一定であると考えられた。

一方，HFT-290（2，4および8 mg）を72時間単回貼付したとき，血清中フェンタニル濃度は24～30時間（中央値）で最高に達し，以降，貼付中にもかかわらず低下が認められた。製剤剥離後の半減期は27～38時間であり，24時間貼付時と同程度の消失半減期を示した。C_{max}および$AUC_{0-\infty}$ともに用量に比例して増加し，またt_{max}および製剤剥離後の$t_{1/2}$はいずれの用量でも大きな差は認められなかった。HFT-290を72時間貼付したとき，貼付中の血清中フェンタニル濃度に関して日内変動が観察された。同様の血中濃度の日内変動は既承認のフェンタニル経皮吸収型製剤やロキソプロフェン経皮吸収型製剤においても報告されている[5)14)]。皮膚温や皮膚pHなどは日内変動することが報告されており[15)]，薬物動態的な日内変動に肝血流量や肝代謝酵素活性などに関する日内変動が関与している可能性も示唆されている[16)]。フェンタニルを経皮投与したとき，体温や外部熱源の上昇で吸収が増加することが報告されており[17)]，また，フェンタニルは主に肝臓で代謝されることを考えると，今回認められた血清中フェンタニル濃度の日内変動はこれらを含む複数の要因により生じている可能性が推察された。

尿中排泄について，フェンタニル，各代謝物およびそれらの総量の尿中累積排泄率は用量によらずほぼ一定であったことから，代謝パターンも用量により変化せず一定であると考えられた。また，尿中の主代謝物はノルフェンタニルであり，*p*-水酸化フェンタニルは抱合体として尿中に排泄されており，尿中代謝物の組成は24時間貼付時と同様の傾向を示していた。

HFT-290を72時間単回貼付したときの剥離後の製剤中に残存するフェンタニルクエン酸塩量から算出した吸収率は約70～80％であり，用量によらずほぼ同程度であった。24時間単回貼付時の吸収率が約60～70％であったことを考えると，貼付後24時間以降は製剤からの吸収はわずかであり，貼付後24時間で製剤からの薬物放出はほぼ終了しているものと推察された。このことは72時間貼付時の血清中フェンタニル濃度が貼付後24時間以降に低下がみられていることとも一致していると考えられる。

HFT-290を3日に1回反復貼付した際にも，貼り替え後24時間から72時間にかけて血清中フェンタニル濃度が低下する傾向がみられた。また，72時間単回貼付成績から予測される血清中フェンタニル濃度（重ね合わせ法によるシミュレーション値）と72時間反復貼付時の実測値はよく一致していることが確認され，反復貼付時の剥離後の製剤中の残存率より算出した吸収率は単回貼付時の吸収率と同程度であったことから，反復貼付による薬物動態的な変化はほとんどないものと考えられた。

既存のリザーバータイプのフェンタニル経皮吸収型製剤（フェンタニルとして2.5mg）を3日に1回貼付したときの血清中フェンタニル濃度はHFT-290（フェンタニルクエン酸塩として2 mg）を1日1回貼付したときの血清中フェンタニル濃度に近似していた。この結果から，HFT-290は既存のリザーバータイプのフェンタニル経皮吸収型製剤と同程度の治療効果が期待できるものと考えられた。

以上の結果から，安定した血清中フェンタニル濃度を得るという観点からはHFT-290を3日に1回貼付するよりも1日1回貼付の用法にすることが好ましいと考えられた。また，製剤からの吸収率は高く，HFT-290の反復貼付において血清中フェンタニル濃度に蓄積性はみられなかったことから，良好な薬物動態特性を示しているものと考えられた。がん疼痛患者を対象としたHFT-290の臨床第Ⅱ相および臨床第Ⅲ相試験において1日1回貼付で有効性，安全性が確認されていることから[18)19)]，HFT-290は患者の状態に応じ貼り替え時に貼付用量を調節でき（増量は2日ごと），安定した疼痛コントロールを得ることができる新しいフェンタニルクエン酸塩含有経皮吸収型製剤となることが期待される。

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総説

新しいフェンタニルクエン酸塩含有経皮吸収型製剤の臨床的有用性

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はじめに

がん疼痛は中等度から高度の慢性的な痛みが長期にわたり持続することから，がん患者のQOLを著しく損なう。わが国においてもモルヒネ製剤に加えフェンタニル経皮吸収型製剤やオキシコドン徐放錠が販売され，WHO方式がん疼痛治療法に整合したオピオイドローテーションの可能性が拡がった。なかでも，患者または医療従事者を対象としたアンケートの結果，フェンタニル経皮吸収型製剤はともに高く評価されており[1)2)]，がん疼痛治療における重要性が増しつつある。

しかしながら，従来のフェンタニル経皮吸収型製剤は3日ごとの貼り替えを基本とするため，患者によっては貼付後1～3日にかけて血中フェンタニル濃度が低下し，鎮痛効果が持続しない可能性が示唆されていた[3)-5)]。また，オピオイド系鎮痛薬から同剤への切り替え換算比では十分な疼痛コントロールが得られていない可能性が示されており，オピオイドローテーションを行う際の適正な換算比の導入が求められている現状があった[6)-8)]。

本稿では，久光製薬株式会社が2010年4月に製造販売承認を取得した初めての1日1回貼付の，フェンタニルクエン酸塩含有経皮吸収型製剤（以下，フェントス®テープ）のがん疼痛患者に対する主な臨床試験成績および臨床的有用性について概説する。

Key Words：フェンタニルクエン酸塩，経皮吸収型製剤，がん疼痛

Ⅰ．フェントス®テープの特長

1．製剤的特長

フェントス®テープは，注射薬として使用されているフェンタニルクエン酸塩を1 cm^2あたり0.2mg含有するマトリクス型の経皮吸収型製剤である。1枚あたりフェンタニルクエン酸塩として1，2，4，6および8 mgを含有する製剤（それぞれ5，10，20，30および40cm^2）5規格を有し，患者ごとの痛みの強さに応じた適切な投与量設定が可能である。フェントス®テープの構造を図1に示した。

フェンタニルクエン酸塩を5 cm^2あたり1 mg含有する低用量規格製剤は，モルヒネ経口換算30mg/日未満などのオピオイド系鎮痛薬低用量使用患者に対して切り替え可能である。さらに，アルコールを含まないマトリクス型製剤であるため，①アルコールに過敏症のある患者にも貼付できる，②薬剤の漏れや飛散リスクがなく，医療事故のリスクを低減することができる，③薬液の単純な抜き取りが困難であり，薬物乱用目的での製剤の悪

Motohiro Matoba（科長），Setsuro Ogawa（主任教授），Masako Iseki（先任准教授）

用を防止することができる，という製剤的特長を有する。一般に，経皮吸収型製剤が途中で剥離・脱落した場合，途中剥離製剤中には使用完了製剤と比較してより高濃度の薬物が残留する。1日1回貼付のため途中剥離が起こりにくいフェントス®テープは，オピオイドを抽出しにくいマトリクス型の製剤構造とあいまって，途中剥離や医療目的以外の使用への可能性が低いメリットを有する。

2．有効性に関する特長

3日ごとの貼り替えを基本とするデュロテップ®パッチ，デュロテップ®MTパッチ（以下，DP）は，貼付期間中に経時的に血中濃度が減少することが指摘されている[3)-5)]。約30％の患者でコントロール不良または無効例がみられ，2日ごとの貼り替えに変更することで疼痛コントロールが得られたとの報告もある[9)]。フェントス®テープは1日1回貼付のため，貼付期間中の血中濃度低下による鎮痛効果減弱の可能性が軽減できると期待され，毎日の貼り替え時に患者の状態に応じて投与量を調整（増量は2日ごと）できるというメリットを有する。

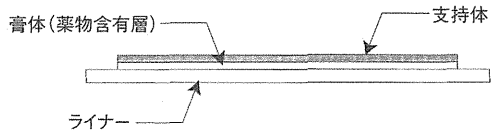

図1．フェントス®テープの断面図

わが国のDPで用いられている換算表は，経口モルヒネ45～134mg/日（中央値90mg）から4.2mg（モルヒネ：フェンタニル＝150：1）の設定となっている。一方，海外では100：1あるいは70：1という換算比が用いられており[10)]，わが国の臨床現場においてDPではモルヒネと同等の鎮痛効果が得られない可能性が示唆されている[6)-8)]。DPの投与量が十分でない場合，モルヒネレスキューの使用回数が多くなりモルヒネによる副作用が出現することも懸念され[6)]，十分な鎮痛効果の得られる換算比の適用が望まれていた。フェントス®テープでは第Ⅱ相臨床試験以降，海外で用いられている換算比と同様に，モルヒネ：フェンタニル＝100：1の換算比を用いて臨床試験を行った。その結果，換算比（表1）の妥当性が検証され，より適切な疼痛コントロールが得られるものと期待される。

Ⅱ．臨床試験成績

1．がん疼痛患者における薬物動態

1）10日間反復貼付試験（第Ⅰ相）[11)]

日本人がん疼痛患者（12例）を対象とし，フェントス®テープ 2 mgまたは4 mg製剤を1日1回10日間貼付した。血清中フェンタニル濃度は5回目剥離時（貼付開始後約120時間）にほぼ定常状態に達した（図2）。また，$AUC_{216-240}$の平均値

