

図1. マイクロサンプリングプローブ™ BC-401Cの外観。外筒(矢頭)と内筒先端の吸収性ロッド(矢印)。



図2. マイクロサンプリング施行時のX線透視像。

実際には通常通りの前処置、キシロカイン噴霧の局所麻酔により検査を開始し、従来からの各種手技(BAL、気管支擦過、TBLBなど)に先立ちBMS法を行っている。これはELF検体への血液混入などを避けるためである。具体的には気管支鏡チャンネルから外筒ごとBMSプローブを挿入し、目的とする部位で内筒を押し進め、ファイバーロッドを気道粘膜に密着させ5~10秒間静止させる。内筒シャフトはステンレス製のためX線透視下にプローブの位置を確認する(図2)。アングルの強いB<sub>1</sub>やB<sub>6</sub>への挿入は困難な場合がある。先端ロッドを外筒内に引き込むことによってチャンネル内での検体のコンタミネーションを防止することができる。ロッドに吸収させたELFは生理食塩水などに抽出させ液性因子を解析する。

採取したELFの重量を測定する場合には、ELFを吸

表2. マイクロサンプリング法の長所と問題点

長所
1) 侵襲性が低く、重症呼吸不全症例でも施行可能
2) 軽時的に反復して施行可能
3) 感染播種などの現病悪化を生じにくい
4) ELFを原液で採取でき定量的評価が可能
5) 任意気道レベルでのELF採取が可能
6) 採取する区域を選択し限局性肺病変に応用可能
問題点
1) 末梢肺野領域の病態を把握できるか?
2) 炎症性細胞の解析が困難
3) ELF採取量が少ない
4) アングルの強いB <sub>1</sub> 、B <sub>6</sub> などの部位には挿入しにくい

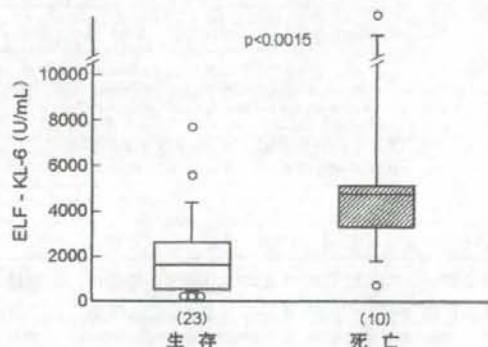


図3. ARDS患者発症時のELF中KL-6値と予後(文献6より一部改変)。

収したプローブを凍結させその状態で湿重量を測定する。生理食塩水で攪拌・抽出させた後にプローブを24時間以上乾燥させ乾燥重量を測定する。湿重量と乾燥重量の差として吸収されたELFの重量が求められる。

また、シャフトの付いたロッドをスピッツ内に固定し、スピッツ自体を遠心すると生理食塩水で希釈することなくELFを回収することができる。われわれはこの方法により得られた高濃度ELFを用いてプロテオーム解析を行っている<sup>5</sup>。

BALと比較した時のBMS法の長所、問題点を表2に列記した。

#### 臨床症例でのBMS法の応用例

以下に臨床例でのBMS法の応用について概略を示す。

##### 1) Acute respiratory distress syndrome (ARDS)

ARDSは敗血症などの重症感染症などを誘因として

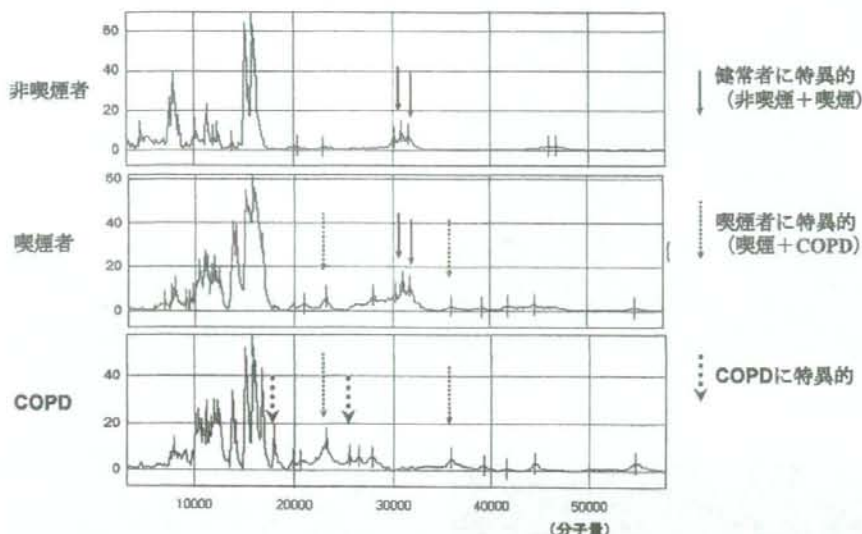


図4. COPD患者ELFのプロテオーム解析. SELDI-MS法 (SAX2)によるELF中蛋白の比較 (文献8より一部改変).

