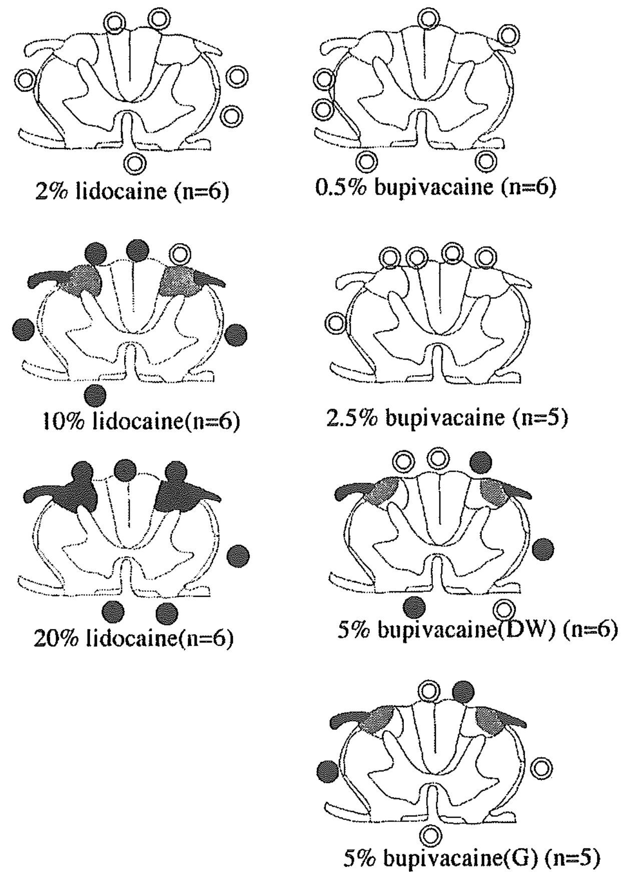

Fig 3. Relevance of the catheter tip and lesion. Lesion in each group was found at the posterior root and posterior white matter. Locations of the catheter tip did not correlate with those of lesions. Black area indicates lesions that show diffuse disruption of both myelin sheath and axonal structure with macrophage infiltration. Gray area indicates lesions that show focal or sporadic disruption of myelin and axonal structure with macrophage infiltration. Closed circles indicate catheter tip with the lesions. Open circles indicate catheter tip without the lesions.

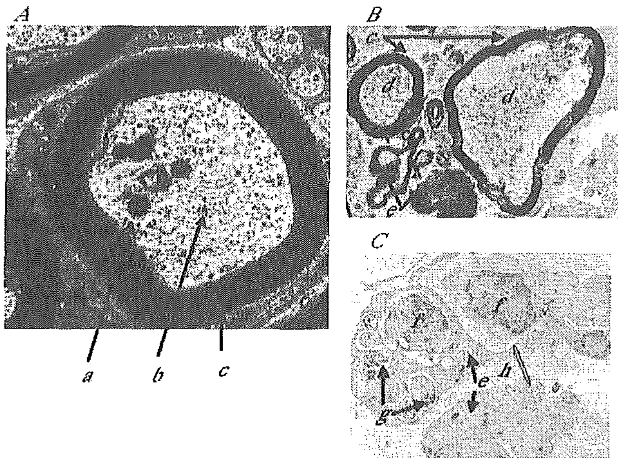

Fig 4. Electron microscopic findings in the posterior root according to treatment. (*A*) Control group: 25% glucose; magnification ×8,000. (*B*) 5% BG group: in the area with mild injury treated with 5% BG. Axonal degeneration is seen and the myelin sheath seems to be intact in some areas; magnification ×8,000. (*C*) 5% BG group: in area with severe injury treated with 5% BG. Both axons and myelin sheaths are degenerated; magnification ×5,000. These histologic features in the 5% BG group are fundamentally identical to those in the 5% BDW, 10% lidocaine, and 20% lidocaine groups. *a*: mitchondria, *b*: neurofilaments and microtubules, *c*: myelin sheath, *d*: normal structures of axoplasm observed in *a* and *b* converted to disintegrated flocculent substance, *e*: fat deposit, *f*: infiltrated macrophage, *g*: myelin figures, *h*: edema space.

group than in the 5% BDW may support the effect of hyperosmolarity of glucose[20] and not neurotoxic effects because no significant difference occurred in the injury score and frequency of the lesions between the 2 groups.

In this study, we administered high doses of local anesthetics at a higher level in comparison with the clinical setting. This method was performed in anticipation of the following benefits. First, we wanted to exclude catheter-induced tissue injury by locating the catheter at a higher spinal level. Second, our high-dose approach facilitates reproduction of rare clinical complications and minimizes the number of sacrificed experimental animals. Third, these histologic findings may reflect neurologic impairment not only after spinal anesthesia but also unintended dural puncture after epidural anesthesia. If the symptoms are sensory dominant, local neurotoxicity may be suggested as one cause of impairment.