表1．オピオイド系鎮痛薬からフェントス®テープへ切り替える際の換算表

		フェントス®テープ1日貼付用量*	1mg	2mg	4mg	6mg
		定常状態における推定平均吸収量（フェンタニルとして）	0.3mg/日	0.6mg/日	1.2mg/日	1.8mg/日
			↑	↑	↑	↑
本剤使用前の鎮痛薬	モルヒネ	経口剤（mg/日）	≦29	30～89	90～149	150～209
		坐薬（mg/日）	≦10	20～40	50～70	80～100
		注射薬/静脈内投与（mg/日）	≦9	10～29	30～49	50～69
		オキシコドン経口剤（mg/日）	≦19	20～59	60～99	100～139
		DP（mg/3日）	2.1	4.2	8.4	12.6

*フェントス®テープ8 mgは初回貼付用量としては推奨されないが，定常状態における推定平均吸収量はフェンタニルとして2.4mg/日に相当する。

はほぼ貼付用量に比例して増加した。製剤剥離後の$t_{1/2}$は貼付用量間で著明な差はなかった(表2)。

2) 用法設定試験(第Ⅱ相)[12]

日本人がん疼痛患者を対象とし，モルヒネ製剤からフェントス®テープ2 mg製剤1日1回9日間貼付(10例)，フェントス®テープ2 mg製剤3日に1回9日間貼付(8例)またはDP 2.5mg(25 μg/時)製剤3日に1回9日間貼付(7例)に切り替えた。血清中フェンタニル濃度の推移は3群間で異なり，フェントス®テープ1日1回貼付では貼付開始後96時間以降の各評価時期において，ほぼ一定の血清中フェンタニル濃度を維持していたのに対し，フェントス®テープ3日に1回貼付では貼り替え後24～72時間にかけて低下する傾向を示した。DP 3日に1回貼付の血清中フェンタニル濃度の推移はフェントス®テープ1日1回貼付に近似し，フェントス®テープ3日に1回貼付と同様に貼り替え後24～72時間にかけてやや低下する傾向を示した(図3)。本試験により，1日1回貼付の妥当性を確認した。

2. がん疼痛患者における有効性

1) DPからの切り替え貼付試験(第Ⅱ相)[13]

6日間以上一定量のDPが投与されている日本人がん疼痛患者(75例)を対象に，試験開始前と同一投与量のDPを3日間貼付後，フェントス®テープの1日1回9日間貼付に切り替えた。最終評価時(終了時または中止時)の有効率，およびフェントス®テープ貼付開始前から最終評価時の疼痛VAS値変化量は表3のとおりであった。フェ

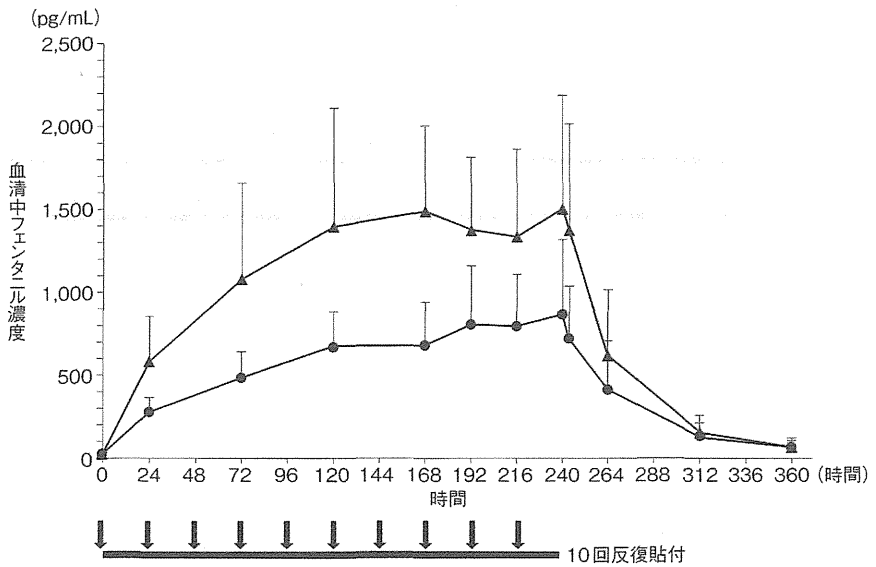

図2. 血清中フェンタニル濃度推移(平均値＋標準偏差)
—●— : 2mg(n=7) —▲— : 4mg(n=5)

表2. 血清中フェンタニル濃度の薬物動態パラメータ

貼付用量	$AUC_{216\text{-}240}$(pg·hr/mL)	剥離後の$t_{1/2}$(時間)
2mg (n = 7)	19,961 ± 9,222	31.3 ± 9.8
4mg (n = 5)	34,102 ± 14,409	25.7 ± 7.0

平均値 ± 標準偏差

ントス®テープ貼付開始前のDP貼付3日間と貼付開始後3日間のレスキュードーズ回数変化量を比較したところ，フェントス®テープ貼付開始後に有意に小さかった(0.3±0.8回/日 vs. 0.0±0.5回/日；p＝0.007，対応のあるt検定)。本試験により，DPから切り替え後9日間貼付により良好な疼痛コントロールが可能であることを確認した。

2) モルヒネ製剤またはオキシコドン経口剤からの切り替え貼付試験(第Ⅲ相)[14]

一定量のモルヒネ製剤(経口モルヒネ換算量として89mg/日以下)またはオキシコドン経口剤(同59mg/日以下)が投与され，24時間の平均疼

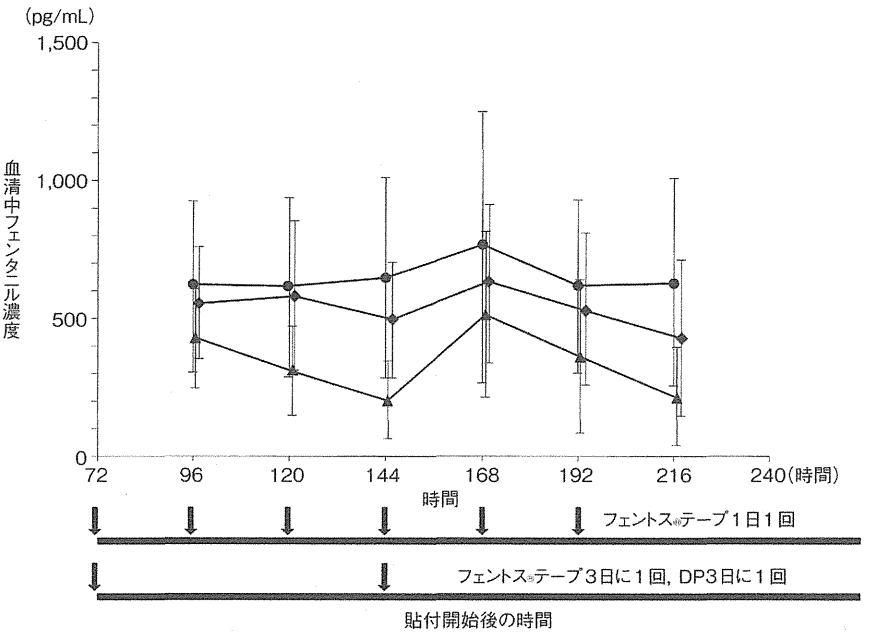

図3．群別の血清中フェンタニル濃度推移(平均値±標準偏差)

●—：フェントス®テープ1日1回(n=10)，▲—：フェントス®テープ3日に1回(n=8)，◆—：DP3日に1回(n=7)
DP群は144時間以降，n=6

表3．DPから切り替えたときの有効率

	全体	a群*	b群†
評価例数(Per Protocol Set)	56	43	13
最終評価時の有効率(有効例数)	83.9％(47)	86.0％(37)	76.9％(10)
フェントス®テープ貼付開始前のDP貼付3日間の平均疼痛VAS値(mm)	21.7±19.5	13.8±11.5	49.3±16.6
最終評価時の疼痛VAS値(mm)	21.0±20.5	15.0±16.3	40.8±21.3
最終評価時の疼痛VAS値変化量(mm)	−0.7±15.1	1.7±13.5	−8.5±17.9

平均値±標準偏差
*a群：フェントス®テープ貼付開始前のDP貼付3日間の平均疼痛VAS値が35mm未満(疼痛コントロールが良好)の患者
†b群：フェントス®テープ貼付開始前のDP貼付3日間の平均疼痛VAS値が35mm以上(疼痛コントロールが不良)の患者

痛VAS値が35mm未満に疼痛がコントロールされている日本人がん疼痛患者(65例)を対象に，試験開始前のオピオイド系鎮痛薬の投与量に基づき，フェントス®テープ1または2 mgの1日1回7日間貼付に切り替えた。フェントス®テープの投与量は7日間一定とした。貼付開始前から最終評価時(終了時または中止時)の疼痛VAS値変化量の平均値±標準偏差は，0.6±16.1mm(95%信頼区間：－3.4～6.6mm)であった。95%信頼区間の上限および下限の絶対値はいずれも，あらかじめ設定された同等性の基準値(15mm以下)の範囲内であった。よって，本試験で用いた用量換算比(モルヒネ：フェンタニル＝100：1およびオキシコドン：フェンタニル＝100：1.5)は妥当であり，切り替え後に疼痛が十分にコントロールできることを検証した(表4)。

