の 3,480 人を対象としたメタ解析[17]では非定型抗精神病薬(オランザピン，リスペリドン，クエチアピン，アリピプラゾール)のプラセボに対する反応率の odds ratio は 1.69，寛解率の odds ratio は 2.00 であることが報告され，その有効性に非定型抗精神病薬の種類による有意な差はないことも示された．このメタ解析はいままでもっともエビデンスが高いとされたリチウムや T3 のメタ解析の実に 12〜13 倍の人数を対象としており，これらの結果からは現在治療抵抗性うつ病に関してもっともエビデンスが高い増強療法は非定型抗精神病薬ということになる．ただし，同時にこの解析における有害事象による治療中断率は 9.1% で odd ratio は 3.91 とプラセボより高く，加えて維持療法時などの長期投与における有効性と安全性は未解明であることには留意する必要がある．抗精神病薬の併用効果の機序はまだわかっていないが，各非定型抗精神病薬の特有の作用や，Zhang ら(2000)の報告する前頭前野におけるノルアドレナリンやドパミンの放出増加作用に関与する可能性がある．

ブロモクリプチン，アマンタジン，プラミペキソールなどのドパミンアゴニストは単独でも抗うつ効果をもつことが報告されており，少数の臨床試験では治療抵抗性うつ病患者に対して有効である可能性が示唆されている．うつ病の病因については従来ノルアドレナリンとセロトニン系が注目され，現在の抗うつ薬が開発されてきたが，従来の薬剤に治療抵抗性があるうつ病ではドパミン系の低下が関与している可能性が示唆されている．このような背景からドパミンアゴニスト，MAO-B 阻害薬である塩酸セレギニン(抗うつ薬との併用は禁忌)，ノルアドレナリン・ドパミン再取込み阻害薬のブプロピオンなどドパミン系を増強する薬剤が，治療抵抗性うつ病の一部に有効である可能性がある．

カルバマゼピン，ラモトリジンなどの抗てんかん薬はやはりいくつかの臨床試験で有効性には一定のエビデンスがある．

その他の増強療法として β-blocker でセロトニン 1A 自己受容体のアンタゴニスト作用をもつピンドロール，セロトニン 1A 受容体作動薬であるタンドスピロン，バルプロ酸，トピラメートなどの抗てんかん薬，交感神経刺激薬であるモダフィニルや，イノシトール，リルゾール，ω-3 脂肪酸，葉酸，ホルモン類などの治療戦略の可能性が存在するが[18]，否定的見解もあり，エビデンスは非常に乏しく有害事象も不明確である．

したがって，現時点では増強療法として上記の非定型抗精神病薬またはリチウムを第一選択とし，それが無効であった場合，順次，甲状腺ホルモン，ドパミンアゴニスト，カルバマゼピンなどを選択肢としていくことが最善と考えられる．

③ 併用療法

Texas Medication Algorithm Project(TMAP)の非精神病性うつ病アルゴリズム 2008 年度版においては，各種の作用機序をもつ新規抗うつ薬による初回治療で部分反応があった場合に，従来の部分反応を変薬により失う可能性を考慮してリチウムや T3 などによる増強療法という選択肢に加えて，SSRI と NaSSA，SNRI と NaSSA の併用など作用機序の異なる新規抗うつ薬どうしの併用を許容している．また，2 種類の治療戦略で改善のない場合には，非定型抗精神病薬による増強療法とともに，SSRI と NaSSA や SSRI と TCA などの併用療法が選択肢のひとつとなっている．そして，4 種類の治療戦略に無反応でかつ電気痙攣療法や迷走神経刺激にも無反応であった場合には薬物相互作用への注意と薬理学的に異なる作用機序の薬剤を使用することを前提に，3 種類の抗うつ薬の併用が選択肢として許容されている．またこのアルゴリズムでは 100 mg 以下のトラゾドンが不眠に対する治療としてどの段階でも許容されている．

また，Star*D 研究における抗うつ剤どうしの併用は，やはり SSRI とブプロピオンや SNRI と NaSSA といった比較的選択性の高い，異なる作用機序の抗うつ薬を用いて広範囲スペクトラムの治療をつくりだす戦略が採用されている．レベル 4 では 3 種類の治療戦略に失敗した患者が MAOI である tranylcypromine 単剤への変更またはベンラファキシンとミルタザピンの併用療法に割り付けられた．HRSD の寛解率と反応率はそれぞれ tranylcypromine 単剤群で 6.9% と 12.1% で，併用

1263-01475

療法群で 13.7%，23.5%であり，不耐性による治療中断率は tranylcypromine 群で 41.4%，併用療法群では 21.6%であった[1]．この研究のほか，ミルタザピンに関しては Carpenter ら(2002)が，抗うつ薬治療に反応しない 20 名の大うつ病または気分変調症患者にミルタザピン 15～30 mg/day の併用療法を行う二重盲検試験を行い，反応率と寛解率はそれぞれミルタザピン群では 64%と 45%，プラセボ群では 20%と 13%であったと報告している．ミルタザピンと同様の後シナプス α_2受容体遮断作用をもつミアンセリンに関しても併用療法の報告が存在する．1 種類以上の治療抵抗性うつ病に関する SSRI とミアンセリンに関する併用療法の効果は，Ferrei ら(2001)の二重盲検試験では明らかな有効性が報告されているが，Licht ら(2002)の二重盲検試験の結果は否定的なものであった．しかし，ミアンセリン併用もミルタザピンと同様 α_2受容体遮断作用によりセロトニン再取込み阻害効果を増強し，抗うつ効果を強める可能性があることに加え，強い抗ヒスタミン作用により強固な不眠に効果を発揮するため，睡眠薬の代用としても臨床的に併用が有用なことがある．SSRI とトラゾドンの併用療法は同様に不眠に効果的であり，Maes ら(1996)による二重盲検試験では，治療抵抗性うつ病にも単剤療法より有効であることが示されている．

TCA と SSRI の併用療法について，Nelson ら(2004)は 39 名のうつ病患者で 6 週間の二重盲検試験を行い，フルオキセチンと TCA であるデシプラミン併用による寛解率は 54%でそれぞれの単独療法よりも優れていることを示している．しかし，Fava ら(2002)はフルオキセチンとデシプラミン併用がフルオキセチンを単純に増量するより効果が劣ることを示しており，評価は定まっていない．

SSRI と TCA や TCA と TCA の併用療法はわが国では多用されているが，大規模な二重盲検試験がなく，薬剤によっては CYP を介した薬物相互作用により血中濃度が何倍にも高まることで，重篤な心血管系副作用やセロトニン症候群の出現のリスクが高まるため，使用がやむをえない場合は薬理学的相互作用を踏まえたうえで慎重に行うべきである．すくなくとも多剤併用にならないよう医師の最善の努力は必要であり，部分効果も乏しい無効な抗うつ薬は早期に中止することが望ましい．上記の知見から，治療抵抗性うつ病に関しての抗うつ薬併用に関してミルタザピン，ミアンセリン，トラゾドンなどが選択肢となりうるが，TCA と SSRI の併用に関しては薬理と相互作用について専門的知識のもと十分な配慮が必要であり，また TCA と TCA の併用はエビデンスが乏しく相互作用もきわめて多いため極力行わないほうがよいと考えられる．

④ 容易に再発し病相を反復するうつ病に対する治療

うつ病がおおむね改善してもまだ軽度の症状が残存する場合は，完全寛解をめざした積極的な薬物療法を行うことで再燃率を低下させることができる．Star*D 研究において各レベルで十分に反応のあった患者の再発に関する 12 カ月の追跡研究によれば，追跡研究導入時に良好に反応したが，寛解していなかった患者の各治療抵抗性レベルでの再発率はそれぞれ 59%，68%，76%，83%であるのに対し，寛解していた患者の各レベルでの再

サイドメモ 2　完全寛解の定義

うつ病治療では完全寛解が治療の最終目標である．完全寛解はうつ病による残遺症状がほとんどなく，心理社会機能が完全に回復した状態と定義することができる．うつ病患者は急性期症状から急速な回復により著しい改善を感じ，評価尺度は大きく改善することが多いが，長期的には軽度の残遺症状を残し心理社会機能は十分な回復に至っていないことが少なくない．寛解と判断された患者の 1/3 に不眠や倦怠感などの残遺症状が存在し，残遺症状のある患者では再発リスクが高いことが示されている．うつ病評価尺度の改善後も自覚的な心理社会機能の回復には時間がかかり，仕事，学業，日常生活などに支障をきたすことがあるため，症状が持続する間は薬物療法を早期に中断せず継続し，認知行動療法などの精神療法を併用することが有効である．今後，抗うつ薬の有効性に関する臨床研究は長期予後や心理社会的機能の評価も含め行われていかなければならない．

263-01475

発率は 33.5%，47.4%，42.9%，50.0%であり，治療抵抗性レベルが高かった患者ほど再発しやすいことを示すとともに，おおむね改善したが，寛解していない患者はより高い再発率を示すことを明らかにしている[1]．

一方，一度は完全寛解するが，すぐに再燃してしまう rapid cycler タイプのうつ病では躁うつ病を視野に入れた病歴聴取や寛解期の気分状態の評価を再度行う必要がある．病相の頻回の反復や急速なうつ病相の出現と回復は前述したような bipolarity の一部である可能性があるからである．真にうつ病で，抗うつ薬を何種類も変更し十分量の抗うつ薬を用いているにもかかわらず容易に頻回に病相を再燃する場合は，積極的にリチウムなどの気分安定薬の併用を考慮する必要がある．リチウムのうつ病における病相予防効果は Souza ら(1991)によるメタ解析で有効性が示されており，その後も多くの臨床試験で有効性が示されている．また，カルバマゼピンもリチウムほどではないが，病相予防効果に関する有効性が報告されている．

2. 修正型電気痙攣療法(mECT)とその他の非薬物療法的戦略

Pagnin ら[19]や UK ECT Review Group[20]のメタ解析によれば，治療抵抗性うつ病を含むうつ病に対して，ECT には薬物療法よりも優れた有効性があることが示されている．アメリカ精神医学会のガイドラインでは，薬物療法抵抗性うつ病は ECT の二次適応となっており，高度の治療抵抗性うつ病でも ECT への反応率は約 70%以上である[21]．Folkers(1997)らは 2 剤以上の抗うつ薬抵抗性であった 39 人を，パロキセチン群と右片側性 ECT 群に割り付け，反応率はパロキセチン群で 28%，ECT 群で 71%であり，HRSD の改善はパロキセチン群で 30%，ECT 群で 60%であったことを示している．

ECT 後の再発は ECT 治療における最大の限界であり，ECT は高い急性期効果を示す一方で，継続療法を行わない場合は高い再燃率を示すことが知られている．ECT コース終了後，維持療法を行わない場合には 6 カ月以内の再燃率は 65～80%と報告されており，治療抵抗性も同様に再燃しやすいことが知られている．ECT 後の維持薬物療法については，ノルトリプチリンとリチウムの併用療法，パロキセチン，イミプラミン，ノルトリプチリンなどの有効性が報告されているが，まだ研究は少ない．すくなくとも再燃予防には十分な維持薬物療法が必要である．