発症する透過亢進型肺水腫であり、依然としてその死亡率は高い。ARDSにおける病態の解明、重症度・治療効果の把握などの目的で当初よりBMS法は開発されてきた。低酸素血症を呈するARDS症例においてBALよりも侵襲の低いBMS法を繰り返し施行することによりいくつかの知見が得られている。ELF中の好中球エラストラーゼ、IL-6、VEGF、KL-6、HMGB1などが上昇していることが判明した<sup>20</sup>。また、興味深いことにARDS患者のうち生存者と死亡者の発症時のELF中KL-6を比較すると死亡者で有意に高く、ARDSの予後を予測しうることも判明した (図3)<sup>9</sup>。

## 2) COPD (Chronic obstructive pulmonary disease) に対する応用

COPDにおける気道炎症のメカニズムには不明な点が多く、臨床経過を反映するバイオマーカーも明らかとなっていない。さらに、有効な抗炎症薬の開発が待たれる点からも、ELFを用いた研究の発展が望まれる。

Komakiら<sup>7</sup>は、BMS法を用いて採取したCOPD患者と健常者のELF中のサイトカイン濃度とxanthine oxidase (XO)活性を比較した。その結果、TNF- $\alpha$ およびIL-1 $\beta$ 濃度はXO活性と相関し、いずれもCOPD患者で健常者よりも高値であった。さらにXO活性は%FEV<sub>1.0</sub>と負の相関を示し、XOによるスーパーオキシド産生の気道炎症との関連が示唆された。

図4にはBMS法を用い、COPD患者、健常喫煙者およ

び非喫煙者の末梢気道よりELFを採取し、SELDI-MS法によりプロテオーム解析を行った結果を示した<sup>8</sup>。Preliminaryな結果ではあるが、BMS法により採取した検体のSELDI-MS法による解析により、喫煙により誘導される蛋白とCOPD発症にともない誘導される蛋白のピークが検出可能であることが示唆された。将来的には、気腫優位型、気道病変優位型などの病型との関連、ステロイド反応性など治療効果との関連などが検討可能になるものと期待される。

## 3) 末梢型肺腫瘍に対するBMS法の応用 ELF中腫瘍マーカー測定による補助診断

肺野小型孤立性陰影の発見頻度が増加しているが、肺癌と良性病変との鑑別は必ずしも容易ではない。確定診断のためには気管支鏡肺生検ならびに擦過細胞診などが行われているが、末梢性小型病変ではその診断率は高いとはいえない。一方、肺癌の補助診断には血液中の各種腫瘍マーカーの測定が有用であるが、これは腫瘍組織で大量のマーカーが産生されることによって血中での測定が可能になるためと考えられる。われわれはBMS法を肺癌の診断に応用し、高値を示すと予想される腫瘍組織あるいは腫瘍周囲組織中の腫瘍マーカーを測定し、病理診断の補助手段として診断率の向上を目指している<sup>9</sup>。

末梢肺腫瘍症例に対してX線透視下に腫瘍またはその近傍 (約2cm程度) からBMS法によりELFを採取し、腫瘍マーカー (CEA, CYFRA, SLXなど) をELISA

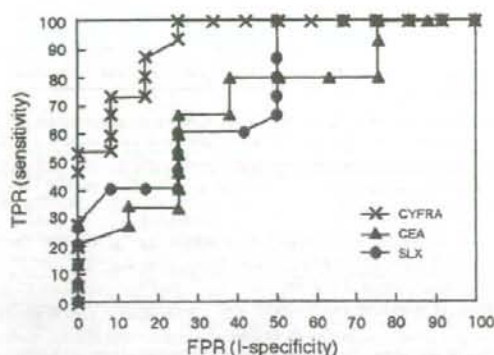


図5. 末梢肺癌例でのROC曲線 (Receiver operating characteristic curve). CYFRAおよびCEAで左肩上がりの至適曲線を示した (文献9より一部改変)。

法で測定した。腫瘍径が8~28 mm、平均16 mmの腺癌症例 (手術により肺腺癌の診断あり) に対してBMS法を行ったが、腫瘍マーカー測定では、腫瘍周囲と健常側のCEAとCYFRAはいずれも腫瘍側で上昇していた。腫瘍マーカー値よりROC曲線 (Receiver operating characteristic curve, 縦軸に真陽性率、横軸に偽陽性率をプロットしたもの) を描くとCYFRAおよびCEAで左肩上がりの至適曲線を示した (図5)。BMS法にともない出血、気胸などの合併症は認めなかった。今回のわれわれの肺腺癌症例の経験では腫瘍から周囲のELF中に放出された腫瘍マーカーをBMS法により検出し高値を示したと考えられ、特にELF中のCYFRA測定が末梢肺腺癌の補助診断として有効であった。

#### 4) ゲフィチニブ投与肺腺癌例でのELF中マーカー測定による抗腫瘍効果の評価

ゲフィチニブ投与症例で薬剤性肺障害の予知、抗腫瘍効果の評価を目的としてBMS法によるELF中各種マーカーの分析を行った。ゲフィチニブ投与前と投与後2週目の2回に腫瘍近傍などからELFを採取しCEA、KL-6、SP-A、VEGF、IL-8の各項目を測定した。

15例 (男性7、女性8、平均年齢68歳) を対象としてELFを採取したが、幸いなことに薬剤性肺障害の発症はなかったため、肺障害予知に関する知見は得られていない。ゲフィチニブの治療効果あり群 (CR-SD症例、9例) と効果なし群 (PD症例、6例) に分けて検討したところ、効果あり群でELF中のCEA、KL-6、SP-Aはいずれも有意に減少、効果なし群で有意に増加していた。一方、血清中ではCEAが効果あり群で減少したが、その他のマーカーは有意な変動を示さなかった。図6にELF中KL-6の変化を示した。また、VEGF、IL-8はELF中、