3．がん疼痛患者における安全性成績

第Ⅰ相～第Ⅲ相臨床試験(計8試験)において，オピオイド系鎮痛薬から切り替えて使用した患者(413例)のうち，236例(57.1%)に副作用がみられた。主な副作用は傾眠(12.6%)，悪心(11.6%)，嘔吐(10.4%)，便秘(9.9%)などであり，副作用の発現状況は既存のオピオイド系鎮痛薬と同様であった。また，貼付部位において発現率1.0%以上の副作用は，適用部位そう痒感(7.0%)，適用部位紅斑(4.4%)であった。このほかオピオイドに特有の重要な副作用として，呼吸抑制が0.5%(2例)発現した。呼吸抑制はいずれも高度で，うち1例は重篤であった。呼吸抑制はいずれも増量後に発現していた。

ほかのオピオイド系鎮痛薬からフェントス®テープに切り替えた場合または投与量を増量した際，フェンタニルの血中濃度が徐々に上昇するため，その間のさらなる増量により著しく血中濃度が上昇し，呼吸抑制などの重篤な有害事象を引き起こす可能性がある。よって初回貼付後および増量後に少なくとも2日間(48時間以上)は同一用量を貼付し，注意深く増量する必要がある。

おわりに

1日1回貼付のフェントス®テープは，貼付期間中ほぼ一定の血中フェンタニル濃度を維持でき，血中フェンタニル濃度低下による鎮痛効果減弱の可能性が低いと考えられた。また，24時間ごとに痛みと副作用の有無を確認し，時刻を決めて規則正しく投与するというオピオイド鎮痛薬服用の基本原則(by the clock)を守りやすいといった，臨床上のメリットを有すると考えられた。さらに，海外で用いられている換算比と同様に，モルヒネ：フェンタニル＝100：1の換算比による切り替えが可能であり，切り替え後に十分な鎮痛効果が期待できる。

以上より，フェントス®テープは従来のDPの問題点を改善するとともに，日本人がん疼痛患者に新たな選択肢を提供し，QOL向上に寄与するものと考えられた。

表4．モルヒネ製剤またはオキシコドン経口剤から切り替えたときの疼痛VAS値変化量

前治療オピオイド系鎮痛薬	全体	モルヒネ製剤		オキシコドン経口剤	
貼付用量	1または2mg	1mg	2mg	1mg	2mg
評価例数(Full Analysis Set)	65	13	16	17	19
貼付開始前の疼痛VAS値(mm)	13.1±9.9	16.5±11.6	11.9±10.7	11.5±8.8	13.3±9.0
最終評価時の疼痛VAS値(mm)	13.8±16.4	19.2±17.5	18.6±25.3	7.5±7.7	11.6±9.3
最終評価時の疼痛VAS値変化量(mm)	0.6±16.1	2.7±14.9	6.7±27.4	－4.1±6.6	－1.7±7.3

平均値±標準偏差

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Small temperature variations alter edaravone-induced neuroprotection of cortical cultures exposed to prolonged hypoxic episodes

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Background. Edaravone, a free radical scavenger, has been shown to be neuroprotective *in vivo* and *in vitro*. However, the impact of small temperature variations on its neuroprotective actions remains unknown.

Methods. We examined the degree of neuroprotection conferred by various concentrations of edaravone on cortical cultures exposed to prolonged hypoxia (24 h) under three conditions: mild hypothermia (32°C), normothermia (37°C), and mild hyperthermia (39°C). The survival of cortical neurones from E16 Wistar rats (SR) was evaluated using photomicrographs taken before and after exposure to hypoxia.

Results. The mean survival of neurones exposed to hypoxia at normothermia was 14.7 (SEM 1.8)%. The addition of 50 μM edaravone significantly improved the mean survival to 40.5 (4.7)%. This improvement was noted at higher doses of edaravone (5 μM ≤) but not at lower doses (≤500 nM). With mild hypothermia and prolonged hypoxia without edaravone, neuroprotection was significantly improved with a mean survival of 63.0 (5.2)%. This neuroprotective effect was not enhanced with the addition of edaravone, even at the highest dose. Hypoxia-induced neurotoxicity was aggravated by mild hyperthermia as reflected by a mean survival of 9.1 (2.1)%. However, higher concentrations of edaravone inhibited the deleterious effect of mild hyperthermia, thereby demonstrating a significant neuroprotective effect. The survival of neurones subjected to both hyperthermia and edaravone was the same as that of neurones exposed to normothermia and edaravone.

Conclusions. Temperature is a potential factor in determining whether edaravone confers a neuroprotective effect when applied during prolonged hypoxic insults.

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Acute hypoxic injury to the central nervous system (CNS) triggers a cascade of biochemical events that provoke prolonged secondary injury to the surrounding tissues. Even small variations in the CNS temperature are known to critically affect the neurological and histological outcome of hypoxic injury. The use of hypothermia is a proven potent neuroprotective tool for improving the outcome of cerebral ischaemia. The neuroprotection in the CNS conferred by hypothermia is attributed to a reduction in the cerebral metabolic rate (CMR), decreased release of neurotransmitters, attenuation of *N*-methyl-D-aspartate (NMDA) receptor activity, reduction in intracellular calcium influx, decreased lipid peroxidation, and reduced production of reactive oxygen species (ROS).[1] We previously reported that mild (32°C) and moderate (27°C) hypothermia conferred as much neuroprotection as deep (22°C) hypothermia in primary cultured neurones when subjected to long-lasting hypoxia. In addition, profound (17°C) hypothermia does not offer any greater protection to the neuronal culture compared with that offered by mild, moderate, or deep hypothermia during hypoxic insults for 24 or 48 h.[2]

In contrast to hypothermia, hyperthermia has been shown to increase the vulnerability of certain neurones to infarction. For example, in a model of hypoxic insult to rat

hippocampal slices, hyperthermia significantly worsened the electrophysiological recovery after 3 min of hypoxia exposure and accelerated ATP depletion.[3] There are many temperature-dependent processes associated with neuronal injury such as the release of glutamate from synaptic vesicles,[4,5] the intracellular accumulation of Ca^{2+},[6] the modulation of calcium-calmodulin-dependent protein kinase,[7] the production of free radicals,[8] and Zn^{2+} translocation from neuronal terminals into postsynaptic neurones.[9]

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a potent synthetic free-radical scavenger, has been used during the early phase of an acute stroke in order to rescue the ischaemic penumbra in the brain tissue. Edaravone has the ability to eradicate free radicals including hydroxyl radicals. This eradication results in edaravone's antioxidant activities. Several *in vivo* and *in vitro* studies have revealed that the administration of edaravone may possibly lessen cerebral damage resulting from ischaemic insults. These studies have indicated the efficacy of edaravone in diminishing oedema formation,[10] in inhibiting oxidative tissue damage,[11] and in inhibiting ROS generation,[12] thereby delaying the development of cerebral infarcts. Moreover, studies indicate that edaravone improves one's functional outcome after an acute ischaemic stroke.[13] However, little is known about the sensitivity of edaravone-induced neuroprotection to temperature during hypoxia. Therefore, the object of the present study is to explore whether small variations in temperature would influence the neuroprotective effect of edaravone on cortical cultures exposed to prolonged hypoxic episodes.

Methods

Chemical reagents

The chemicals used in this study were obtained from the following sources: Dulbecco's modified Eagle's medium (DMEM) from Nissui Pharmaceutical (Tokyo, Japan); 5-fluoro-2′-deoxyuridine (5-FU), poly-L-lysine, streptomycin, penicillin, and anti-microtubule-associated protein 2 (MAP2) from Sigma–Aldrich (St Louis, MO, USA); trypsin from Difco Lab (Detroit, MI, USA); fetal calf serum (FCS) from ICN Biochemicals (Costa Mesa, CA, USA); horse serum (HS) from Gibco BRL (Carlsbad, CA, USA); and anti-glial fibrillary acidic protein (GFAP) antibody from Dako (Carpinteria, CA, USA). Edaravone was kindly provided by Tanabe Mitsubishi Pharm Corporation (Osaka, Japan).

Cell culture

All animals were treated in strict accordance with the institutional and NIH guidelines for the care and treatment of laboratory animals. The study protocol was approved by the Animal Care Committee at the Osaka University Graduate School of Medicine.

Primary cultures of cortical neurones were prepared as described previously.[14–16] Briefly, rat fetuses were removed at embryonic day 16 from anaesthetized pregnant Wistar rats, which were obtained from Nihon SLC (Hamamatsu, Japan). The fetal rat brains were examined under a microscope. Cerebral cortical neurones were treated with 0.25% trypsin at 37°C for 20 min and triturated with a Pasteur pipette. Dispersed cells were diluted to a concentration of 1.0×10^6 cells ml^{-1} in DMEM, which contained 8% FCS and 4% HS, 50 $\mu g\ ml^{-1}$ streptomycin, and 50 IU ml^{-1} penicillin. This suspension was placed on a poly-L-lysine-coated 35 mm diameter tissue culture dish (1.5 ml per well) with a 2 mm grid (Nunc, Naperville, IL, USA) used to observe the same neurones in a given area over time.[16]

After 4 days in culture, cells were treated with 5 $\mu g\ ml^{-1}$ of 5-FU for 3 days to prevent proliferation of non-neuronal cells. The neurones were maintained in DMEM containing 8% FCS and 4% HS in an atmosphere of 5% CO_2 and 95% air, and under 100% humidity at a temperature of 37°C. The medium was changed twice weekly. All subsequent experiments were carried out after 13–14 days in culture.