適切かつ十分な薬物療法にもかかわらず，再燃，再発を繰り返す場合は，低頻度の ECT を定期的に繰り返す維持 ECT(maintenance ECT)も選択肢となりうる．維持 ECT の目標は，再燃を防ぐために十分な頻度で ECT を行い，寛解状態を保つことであり，薬物抵抗性で ECT に反応するが，再燃，再発を繰り返す症例に適している．維持 ECT のガイドラインは存在しないが，Schwarz らは，維持 ECT の施行基準を示し平均 7 回の繰り返す入院，10 回の薬物療法の失敗，5 種類の向精神薬，ECT への高い反応性などをあげている．Gagne ら(2000)は，5 年間の追跡調査を行い，抗うつ薬と維持 ECT の併用群での寛解維持率は 2 年後，5 年後それぞれ 93%，73%と良好であったが，抗うつ薬単独群では 52%，18%と低いことを示している．この研究においては ECT 群のほうが過去の薬物療法抵抗性レベルが高かったが，高い寛解維持率を示している．再燃の兆候がみられた場合は，

サイドメモ 3　修正型電気痙攣療法

電気痙攣療法(electroconvulsive therapy：ECT)の歴史は古く，わが国では 1939 年より ECT の報告があり，うつ病に対しても高い有効性が確認されている治療である．麻酔や筋弛緩薬を使わず施行する従来型 ECT では副作用も多く，1950 年代になると，静脈麻酔薬と筋弛緩薬，呼吸循環管理を用いた修正型電気痙攣療法(modified ECT：mECT)が施行されるようになった．わが国でも，1980 年代に mECT が普及し，高齢患者や身体合併症患者に対しても安全な ECT を提供できるようになった．さらに，定電流短パルス矩形波治療器(パルス波治療器)が，わが国で 2002 年に認可され，導入された．パルス波治療器は交流正弦波治療器(サイン波治療器)の 1/3 程度のエネルギー量で痙攣誘発することができ，さらに安全性が向上した．高度の治療抵抗性うつ病に関しても期待できる治療法である．

0263-01475

維持 ECT の予定を早めることで対応可能である．しかし，維持 ECT に関する具体的なガイドラインはなく，安易な維持 ECT 導入は避け，十分なインフォームドコンセントを行い症例ごとに慎重に検討することが望ましい．臨床研究では ECT の作用機序の解明が急務であるが，ECT の無作為化割付試験や維持 ECT の長期効果と副作用，ECT コース終了後の継続薬物療法に関する研究も必要であろう．

また，近年 FDA に認可された迷走神経刺激はわが国ではまだ適応がなく，経頭蓋磁気刺激，深部脳刺激，精神外科においては海外においてもまだ実験的治療の域を出ない．

3. 認知行動療法(CBT)

治療抵抗性うつ病に対する精神療法の有効性については McPherson ら(2005)や Thase ら(2001)による総説があり，エビデンスは十分ではないが，CBT が有効である可能性が示唆されている．

Star*D 研究では初回 SSRI による治療が失敗した場合，CBT を単独で行った群，CBT をシタロプラムに対する増強療法として行った群に割り付けられ，それぞれが薬剤変更群，薬剤増強療法群と比較された．増強療法群での比較ではシタロプラムと CBT の併用が citalopram に対する薬剤増強療法と比較され，CBT を含む治療変更群では CBT 単独群が薬剤変更群と比較された．増強療法群の比較では，認知療法増強群では HRSD 寛解率 23%，反応率 35%で，薬物療法増強群では寛解率 33%，反応率 28%で有意差はなかった．ただし，薬物療法増強群では認知療法による増強群よりもより早期に寛解する傾向があった．治療変更群での比較では認知療法群では寛解率 25%，反応率 22%，薬物療法群では寛解率は 28%，反応率 27%で有意な差はなかったが，認知療法群のほうが異なる抗うつ薬へ変更した群より副作用が少なかった[1]．また，1 種類の SSRI が無効であった青年期うつ病に関する研究では，抗うつ薬に CBT を併用したほうが抗うつ薬単独よりも治療成績がよいことが示されている[8]．これらの研究からは，すくなくとも 1 種類の抗うつ薬への治療抵抗性レベルでは CBT は有効と考えられ，臨床的にもうつ病に特徴的な認知の偏りをもつうつ病や薬効の乏しいストレスへの耐性の低い，いわゆる新型うつ病などには抗うつ薬に併用した CBT が必要かつ有効な場合がある．

おわりに

難治性うつ病に対する治療戦略は現在臨床的な重要課題であるが，多くの治療戦略に関するエビデンスはいまだ乏しく，治療の標準化や治療アルゴリズムの構築のために，今後エビデンスを蓄積していく必要がある．また，研究の標準化のためには，難治性うつ病の定義，反応や寛解の定義，みせかけの治療抵抗性うつ病について十分考慮しなければならない．治療に関してはエビデンスに十分な配慮を行うとともに，各個人にオーダーメイドの治療を提供する必要がある．

当院ではうつ病専門外来とうつ・ストレス専門病棟の連携のもと，2 名の気分障害を専門とする精神科専門医により客観的な評価・診断を行うとともに，治療抵抗性うつ病に対するより高いエビデンスに基づく治療戦略のアドバイスを行っている．当院病棟では年間 1,300 回を超える修正型電気痙攣療法の施行件数があり，個々の症例の特性に応じた急性期 ECT または維持 ECT を行っている．また，専門病棟において多職種チームによる入院認知行動療法，心理社会的治療などの複合的な治療を行い，各個人にオーダーメイドの治療を提供できるように複合的な戦略をとっている．

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Electroconvulsive Therapy as a Potentially Effective Treatment for Severe Serotonin Syndrome
Two Case Reports

To the Editors:

Although many fatal cases had been reported due to serotonin syndrome (SS), the treatment of severe SS is not yet established. Electroconvulsive therapy (ECT) has been reported to be effective against neuroleptic malignant syndrome (NMS). Serotonin syndrome is a similar neurotoxic syndrome; however, there are few reports on the efficacy of ECT for SS. We encountered 2 patients with recurrent major depressive disorder (MDD) in whom ECT was effective against severe SS with malignant catatonia (MC) associated with antidepressants. In both cases, the patients' guardians provided written informed consent for ECT. Atropine sulfate, propofol (1 mg/kg), and succinylcholine (0.8 mg/kg) were used for ECT. Stimulus levels for the ECT were determined by the half-age method, and brief-pulse square wave was delivered using Thymatron System IV (Somatics Inc, Lake Bluff, Ill).

CASE 1

The patient was a 67-year-old man. He was diagnosed with MDD at the age of 51 years. He had experienced 2 recurrences of MDD subsequently and had been treated with mianserin (30 mg/d), amitriptyline (75 mg/d), amoxapine (75 mg/d), clomipramine (75 mg/d), and paroxetine (20 mg/d), separately. But he had developed paroxysmal hot flushes and sweating while taking these antidepressants, except with mianserin.

In October 2006, he had a relapse of MDD and visited our hospital on November 11, 2006, and the administration of paroxetine (20 mg/d) was resumed. On November 30, while the depressive symptoms persisted, transient hyperthymia for 3 hours each day developed. He developed a transient obsessive idea that "I am afraid of being urged to kill people" and also developed hot flushes and marked sweating on December 2. He manifested tremors and muscle rigidity with hyperthymia several times a day. From December 10, he had occasional disorientation. He exhibited psychomotor slowing and needed assistance in walking.

On December 13, he was brought to our emergency department and was immediately hospitalized. Physical examination revealed labile consciousness, pyrexia (37.9°C), elevated blood pressure (153/100 mm Hg), and tachycardia (100 beats/min). The pupils were rather dilated. Marked hot flushes, sweating and pyrexia, and tremors of the fingers were observed. Myoclonus was noted in the face and limbs. Tendon reflexes were generally exaggerated. Paroxysmal episodes of aggravation of generalized muscle rigidity, tremors, and marked myoclonus lasting for several hours were frequently observed, which led to rapid breathing and tachycardia (180 beats/min). He had catatonic syndrome with akinetic mutism and catalepsy and periodically exhibited a stuporous state and confusional state with disorientation. Occasionally, he nodded slightly in response to questions; however, communication was difficult, and sometimes visual hallucinations and delusions were noted. Laboratory examination revealed slight leukocytosis ($10.14 \times 10^3/\mu L$) and slight elevation of the serum alanine transaminase (48 U/L) and blood urea nitrogen (28.9 mg/dL). The serum levels of thyroid hormones and electrolytes were within reference range. Electroencephalography revealed no abnormality, and the basic rhythm was dominated by β-waves. Brain magnetic resonance imaging revealed no abnormality. His limbs and body were restrained, and parenteral nutrition became necessary. Based on the suspicion of SS, paroxetine was decreased on admission and discontinued on the day after the admission.

The score on the SS scale (SSS)[1] was 15. During the confusional state, the generalized muscle rigidity, pyrexia, sweating, tremors, and rapid breathing became even more aggravated, and dyspnea due to muscle rigidity was associated with a low level of oxygen and significant sinus tachycardia, which necessitated daily intravenous administration of diazepam. The benefit of diazepam persists for approximately 20 minutes. The psychiatric symptoms dramatically fluctuated. From 3 days after the admission, he had diarrhea (>10 times a day). The pyrexia was aggravated, and at 5 days after admission, the body temperature reached 38.4°C. But no evidence of complicating infections was detected. Paroxysmal muscle rigidity and myoclonus and the confusional state also gradually worsened. Despite discontinuation of the causal drug, paroxetine, the SSS score eventually increased to 23. Six days after admission, cerebrospinal fluid (CSF) examination was performed to exclude encephalitis, and it revealed no inflammation. Cerebrospinal fluid levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were measured (34.5 and 7.6 ng/mL, respectively), and blood examination revealed increase in the blood levels of the catecholamines, 5-HIAA, and HVA. Administration of paroxetine was discontinued, and dehydration was corrected; however, no symptomatic improvement was obtained. The poor nutritional status due to severe autonomic and neurological symptoms rapidly progressed.

Seven days after admission, ECT was administered. After the first ECT, marked improvement of the sweating and paroxysmal tachycardia was noted. His muscle rigidity and myoclonus improved in terms of the frequency and severity. After the second ECT, rapid improvement of the catatonic symptoms with the confusional state and paroxysmal disorientation was noted. His muscle rigidity, myoclonus, and tremors disappeared completely, and the autonomic nervous symptoms were also scarcely observed. After the third ECT, the agitation also abated, with disappearance also of the autonomic nervous symptoms such as pyrexia and diarrhea. He became alert and clear completely, and oral intake was resumed. After the fifth ECT, a second CSF examination showed increased levels of HVA and 5-HIAA (53.8 and 28.0 ng/mL, respectively). The 3-methoxy-4-hydroxyphenylglycol level in the CSF was not changed (11.9 ng/mL before vs 10.5 ng/mL after the ECT). The blood levels of the catecholamines, 5-HIAA, and HVA also tended to be restored. Ten sessions of ECT were performed for sustained remission of MDD. The time course of changes on total SSS score in response to the ECT sessions is summarized in Figure 1.

Administration of milnacipran was started, with the dose slowly increased up to 100 mg/d. He was finally discharged on January 2007. Until December 2009, he has shown no relapse.

CASE 2

The patient was a 66-year-old man. He was diagnosed with MDD at the age of 44 years. He experienced a recurrence of MDD and was treated with sulpiride

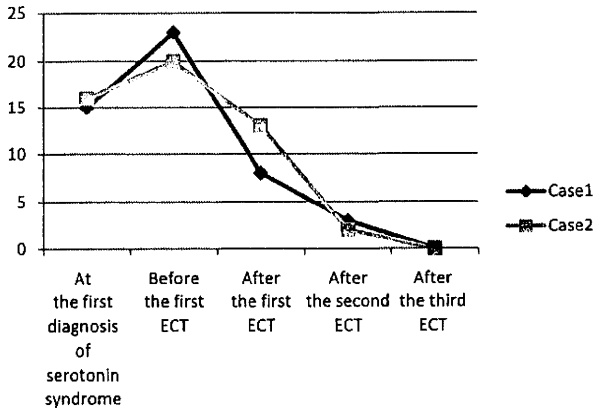

FIGURE 1. The time course of changes in total score on the serotonin syndrome scale in response to ECT sessions in Cases 1 and 2.