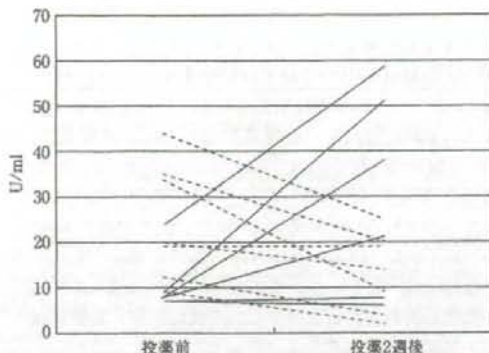


図6. 肺癌ゲフィチニブ投与例での投与前後のELF中KL-6値の変動。治療効果あり群 (点線, n=7) では有意に減少し、治療効果なし群 (実線, n=6) では上昇した。

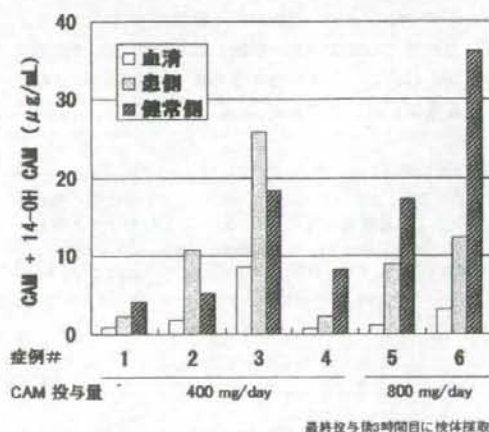


図7. クラリスロマイシン (CAM) 内服投与例でのELF中CAMおよび14-OH CAM濃度。ELF中濃度は800 mg投与例で高い傾向にあった。

血清中とも有意な変動を示さなかった。当初KL-6は腫瘍マーカーとして使用されていたが、BMS法による解析でもCEAのみならずKL-6、SP-AはいずれもELF中で血清中よりも肺癌の病勢 (治療効果) を鋭敏に反映していることが示され、興味深い知見と考えている<sup>10</sup>。

#### 5) 非結核性抗酸菌症での肺内抗菌剤濃度測定

肺感染症に対する抗菌剤治療を行う際には目標とする菌種に対して感受性を有する薬剤を投与することに加え、薬剤が肺組織中へ移行し十分な薬剤濃度を保つことも重要である。その評価法として肺組織、BAL液中など

の抗菌剤濃度測定が行われている。クラリスロマイシン (CAM) を投与中の非結核性抗酸菌症 (NTM) 症例で BMS 法による薬剤濃度測定を試みた。CAM 400 mg/日または 800 mg/日を 7 日間経口投与した 2 群で ELF 中の CAM および 14-OH CAM (代謝された活性型 CAM) 濃度を測定した (図 7)。両者の血清中濃度に差はなかったが、ELF 中濃度は 800 mg 投与で高い傾向にあった。また、ELF 中濃度は健常側で患側よりも高い傾向にあった。これらから、NTM 症では病巣部と健常部では薬物の肺組織移行が異なり、これが治療に影響をおよぼす可能性が示唆された。

#### まとめ

BMS 法の実際と各種肺疾患に対する応用例を示した。びまん性肺疾患のみならず肺腫などの限局性肺疾患でも本法は有用であった。BMS 法は特に高度な技術を必要とする手技ではないが、侵襲が低く重大な合併症の発生もないため広く各種肺疾患の診断、病態解析などに使用可能と考えられる。今後、様々な疾患での臨床応用が期待される気管支鏡手技である。

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[原 著]

ラット敗血症モデルに対する  
抗 HMGB1 療法の有効性に関する検討

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## ラット敗血症モデルに対する 抗 HMGB1 療法の有効性に関する検討

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### 要 旨

HMGB1は、エンドトキシンショックや出血性ショックによる臓器不全の発症における後期メディエーターとして注目されている。今回われわれは、重症敗血症に対する抗HMGB1抗体投与の有用性に関して検討した。盲腸結紮切断モデルラットを用いて、閉腹直後に抗HMGB1抗体を皮下投与した中和抗体投与群の10日生存率、血清HMGB1値をコントロール抗体投与群と比較した。さらに、モデル作製24時間後における盲腸、肺の病理組織学的検討を行った。中和抗体投与群においてモデル作製20時間後および32時間後の血清HMGB1濃度が有意に抑制され、盲腸および肺の両方において著明に炎症所見およびHMGB1の発現が抑制された。10日生存率は有意に改善した。HMGB1が、ラット敗血症の病態進展に関するkey mediatorであること、および、重症敗血症に対する抗HMGB1療法の有用性が明らかとなった。

索引用語：HMGB1, 敗血症, 盲腸結紮穿孔モデル, 抗HMGB1抗体, ラット

### はじめに

生体反応は、古典的な神経・内分泌系による制御に加え、サイトカインをはじめとするさまざまなメディエーターによって調節されている<sup>1,2</sup>。侵襲後の高サイトカイン血症により全身性炎症反応症候群 (systemic inflammatory response syndrome: SIRS) の病態が誘発され、それが持続してサイトカ