Immunohistochemical assessment

In order to confirm the purity of the neuronal culture, cells were immunostained with anti-MAP2 or anti-GFAP antibody before and after the experiment. More than 97% of the cells expressed MAP2 and less than 2% expressed GFAP regardless of the duration of the experiments. This demonstrated that most of the cells in our cultures were neurones.[1]

Cytotoxicity

Neurotoxicity was investigated using Shibuta's model, as described previously.[1,14–16] Before each experiment, the culture medium was replaced with fresh medium but lacking FCS and HS. A hypoxic atmosphere was maintained in a special anoxic incubator (ASTEC water jacket type multi-gas incubator, APM-30D; Fukuoka, Japan). In this incubator, the ambient temperature and concentration of oxygen could be precisely controlled. Before the experiments, we used a gas analyser (ABL 620, Radiometer Copenhagen Trading Co., Denmark) to measure changes in the partial pressure of oxygen in the medium. The oxygen concentration was then adjusted in the incubator to ensure that the medium was maintained in a hypoxic atmosphere. During the hypoxic periods, neurones were maintained in an atmosphere consisting of 5% CO_2 and 94–95% nitrogen under 100% humidity. Cultured neurones were exposed to hypoxic insults without reoxygenation after the hypoxic insults and survival was evaluated. Experiments were conducted by examining the effects of three different temperatures and various concentrations of edaravone. The survival of neurones was analysed after a 24-h period of hypoxia. Assessment of survival was carried out at the end of each experiment.

The experimental groups were as follows: Group A—normothermia, 37°C; Group B—mild hypothermia, 32°C; Group C—mild hyperthermia, 39°C; Group D—normothermia (37°C) with edaravone at five concentrations ranging from 5 nM to 50 μM; Group E—mild hypothermia (32°C) with edaravone at five concentrations ranging from 5 nM to 50 μM; Group F—mild hyperthermia (39°C) tested with edaravone at five concentrations ranging from 5 nM to 50 μM.

There were 18 groups altogether from which 185 cortical culture dishes were derived. In clinical practice, hypothermia is commonly divided into four groups: (i) mild hypothermia (32–35°C); (ii) moderate hypothermia (26–31°C); (iii) deep hypothermia (20–25°C); and (iv) profound hypothermia (14–19°C).[17] Polderman[18] concluded that, on the basis of available evidence, patients should be cooled to 32–34°C. Therefore, we chose 32°C as the lowest temperature that we could clinically use for hypothermic therapy. On the other hand, 39°C is a level typical for febrile adults or neonates.[19]

As control conditions, we investigated neuronal survival in 50 cortical culture dishes at three different temperatures (37°C, 32°C, 39°C) exposed to a normoxic atmosphere for a 24-h period with and without 50 μM edaravone.

We used the Shibuta's model, which was described in our previous studies,[16] to evaluate cytotoxicity using a photomicroscopy system (Axiovert 25, Carl Zeiss, Germany; Canon EOS10D, Japan). The traditional assay of lactate dehydrogenase release is a well-established method to investigate cell viability. However, our model of counting cells with microphotographs is the most cost-effective method of determining cell viability in our institution. Three or four photomicrographs were made of each well shortly before exposing the cells to hypoxia and at the end of the experiment. The grid arrangement of the dish helped in determining the specific location of each well. The cells were exposed to 0.4% trypan blue with phosphate-buffered saline to stain non-viable cells and photomicrographs were again taken at the exact area as performed before the experiment. Viable neurones were readily distinguished from non-viable neurones. Non-viable neurones were either stained with trypan blue or disappeared from the culture dish, whereas viable neurones remained unstained. Approximately 500–1000 viable neurones per culture dish were subjected to manual counting. A second observer blinded to the arrangement of photographs, study design, and treatment protocol, replicated all manual counts to ensure count accuracy and minimal inter-observer variability. Survival rates were calculated using the following formula: 100 × the number of unstained cells at the end of the experiment divided by the total number of whole cells shortly before the experiment per grid area.

Statistical analysis

The results are expressed as the percentages of the mean and the standard error of the mean (SEM). The differences between the means were assessed using analysis of variance followed by Fisher's protected least significant difference (PLSD) test with P-values <0.05 considered significant.

Results

In Group A, the mean survival of the neurones was 14.7 (SEM, 1.8)% after exposure to 24 h of hypoxia at normothermia without the administration of edaravone. In Group D, the survival did not improve considerably when hypoxic neurones exposed to normothermia were administered low concentrations (5, 50, 500 nM) of edaravone. However, significant neuroprotection was achieved in this group at the higher concentrations (5 and 50 μM) of edaravone. Neuronal survival in Group D at the higher concentrations was 26.7 (4.7)% at 5 μM and 40.5 (4.7)% at 50 μM. These are statistically significant differences ($P<0.01$) from Group A, as shown in Figs 1 and 2.

As a control condition, cortical neurones were exposed to normoxia in a normothermic condition for 24 h with and without 50 μM edaravone. The survival of cells exposed to edaravone was approximately 96%, indicating it had no lethal effect.

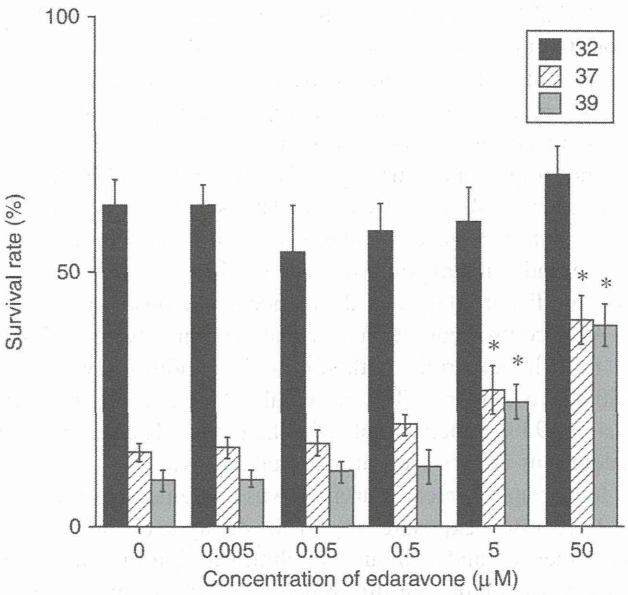

Fig 1 The survival of cultured neurones exposed to hypoxia at different concentrations of edaravone and temperatures. Each bar represents the mean (SEM) percentage. The higher concentrations of edaravone (≥5 μM), but not the lower concentrations (≤500 nM), improve the survival of neurones at both normothermia (37°C) and hyperthermia (39°C) after exposure to prolonged hypoxia. With mild hypothermia (32°C) and prolonged hypoxia, survival significantly improved, but this neuroprotective effect was not enhanced with the addition of edaravone, even at the highest concentration. The differences between the means were assessed using analysis of variance followed by Fisher's PLSD-test with P-values <0.05 considered significant. *$P<0.05$ indicates a significant difference from having no application of edaravone at the same temperature.

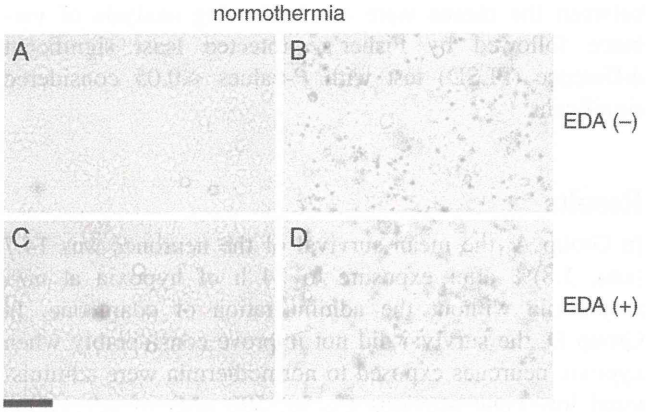

Fig 2 Photomicrographs of neurones in cortical cultures at normothermia (37°C). Without edaravone, approximately 85% of neurones after a 24 h exposure to hypoxia died, disappeared, or were stained with trypan blue at the same location in the photomicrographs. The image shows the neurones shortly before exposure to hypoxia (A) and after the experiment (B). An increased number of viable neurones was observed after exposure to 24 h of hypoxia with the application of 50 μM edaravone. The image shows the neurones shortly before exposure to hypoxia (C) and after the experiment (D). Scale bar=100 μm.

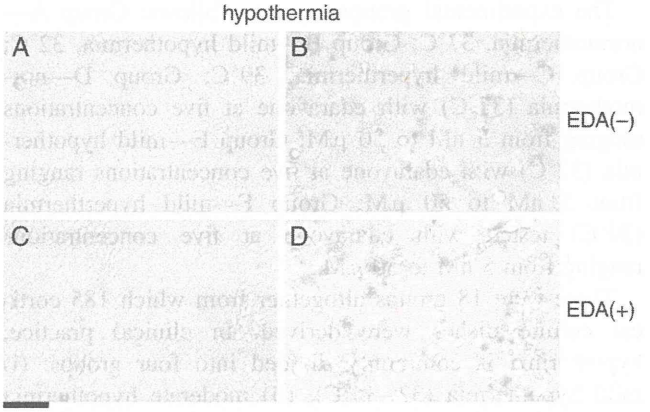

Fig 3 Photomicrographs of cortical neurones in culture at mild hypothermia (32°C). With mild hypothermia, administration of edaravone did not significantly influence the neuronal survival (which was approximately 65%). Photographs were taken shortly before the exposure to hypoxia without edaravone (A) and shortly before exposure to hypoxia with 50 μM edaravone (C); at the end of the experiment without edaravone (B), and at the end of the experiment with 50 μM edaravone (D). Scale bar=100 μm.