(300 mg/d). In March 2007, he developed depressive mood, hypobulia, insomnia, palpitations, and difficulty in swallowing. Several examinations in an internal medicine clinic revealed no abnormality, and he was referred to a psychiatric clinic. Sulpiride (150 mg/d) was prescribed, with no effect. He lost 9 kg over 2 months because of loss of appetite. Paroxetine (20 mg/d) was added to the treatment, with no effect. He was referred to our hospital on June 2007. He was diagnosed with MDD and was hospitalized. A high level of anxiety, agitation, depressive mood, hypobulia, loss of appetite, and insomnia were noted. His vital signs were normal.

We discontinued sulpiride and instead increased the dose of paroxetine to 40 mg/d, with no effect. We planned to switch the medication from paroxetine to clomipramine and started clomipramine in combination with paroxetine on July 24. The dose of clomipramine was gradually increased and reached 150 mg/d on August 8; however, transient hyperthymia and tremors of the fingers appeared on August 10. Thus, the dose of clomipramine was decreased and discontinued on August 12, but he developed pyrexia (37.8°C), sweating, tachycardia (120 beats/min), elevated blood pressure (156/106 mm Hg), hot flushes, generalized muscle rigidity, tremors (especially of the fingers), and myoclonus of the face. The tendon reflexes were generally exaggerated. Auditory and visual hallucinations and delusions appeared. With marked agitation and excitement, he suddenly made an attempt at suicide saying, "Please let me die," and tried to escape from the isolation room. He presented labile consciousness disturbance. The blood test results revealed no abnormalities, except for slight anemia (hemoglobin, 12.8 g/dL) and slight elevation of C-reactive protein (0.6 mg/dL); the thyroid hormones and electrolytes were also normal. Brain magnetic resonance imaging and electroencephalography revealed no abnormality.

Based on the dramatic appearance of the psychiatric symptoms, autonomic nervous symptoms, and neurological symptoms, we made the diagnosis of SS. The SSS[1] score was 16. All the drugs were discontinued. On August 14, he had developed marked generalized muscle rigidity and sweating. Because the muscle rigidity was so intense, he bit his tongue, necessitating the use of a mouth gag. The muscle rigidity was alleviated by intravenous administration of diazepam (17.5 mg/d), but once the effect wore off, muscle rigidity with remarkable autonomic nervous symptoms appeared again. Therefore, he was administered a continuous drip infusion of midazolam (10 mg/h). Despite the patient being under continuous sedation, frequently he exhibited generalized muscle rigidity and myoclonus lasting for several hours, which led to reduction of the oxygen level, sinus tachycardia, and hypertension. He repeatedly manifested the following psychiatric states: depressive state with intense anxiety and suicidal idea, transient hypomanic state, stupor with no spontaneous behavior, and confusional state with disorientation. The SSS score was 20, indicating aggravation.

On August 16, ECT was started. After the first ECT, stupor was palliated. Sweating and tachycardia improved. Muscle rigidity and myoclonus disappeared. After the second ECT, although the depressive symptoms and hallucinations/delusions still persisted, he could follow instructions. The autonomic nervous symptoms disappeared. After the third ECT, he could consume all his food. After the fifth ECT, the depressive symptoms remitted. The time course of changes on total SSS score in response to the ECT sessions in Case 2 is summarized in Figure 1. Thereafter, the administration of sertraline was started, slowly increasing the dose up to 50 mg/d. He was discharged on October 2007. Until December 2009, he has shown no relapse.

DISCUSSION

Both cases exhibited various psychiatric symptoms, in addition to manifesting diverse neurological and autonomic symptoms. Serotonin syndrome is sometimes viewed as MC,[2] and both cases presented also MC with SS induced by antidepressant.

Our Cases 1 and 2 fulfilled criteria 9 and 7 of SS, respectively, of the 10 criteria proposed by Sternbach,[3] and both cases fulfilled all the 9 major criteria of the modified criteria proposed by Radomski et al[4]; therefore, we considered SS as being a valid diagnosis. Although both cases did not exhibit elevation of serum creatine phosphokinase, they satisfied the criteria for NMS proposed by Levenson.[5] Nonconvulsive status epilepticus and delirium were ruled out based on the electroencephalographic findings.

In Case 1, the responsible drug for SS with MC was considered to be paroxetine. The patient developed nervous hypersensitivity, aggravation of anxiety, and transient hyperthymia after the start of paroxetine, which suggests that he had developed very mild SS. Typically, SS has been reported to develop within 24 hours of the start of administration or increasing the dose of antidepressants. However, in this case, dehydration was caused by reduced intake of food and fluids, which may result in elevation of the serum level of paroxetine, and induces severe SS with MC.

In Case 2, SS with MC may be caused by the enhancement of the serotonergic activity due to the combination of clomipramine and paroxetine as well as by the elevated concentrations of both drugs due to the competitive inhibition of CYP2D6. In neither case did the patient take health foods such as tryptophan or St John's wort. Serotonin syndrome is considered to be ameliorated in approximately 70% of the cases within 24 hours of discontinuation of the responsible drug,[6] although some cases with protracted symptoms are known.[7] Both cases presented such protracted SS with MC.

It is known that, in some severe or protracted SS, the patient could die of disseminated intravascular coagulation, renal impairment, acidosis, acute res piratory distress syndrome, convulsive seizures, ventricular tachycardia, and so on;

23 fatal cases had been reported by 1999,[8] but the treatment of severe SS is not yet established. Efficacy has been demonstrated against NMS,[9] and some researchers indicate that ECT should be adopted more often in cases that present MC with intensive autonomic nervous symptoms.[10] Electroconvulsive therapy might be also effective for the treatment of SS with MC, which is similar to neurotoxic syndrome. But to date, there are only 2 reports of ECT for the treatment of SS. In a case report, ECT was effective in treating a patient in whom the differential diagnosis between NMS and SS was difficult,[11] and in the other, protracted SS was improved by 4 sessions of ECT.[12]

In our cases, the autonomic nervous, neurological, and psychiatric symptoms improved dramatically after ECT, and rapid improvement based on the SSS was also noted. These results suggest that ECT may be effective in the treatment of SS with MC. However, according to a case report, ECT administered in combination with lithium and mirtazapine induced a transient SS.[13] Therefore, involuntary application of ECT for patients with SS, without discontinuation of the causal drugs, should be avoided.

The mechanism underlying the effect of ECT is still unknown. In Case 1, elevated blood levels of catecholamines, except for adrenaline, and HVA and 5-HIAA were observed before ECT, and elevated blood concentrations of noradrenaline, dopamine, and serotonin were suggested, but these abnormalities had improved after remission. Moreover, in the CSF, the levels of HVA and 5-HIAA were increased after remission. In the previous 4 cases of SS reported, it has been observed that the 5-HIAA and HVA levels in the CSF decreased during the active phase of SS.[14] Possibly at the acme of SS, excessive inhibition of presynaptic serotonin reuptake occurs because of the serotonin reuptake effect of the drug, with increase in the serotonin level at the synaptic gap. However, because the presynaptic serotonin level is decreased, the amount of serotonin degraded by monoamine oxidase might also decrease, resulting in a reduction of the 5-HIAA level in the cerebrospinal fluid. Electroconvulsive therapy might correct this excessive inhibition of serotonin reuptake.

AUTHOR DISCLOSURE INFORMATION

All the authors declare that they have no conflicts of interest and that they do not have any funding source relevant to the case report. Consent for publication in print and electronically was obtained from the patients.

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Major depression: what caused the crisis?

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In July, 2008, a 67-year-old woman with refractory depression was referred to our institute. In 2006, she had a thymectomy for thymoma. In January, 2008, after experiencing family discord, she lost her appetite, and her bodyweight decreased by 5 kg in 1 month. She became pessimistic and self-recriminating and made several suicide attempts. CT showed no evidence of a recurrence of the thymoma. Neurological examination showed only slight muscle weakness of her limbs, but the cause of her anorexia remained unclear despite further in-hospital examinations such as gastrointestinal tract endoscopies and systemic contrast-enhanced CT. She was diagnosed as having depression and was transferred to a regional psychiatric hospital, where she was treated sequentially with sertraline, paroxetine, clomipramine, and nortriptyline, and augmentation lithium. These treatments were ineffective, and her body weight decreased from 60 kg to 33·5 kg. She was then transferred to us.

Her depressed mood, decreased interests, hypogeusia, anorexia, insomnia, anxious restlessness, decreased energy and fatigue, guilty feelings, poor concentration, and suicidal ideation persisted. She fulfilled the DSM-IV diagnostic criteria for major depressive disorder. Her total score on the 17-item Hamilton Depression Rating Scale (total-HDRS) was 40. She could walk and had no ocular and bulbar symptoms, but neurological examinations showed mild proximal muscle weakness and atrophy of her limbs. Blood test results indicated hypoalbuminaemia (albumin 3·4 g/dL); other investigations including CT chest, tumour markers, electroencephalography, and brain MRI were normal. Ten sessions of electroconvulsive therapy (ECT) were done in September, but the depressive symptoms persisted. She stopped taking medication, other than quetiapine as required (prescribed by us), but at the end of October, she suddenly developed impaired consciousness with a reduced respiratory rate. A blood gas analysis showed carbon dioxide narcosis, and she was immediately placed on a ventilator. No evidence of pulmonary disease was found, and a diagnosis of myasthenic crisis was made on the basis of a high acetylcholine receptor antibody (AchR-Ab) titre (120 nmol/L), waning on the Harvey-Masland test, and a history of thymoma. She was treated with plasmapheresis and immunoadsorption followed by prednisolone treatment (maximum dose, 50 mg/day). Her respiratory function subsequently improved, and she was extubated. In December, she was treated with pyridostigmine (180 mg/day). As the anti-AchR-Ab titre decreased, total-HDRS score improved substantially without antidepressant therapy (figure). Her long-lasting depressive symptoms improved completely, and her bodyweight recovered to 40 kg; she was discharged in July, 2009. When last seen in September, 2009, both her depression and myasthenia gravis were in remission.