インバランスが破綻したとき、多臓器不全 (multiple organ dysfunction syndrome: MODS) や死へと至る<sup>1,2</sup>。近年、敗血症性ショック、急性肺障害、出血性ショックによる臓器不全発症における重要な後期メディエーターとしてHigh mobility group box chromosomal protein 1 (HMGB1) が注目されている<sup>3-7</sup>。HMGB1はDNA結合蛋白であり、重症病態下において、壊死細胞の核内から受動的に分泌

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される経路、および、活性化されたマクロファージや血小板から能動的に分泌される経路の二つの経路を介して血清中に出現し、Receptor for advanced glycation endproducts (RAGE) を介して炎症を増悪させたり細胞遊走を促進したりする作用を有する<sup>3-7</sup>。SIRS や MODS における HMGB1 の動態、病態生理への関与についてはこれまで不明であった。

われわれは、この点に着目し、消化器癌手術の中でも開胸開腹操作を要し、広範なリンパ節郭清を伴うため、極めて侵襲が高度である食道癌根治術における血清 HMGB1 濃度の臨床的意義について検討を行った<sup>8</sup>。その結果、高度な外科的侵襲によって血清 HMGB1 濃度が一過性に上昇し、合併症発症例では高値が遷延することが判明した<sup>8</sup>。術後の高 HMGB1 血症が食道癌術後合併症発症の一因となっている可能性が示唆され、周術期における抗 HMGB1 療法施行により、術後合併症発生を予防、軽減できる可能性に関する検討が期待された<sup>8</sup>。

消化器外科領域で高率に SIRS や MODS を呈する重症病態として、食道癌根治術等の高度侵襲手術後や、消化管穿孔による腹膜炎・敗血症等の重症腹腔内感染症が挙げられる<sup>9</sup>。食道癌根治術後の状態を動物モデルにて再現することは困難であるため、ヒトの敗血症に最も近い病態を再現できることが知られている盲腸結紮穿孔による腹膜炎・敗血症モデルラットを用いて SIRS 病態への HMGB1 の関与、および、HMGB1 中和による病態進展の予防・軽減効果について検討を行った<sup>10-12</sup>。

## 1. 材料・方法

Sprague-Dawley ラット (8 週齢, 雄, 250 ~ 300 g) を用いて、盲腸血流を温存した改良型盲腸結紮穿孔ラットを作製した。ケタミン (40 mg/kg i.m.) 麻酔下に正中切開にて 2 cm 開腹し、盲腸根部を盲腸壁のみ結紮し、盲腸先端に 5 mm の穿孔を加え、元の位置に盲腸を戻して、全層一層縫合にて閉腹した。閉腹直後に生理的食塩水を 5.0 mL/kg 皮下注した。抗生剤投与は行わなかった (図 1)。

ブタ甲状腺から抽出した HMGB1 を抗原とし、ニワトリに免疫して、卵黄から抗 HMGB1 ポリクローナル IgG を精製し、中和抗体を作製した<sup>13</sup>。

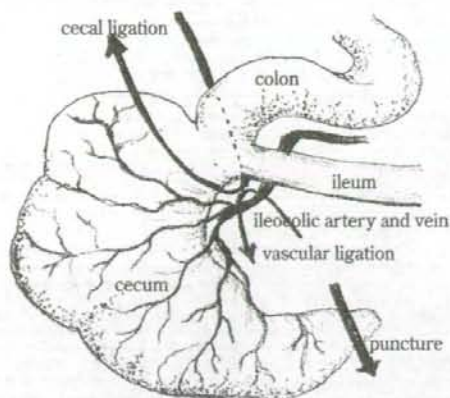


図 1 Modified cecal ligation and puncture model

モデル作製 15 分後に抗 HMGB1 抗体 10 mg/kg を皮下投与した群を中和抗体投与群 (n=11)、コントロール抗体 10 mg/kg を皮下投与した群をコントロール群 (n=11) とした。中和抗体投与量は、*in vitro* において 3 ng/mL の HMGB1 (コントロール群におけるモデル作製 24 時間以内の最高平均濃度) を 24 時間以内に完全に失活し得る最小限の量とした。4 時間ごとに 10 日目まで生存を観察した。術前、4, 8, 20, 32, 48 時間後、3, 4, 5, 6 日後の計 10 点にて頸静脈から 0.6 mL ずつ採血し、ただちに血清を分離して -80°C にて凍結保存し、血清 HMGB1 濃度を ELISA 法 (シノテスト社製) にて測定した<sup>14</sup>。さらに、中和抗体投与群 (n=3)、コントロール群 (n=3) を作製し、24 時間後に屠殺して盲腸、肺を摘出・固定した。盲腸の Hematoxylin-Eosin (H&E) 染色、HMGB1 免疫染色 (シノテスト社製マウス抗 HMGB1 モノクローナル抗体、ニチレイ社製ヒストファインシンプレステイン MAX-PO (M) キット使用)、および、肺の H&E 染色、Elastica-Masson (EM)、HMGB1 免疫染色を行い、病理組織学的検討を行った。

2 群間における生存率の比較は、Kaplan-Meier 法および log-rank 検定を用いて行った。各時点における血清 HMGB1 濃度の 2 群間比較は、Mann Whitney U 検定を用いて行った。いずれも  $p < 0.05$  をもって有意差とした。統計処理には、Statview 5.0 および SAS statistical analysis package (SAS Institute) を使用した。