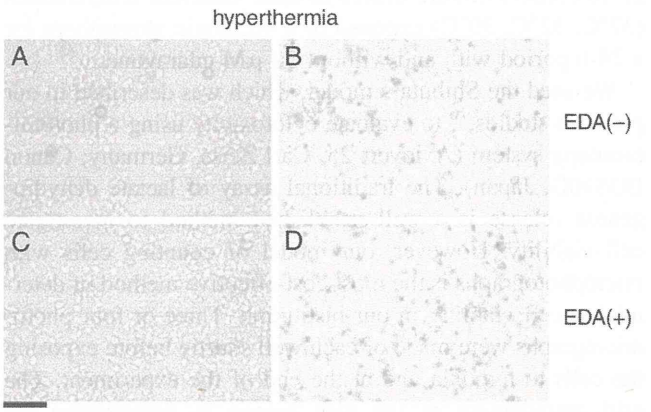

Fig 4 Photomicrographs of cortical neurones in culture with mild hyperthermia (39°C). Without edaravone, approximately 90% of the neurones after exposure to hypoxia were dead, had disappeared or were stained with trypan blue in the same locations in the photomicrographs. The image shows the neurones shortly before exposure to hypoxia (A), and after the experiment (B). An increased number of viable neurones after exposure to hypoxia was observed with the application of 50 μM edaravone. The image shows neurones shortly before exposure to hypoxia (C) and after the experiment (D). Scale bar=100 μm.

In Group B, a significant neuroprotection was achieved with a 24 h period of hypoxia at mild hypothermia, even without edaravone administration. The mean survival was 63.0 (5.2)%. This was a significant difference ($P<0.01$) from Group A, as shown in Figs 1 and 3.

The SR of neurones exposed to mild hypothermia (32°C) and various concentrations of edaravone was analysed in Group E. As shown in Figs 1 and 3, edaravone did not impact the neuroprotective effect of mild hypothermia during 24 h of hypoxia. The survival of neurones exposed to various concentrations (5 nM–50 μM) of edaravone and mild hypothermia were 58–68% in all groups. No significant intergroup differences were observed.

As a control condition, cortical neurones were exposed to mild hypothermia with normoxic conditions with and without edaravone. The survival of cells was approximately 95% independent of edaravone. There were no deleterious effects seen in neuronal survival.

In Group C, mean survival was extremely low at 9.1 (2.1)%, after exposure to 24 h of hypoxia with mild hyperthermia and without the administration of edaravone. This was a significant difference ($P<0.05$) from Group A, as shown in Figs 1 and 4. In Group F, neuronal survival at mild hyperthermia did not improve considerably when the neurones were exposed to low concentrations (5, 50, and 500 nM) of edaravone. At concentrations of 5 nM and 500 nM, the mean survival with mild hyperthermia were significantly lower ($P<0.05$) compared with survival with normothermia (i.e. Group A). However, significant neuroprotection of hypoxic neurones under mild hyperthermia was achieved at higher concentrations (5 and 50 μM) of edaravone. In this case, mean survival at 5 μM was 24.5 (3.3)% and at 50 μM was 39.5 (3.5)%. These were significantly different ($P<0.01$) from the survival in Group C, as shown in Figs 1 and 4. No significant differences were seen in the survival of neurones in Group D and Group F when exposed to higher concentrations of edaravone.

As a control condition, cortical neurones were exposed to mild hyperthermia at normoxia both with and without edaravone. The survival of cells was approximately 92%, which was independent of the administration of edaravone. There was no significant difference between the survival of neurones exposed to normoxia with either normothermia or hypothermia.

Discussion

These results demonstrate that a small variation in the temperature of cultured neurones can affect whether edaravone confers neuroprotection when utilized during prolonged hypoxia. In our *in vitro* experiments, the neuroprotective effect of mild hypothermia was greater than the neuroprotective effect of edaravone used at clinical concentrations and independent of edaravone.

On the other hand, hypoxia-induced neurotoxicity was aggravated by mild hyperthermia. However, edaravone used at clinical concentrations conferred a significant neuroprotective effect; suppressed the deleterious effect of mild hyperthermia, and maintained the survival of neurones at the same level as with normothermia. These experimental findings might be clinically important as stroke patients typically experience a range of brain and body temperatures.

A limitation of this experiment is that the neuroprotective effect of cortical neurones *in vitro* may not necessarily correlate with their neuroprotective effect *in vivo*. Another limitation is that the response of rat fetal neurones to experimental conditions does not always mirror the response of adult neurones under the same conditions. Cultures of embryonic and fetal mammalian CNS neurones have been used in many experiments because of their reliability. However, the *in vitro* maintenance of neurones of adult mammals has hitherto been largely unsuccessful.

Edaravone, a potent free-radical scavenger, has been used in clinical practice during the early phase of acute stroke to reduce neuronal damage. Acute traumatic, ischaemic, or hypoxic injuries to the CNS provoke a cascade of biochemical events that result in a prolonged secondary injury to neurones adjacent to the site of the local injury. Excessive stimulation of excitatory amino acid receptors in these pathological conditions might trigger the production of free radicals such as superoxide anion (O_2^-). Superoxide is rapidly converted to a hydroxyl radical ($-OH^-$) in the presence of Fe^{2+}. Moreover, superoxide reacts with nitric oxide to produce peroxynitrite ($ONOO^-$). Peroxynitrite is protonated to form peroxynitrous acid, an unstable species. In shock, inflammation, oxidative stress, and the formation of nitric oxide (NO), $ONOO^-$, and superoxide radicals could produce neuronal cellular damage.[20] Therefore, scavenging these free radicals would attenuate neuronal injury and improve the outcome of cerebral ischaemia.

Previous studies have shown that edaravone can eradicate the hydroxyl radical ($-OH^-$) and inhibit $-OH^-$-dependent and $-OH^-$-independent lipid peroxidation.[21] In addition, edaravone inhibits both water-soluble and lipid-soluble peroxyl radical-induced peroxidation systems, non-enzymatic lipid peroxidation,[22] and lipoxygenase and NO-induced activation of mitogen-activated protein (MAP) kinases.[23] Other mechanisms by which edaravone might offer neuroprotective effects are by reducing Ca^{2+} overload,[24] regulating NO synthase (NOS), and improving cerebral blood flow by upregulating eNOS expression and downregulating nNOS and iNOS expression.[25]

In the present study, high doses of edaravone (5 and 50 μM) appreciably improved the mean survival of hypoxic cortical neurones cultured at normothermia, demonstrating a neuroprotective effect against hypoxic insult. The concentrations of edaravone used in this study ranged between 5 nM and 50 μM; these concentrations are comparable with the serum concentrations observed in patients who receive edaravone i.v. for the treatment of acute embolic strokes.[26,27]

It is well known that neuroprotective mechanisms of hypothermia include reductions in CMR, excitatory neurotransmitter release, and intracellular Ca^{2+} influx. The degree of neuroprotection that mild hypothermia confers against hypoxia has been extensively studied.[1,2,17,28–30] In this study, considerable neuroprotection was elicited at mild hypothermia. This finding is compatible with many previous *in vivo* and *in vitro* studies demonstrating the extremely deleterious effects of normothermia compared with that of hypothermia during hypoxic insults.[2,28] We have previously demonstrated the advantages of mild hypothermia in reducing neuronal death; we also suggested that hypothermic injury associated with profound hypothermia might possibly be caused by enhanced glutamate- and NMDA-induced excitotoxicity.[2] Moreover, compared with profound and deep hypothermia, mild hypothermia could easily be administered in patients as it has fewer adverse effects. Therefore, we performed these experiments at 32°C, as the lowest temperature that we could clinically use for hypothermic therapy,[18] instead of at deep and profound hypothermia.

Although a number of reports have validated the neuroprotective benefits of mild hypothermia,[1,2,28] a fair number of studies have exhibited contradictory findings as well.[31–33] Our results clearly indicate that cooling to mild hypothermia (32°C) confers considerable protection to neurones exposed to prolonged hypoxia.