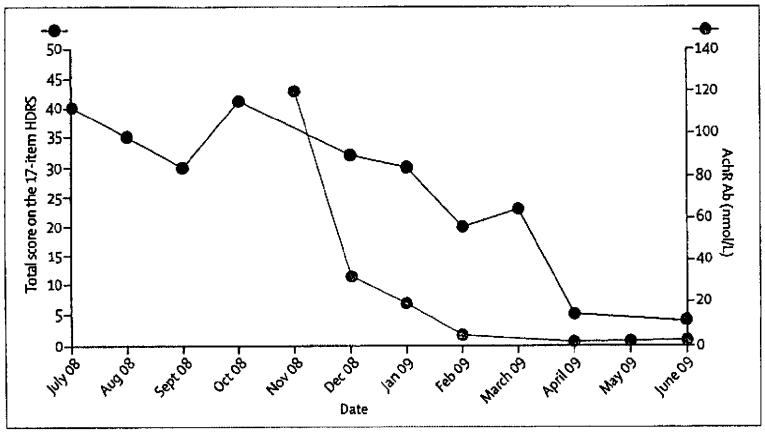

Figure: Changes in AchR-Ab titres and total-HDRS

The pathology of depressive symptoms associated with myasthenia gravis, including the hypothalamo-pituitary-adrenal axis dysfunction resulting from chronic stress and central cholinergic deficit, is controversial and remains to be elucidated.[1] Although some patients with major depressive disorder complicated with myasthenia gravis improve after ECT,[2] the potential to misdiagnose myasthenia gravis as depression has been highlighted.[3] 20% of people with myasthenia gravis are initially diagnosed as having a psychiatric disorder,[4] and improvements in depressive symptoms associated with improvements in myasthenia gravis have been reported.[1,5] Whether depressive symptoms in individual cases are attributable to myasthenia gravis or major depressive disorder should be investigated. Since the AchR-Ab titre and the depressive symptoms improved over time in our case, we concluded that the patient's depressive symptoms could predominantly be attributed to myasthenia gravis. When managing treatment-resistant depressive patients, the medical history must be sufficiently considered.

Contributors

All the authors participated in the management of the patient. NO wrote the Case Report. Written consent to publish was obtained.

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Rapid Antidepressant Effect of Ketamine Anesthesia During Electroconvulsive Therapy of Treatment-Resistant Depression

Comparing Ketamine and Propofol Anesthesia

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Background: Reports of the superiority of the antidepressant effect of ketamine during the conduct of electroconvulsive therapy (ECT) have been limited. We conducted an open-label trial of ketamine to determine whether ketamine as the anesthetic during ECT would provide a greater antidepressant effect than the antidepressant effect obtained with propofol.

Methods: Between April 2006 and April 2007, 31 inpatients with treatment-resistant depression gave written consent for ECT and to participate in this study. An anesthesiologist who was unaware of the mental symptoms of the subjects assigned them to receive propofol or ketamine anesthetic according to the preferences of the patients, and the patients underwent 8 ECT sessions for 4 weeks. The Hamilton Depression Rating Scale (HDRS) was valuated before ECT and after the completion of the second, fourth, sixth, and eighth ECT sessions.

Results: The HDRS scores improved earlier in the ketamine group, with decreases in HDRS scores that were significantly greater in the ketamine group.

Conclusions: The results suggested that it is possible to improve symptoms of depression earlier by using ketamine anesthesia.

Key Words: treatment-resistant depression, antidepressant response, ketamine, electroconvulsive therapy (ECT)

(*J ECT* 2009;00: 00–00)

The Sequenced Treatment Alternatives to Relieve Depression study demonstrated positive outcomes in no more than 30% of the patients even after 4 adequate treatment options had been performed.[1] There are limits to the therapeutic efficacy of the treatment of depression with the current antidepressant agents that enhance serotonin, noradrenaline, or dopamine nerve function. As a result, attention is being focused on glutamate pathway dysfunction as a novel pathophysiology of depression and on the antidepressant effect of the *N*-methyl-D-aspartate (NMDA) receptor antagonists.[2] The results of animal experiments in learned helplessness rats, a traditional model of depression, have suggested that NMDA receptor antagonists may have anxiolytic and antidepressant actions.[3–6] In recent years, it has been reported that lamotrigine[7] and riluzole,[8] which inhibit glutamate release, exhibit an antidepressant effect in humans as well and studies have been reported in which the NMDA receptor antagonist ketamine also exerted an antidepressant effect in humans when a single dose was infused intravenously.[9–10]

The principal action of ketamine is its NMDA receptor antagonist action, and it blocks the calcium-ion influx that occurs when glutamate binds to the NMDA receptor. Ketamine suppresses the cerebral cortex and thalamus and converts the brain waves in the electroencephalogram (EEG) to slow waves, but it is a dissociative anesthetic that exhibits a stimulant action on the cerebral limbic system. Ketamine is covered by the national health insurance system as a general anesthetic in Japan, and there are reports that no significant adverse events have been observed even when used as an anesthetic during electroconvulsive therapy (ECT).[11,12]

There have been few studies on the superiority of the antidepressant effect of ketamine anesthesia during ECT in earlier research, and there have been only a few case reports in which a rapid antidepressant effect of ketamine anesthesia has been inferred when used during ECT. Ostroff et al[13] reported the case of a 47-year-old woman with a depressive state in schizoaffective disorder. The patient was resistant to treatment with numerous antidepressant drugs and mood-stabilizing drugs, and bilateral ECT was pursued by inducing anesthesia with ketamine at 0.5 mg/kg. Electroconvulsive therapy was performed 6 times, and a rapid improvement in her symptoms of depression was observed after completion of the first ECT session. In addition, Goforth et al[14] reported a case in which they observed rapid improvement in symptoms starting with completion of the first ECT session when they performed ECT using ketamine at 1.5 mg/kg intramuscularly in a 54-year-old male patient with psychotic major depression.

However, ECT itself often has a rapid antidepressant effect beginning with the completion of the first session, and it remained unknown whether the rapid antidepressant effect in these case reports was actually an effect of ketamine.

We therefore conducted an open-label trial to determine whether a greater antidepressant effect would be obtained by using ketamine as the anesthetic during the performance of ECT than by using propofol.

MATERIALS AND METHODS

Subjects

We screened 52 patients with treatment-resistant depression who had consented to ECT while inpatients in the Department of

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Psychiatry of the National Center Hospital of Neurology and Psychiatry during the period between April 2006 and April 2007. All patients fulfilled the diagnostic criteria for major depression according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, per the Structured Clinical Interview for DSM-IV Axis I Disorders, and they had failed to respond to at least 2 adequate drug therapies for their current depression episode with a total score of 20 or higher on the 17-item Hamilton Depression Rating Scale (HDRS).

The exclusion criteria for this study were (1) complication by any serious physical disease, such as cardiovascular disease, cerebrovascular disorder, intracranial hypertension, respiratory tract disease, and severe fracture; (2) hypertension, glaucoma, arterial aneurysm, or cerebrovascular malformation; (3) presence of a foreign body, such as a pacemaker, intracranial electrode, and clips; (4) history of seizures; (5) history of substance abuse or dependence, including alcohol abuse; (6) status 4 or 5 evaluated according to the criteria of the American Society of Anesthesiologists; (7) history of serious adverse effects related to anesthetics, for example, allergy; (8) concomitant presence of a mental disorder other than major depression, such as dementia and bipolar disorder; (9) pregnancy; (10) being a minor; and (11) any other reason that the attending physician judged to make performing ECT inappropriate from a therapeutic standpoint.

All of screened patients underwent detailed pre-ECT examinations (psychiatric interview and physical examination, blood examination, chest x-ray, electrocardiography, and brain computed tomography, and if the results of the tests fulfilled any of the exclusion criteria, the patient was excluded from participation in the study).

Fifty-two patients were screened, and 31 of them (16 men and 15 women, aged 32–78 years old) who did not meet any of the exclusion criteria and from whom written consent for the study was obtained participated in this study (Fig. 1).

Electroconvulsive Therapy Administration

An anesthesiologist with no knowledge of the mental symptoms of the subjects assigned them to a propofol anesthesia group (N = 20, 10 men and 10 women) or a ketamine anesthesia group (N = 11, 5 men and 6 women) according to the preference of the patients. The anesthetic consisted of intravenous atropine sulfate (0.25 mg) with either ketamine (0.8 mg/kg at the first ECT session) or propofol (0.8 mg/kg at the first ECT session) by intravenous bolus. The quantity of the anesthetic agent was revised in the subsequent sessions in consideration of the initial anesthetic effect. Succinylcholine (1 mg/kg) was given intravenously as a muscle relaxant after induction of anesthesia. Thymatron System IV (Somatics Inc, Lake Bluff, Ill) was used, and brief pulse ECT was performed twice a week for a total of 8 times. The seizure threshold was determined by the half-age method during the first ECT session. The dose of anesthetic, the stimulation intensity, and the seizure duration on the EEG were recorded for each ECT session.

No changes in oral medication, including antidepressant drugs, were made between before the start of ECT and the completion of the ECT sessions.

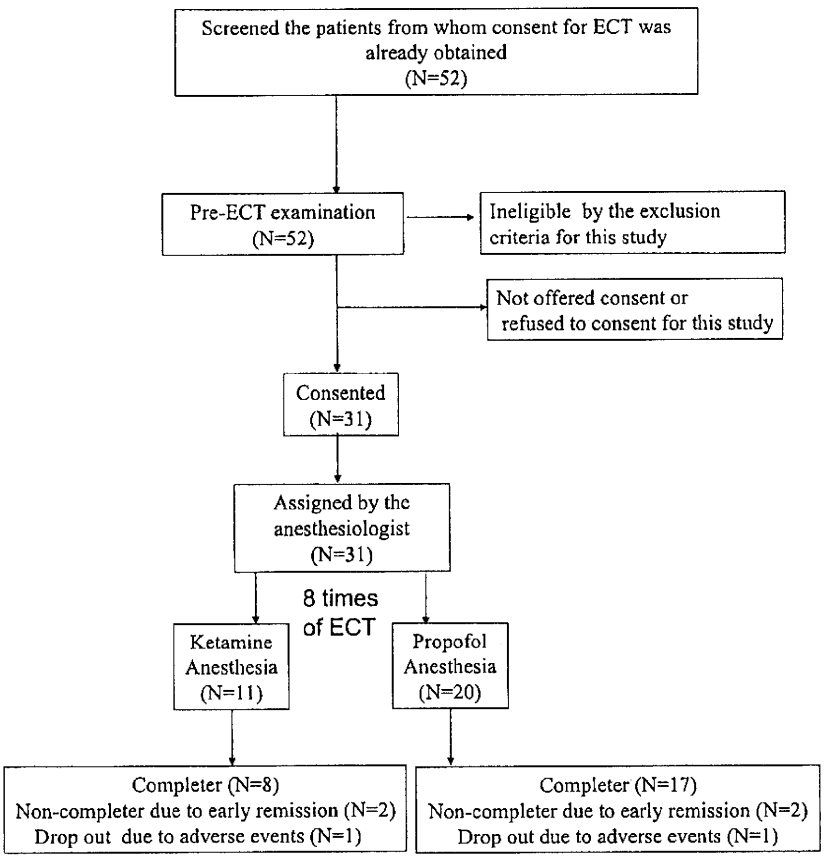

FIGURE 1. Participant flow of this study.

Measures

The 17-item HDRS was used as the primary end point to evaluate improvement in the symptoms of depression, administered by a psychiatrist before the start of ECT and on the day of completion of the second (1 week later), fourth (2 weeks later), sixth (3 weeks later), and eighth sessions (4 weeks later). Adverse events were collected by spontaneous report at every ECT session and during hospitalization.

Statistical Analysis

The statistical analysis was performed with the SPSS 16.0 software (SPSS Inc, Chicago, Ill), and the nonpaired *t* test and Pearson χ^2 test were used in the baseline analysis. Differences between the 2 groups in mean values for stimulus intensity and seizure duration on the EEG were analyzed for each session by the nonpaired *t* test. To analyze the differences in antidepressant effect between the 2 groups, we calculated the decrease in total score on the 17-item HDRS (δ HDRS-17) between the baseline and the completion of each ECT session. Then, the differences were analyzed between the mean δ HDRS-17 values in the propofol and ketamine groups after the completion of each ECT session by the nonpaired *t* test. Adverse events were analyzed by the Pearson χ^2 test.