Several *in vitro* studies[8-10,21] have demonstrated that lidocaine and bupivacaine induce irreversible loss of nerve conduction and membrane action potential, but these changes were observed only at high concentrations of the drugs used.[8,22,23] Our model also showed histologic and functional deterioration only at high concentrations, similar to those in previous animal studies.[12,24-26] Thus, the clinically recommended doses of local anesthetics appear to be relatively safe. Nevertheless, patients with neurologic symptoms induced by local anesthetics have been reported.[1-4] Knowing the site of the primary lesion induced by the drug is important for distinguishing the cause of complications. However, no consensus exists with regard to the primary lesion of local neurotoxicity, even in animal studies. Some investigators reported that neurotoxic lesions induced by intrathecal lidocaine and bupivacaine were limited to the nerve roots,[12] others reported that they were found only in the spinal cord,[27,28] and still others reported them in both.[24,25] Morphologic lesions have also been reported, including necrosis of the nerves,[24,29,30] spinal cord,[24] and subpial membrane,[28] vacuolation of subpial membrane or white matter,[24,28,31] and cytoplasmic vacuolation in the neurons of the anterior and posterior horns.[27,31] In this regard, the present study clearly demonstrates that the primary sites of anesthetic-induced damage are the posterior roots. Yamashita et al.[31] demonstrated that lidocaine induces a more severe form of neurotoxicity than does bupivacaine after intrathecal administration in rabbits. They demonstrated the presence of vacuolated lesions in the posterior white matter and anterior horn of the spinal cord but not in the cauda equina. However, whether the vacuolation appeared as a result of degeneration of the axon or myelin sheath is equivocal. Our electron microscopic studies reveal that axons of the posterior root are the primary target of drug neurotoxity, which extends to the posterior white matter. These results have some significant meanings. First, knowledge of whether the neurotoxic lesion is axonal degeneration or demyelination in nature may make possible the estimation of prognosis of neuronal injury, including potential recovery of neurologic function. Neurotoxic damage commences in the posterior root, and the dorsal ganglia are free from the neurotoxic changes caused by the anesthetic drugs because axonal degeneration in the posterior root generally progresses antegradely rather than retrogradely. Moreover, the dorsal ganglion is not directly immersed in cerebrospinal fluid. Second, therefore, axons of the posterior roots should be the main subject of further studies designed for analysis of the neurotoxicity of the 2 anesthetics. Third, focally degenerated nerve fibers in the posterior root just at the entrance into the spinal cord could likely regenerate by axonal sprouting of the mother neurons in the dorsal ganglia.

In summary, we compared the neurotoxicity of

intrathecal lidocaine with that of bupivacaine by histologic and functional examinations. The primary site and the extent of neurotoxic lesions caused by intrathecal lidocaine and bupivacaine were virtually identical, although the extent of neurotoxicity was worse in lidocaine than in bupivacaine when compared at pharmacologically equivalent potencies.

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Patient-controlled epidural analgesia during labor using ropivacaine and fentanyl provides better maternal satisfaction with less local anesthetic requirement

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Abstract
Purpose. To test the hypothesis that patient-controlled epidural analgesia (PCEA) using ropivacaine and fentanyl provides better maternal satisfaction and less anesthetic requirement than conventional continuous epidural infusion (CEI) during labor, we studied 58 uncomplicated parturients (singleton, vertex presentation).
Methods. After establishing effective epidural analgesia with 11 ml of 0.2% ropivacaine, all parturients were randomly divided into one of two groups: the PCEA group (n = 29) or the CEI group (n = 29). In the PCEA group, the pump was initiated to deliver a basal infusion at 6 ml·h^{-1} and a demand dose of 5 ml; the lockout interval was 10 min, and there was a 31 ml·h^{-1} limit. The drugs used were 0.1% ropivacaine + fentanyl 2 μg·ml^{-1}. In the CEI group, epidural analgesia was maintained with the same solution as the PCEA group at a constant rate of 10 ml·h^{-1}. If parturients requested additional analgesia in the CEI group, we added 8 ml of epidural 0.2% ropivacaine without fentanyl.
Results. Parturients' demographic data, such as duration of labor, mode of delivery, Apgar score, and umbilical arterial pH did not differ between the two groups. However, the hourly requirement of ropivacaine was significantly less in the PCEA group than in the CEI group (9.3 ± 2.5 vs. 17.6 ± 7.6 mg·h^{-1}; $P < 0.05$). Parturients' satisfaction assessed by the Visual Analogue Scale tended to be higher in the PCEA group than in the CEI group. Side effects such as nausea, hypotension, and itching were similar for the two groups.
Conclusion. We found that PCEA was an effective means of providing optimal analgesia, with better satisfaction during labor and less local anesthetic requirement.

Key words Labor analgesia · Patient-controlled epidural analgesia · Ropivacaine · VAS satisfaction

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Introduction

Patient-controlled epidural analgesia (PCEA) for labor has become popular in Europe and the United States [1]. However, the adequate concentration of local anesthetics or opioids and their infusion rate, the lockout time, and the bolus dose for PCEA remain controversial. Previous studies using bupivacaine or ropivacaine [2–6] demonstrated that PCEA provided sufficient analgesia with less local anesthetic requirement than conventional continuous epidural infusion (CEI) analgesia. In this study, we hypothesized that the PCEA using ropivacaine and fentanyl for labor also provides better maternal satisfaction with less local anesthetic requirement than CEI. We chose ropivacaine because it is less toxic to the cardiovascular and central nervous systems than bupivacaine.

Materials and methods

After obtaining approval of the institutional investigation committee and informed consent from each parturient, we studied 58 primiparous women with American Society of Anesthesiologists (ASA) physical status I who requested analgesia for childbirth at term with singleton and vertex presentation of the fetus. Exclusion criteria were multiparity, preeclampsia, pregnancy-induced hypertension, multiple gestation, and any contraindication to epidural analgesia such as a bleeding disorder.

Labor was induced electively by infusion of oxytocin at adjusted 2.5 mU·min^{-1} under fetal heart rate monitoring. The infusion rate of oxytocin was adjusted so measured Montevideo units reached 100–150 for the first half of the first stage, 150–200 for the last half of the first stage, and 200–300 for the second stage of labor.

During labor, conventional cardiotocography was used for monitoring to ensure fetal well-being and to