We have previously shown using the *in vitro*[28,34] studies that the combined use of anaesthetic drugs and hypothermia enhances neuroprotection, more than what would occur if either were applied individually during prolonged hypoxia. Moreover, we have demonstrated that maximal neuroprotection during exposure to 24 h of hypoxia was achieved with a clinically relevant combination of hypothermia (32°C) and thiopental. The survival of cultured neurones subjected to this combination was approximately 90% and was significantly higher than the survival offered by either of these two agents alone. Similarly, we expected that the combined administration of mild hypothermia and edaravone would show additive neuroprotective effects as the neuroprotective mechanism of each is independent of the other. Referring to the administration of edaravone, a few reports have disclosed adverse effects in patients such as acute renal failure and fulminate

hepatitis. In consideration of these adverse effects, the use of edaravone in extremely high doses might not be clinically useful.[35 36]

The pattern for thiopental-induced neuroprotection was similar to that of edaravone at the same temperature. We hypothesize that the combined use of mild hypothermia and low-dose edaravone might confer considerable neuroprotection without causing undesirable effects. Surprisingly, the current study failed to demonstrate an additional increase in cortical neurone survival with mild hypothermia even at the highest dose of edaravone. It is difficult to determine why—in contrast to thiopental—edaravone failed to enhance the neuroprotection conferred by mild hypothermia. One reason might be that, in order to elicit neuroprotective effects as reported by Wu and colleagues[24] and Ikegami and colleagues,[37] edaravone might be needed at higher concentrations *in vitro* than is appropriate for use clinically. Based on the present findings, the neuroprotective effect of mild hypothermia is much greater than that of edaravone, and neuroprotection by edaravone is too small to augment the protective effects of mild hypothermia. Another reason might be that thiopental has several mechanisms for neuroprotection—other than as a free-radical scavenger—such as a reduction in CMR, Na^+ channel blockade, glutamate receptor blockade, inhibition of Ca^{2+} influx, or potentiation of GABAergic activity.[38–40]

The cerebral cortex is a brain region that is very sensitive to temperature manipulation.[41] Our results also show that the survival of neurones after exposure to hypoxia at mild hyperthermia and without the administration of edaravone (39°C) was significantly lower than that of neurones exposed to these same conditions at normothermia. Temperature-dependent neuronal death has been attributed to the release of glutamate, intracellular accumulation of Ca^{2+}, Zn^{2+} translocation, and the production of free radicals.[9]

In our experiments, edaravone administered during mild hyperthermia and at higher doses of 5 and 50 μM markedly improved the survival of cultured cortical neurones, and at normothermia. These experimental findings may be clinically important as stroke patients might present with variable body temperatures.

To our knowledge, this is the first report that compares the protective effect of different temperatures at various concentrations of edaravone on hypoxia-induced neurotoxicity in cultured cortical neurones. Although edaravone did not demonstrate an enhanced neuroprotective effect at mild hypothermia, this novel free-radical scavenger showed significant neuroprotection at both normothermia and mild hyperthermia. The induction of hypothermia requires special equipment[42] and a time frame to attain a particular hypothermic temperature. In contrast, edaravone can be administered quite easily and has a relatively wider therapeutic time window. Zhang and colleagues[22] reported that even though edaravone administration was delayed until 6 h after reperfusion, it nevertheless significantly improved neurological function and reduced the infarct volume. Initial therapy with edaravone might extend the therapeutic window until mild hypothermia can be administered. In conclusion, the temperature of the CNS is a potential factor in determining whether edaravone confers a neuroprotective effect when applied during prolonged hypoxic insult.

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Pregnancy Does Not Enhance Volatile Anesthetic Sensitivity on the Brain

An Electroencephalographic Analysis Study

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ABSTRACT

Backgrounds: Parturients are thought to be more sensitive to inhalational anesthetics because their minimum alveolar concentration is decreased. However, this conventional theory may be wrong, because, according to recent animal studies, minimum alveolar concentration indicates anesthetic effect on the spinal cord but not on the brain. The aim of this electroencephalographic study was to investigate the differences in the hypnotic effect of sevoflurane on parturients and nonpregnant patients.

Methods: Fifteen parturients undergoing cesarean section and 15 patients undergoing elective gynecologic surgery were enrolled. Anesthesia was induced with 4 mg/kg thiopental, 2 μg/kg fentanyl, and 2 mg/kg suxamethonium or 0.15 mg/kg vecuronium. Anesthesia was maintained with sevoflurane and fentanyl. The electroencephalographic signals, obtained from the bispectral index monitor, were recorded on a computer. We calculated 95% spectral edge frequency, amplitude, and bicoherence using custom software (Bispectrum Analyzer for bispectral index). After confirming that end-tidal sevoflurane had reached equilibrium, we measured electroencephalographic parameters of sevoflurane at 2.0 and 1.5% during surgery and at 1.0 and 0.5% after surgery.

Results: With the decrease of end-tidal sevoflurane concentration from 2.0 to 0.5%, 95% spectral edge frequency, amplitude, bispectral index, and bicoherence values changed dose-dependently in pregnant and nonpregnant women ($P < 0.0001$). However, there were no significant differences in those electroencephalographic parameters in pregnant and nonpregnant women.

Conclusions: This electroencephalographic study has shown that pregnancy does not enhance hypnotic effect of sevoflurane. These results suggested that the decrease in minimum alveolar concentration during pregnancy does not mean an enhanced volatile anesthetic effect on the brain.

What We Already Know about This Topic

- Minimum alveolar concentration (MAC) is decreased during pregnancy
- The incidence of intraoperative awareness is increased during cesarean section.

What This Article Tells Us That Is New

- No differences in electroencephalographic measures during sevoflurane anesthesia were found between end-term pregnant and nonpregnant patients.
- MAC may not be a correlate of anesthetic depth.

THE application of light general anesthesia has been encouraged in cesarean section to avoid neonatal depression and uterine atony,[1] because the supposition has been that minimum alveolar concentration (MAC) decreases during pregnancy. In 1974, Palahniuk and Shnider[2] found that MAC of halothane, methoxyflurane, and isoflurane in pregnant ewes decreased by 25–40% compared with that in nonpregnant ewes. From this finding, they proposed that parturients require a smaller amount of volatile anesthetics than do nonpregnant women. Thereafter, a 30% decrease in MAC of volatile anesthetics was identified in first trimester parturients.[3] The incidence of intraoperative awareness during cesarean section has been reduced by the improvement of anesthesia technique.[1] However, the incidence of intraoperative awareness during cesarean section performed under general anesthesia is still 0.4%,[4] which is

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higher than the rate in nonpregnant women undergoing general anesthesia for surgeries (0.2%).[5] Thus, patients undergoing cesarean section may have increased risk of intraoperative awareness, as occurs in patients having cardiac surgery and trauma surgery. However, it remains unknown why intraoperative awareness occurs commonly in parturients despite the fact that their MAC is low and anesthetic sensitivity is high.

MAC still does indicate anesthetic efficacy, but for movement,[6] however, this established theory has recently been challenged. Rampil *et al.*[7] showed no change in MAC before and after the removal of the forebrain in mice. Antognini *et al.*[8,9] reported a MAC of 0.8% in goats that had been administered isoflurane in the lower body using separate extracorporeal circulation, but a MAC of 2.9% (*i.e.*, greater than 3-fold increase) when isoflurane had been selectively given to the brain. These results suggest that the anesthetic efficacy indicated by MAC mainly reflects its effect on the spinal cord, not on the brain. Therefore, it is likely that MAC is not a good indicator of unconsciousness or amnesia. If the sensitivity to inhalational anesthetics on the brain is not enhanced by pregnancy, current general anesthetic procedures in cesarean section should be reviewed.

According to the current definition of anesthetic depth, anesthesia consists of hypnosis and analgesia.[10] Intravenous and inhalational anesthetics induce hypnosis, whereas opioid and local anesthetics induce analgesia.[10] Electroencephalographic monitoring techniques during anesthesia, such as the bispectral index (BIS), are considered an indicator of the hypnotic effect of volatile and intravenous anesthetics on the brain.[10] In patients who are awake, the electroencephalogram usually consists of low-amplitude fast waves. Clinical doses of volatile anesthetics induce dose-dependent decreases in the electroencephalogram frequency and the BIS and dose-dependent increases in the amplitude and bicoherence as a result of phase consistency (*i.e.*, enhanced synchrony).[11–13] In this study, electroencephalograms were obtained from parturients during cesarean section and from nonpregnant women during gynecological abdominal surgery at 2.0 to 0.5% sevoflurane expiratory concentrations. By comparing the electroencephalographic parameters in the two groups, we investigated whether a decreased MAC in parturients indicates an enhanced anesthetic effect on the brain or not.

Materials and Methods

Patients

The subjects were 15 full-term pregnant patients (aged 23 to 38 yr) who underwent a scheduled cesarean section under general anesthesia (pregnant group) and 15 patients (aged 21 to 41 yr) who underwent a scheduled gynecological surgery (nonpregnant group). All of the patients gave written informed consent and were approved by the institutional review board (Osaka University Hospital, Suita, Osaka, Japan). All of the patients had an American Society of Anesthesiologists physical status of I or II. Patients with a history of mental or neurologic disorders and patients treated with central nervous system drugs, such as sedatives and antidepressants, were excluded. Patients with multiple-fetus pregnancies, placenta previa, or other complications were also excluded. The pregnant group consisted of seven patients with idiopathic thrombocytopenia purpura: five were treated with steroids before surgery; four were given heparin for thrombosis in the lower extremity before surgery; two refused spinal anesthesia; and two patients has a history of spinal surgery. The nonpregnant group consisted of seven patients undergoing myomectomy, five patients undergoing ovarian cystectomy, and three patients undergoing hysterectomy. Patients who underwent laparoscopic surgery were excluded. For intraoperative monitoring, we used electrocardiogram, noninvasive blood pressure, pulse oximetry, and a respiratory monitor (M2360A; Hewlett Packard, Palo Alto, CA).