RESULTS

Baseline Analysis

As shown in Table 1, in the baseline analysis, the mean (SD) age was 59.3 (13.5) years in the ketamine group and 55.1 (15.4) years in the propofol group; the duration of the current depression phase was 2.8 (2.1) years in the ketamine group and 2.7 (2.0) years in the propofol group; the number of previous adequate antidepressant trials was 6.5 (2.7) in the ketamine group and 6.7 (2.2) in the propofol group; there was a history of ECT in 2 patients in the ketamine group and in 3 patients in the propofol group; the δ HDRS-17 was 31.9 (4.5) in the ketamine group and 30.3 (5.4) in the propofol group; and none of the differences between the 2 groups were significant.

The mean anesthetic dose was 0.86 mg/kg (40–60 mg) in the ketamine group and 0.94 mg/kg (34–84 mg) in the propofol group.

TABLE 1. Baseline Characteristics of the Ketamine and Propofol Groups

	Ketamine Group (n = 11)	Propofol Group (n = 20)	*P*
Male	5 (45%)	10 (50%)	0.893
Age, yr	59.3 (13.5)	55.1 (15.4)	0.469
Duration of current depressive episode, yr	2.8 (2.1)	2.7 (2.0)	0.982
No. failures of adequate therapy	6.5 (2.7)	6.7 (2.2)	0.843
Positive history of ECT in past episodes	2 (18%)	3 (15%)	0.187
δ HDRS-17 before the ECT session	31.9 (4.5)	30.3 (5.4)	0.436
Medication			
SSRI	6 (55%)	10 (50%)	0.809
TCA	5 (45%)	10 (50%)	0.809
AAP	3 (27%)	6 (30%)	0.873
BZP	4 (36%)	4 (20%)	0.319

AAP indicates atypical antipsychotic; BZP, benzodiazepine; SSRI, serotonin-selective reuptake inhibitor; TCA, tricyclic antidepressant.

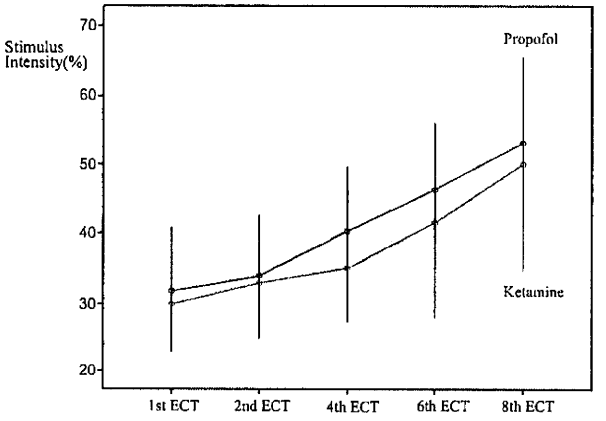

FIGURE 2. Stimulus intensity (in percent) in each ECT session (sessions 1, 2, 4, 6, and 8).

Noncompleters and Dropouts

Because depressive symptoms remitted completely with the second or third ECT session, ECT was completed in 6 sessions in 2 patients in the propofol group and 2 patients in the ketamine group. These patients were included in the statistical analysis of antidepressant effect. Two patients dropped out, 1 in the propofol group because ECT was discontinued after 2 sessions because of strong delirium and another in the ketamine group because the patient strongly complained of sense of fears with hallucinations upon awakening from anesthesia, and the anesthetic was switched to thiopental. Both dropouts were included in the analysis of adverse events, but they were excluded from the analysis of antidepressant effect (Fig. 1).

Stimulus Intensity and Seizure Duration on the EEG

Gradual increases in stimulus intensity were necessary in both groups as the number of ECT sessions increased. Stimulus intensity tended to be lower in the ketamine group in the series of sessions as a whole, but no significant differences were observed according to the nonpaired *t* test at any of the points (Fig. 2).

Seizure duration on the EEG tended to be longer in the ketamine group in the series of sessions as a whole, but the results of the analyses by the nonpaired *t* test showed significant differences only in the first ($P = 0.015$) and sixth sessions

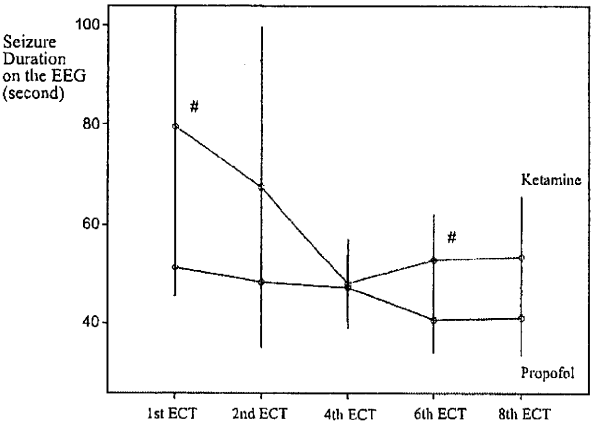

FIGURE 3. Seizure duration on the EEG (second) during each ECT session (sessions 1, 2, 4, 6, and 8).

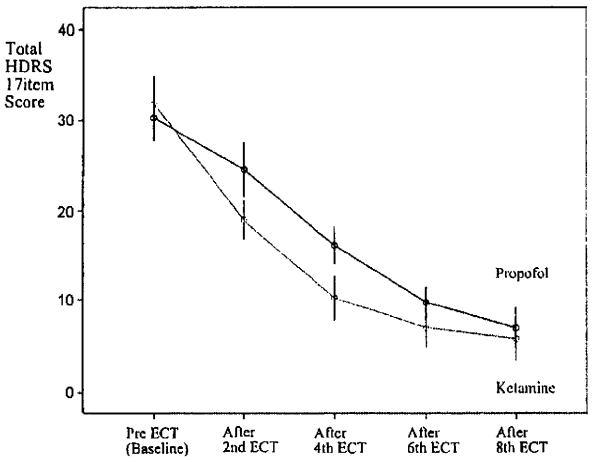

FIGURE 4. Total score on the 17-item HDRS before the start of ECT (pre-ECT) and after sessions 2, 4, 6, and 8.

($P = 0.027$; # in Fig. 3). The differences in the second, fourth, and eighth sessions were not significant (Fig. 3).

Antidepressant Effect

Decreases in the δ HDRS-17 were observed in both groups while the number of ECT sessions increased (Fig. 4).

The decrease in the δ HDRS-17 was significantly greater in the ketamine group than in the propofol group after the completion of the second ($P = 0.000$) and fourth sessions ($P = 0.000$; # in Fig. 5), but the differences after the completion of the sixth ($P = 0.086$) and eighth sessions ($P = 0.360$) were not significant (Fig. 5).

Adverse Events

The adverse events in the ketamine and propofol groups were headache (36% vs 40%), nausea (9% vs 15%), angialgia at the site of injection of the anesthetic (0% vs 45%), hypertension during the ECT session (55% vs 20%), sense of fears with hallucinations upon awakening from anesthesia (27% vs 0%), brief delirium within 1 hour after awakening (9% vs 15%), and prolonged delirium longer than 1 hour (0% vs 5%; Table 2).

Angialgia was significantly more common in the propofol group ($\chi^2_1 = 6.975$, $P = 0.008$), and intrainterventional hypertension ($\chi^2_1 = 3.876$, $P = 0.049$) and sense of fears with hallucinations upon awakening from anesthesia ($\chi^2_1 = 6.039$, $P = 0.014$) were more common in the ketamine group, but there were no serious adverse effects in either group.

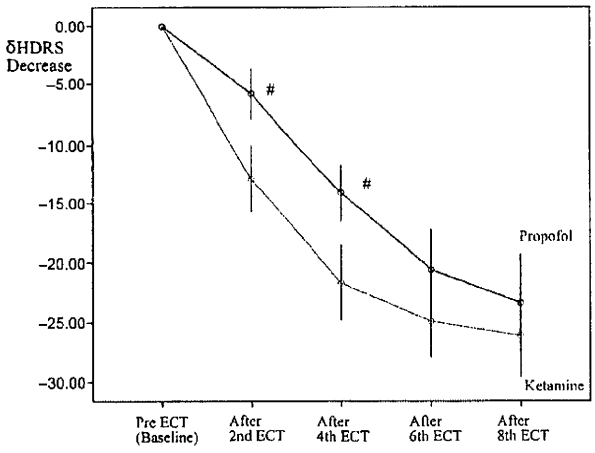

FIGURE 5. Change in δ HDRS-17 between the baseline (pre-ECT) and after sessions 2, 4, 6, and 8.

TABLE 2. Adverse Events

	Ketamine Group (n = 11), n (%)	Propofol Group (n = 20), n (%)	P
Headache	4 (36)	8 (40)	0.842
Nausea	1 (9)	3 (15)	0.639
Angialgia	0 (0)	9 (45)	0.008
Hypertension during ECT	6 (55)	4 (20)	0.049
Sense of fear with hallucinations upon awakening from anesthesia	3 (27)	0 (0)	0.014
Brief delirium (within 1 h)	1 (9)	3 (15)	0.639
Prolonged delirium (longer than 1 h)	0 (0)	1 (5)	0.451

DISCUSSION

According to the report of Berman et al[9] of a ketamine-versus-saline, placebo-controlled, double-blind, single-dose study in depressed patients, a significant decrease in the depression scale score was observed within 3 hours in the group of 9 treatment-resistant patients who were infused with ketamine at 0.5 mg/kg intravenously, and the decrease persisted for 72 hours. In 2006, a placebo-controlled double-blind crossover study was conducted in 18 nonpsychotic recurrent–major depression inpatients who were resistant to 2 adequate antidepressant drugs.[10] A significant improvement in depression was observed in the ketamine group within 2 hours, and the difference was maintained for an entire week. Moreover, 71% of the 17 subjects who received a single dose of ketamine responded, and 29% fulfilled the remission criteria. The adverse events in this study, that is, perceptual disturbance, confusion, elevation of blood pressure, euphoria, dizziness, and increased libido, were found to be more common in the ketamine group than in the placebo group, but no serious adverse effects were observed. These studies showed that ketamine has a clinical antidepressant effect when administered in a single dose, but until now, there has been no clinical research showing its superiority from the standpoint of an antidepressant action during ECT anesthesia.

On the other hand, ketamine is a general anesthetic that has been routinely used, and it has long been used as an anesthetic during the conduct of ECT.[12]

In addition, possessing a seizure-inducing action and increasing seizure duration are known as distinctive properties of ketamine as an ECT anesthetic. Nonbarbiturate anesthetics, including propofol, and barbiturate anesthetics, including thiopental, which are commonly used as ECT anesthetics, have an anticonvulsant action, and sometimes seizure induction is inadequate. According to a report on 471 patients who underwent ECT with methohexital, 72 (15%) required the maximal stimulation intensity, but in 24 (33%) of the 72 patients, seizure duration was insufficient or no seizure occurred at all even at the maximal stimulation intensity.[15] Switching to ketamine anesthesia has been found to be a useful method as a seizure induction technique when seizure induction at the maximal stimulation intensity is inadequate during ECT for which an

anesthetic that has such an anticonvulsant action has been used for anesthesia. There is a report of a switch to ketamine in 36 patients in whom seizure induction was inadequate or who were intolerant at the maximal stimulation intensity with methohexital, and seizure duration increased in 83% without any significant adverse effects.[16]

Moreover, in recent years, attention has been focused on the possibility of a cognitive function–preserving action by ketamine anesthesia during the conduct of ECT. The possibility that it suppresses excitotoxicity and has a neuroprotective action in relation to hippocampal synaptic plasticity and so on has been shown in an ECT rat model of ketamine anesthesia.[17] A cognitive function–preserving action during ECT in humans has also been reported in a small group of subjects,[18] and it has been suggested that ketamine anesthesia may reduce the cognitive impairment caused by ECT.[19]

Thus, despite the advantage of the antidepressant action that ketamine itself possesses and the advantages of its seizure-inducing action and cognitive function–preserving action during ECT, little attention has ever been paid to the superiority of the antidepressant effect of ketamine in ECT.