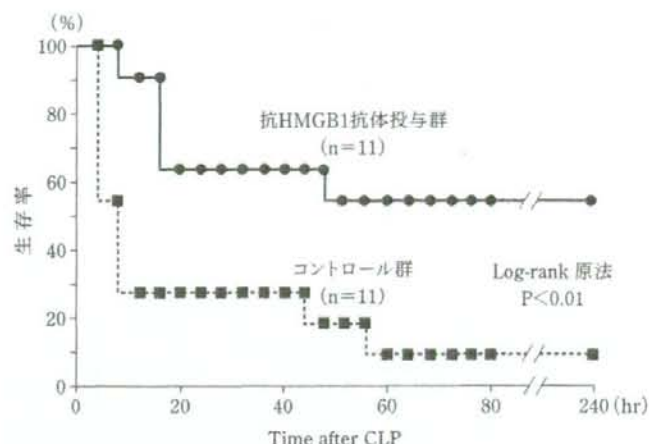


図2 Treatment with anti-HMGB1 antibodies significantly improved survival in CLP rats.

Data are shown as percentages of animals surviving (n=11 in each group). The whole period of follow up is compared with a Kaplan-Meier analysis and log rank test ( $p<0.01$ ), ●, anti-HMGB1 antibody-treated group; ■, controls.

なお、これらの動物実験は慶應義塾大学医学部倫理委員会の許可のもと、無用の苦痛を動物に与えないような手段を用いて施行した。

## II. 結 果

10日生存率はコントロール群と比較して中和抗体投与群において有意に改善した(中和抗体投与群; 55%, 6/11, コントロール抗体投与群; 9.1%, 1/11;  $p<0.01$ ) (図2)。

血清 HMGB1 濃度に関しては、中和抗体投与群においてコントロール群に比してモデル作製20時間後および32時間後に有意に抑制された ( $p<0.05$ ) (図3)。

コントロール群においてはモデル作製4時間後に著明な血清 HMGB1 濃度の上昇を認めた (n=11; median 2.7 ng/mL, interquartile range 0 ~ 11.5 ng/mL) のに対し、中和抗体投与群においてはあまり上昇しなかった (n=11; median 0.2 ng/mL, interquartile range 0 ~ 0.55 ng/mL) が、有意差は認めなかった。特に、モデル作製4時間後までに死亡したコントロール群のラットにおいては、血清 HMGB1 濃度が著明に高値であった (n=5; median 18.5 ng/mL, interquartile range 3.6 ~ 82 ng/mL)。ラットが死亡する直前の血清 HMGB1 濃度に関しても、中和抗体投与群において有意に低値

であった(図4)。血清 HMGB1 濃度の経時変化は、立毛、寡動、探索行動の低下、食欲低下などのラット敗血症症状と関連する傾向を示した。コントロール群11匹中8匹は、モデル作製8時間後までにこれらの敗血症症状を呈し、12時間以内に死亡したのに対し、中和抗体投与群は、より活動的で、死亡が発生する時間帯がコントロール群に比べて遅れる傾向を認めた(図2)。

モデル作製24時間後における盲腸病理組織所見に関しては、コントロール群において、盲腸漿膜側を中心とした高度の炎症細胞浸潤を認め、同部に多数の HMGB1 陽性細胞を認めたのに対し、中和抗体投与群においては、漿膜側の炎症が軽度で、HMGB1 陽性細胞もほとんど認めなかった(図5)。肺病理組織所見に関しては、コントロール群において、肺胞間質への著明な炎症細胞浸潤を認め、いわゆる septic lung の状態を呈しており、HMGB1 陽性細胞を多数認めたのに対し、中和抗体投与群においては、炎症細胞浸潤や HMGB1 陽性細胞数がコントロール群と比較して少なかった(図6)。

## III. 考 察

本研究において、抗生剤投与を併用せずに抗 HMGB1 抗体投与のみによって盲腸結紮ラット



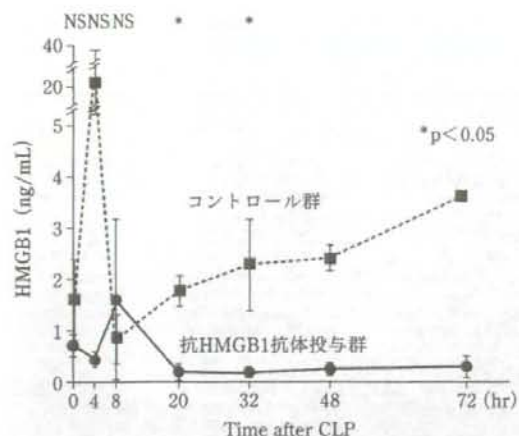


図3 Serum HMGB1 concentrations of the animals treated with anti-HMGB1 antibodies were significantly lower than in controls at POH 20 and POH 32.

Data are shown as Means  $\pm$  SEM of 11 rats in each group. The comparisons between the data at every time point were made with a nonparametric Mann Whitney U test ; with p values < 0.05 considered to indicate a statistically significant difference. ● , anti-HMGB1 antibody-treated group ; ■ , controls.