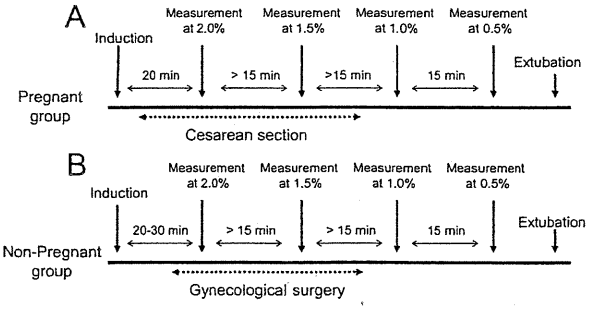

Fig. 1. A schematic summary of the study protocol in pregnant group (*A*) and nonpregnant group (*B*). Electroencephalographic measurements at sevoflurane concentration of 2.0 and 1.5% were done during surgery and at 1.0 and 0.5% after surgery.

Anesthetic Protocols

Pregnant Group (Cesarean Section). No premedication, except 150 mg of ranitidine orally the night before and on the morning of surgery, was administered. After preoxygenation, rapid-sequence induction with cricoid pressure was performed using 4 mg/kg thiopental, 2 μg/kg fentanyl, and 0.15 mg/kg vecuronium, or 2 mg/kg suxamethonium. After tracheal intubation, the patient was placed on mechanical ventilation with sevoflurane at an end-tidal concentration of 3% in 4 l of air and 2 l of oxygen. End-tidal carbon dioxide concentration was maintained between 30 and 35 mmHg. Immediately after tracheal intubation, cesarean section was started. After tracheal intubation, 3.0% sevoflurane was administered for 10 min, followed by 2 to 2.5% sevoflurane for 10 min to maintain end-tidal sevoflurane concentration at 2.0%. After that, 1.5 to 2.0% sevoflurane was administered to maintain end-tidal sevoflurane concentration at 1.5% for 15 min. The electroencephalographic parameters were recorded at the end of the 2.0 and 1.5% sevoflurane administration periods (fig. 1A). Nitrous oxide was not given. Patients who were given suxamethonium were administered 4 to 6 mg vecuronium after delivery. Immediately after delivery, 5 units of intravenous oxytocin and 2 μg/kg intravenous

fentanyl was administered for 5 min. We also administered 1 μg/kg additional fentanyl every 30 min during operation. Patients with hypotension were treated with intravenous ephedrine. After completion of surgery, we administered sevoflurane at expiratory concentrations of 1.0 and 0.5% for 15 min at each concentration (fig. 1A). Electroencephalographic parameters were recorded at the end of the 1.0 and 0.5% sevoflurane administration periods. Sevoflurane administration protocol was obtained from computer simulation using the software Gas Man® (Med Man Simulations, Boston, MA).

Nonpregnant Group (Gynecological Surgery). No premedication, except 150 mg of ranitidine orally the night before and on the morning of surgery, was administered. After establishing an intravenous route, 4 mg/kg thiopental, 2 μg/kg fentanyl, and 0.15 mg/kg vecuronium were administered for induction of general anesthesia. After tracheal intubation, sevoflurane, fractional inspired oxygen tension, and end-tidal carbon dioxide were maintained in the same manner as the cesarean section group. Surgery was started 10 to 15 min after tracheal intubation in all patients. We administered 2 μg/kg fentanyl before incision, and an additional 1 μg/kg fentanyl was given every 30 min during the operation. Electroencephalographic parameter recording was also performed at the end of the 2.0 and 1.5% sevoflurane administration period (fig. 1B). Patients with hypotension during surgery were treated with intravenous ephedrine. After completing surgery, sevoflurane was administered at an expiratory concentration of 1.0 and 0.5% for 15 min (fig. 1B). Electroencephalographic parameters were recorded at the end of each sevoflurane administration period.

Electroencephalographic Monitoring

For electroencephalographic recordings, we used the BIS® A-1050 monitor (Aspect Medical Systems, Natick, MA). Three-point electroencephalographic sensors were attached to the forehead. Automatic electrode impedance check was done in all subjects. Raw data, including electroencephalographic waveforms, BIS, and other parameters, were obtained from the BIS A-1050 monitor *via* a RS232 cable connected to a laptop computer and analyzed with custom software (Bispectrum Analyzer for BIS).[12,13] Using this software, we calculated the 95% spectral edge frequency (SEF95), amplitude, and bicoherence. Bicoherence is an indicator of electroencephalographic synchrony, and volatile anesthetics are known to increase the peak heights of bicoherence at 3–5 Hz (pBIC-low) and 5–10 Hz (pBIC-high) in a dose-dependent manner.[12,13] In this study, to evaluate the difference in response to sevoflurane, we compared the changes in electroencephalographic parameters (SEF95, amplitude, BIS, and bicoherence) in the pregnant and nonpregnant women at sevoflurane concentrations of 2.0 to 0.5%.

Statistical Analysis

A pilot study was performed in 10 patients (n = 5 for each group). The pilot study showed that BIS has a larger SD (50 ± 9 at 1.5% sevoflurane in nonpregnant group) than the other parameters (SEF and amplitude). Based on BIS data from the pilot study, a sample size of 13.75 in each group was considered to have 80% power to detect a difference in means of 20% (specifically, because BIS value mean in nonpregnant women was 50, the difference in mean was 10), assuming that the common SD was 9 using a two-group *t* test with a 0.05 two-sided significance level. Consequently, the number of subjects was specified to 15 patients per group. The patient characteristics, including age, height, weight, surgical time, and hemodynamic data, were compared using an unpaired *t* test. SEF95, amplitude, BIS value and bicoherence (pBIC-low and pBIC-high) at 2.0% to 0.5% concentrations of sevoflurane were analyzed by a two-way analysis of variance. The model included the main effects, group (pregnant/nonpregnant), and sevoflurane concentration (0.5, 1, 1.5, or 2%), and their interaction. These electroencephalographic parameters in the two groups were also compared using an unpaired *t* test. A *P* value of less than 0.05 was considered statistically significant (two-sided). All statistical analysis were performed by SAS Release 9.2 (SAS Institute Inc., Cary, NC).

Table 1. Patient Characteristics

Characteristic	Pregnant Group (n = 15)	Nonpregnant Group (n = 15)	*P* Value
Age (yr)	31 ± 6	32 ± 4	0.52
Height (cm)	160 ± 5	159 ± 5	0.72
Weight (kg)	64.5 ± 7.0	58 ± 8	0.78*
(Weight before pregnancy (kg))	(59 ± 8)	—	—
Gestational age (wk)	39 ± 0.4	—	—
Surgical time (min)	71 ± 17	77 ± 23	0.43

Data are expressed as mean ± SD.

* *P* value between weight before pregnancy in pregnant group and weight in nonpregnant group.

Results

The patient's characteristics are shown in table 1. No significant differences between the pregnant and nonpregnant groups were found in age, height, and nonpregnant weight. Maternal and neonatal data are shown in table 2. Table 3 shows the hemodynamic data in each group. In the pregnant group, heart rate was significantly higher than in the nonpregnant group ($P < 0.01$) during study period. The average ephedrine doses for treatment of hypotension in the nonpregnant and pregnant groups were 6.3 ± 2.5 (n = 4) and 7.0 ± 2.7 mg (n = 5), respectively. Seven parturients were given suxamethonium at induction of general anesthesia.

Typical electroencephalographic waveforms of the two groups are shown in figure 2. There was no problem in the quality of electroencephalographic signals in the two groups. The signal quality index was 0.8 or higher. Reducing the sevoflurane concentration in 0.5 percentage point increments (from 2.0 to 0.5%) decreased the electroencephalographic amplitude and increased the electroencephalographic frequency in both groups.

Table 2. Maternal and Neonatal Data

Maternal Data	n = 15
Uterine Incision Delivery Time (s)	91 ± 43
Blood Loss with Amniotic Fluid (ml)	1,130 ± 480
Umbilical A	
pH	7.32 ± 0.07
Pao_2 (mmHg)	27 ± 8.6
$Paco_2$ (mmHg)	52 ± 4.3
Base Excess (mM)	−1.1 ± 1.7
Apgar scores at 1 min	
8–10	10
<8	5
Apgar scores at 5 min	
8–10	13
<8	2

Data are expressed as mean ± SD except for Apgar scores.

Table 4 shows the electroencephalographic parameters and the differences between the two groups at 2.0, 1.5, 1.0, and 0.5% sevoflurane concentrations. Figures 3, 4, and 5 show the changes in SEF95, amplitude, and BIS value at 2.0 to 0.5% sevoflurane concentrations, respectively. In the pregnant group, the reduction in sevoflurane concentration in 0.5 percentage-point decrements from 2.0 to 0.5% caused changes in the frequency, the BIS, and the amplitude. To be specific, SEF95 increased from (mean ± SD) 13.8 ± 2.2 to 19.7 ± 2. 6 Hz, the BIS increased from 40.7 ± 6.7 to 79.2 ± 6.2, and the amplitude reduced from 14.6 ± 2.8 to 7.6 ± 0.7 μV. In addition, in the nonpregnant group, the SEF95 increased from 13.4 ± 1.5 to 21.0 ± 2.7 Hz, the BIS increased from 37.8 ± 5.6 to 82.4 ± 6.7, and the amplitude reduced from 15.1 ± 2.4 to 7.1 ± 0.8 μV.

pBIC-low and pBIC-high (which indicate electroencephalographic synchrony) were 33.3 ± 7.7 and 37.9 ± 7.3, respectively, at 2.0% sevoflurane concentration in the pregnant group. They decreased to 20.7 ± 5.9 and 19.6 ± 6.2, respectively, at 0.5%. In the nonpregnant group, pBIC-low and pBIC-high were 36.4 ± 9.2 and 40.8 ± 6.8 at 2.0% sevoflurane concentration. This decreased to 17.3 ± 6.3 and 20.2 ± 6.2, respectively, at 0.5%.