Our results showed that until the completion of the fourth ECT session, the δ HDRS-17 was statistically significantly higher in the ketamine group than in the propofol group. This finding suggests that ketamine anesthesia has an early antidepressant effect during ECT that was superior to propofol anesthesia and the effect may come from the antidepressant effect of ketamine itself.

However, the results of the statistical analysis showed that from the sixth ECT session onward, the antidepressant effect in the propofol group had caught up to the antidepressant effect in the ketamine group. That seems to have been due to the influence of the antidepressant effect of ECT itself being sufficiently expressed in both groups while the number of ECT session increased and the differences between the δ HDRS-17 in the 2 groups become smaller.

Because of this, ketamine may be useful when an early antidepressant effect is needed clinically in severe cases in which, for example, a suicide attempt is imminent clinically.

No serious adverse events were observed in either group in this study, but hypertension and sense of fears with hallucinations upon awakening from anesthesia were significantly more common in the ketamine group. Especially because of hypertension, some sort of arrangement, such as using an appropriate antihypertensive agent, seems necessary during the delicate anesthesia management of ECT.

Furthermore, in this study, there was not the case that showed a dependence of ketamine clinically after ECT session and serious psychedelic effect induced by ketamine, but we should pay enough attention to the dependence and the psychedelic properties of ketamine when we use it as an anesthetic agent of ECT.

The limitations of this study were that it was an open trial and the number of subjects was small. A large-scale double-blind trial is anticipated in the future to verify the superiority of the antidepressant effect of ketamine anesthesia in ECT.

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Pramipexole for stage 2 treatment-resistant major depression: An open study

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ABSTRACT

Objective: To examine the effectiveness and safety of adjunctive pramipexole in the treatment of stage 2 treatment-resistant major depressive disorder.

Methods: This study included patients with moderate or non-psychotic severe major depressive disorder according to DSM-IV-TR criteria despite at least two adequate treatment trials with antidepressants from different pharmacological classes. Pramipexole 0.25 to 2 mg daily was added to antidepressant therapy. Previous treatments were continued unchanged, but no new treatments were allowed. We conducted assessments at baseline and at weeks 2, 4, 6, and 8. We defined response as a 50% or greater reduction on the Montgomery–Åsberg Depression Rating Scale (MADRS).

Results: Ten patients (4 men, 6 women) aged 43.7 ± 11.4 years received pramipexole at mean dose of 1.3 ± 0.6 mg/d. Mean MADRS scores improved significantly from baseline to endpoint (mean differences = 11.4, 95% CI [4.1, 18.7], $P = 0.0064$). At the endpoint, six of 10 (60%) were responders on MADRS (≥50% reduction). Two patients (20%) terminated early due to mild somatic and psychiatric adverse effects.

Conclusion: These preliminary data suggest that the addition of pramipexole to antidepressant treatment may be effective and well tolerated in patients with stage 2 treatment-resistant major depressive disorder.

1. Introduction

Treatment-resistant major depression is a major issue in clinical practice, and the search for new, more effective treatments is ongoing. In general, treatment-resistant major depression is defined as the persistence of significant or moderate depressive symptoms despite at least two treatment trials with antidepressants from different pharmacological classes [stage 2 major depression according to the staging of depression based on prior treatment response proposed by Thase and Rush (1995)]. Each prior treatment must have been used in an adequate dose for an adequate period (i.e., a minimum of the equivalent of 150 mg of imipramine for 4 weeks) (Thase and Rush, 1995). The prevalence of treatment-resistant major depression is estimated to be 5–10% among all patients with major depression (Inoue et al., 2002). Nevertheless, most studies have investigated non-responders to single antidepressant trials [stage 1 major depression by Thase and Rush (1995)] and defined these patients as having treatment-resistant major depression (Thase and Rush, 1995). Furthermore, because of short treatment periods and small doses of antidepressants in several studies, there has been little evidence regarding effective therapy for stage 2 treatment-resistant major depression (Stimpson et al., 2002). Electroconvulsive therapy, lithium augmentation, and thyroid augmentation are recommended as treatment options in the World Federation of Societies of Biological Psychiatry Guidelines for Biological Treatment of Unipolar Depressive Disorders (Bauer et al., 2002), and have some evidence for stage 1 depression, but unexpectedly, little evidence for stage 2 treatment-resistant major depression has been noted (Stimpson et al., 2002). It is noteworthy that growing evidence for the treatment of stage 2 treatment-resistant major depression has shown clinical efficacy for atypical antipsychotic drugs (olanzapine and aripiprazole) as adjunctive therapy in large-scale randomized clinical trials (Thase et al., 2007; Marcus et al., 2008). Moreover, adjunctive repetitive transcranial magnetic stimulation was shown to be effective for stage 2 major depression in a small randomized clinical trial (Fitzgerald et al., 2006), whereas monotherapy with repetitive transcranial magnetic stimulation has been proven effective only for stage 1 major depression (O'Reardon et al., 2007).

A growing number of studies report abnormalities in the dopaminergic system in major depression, and the efficacy of pro-dopaminergic drugs, including dopamine receptor agonists, for major depression have been reported (Papakostas, 2006). The idea that major depression resistant to treatment with multiple serotonergic- and noradrenergic-based antidepressants may be responsive to pro-dopaminergic drugs is rational in terms of the mechanism of action of

Abbreviations: MADRS, Montgomery-Åsberg Depression Rating Scale; GAF, Global Assessment of Functioning; HDRS, Hamilton Depression Rating Scale; LOCF, last-observation-carried forward; CI, confidence interval; SSRI, selective serotonin reuptake inhibitor; DSM-IV-TR, Diagnostic and statistical manual of mental disorders, 4th edition, text revision.

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these drugs. Several studies have reported that dopamine receptor agonists (bromocriptine, pergolide, pramipexole, and ropinirole) are effective for stage 1 major depression that fails to respond to at least a single adequate conventional antidepressant treatment trial (Inoue et al., 1996; Izumi et al., 2000; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005). As mentioned above, the clinical efficacy of dopamine receptor agonists for stage 2 treatment-resistant major depression has not been assessed.

This pilot prospective, open study was undertaken to investigate the efficacy and safety of pramipexole in patients with stage 2 treatment-resistant major depression.

2. Methods

2.1. Subjects

We conducted an 8-week, open-label trial of pramipexole for patients with stage 2 treatment-resistant major depression at the Department of Psychiatry, Hokkaido University Hospital, Sapporo, Japan. The inclusion period started in June 2005 and ended in October 2008.

We included patients of both sexes, aged 20 to 70 years, with a diagnosis of moderate or non-psychotic severe major depressive disorder according to DSM-IV-TR criteria despite at least two treatment trials with antidepressants from different pharmacological classes, each used in an adequate dose for an adequate time period (i.e., a minimum of the equivalent of 150 mg of imipramine for 4 weeks) (Thase and Rush, 1995). Patients with scores of 20 or greater on the Montgomery–Åsberg Depression Rating Scale (MADRS, 10 items) (Montgomery and Åsberg, 1979) or scores of 60 or less on the Global Assessment of Functioning (GAF) Scale (even if MADRS scores were less than 20) were included. Patients with organic brain syndrome, schizophrenia, bipolar or schizoaffective disorder, severe physical illness, a history of substance use, or marked suicidality were excluded. All subjects provided written informed consent, and the trial was approved by the institutional review board of Hokkaido University Graduate School of Medicine.

2.2. Intervention and measurements

Subjects entering this study were prescribed pramipexole. All took one or two antidepressants, and doses of these drugs were held constant throughout the study. Pramipexole administration was started at 0.125 mg twice daily and increased 0.25–0.5 mg/day every 7 days to a target range of 0.5–2 mg/day. Higher doses (up to 3 mg/day) were permitted as needed. Dose escalations continued until 1) achievement of the primary endpoint (defined as a reduction of 50% or more from baseline in MADRS score), 2) drug intolerance, or 3) the 8-week protocol completion. Dosages were adjusted individually for patients.

Clinical assessments of adverse events and drug compliance were performed at each visit (every day for three inpatients) by trained psychiatrists at baseline and weeks 2, 4, 6, and 8. Outcomes were assessed using the MADRS score, the 17-item Hamilton Depression Rating Scale (HDRS) (Williams, 1988), and the GAF scale. The primary efficacy measure was the MADRS score. Secondary efficacy measures were the 17-item HDRS and GAF scores. Spontaneously reported adverse events were recorded at each visit.

2.3. Data analysis

All analyses were carried out on an intent-to-treat basis. Longitudinal efficacy outcomes (MADRS, HDRS, and GAF) were analyzed using paired *t*-tests comparing baseline and last-observation-carried forward (LOCF) results, with α set at 0.05; all tests were 2-tailed.

The primary outcome was defined as treatment response based on a ≥50% reduction in MADRS score over 8 weeks using LOCF methodology. Remission was defined as MADRS score<10 at the last visit (LOCF).

Secondary outcomes were determined using HDRS and GAF scores. Secondary treatment response was defined as a ≥50% reduction in HDRS score or a 10-point improvement (increase) in GAF score. Functional recovery was defined as GAF score>70 (Haykal and Akiskal, 1999; Furukawa et al., 2001). Changes in scores from baseline to final study visit were calculated for the MADRS, HDRS, and GAF. Pearson's correlation coefficients between GAF changes and MADRS or HDRS changes were calculated to assess potential predictors of functional improvement.

All continuous data are presented as means with standard deviations or 95% confidence intervals (CIs).

3. Results

Clinical and demographic characteristics of subjects are shown in Tables 1 and 2. All patients were diagnosed with non-psychotic major depressive disorder, moderate ($n=9$) or severe ($n=1$) with melancholic features. The mean peak dose of pramipexole was 1.3 mg/day (SD = 0.6). Eight of 10 patients (80%) completed the 8-week trial. Two patients discontinued the trial due to lack of efficacy and adverse events. As shown in Table 2, all 10 patients took one or two concurrent antidepressants.

Six of 10 patients (60%) were judged to be treatment responders based on the MADRS (≥50% reduction). Among the 10 patients, MADRS scores improved statistically significantly from baseline to the primary endpoint (mean difference = 11.4, 95% CI [4.1, 18.7], $P=0.0064$). Six patients achieved a MADRS score<10 at last visit (LOCF), yielding a 60% remission rate. As seen in Fig. 1A, this improvement was seen in week 2 and remained statistically significant throughout the study and at endpoint (LOCF). Eight items on the MADRS showed significant mean changes from baseline to endpoint (LOCF): apparent sadness, 1.90 (95% CI 0.92, 2.88), $P=0.0018$; reported sadness, 1.30 (95% CI 0.47, 2.13), $P=0.0063$; inner tension, 1.30 (95% CI 0.40, 2.20), $P=0.0095$; reduced sleep, 1.40

Table 1
Baseline characteristics of 10 patients with major depressive disorder.