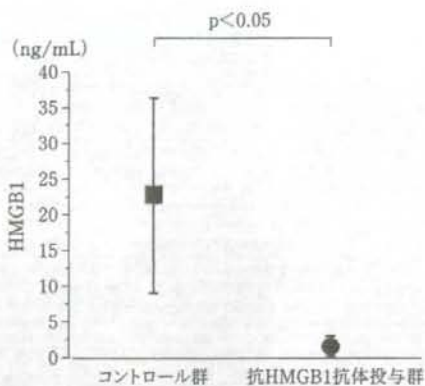


図4 To assess the efficacy of treatment with anti-HMGB1 antibodies, an analysis is performed with the "last observation carried forward" method.

The levels of the animals treated with anti-HMGB1 antibodies are significantly lower than those of controls (p < 0.05 by a nonparametric Mann Whitney U test).

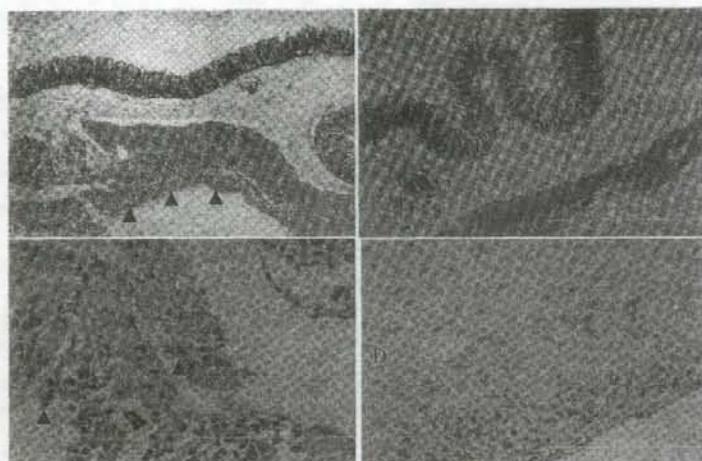


図5 Histological and immunohistochemical findings in the cecum.

- A : H&E staining in controls (original magnification, 40  $\times$ ).
- B : Immunohistochemical staining of HMGB1 in controls (original magnification, 400  $\times$ ).
- C : H&E staining in anti-HMGB1 antibody-treated group (original magnification, 40  $\times$ ).
- D : Immunohistochemical staining of HMGB1 in anti-HMGB1 antibody-treated group (original magnification, 400  $\times$ ).

の血清 HMGB1 濃度を低下させ、炎症局所および遠隔臓器障害を軽減し、生存率を有意に改善できること、および HMGB1 は後期メディエーターといわれ

ているが、モデル作製 4 時間後という比較的早期においてさえ血中濃度が著明に上昇し得ることを明らかにした。

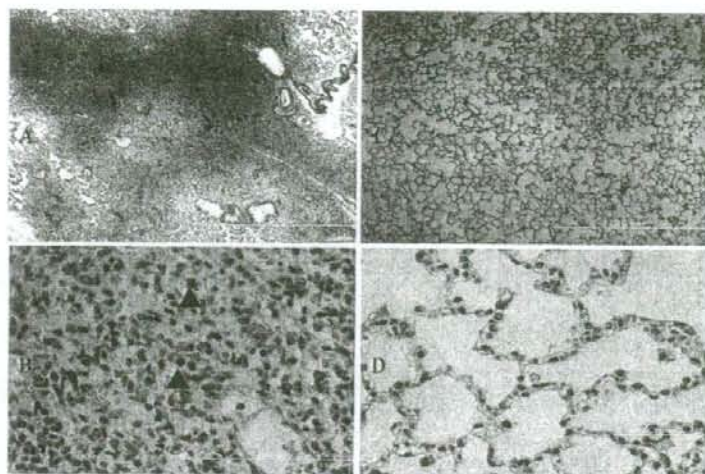


図6 Histological and immunohistochemical findings in the lung.

- A: Elastica-Masson staining in controls (original magnification, 40×).  
 B: Immunohistochemical staining of HMGB1 in controls (original magnification, 400×).  
 C: Elastica-Masson staining in anti-HMGB1 antibody-treated group (original magnification, 40×).  
 D: Immunohistochemical staining of HMGB1 in anti-HMGB1 antibody-treated group (original magnification, 400×).

今回、敗血症モデル作製15分後に抗HMGB1抗体を投与し、敗血症に伴う臓器障害を軽減することができた。われわれの以前の研究結果では、HMGB1は後期メディエーターであり、食道癌根治術後、IL-1 $\beta$ やIL-6に遅れて血中に出現するため、メディエーター修飾療法の標的として、炎症性サイトカインより幅広い治療のタイミングを有すると考えられる<sup>8</sup>。副腎皮質ステロイドやウリナスタチン、メシル酸ガベキセート等、これまで食道癌根治術後におけるメディエーター修飾療法として有用性が報告されてきた薬剤はすべて術前投与のみが有効であった<sup>15-17</sup>。本研究において、モデル作製後の投与により血中濃度、臓器障害、生存率を改善できたことから、食道癌術前あるいは術後早期における抗HMGB1療法の施行によって、食道癌術後臓器障害を予防・軽減できる可能性が考えられる。