The results of two-way analysis of variance showed that sevoflurane concentration effect was significant ($P < 0.0001$) for each electroencephalographic parameter (table 5). However, the group effect (pregnant/nonpregnant) and the interaction were not significant for each electroencephalographic parameter (table 5). The unpaired t test also showed that electroencephalographic parameters in the pregnant and nonpregnant groups at each sevoflurane concentration were not significantly different (table 4). These results imply that each electroencephalographic parameter changed dose-dependently according to sevoflurane concentration but was unaffected by pregnancy.

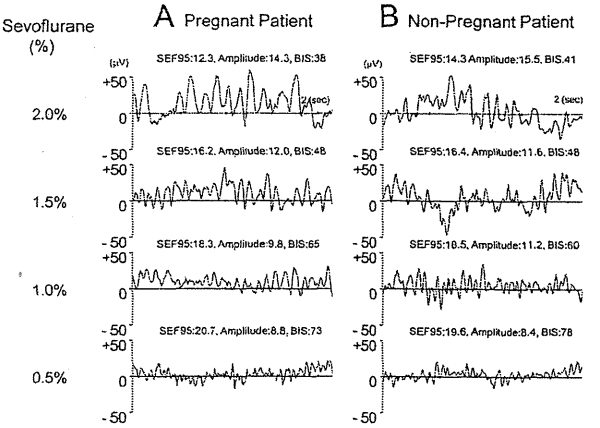

Fig. 2. The typical electroencephalographic wave forms at 2.0, 1.5, 1.0, and 0.5% sevoflurane concentration in the pregnant (*A*) and nonpregnant (*B*) groups. The reduction in sevoflurane concentration from 2.0 to 0.5% changes electroencephalograms from a high-amplitude slow wave to a low-amplitude fast wave. BIS bispectral index; SEF95 = 95% spectral edge frequency.

A BIS value greater than 60 at 1.5% sevoflurane concentration was observed in two patients (one each from the pregnant and nonpregnant groups). A BIS value greater than 60 at 1.0% sevoflurane concentration was observed in 10 patients from the nonpregnant group and 8 patients from the pregnant group. The patients from the two groups were interviewed on surgery day and the following day but none had intraoperative memory.

Discussion

During non–rapid eye movement sleep, as the stages of sleep progress, the electroencephalogram pattern changes from a low-amplitude fast wave to a high-amplitude slow wave.[14]

Table 3. Hemodynamic Data

Groups	Sevoflurane (%)				
	Control	2.0	1.5	1.0	0.5
Pregnant (n = 15)					
MBP (mmHg)	85.8 ± 8.2	95.3 ± 12.0	84.3 ± 10.3	81.0 ± 8.5	83.4 ± 12.3
HR (beats/min)	88.3 ± 16.7*	93.2 ± 14.3*	92.2 ± 13.3*	87.3 ± 10.2*	88.3 ± 11.2*
Nonpregnant (n = 15)					
MBP (mmHg)	87.2 ± 11.8	97.2 ± 11.5	85.3 ± 12.8	80.3 ± 7.8	80.2 ± 10.4
HR (beats/min)	73.2 ± 11.3	77.3 ± 13.5	72.3 ± 11.7	69.5 ± 10.5	71.5 ± 12.0

Data are mean ± SD.

* Significant difference from nonpregnant group ($P < 0.01$).

HR = heart rate; MBP = mean blood pressure.

Table 4. The Electroencephalographic Parameters and the Differences between Two Groups at 2.0, 1.5, 1.0, and 0.5% Sevoflurane Concentrations

Parameters and Sevoflurane Concentration	Pregnant Group (n = 15)	Nonpregnant Group (n = 15)	Difference	*P* Value
SEF95				
2%	13.8 ± 2.2Hz	13.4 ± 1.5Hz	−0.5	0.515
1.5%	16.1 ± 1.9Hz	16.5 ± 2.3Hz	0.4	0.571
1.0%	18.3 ± 3.0Hz	19.8 ± 2.3Hz	1.6	0.122
0.5%	19.7 ± 2.6Hz	21.0 ± 2.7Hz	1.4	0.179
Amplitude				
2%	14.6 ± 2.8μV	15.1 ± 2.4μV	0.5	0.579
1.5%	12.1 ± 2.0μV	12.7 ± 2.1μV	0.5	0.489
1.0%	9.5 ± 2.2μV	9.1 ± 1.4μV	−0.4	0.538
0.5%	7.6 ± 0.7μV	7.1 ± 0.8μV	−0.4	0.123
BIS				
2%	40.7 ± 6.7	37.8 ± 5.6	−2.9	0.211
1.5%	48.5 ± 8.4	50.2 ± 7.4	1.8	0.544
1.0%	59.5 ± 8.9	62.4 ± 5.0	2.9	0.277
0.5%	79.2 ± 6.2	82.4 ± 6.7	3.3	0.180
pBIC-Low				
2%	33.3 ± 7.7	36.4 ± 9.2	3.1	0.313
1.5%	30.3 ± 8.8	35.1 ± 10.9	−0.1	0.266
1.0%	24.6 ± 6.8	24.5 ± 9.7	4.8	0.969
0.5%	20.7 ± 5.9	17.3 ± 6.3	−3.3	0.142
pBIC-High				
2%	37.9 ± 7.3	40.8 ± 6.8	3.0	0.253
1.5%	37.8 ± 7.0	41.6 ± 6.9	3.8	0.146
1.0%	28.3 ± 12.0	29.3 ± 10.3	1.0	0.816
0.5%	19.6 ± 6.2	20.2 ± 6.2	0.5	0.842

Data are expressed as mean ± SD. There were no significant differences between the groups as determined by unpaired *t* test.
BIS = bispectral index; pBIC-high = peak heights of bicoherence at 5–10 Hz; pBIC-low = peak heights of bicoherence at 3–5 Hz; SEF95 = 95% spectral edge frequency.

Patients given volatile anesthetics at clinical concentration also reveal a similar electroencephalographic pattern. Therefore, the level of hypnosis can be presumed from these anesthetic-induced electroencephalographic changes.[10] From this aspect, electroencephalogram is thought to be a reliable monitor of the hypnotic effects of anesthetics.

In our study, values for the electroencephalographic parameters SEF95, amplitude, BIS, and bicoherence changed in a dose-dependent manner in the 2.0% to 0.5% sevoflurane concentration range in both the pregnant and nonpregnant groups. If pregnancy enhances the hypnotic effect of volatile anesthetics, electroencephalographic parameters of the pregnant group at respective sevoflurane concentrations would be expected to change more significantly than in the nonpregnant group. However, no significant differences were found in the SEF95, amplitude, BIS, and bicoherence values in the two groups.

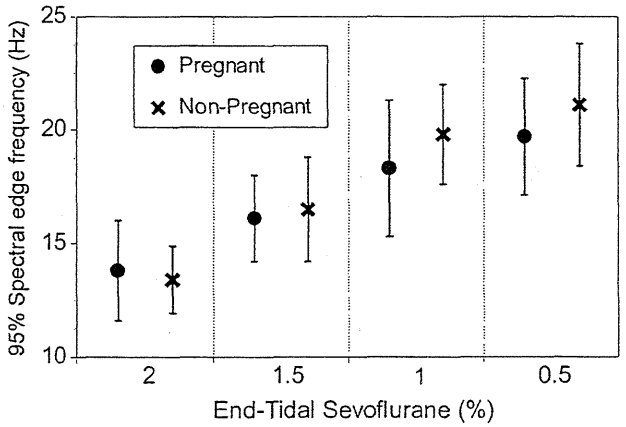

Fig. 3. 95% Spectral edge frequency at 2.0, 1.5, 1.0, and 0.5% sevoflurane concentrations in the pregnant and nonpregnant groups. Data are mean values with SD. There was no significant difference between groups.

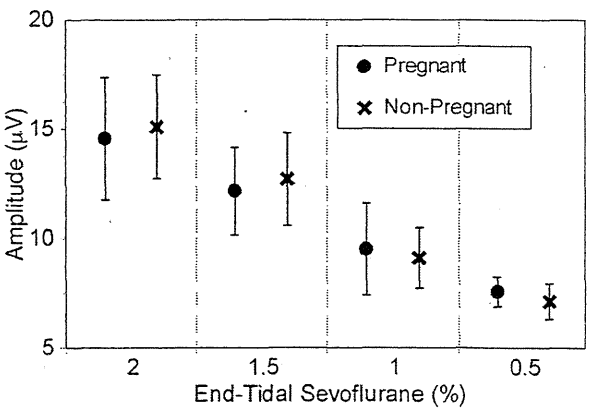

Fig. 4. Amplitude at 2.0, 1.5, 1.0, and 0.5% sevoflurane concentrations in the pregnant and nonpregnant groups. Data are mean values with SD. There was no significant difference between groups.