Characteristic	Value
Diagnosis	
Major depressive disorder, single episode, *n* (%)	6 (60)
Major depressive disorder, recurrent, *n* (%)	4 (40)
Sex	
Female, *n* (%); male, *n* (%)	4 (40); 6 (60)
Age at entry, mean ± SD (yr)	43.7 ± 11.4
Range	29 – 64
Marital status	
Married, *n* (%); single, *n* (%)	7 (70); 3 (30)
Employment status	
Employed, *n* (%); unemployed, *n* (%)	7 (70) ; 3 (30)
Education, mean ± SD (yr)	13.7 ± 2.4
Length of current major depressive episode, mean ± SD (yr)	2.3 ± 1.3
Age at onset of first episode, mean ± SD (yr)	39.6 ± 11.5
Depression episodes, life time, *n* (%)	
1 episode	6 (60)
2 episodes	4 (40)
Patients with failed adequate antidepressant trials, *n* (%)	
2 trials	5 (50)
3 trials	3 (30)
4 trials	1 (10)
5 trials	1 (10)
Baseline MADRS score, mean ± SD	23.9 ± 7.0
Baseline HDRS score, mean ± SD	16.4 ± 4.4
Baseline GAF score, mean ± SD	46.2 ± 8.8

MADRS = Montgomery–Åsberg Depression Rating Scale, HDRS = 17-item Hamilton Depression Rating Scale, GAF = Global Assessment of Functioning.

Table 2
Clinical data of 10 patients with treatment-resistant depression treated with pramipexole.

Subjects	Age	Sex	MADRS Response	Previous antidepressant treatment for current episode (maximal dose and duration, weeks [W])
1	52	M	Responder	Milnacipran (150 mg, 8 W), amitriptyline[a] (150 mg, 4 W), clomipramine (150 mg, >8 W), imipramine (150 mg, 4 W)
2	37	M	Responder	Amoxapine[a] (300 mg, >8 W), clomipramine[a] (150 mg, >8 W), milnacipran (150 mg, 6 W), imipramine (200 mg, 8 W), paroxetine (40 mg, >8 W)
3	41	M	Non-responder	Mianserin[a] (60 mg, >8 W), paroxetine (40 mg, 7 W), milnacipran (200 mg, 8 W), amitriptyline[a] (150 mg, >8 W)
4	56	F	Responder	Milnacipran (100 mg, 8 W), clomipramine[a] (150 mg, 7 W), imipramine[a] (200 mg, >8 W), mianserin (60 mg, 8 W)
5	33	M	Non-responder	Nortriptyline[a] (150 mg, 8 W), sertraline[a] (100 mg, 8 W), paroxetine (40 mg, 8 W)
6	51	M	Responder	Amoxapine[a] (150 mg, >8 W), imipramine[a] (300 mg, 6 W), paroxetine (40 mg, 8 W)
7	39	F	Responder	Imipramine[a] (150 mg, >8 W), amitriptyline (150 mg, 8 W), paroxetine[a] (40 mg, >8 W)
8	64	F	Non-responder	Fluvoxamine (150 mg, 8 W), milnacipran (100 mg, 8 W), mianserin[a] (30 mg, 6 W)
9	29	F	Responder	Amitriptyline[a] (150 mg, 8 W), mianserin (60 mg, 8 W)
10	35	M	Non-responder	Maprotiline[a] (150 mg, 6 W), sertraline[a] (100 mg, 6 W)

MADRS = Montgomery–Åsberg Depression Rating Scale, M = male, F = female.
[a] Concurrent antidepressant.

(95% CI 0.04, 2.76), $P = 0.0445$; concentration difficulties, 1.70 (95% CI 0.74, 2.66), $P = 0.0030$; lassitude, 1.50 (95% CI 0.37, 2.63), $P = 0.0150$; inability to feel, 1.60 (95% CI 0.63, 2.57), $P = 0.0046$; and pessimistic thoughts, 1.10 (95% CI 0.18, 2.02), $P = 0.0243$). Our subjects took tricyclic or tetracyclic antidepressants with pramipexole, and three subjects took SSRIs with tricyclic or tetracyclic antidepressants

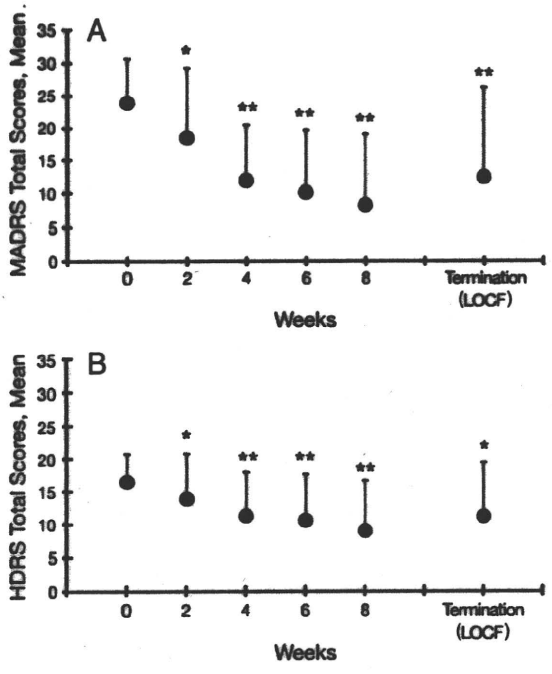

Fig. 1. MADRS (A) and HDRS (B) scores with pramipexole treatment ($n = 10$). Error bars represent SD. *$P<0.05$ and **$P<0.01$ indicate statistical significance from baseline to given point, using paired t test. Termination represents LOCF. MADRS = Montgomery–Åsberg Depression Rating Scale, HDRS = 17-item Hamilton Depression Rating Scale, LOCF = last observation carried forward.

(Table 2). Accordingly, most of them had received pharmacological treatments producing enough inhibition of both noradrenaline and serotonin reuptake. It is difficult to say what kinds of antidepressants are appropriate for adjunctive pramipexole therapy.

HDRS scores also decreased at week 2, and this improvement remained statistically significant throughout the study and at endpoint (LOCF) [mean differences = 5.2, 95% CI 1.4, 9.0, $P = 0.0135$] (Fig. 1B). Five of 10 patients (50%) were judged to be treatment responders based on the HDRS (≥50% reduction). Five patients achieved a HDRS score ≤7 at last visit (LOCF), yielding a 50% remission rate. One patient, who was a responder and remitter on the MADRS (subject 1 in Table 2) was only a partial responder (27% reduction) on the HDRS, because physical symptoms remained and kept the HDRS score high. Five items of the HDRS showed statistically significant mean differences from baseline to endpoint (LOCF): depressed mood, 1.00 (95% CI 0.11, 1.89), $P = 0.0319$; work and activities, 1.30 (95% CI 0.54, 2.06), $P = 0.0037$; psychomotor retardation, 0.4 (95% CI 0.03, 0.76), $P = 0.0368$; anxiety psychic, 0.90 (95% CI 0.37, 1.43), $P = 0.0039$; and somatic symptoms general, 0.60 (95% CI 0.10, 1.10), $P = 0.0239$.

GAF scores improved significantly from baseline to endpoint [mean difference = 13.5, 95% CI (5.1, 21.9), $P = 0.0055$], with six of 10 patients (60%) showing a GAF-based treatment response. Two patients were judged as achieving functional recovery defined as a GAF score>70. GAF score changes increased as a function of the improvements of depressive symptoms, HDRS scores, and MADRS scores [HDRS, $r = -0.73$, 95% CI (−0.93, −0.18), $P = 0.0174$; MADRS, $r = -0.80$, 95% CI (−0.95, −0.35), $P = 0.0050$].

Two patients (20%) discontinued treatment because of lack of efficacy and side effects ($n = 1$ at week 6, nausea and appetite loss; subject 8 in Table 2) and psychiatric side effects ($n = 1$ at week 2, irritability and buying sprees, which did not fulfill the criteria of hypomanic or manic episode of DSM-IV-TR; subject 5 in Table 2). Reported side effects were nausea, appetite loss, sea sickness-like symptoms, and consciousness of something non-existent [Leibhaftige Bewusstheit; Jaspers (1973)] ($n = 1$ each) in four patients. All side effects were mild and improved by pramipexole termination, dose reduction, or use of an antiemetic agent.

4. Discussion

In this open-label, nonrandomized, prospective study, we found that pramipexole added to antidepressants seemed to be effective for improving stage 2 treatment-resistant major depression in six of 10 patients (60%) based on the primary outcome (MADRS score). Statistically significant improvements in depressive symptoms were seen overall in the entire sample. Using secondary outcome measures (HDRS and GAF), similarly high response rates were observed (50–60%), indicating that pramipexole improved not only depressive symptoms, but also psychosocial function. No serious side effects were seen, which is consistent with previous studies (Inoue et al., 1996; Izumi et al., 2000; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005), and the discontinuation rate was relatively low (2 of 10 patients [20%]). Two of the 10 patients were not able to complete the open-label trial due to intolerable side effects, but among the remaining eight patients, pramipexole was reasonably well tolerated.

Pramipexole improved total MADRS scores. We analyzed whether improvements differed among the sub-items of this scale. Eight of 10 items of the MADRS showed significant improvements. Accordingly, it does not appear that pramipexole improves only a certain subset of depressive symptoms. Improvements in the 17-item HDRS ($HDRS_{17}$) were seen in five sub-items, all of which were symptoms of so-called "Bech's, $HDRS_6$", which is more clearly unidimensional and more sensitive to changes than the $HDRS_{17}$ (Carmody et al., 2006). Both the MADRS and the $HDRS_6$ are related highly to the core concept of depression and had acceptable effect sizes in two clinical trials

including highly treatment-resistant and non-treatment-resistant patients with major depression, respectively (Carmody et al., 2006). Hence, our data on changes in sub-items over time are in good agreement with a previous study (Carmody et al., 2006).

The antidepressant effects of pramipexole were first observed in several animal models and, later, in a series of controlled and uncontrolled clinical studies in which pramipexole was used as monotherapy or augmentation therapy in patients with bipolar and unipolar depression (Aiken, 2007). A randomized controlled trial of pramipexole compared three doses of pramipexole to fluoxetine and placebo in 174 subjects with non-refractory unipolar major depression (Corrigan et al., 2000). At 8 weeks, pramipexole performed comparably to fluoxetine and significantly better than placebo; pramipexole 1.0 mg per day resulted in significant improvement over baseline compared with the placebo group. In addition, six previous studies have reported that dopamine receptor agonists, including pramipexole, in addition to antidepressants improved major depressive disorder refractory to at least one standard antidepressant trial (Bouckoms and Mangini, 1993; Inoue et al., 1996; Izumi et al., 2000; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005). However, two studies did not define treatment-resistant depression clearly (Bouckoms and Mangini, 1993; Sporn et al., 2000). Four studies included not only major depressive disorder, but also major depressive episode associated with bipolar disorder (Bouckoms and Mangini, 1993; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005). Although these six studies might include stage 2 treatment-resistant major depressive disorder as defined by Thase and Rush (1995), the efficacy of dopamine agonists for stage 2 major depressive disorder remains unclear. Our study suggests that a dopamine receptor agonist may be an effective adjunctive therapy for stage 2 treatment-resistant major depressive disorder.