HMGB1は後期メディエーターと呼ばれている<sup>3-7</sup>。Wang Hら<sup>3,5,18</sup>の報告によると、血清HMGB1濃度は、LPS投与マウスにおいてはモデル作製8時間後から、盲腸結紮穿刺マウスにおいてはモデル作製18時間後から上昇し、いずれのモデルにおいてもモデル作製24時間後に抗HMGB1抗体を投与しても生存率を有意に改善することができた。

われわれの改良型盲腸結紮穿刺ラットにおいて、モデル作製4時間後という早い時点から血清HMGB1濃度が上昇した原因として、種差や抗生剤を投与していなかったこと等が考えられる。一方で、肝虚血再灌流モデルマウスにおいては、血清HMGB1濃度はモデル作製6時間後から上昇したという報告もあり、種差や抗生剤非投与のみならず、侵襲の種類や程度によって血清中にHMGB1が出現してくるタイミングは変わり得ると考えられる<sup>19</sup>。今回は採血量の問題からTNF $\alpha$ やIL-1 $\beta$ 、IL-6等の炎症性サイトカインは測定しなかったが、おそらく、いずれのモデルにおいても炎症性サイトカインに遅れてHMGB1が血清中に出現してくるというsequenceは普遍的であり、その意味でHMGB1は後期メディエーターであると考えられる<sup>5</sup>。

従来の盲腸結紮穿刺モデルは、盲腸根部にて栄養血管を含む腸間膜ごと集族結紮し、先端に穿孔を加えて作製され、腹膜炎の治療として抗生剤投与を併用していた<sup>10-12</sup>。すなわち、壊死した盲腸壁および腹膜炎によって活性化された炎症細胞の二つの異なるHMGB1産生源が考えられ、これが盲腸結紮穿刺モデルの重症度コントロールを困難にしていた原因と思われる。われわれは、盲腸壁と盲腸血流を分離

して結紮/温存することによって、抗生剤を使用せずに盲腸結紮穿孔モデルの重症度を安定化することに成功した。本研究では、壊死や抗生剤の影響を除き、腹膜炎の炎症によって産生された HMGB1 のみによる現象を観察した。結果、比較的早期から血清中に HMGB1 が出現し、特異的修飾によって敗血症の病態を改善し得た。したがって、sequence とは別に、絶対的時間軸に関して、HMGB1 は壊死を伴わない炎症反応における early mediator の機能も有している可能性が考えられる。今後、皮下投与された抗体の血中への移行率や半減期、抗体を経静脈投与した場合などについて検討を行うことによって、モデルの敗血症病態において HMGB1 が重要な機能を果たしている時間帯をより詳細に解析できると考えられる。

HMGB1 によって臓器不全が誘導されるメカニズムとして、RAGE-NF $\kappa$ B 系を介する炎症増悪・細胞遊走促進作用の他に、toll-like receptor-2, 4 を介する作用も報告されている<sup>20</sup>。HMGB1 は、炎症局所では、本来生体防御に重要な役割を果たしているが、炎症局所で過剰となり流血中に溢れ出した HMGB1 が臓器障害を引き起こすと考えられる<sup>7,20</sup>。

抗 HMGB1 抗体を投与しても死に至るラットが存在した理由については、抗生剤投与や病巣切除などの原因に対する治療が施されていなかったこと、炎症反応には多種多様な液性・細胞性因子が関与しており、単一 mediator の制御のみですべての炎症反応を制御しきれるとは考えにくいことが考えられる<sup>1,2,5,7,20</sup>。

## 結 論

以上より、重症敗血症に対する抗 HMGB1 療法の有用性が preclinical に明らかとなった。また、炎症反応において HMGB1 が比較的早期から重要な機能を果たしている可能性が示唆された。HMGB1 吸着カラムを用いた急性血液浄化療法、HMGB1 の A-Box 蛋白や抗 B-Box 抗体等、ヒトに応用可能な抗 HMGB1 療法の開発が期待される<sup>5</sup>。

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[Original Article]

Therapeutic Effects of Anti-High-Mobility Group Box Chromosomal Protein 1 Antibodies in Rats with Sepsis

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Abstract

High-mobility group box chromosomal protein 1 (HMGB1) has recently been shown to be an important late mediator of endotoxin shock, intraabdominal sepsis, and acute lung injury, and a promising therapeutic target of severe sepsis. We sought to investigate the effect of antibodies to HMGB1 for severe sepsis in a rat cecal ligation and puncture (CLP) model. Adult male Sprague-Dawley rats underwent CLP, and then were randomly divided into two groups: treatment with anti-HMGB1 polyclonal antibodies, and nonimmune IgG-treated controls. The serum HMGB1 concentrations were measured at 10 time points (preoperatively, and at postoperative hour 4, 8, 20, 32, and 48, and postoperative days 3, 4, 5, and 6). Hematoxylin-Eosin staining, Elastica-Masson staining, and immunohistochemical staining for HMGB1 were performed on the cecum and the lung to assess pathological changes 24 h after the CLP procedure. Treatment with anti-HMGB1 antibodies significantly increased survival. The serum HMGB1 concentrations at postoperative hour 20 and 32 of anti-HMGB1 antibody-treated animals were significantly lower than those of controls. Treatment with anti-HMGB1 antibodies markedly diminished the pathological changes and the number of HMGB1 positive cells in the cecum and the lung. The present study demonstrates that anti-HMGB1 antibodies are effective in the treatment of severe sepsis in a rat model, supporting the relevance of HMGB1 eradication therapy for severe sepsis.