The clinical efficacy of pramipexole for treatment-resistant bipolar depression is clearer than that for treatment-resistant unipolar depression (Goldberg et al., 2004; Zarate et al., 2004). In addition to the open trials with dopamine receptor agonists described above (Bouckoms and Mangini, 1993; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005), two preliminary randomized, double-blind, placebo-controlled trials were undertaken for treatment-resistant bipolar depression among patients with non-response to at least two adequate trials of standard antidepressants with concomitant mood stabilizers during the current depressive episode (Goldberg et al., 2004) or non-response to at least one adequate antidepressant trial regardless of concomitant mood stabilizer (Zarate et al., 2004). Both studies showed significant antidepressant effects of pramipexole. Because bipolar disorder is one of the most common reasons for treatment-resistant major depression (Sharma et al., 2005; Inoue et al., 2006), the clinical efficacy of pramipexole suggests that pramipexole may be effective for unrecognized bipolar depression that has been treated as treatment-resistant major depression.

This study does not prove efficacy or safety of this agent because it was nonrandomized and uncontrolled, but it does apply a prospective outcomes assessment, with no changes allowed in any other treatments. Thus, it provides useful pilot data that tend to support performing randomized studies of pramipexole in stage 2 treatment-resistant major depressive disorder. However, two limitations should be noted. First, open-label case series tend to over-estimate the effectiveness of novel interventions. Second, this study examined the short-term efficacy of pramipexole. Future studies examining long-term efficacy are needed because a high proportion of patients with more advanced levels of treatment-resistant major depression relapsed after responding to later stage therapies in STAR*D (Rush et al., 2006).

5. Conclusion

Pramipexole may have benefit for stage 2 treatment-resistant major depressive disorder. Further studies to confirm the clinical efficacy of pramipexole are warranted.

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Neuropharmacology and Analgesia

Combined treatment with MAO-A inhibitor and MAO-B inhibitor increases extracellular noradrenaline levels more than MAO-A inhibitor alone through increases in β-phenylethylamine

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ABSTRACT

Monoamine oxidase inhibitors (MAO inhibitors) have been widely used as antidepressants. However, it remains unclear whether a difference exists between non-selective MAO inhibitors and selective MAO-A inhibitors in terms of their antidepressant effects. Using in vivo microdialysis methods, we measured extracellular noradrenaline and serotonin levels following administration of Ro 41-1049, a reversible MAO-A inhibitor and/or lazabemide, a reversible MAO-B inhibitor in the medial prefrontal cortex (mPFC) of rats. We examined the effect of local infusion of β-phenylethylamine to the mPFC of rats on extracellular noradrenaline and serotonin levels. Furthermore, the concentrations of β-phenylethylamine in the tissue of the mPFC after combined treatment with Ro 41-1049 and lazabemide were measured. The Ro 41-1049 alone and the combined treatment significantly increased extracellular noradrenaline levels compared with vehicle and lazabemide alone. Furthermore, the combined treatment increased noradrenaline levels significantly more than Ro 41-1049 alone did. The Ro 41-1049 alone and the combined treatment significantly increased extracellular serotonin levels compared with vehicle and lazabemide alone, but no difference in serotonin levels was found between the combined treatment group and the Ro 41-1049 group. Local infusion of low-dose β-phenylethylamine increased extracellular noradrenaline levels, but not that of serotonin. Only the combined treatment significantly increased β-phenylethylamine levels in tissues of the mPFC. Our results suggest that the combined treatment with a MAO-A inhibitor and a MAO-B inhibitor strengthens antidepressant effects because the combined treatment increases extracellular noradrenaline levels more than a MAO-A inhibitor alone through increases in β-phenylethylamine.

1. Introduction

Non-selective MAO inhibitors were first developed as antidepressants. Subsequently, selective MAO inhibitors such as selective monoamine oxidase A (MAO-A) inhibitor (clorgyline) and selective monoamine oxidase B (MAO-B) inhibitor (selegiline) were introduced. However, irreversible MAO inhibitors entail risks of causing hypertensive attacks after consumption of tyramine-rich food (Blackwell et al., 1967) and of causing serotonin syndrome in cases of co-administration of non-selective MAO inhibitors and tricyclic antidepressants (TCA) or selective serotonin reuptake inhibitors (SSRIs) (Schuckit et al., 1971; Ananth and Luchins, 1977; Sternbach, 1991). Consequently, MAO inhibitors have been used only infrequently as the first-line antidepressant of the depression treatment for the reasons described above (Lam et al., 2009).

Reversible monoamine oxidase A inhibitors (RIMAs) were developed later. Moclobemide, an RIMA, has an antidepressant effect that is equal to that of SSRIs and different side effect profiles from SSRIs (Papakostas and Fava, 2006). In several countries, RIMAs are used for the treatment of depression. Now, RIMAs are recognized as important antidepressants. They are used as first-line antidepressants for the treatment of depression (Lam et al., 2009).

Several reports have described that the MAO-A inhibition contributes to the mechanism of antidepressant effects of MAO inhibitors more than MAO-B inhibition (Lipper et al., 1979; Mann et al., 1989). Moreover, Larsen et al. (1991) reported that RIMA has equal antidepressant effects to those of irreversible MAO inhibitors. However, Lotufo-Neto et al. (1999) examined antidepressant effects of MAO inhibitors in a meta-analysis and described the possibility that non-selective MAO inhibitors are more effective than RIMA. Consequently, it is likely that MAO-B inhibition also contributes to an

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antidepressant effect. Nevertheless, no consensus has been reached on the matter.

For this study, to examine the pharmacological mechanism of antidepressant effects of MAO-A and MAO-B inhibitors, we measured extracellular noradrenaline and serotonin levels after administration of Ro 41-1049, an RIMA, and/or lazabemide, a reversible MAO-B inhibitor in the medial prefrontal cortex (mPFC) of rats using the in vivo microdialysis method. A main substance of MAO-B, β-phenylethylamine, exists in the brain; it is related to catecholamine release (Mesfioui et al., 1998; Nakamura et al., 1998; Burchett and Hicks, 2006). Accordingly, we also measured extracellular noradrenaline and serotonin levels after local infusion of β-phenylethylamine to the mPFC of rats. In addition, the concentrations of β-phenylethylamine in the tissues of the mPFC after administration of Ro 41-1049 and lazabemide were measured.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 180–280 g were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were housed in groups of four and maintained on a 12 h light–dark cycle (light phase: 06:30–18:30) in a temperature-controlled environment (22 ± 1 °C) with free access to food and water. Experiments began after a 10-day period of acclimatization. All procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee. They complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.2. Drugs

After dissolution in saline, Ro 41-1049 (N-(2-aminoethyl)-5-(3-fluorophenyl)-4-thiazolecarboxamide hydrochloride) (Research Biochemical Inc., Natick, U.S.A.) and lazabemide (N-(2-aminoethyl)-5-chloro-2-pyridinecarboxamide hydrochloride) (F. Hoffman-La Roche Ltd., Switzerland) were injected intraperitoneally (i.p.) at a volume of 1 ml/kg. Then β-phenylethylamine (Sigma Chemical Co., St. Louis, U.S.A.) was dissolved in artificial cerebrospinal fluid (CSF) and was thereafter administered from microdialysis probes (reverse-dialysis). The doses of the selective MAO-A and MAO-B inhibitors were chosen, respectively, to inhibit MAO-A and MAO-B fully and selectively (Da Prada et al., 1990).

2.3. Microdialysis procedures

2.3.1. Surgery and perfusion

Experiments were performed according to a procedure described in a previous report (Kitaichi et al., 2004). Briefly, rats were implanted stereotaxically under pentobarbital anesthesia (30 mg/kg i.p.) using an AG-4 guide cannulae (Eicom Corp., Kyoto, Japan) leading to the surface of the mPFC at the following coordinates relative to the bregma: A + 3.2, ML + 0.8, DV + 1.0 mm. Dialysis probes with 0.22 mm outer diameter (A-I-4-03; Eicom Corp.) were then inserted into the guide cannulae so that 3.0 mm of the probe was exposed to the tissue of the mPFC. Rats were housed individually after these operations.

Experiments were performed using freely moving rats. On the following day, 24 h after surgery, perfusion was started using artificial CSF (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) at a flow rate of 1 μl/min. Following initial perfusion for 2 h, dialysate samples were collected in sample vials containing 50 μl of 0.05 M acetic acid every 40 min for 440 min.

2.3.1.1. Experiment 1: Acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on extracellular noradrenaline and serotonin concentrations. Rats received a single injection (i.p.) of vehicle, Ro 41-1049 (30 mg/kg), lazabemide (10 mg/kg), or the combination of Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg), 200 min after the first dialysate samples were collected. Extracellular noradrenaline and serotonin levels were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) (Eicom Corp.).

2.3.1.2. Experiment 2: Local infusion of β-phenylethylamine (0, 10, and 100 μmol/l) into the mPFC on extracellular noradrenaline and serotonin concentrations. Rats received local infusion of β-phenylethylamine (0, 10, and 100 μmol/l) via reverse microdialysis into the mPFC (local reverse-dialysis) during 0–240 min, 200 min after the first dialysate samples were collected. Extracellular noradrenaline and serotonin levels were determined using HPLC-ECD (Eicom Corp.).

2.3.2. Analytical procedures for noradrenaline

The HPLC system consisted of a liquid chromatograph pump (EP-300; Eicom Corp.), a degasser (DG-300; Eicom Corp.), a reverse phase ODS column (Eicompak CA-5ODS 150 2.1 mm; Eicom Corp.), an ECD-300 electrochemical detector (Eicom Corp.), and a data acquisition system (PowerChrom; AD Instruments Pty. Ltd., Sydney, Australia). For the noradrenaline analysis, 30 μl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 5% (v/v) methanol, 50 mg/l Na_2EDTA and 500 mg/l L-octanesulfonic acid. Separations were conducted at 25 °C with a flow rate of 0.23 ml/min. The electrochemical detector was set at an oxidation potential of 550 mV. Noradrenaline standard solutions were injected every working day and the peak heights for the standard were used for comparison to determine the amount of noradrenaline in the samples.

2.3.3. Analytical procedures for serotonin

To determine serotonin concentrations, the same equipment as that used for the noradrenaline analysis with the exception of a different reverse phase ODS column, an Eicompak PP-ODS 30 4.6 mm (Eicom Corp.) was used. For serotonin analysis, 20 μl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 1% (v/v) methanol, 50 mg/l Na_2-EDTA and 500 mg/l sodium L-decanesulfonate. Separations were conducted at 25 °C with a flow rate of 0.5 ml/min. The electrochemical detector was set at an oxidation potential of 400 mV. Standard solutions for serotonin were injected every working day, and the peak heights for the standards were used for comparison to determine the amount of serotonin in the samples.

2.4. Experiment 3: Effect of acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on β-phenylethylamine concentrations in the mPFC

Rats were administered vehicle, Ro 41-1049 (30 mg/kg), lazabemide (10 mg/kg) or the combination of Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg). All rats were killed by decapitation 4 h after drug administration. Brains were quickly removed and frozen at −80 °C. We entrusted the measurement of β-phenylethylamine concentrations of the mPFC to S-Medical Service Inc. (Tokyo, Japan). β-Phenylethylamine was measured using gas chromatography–mass spectrometry.

2.5. Statistical analysis

All data are given as the mean values ± S.E.M. of individual rats from each group. The noradrenaline and serotonin contents of dialysate samples were expressed as absolute values (pg/fraction).

In experiment 1, to investigate the combined effect of Ro 41-1049 and lazabemide (2×2 design) on extracellular noradrenaline and serotonin concentrations, repeated measures analysis of variance (ANOVA) for absolute values was used during the 0–240 min interval after MAO inhibitors administration. The respective areas under the curve for the 0–240 min periods were compared among the four groups using one-way ANOVA, followed by Duncan's test. Differences