**Key words:** HMGB1, Sepsis, Cecal ligation and puncture, Anti-HMGB1 antibody, Rat

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## Neutrophil elastase inhibitor improves postoperative clinical courses after thoracic esophagectomy

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**SUMMARY.** Sivelestat sodium hydrate is a selective inhibitor of neutrophil elastase (NE), and is effective in acute lung injury associated with systemic inflammatory response syndrome (SIRS). The effect of Sivelestat for postoperative clinical courses after transthoracic esophagectomy was investigated. Consecutive patients with carcinoma of the thoracic esophagus who underwent transthoracic esophagectomy between 2003 and 2004 were assigned to the Sivelestat-treated group ( $n = 18$ ), and those between 1998 and 2003 were assigned to the control group ( $n = 25$ ). The morbidity rate, duration of postoperative SIRS, mechanical ventilation, and intensive care unit (ICU) stay, and the sum of the sequential organ failure assessment scores at all time points after the operation were compared. Serum NE activities and serum concentrations of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and high mobility group box chromosomal protein 1 (HMGB1) were measured. Postoperative complications developed in three patients in the control group, and one in the Sivelestat-treated group. The durations of SIRS, mechanical ventilation, and ICU stay were significantly shorter in the Sivelestat-treated group. Even in patients without complications, the durations of mechanical ventilation, and ICU stay were also significantly shorter, and the arterial oxygen pressure/fraction of inspired oxygen ratio at postoperative day 1 was significantly higher in the Sivelestat-treated group. Serum NE activities and serum concentrations of IL-1 $\beta$ , IL-6, and HMGB1 were significantly suppressed in the Sivelestat-treated group. Postoperative Sivelestat treatment after transthoracic esophagectomy improves the condition of SIRS and postoperative clinical courses, even in patients without complications.

**KEY WORDS:** acute respiratory distress syndrome, complications, esophageal cancer, esophageal surgery, inflammatory mediators, surgery.

### INTRODUCTION

The surgical treatment of esophageal cancer, which requires thoracotomy manipulation, is one of the most invasive operations in gastrointestinal surgery and the frequency of postoperative complications is still relatively high.<sup>1,2</sup> Despite advances in anesthesia, operative techniques, and postoperative

management, peri-operative mortality rates continue to range from 3% to 10%.<sup>3–13</sup> In particular, pulmonary complications, which occur in approximately 30% of cases, are among the most frequent to develop after esophagectomy.<sup>3–13</sup> Surgical procedures for esophageal cancer induce a systemic inflammatory response syndrome (SIRS) characterized by the overproduction of pro-inflammatory cytokines, which produces excessive stress and may trigger postoperative complications.<sup>2,14</sup> Moreover, the lung is one of the main target organs of neutrophil sequestration in the condition of SIRS.<sup>15–18</sup> These facts lead to the hypothesis that a drug which can relieve SIRS,

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control neutrophil function and decrease pulmonary complications might improve postoperative clinical courses after transthoracic esophagectomy.

Sivelestat sodium hydrate, sodium N-(2-[4-(2,2-dimethylpropionyloxy) phenylsulfonamino] benzoyl) aminoacetate tetrahydrate (Elaspol, ONO-5046-Na[C<sub>20</sub>H<sub>21</sub>N<sub>2</sub>NaO<sub>7</sub>S-Na-4H<sub>2</sub>O, molecular weight, 528.51]; Ono Pharmaceutical Co., Osaka, Japan), is a synthetic, specific, low molecular weight neutrophil elastase (NE) inhibitor.<sup>19-21</sup> The drug is intravenously active and competitively inhibits the activity of NE in humans, hamsters and dogs, but it does not affect other proteases such as plasmin, thrombin, kallikrein, cathepsin B, or collagenase I.<sup>20</sup> The efficacy of Sivelestat on acute lung injury (ALI) has been demonstrated in several investigations.<sup>19,20,22-24</sup> The use of the drug in humans has been approved in Japan for cases of ALI associated with SIRS, although there have been conflicting conclusions regarding its benefits.<sup>20,21,25</sup>

The present study was designed to investigate the effects of Sivelestat on the postoperative clinical course after transthoracic esophagectomy.

## MATERIALS AND METHODS

### Patients

The study group consisted of 43 consecutive patients with carcinoma of the thoracic esophagus (median age, 58 years [interquartile range: 53-66]; male/female ratio, 35/8; pathologic stage by the International Union against Cancer classification of I : II<sub>A</sub> : II<sub>B</sub> : III : IV<sub>A</sub> = 13 : 8 : 3 : 15 : 4) who underwent transthoracic esophagectomy without preoperative treatment between 1998 and 2004 at Keio University Hospital, Tokyo, Japan. The patients between 2003 and 2004 were assigned to the Sivelestat-treated group (*n* = 18), and those between 1998 and 2003 were assigned to a control group as a historical

control (*n* = 25) (Table 1). Only one surgical team performed all the operations in the present study. The definition of SIRS was taken from the report by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference<sup>26</sup> and the definition of ALI was taken from the report by the American European Consensus Conference on acute respiratory distress syndrome (ARDS).<sup>27</sup>

Both the acute physiology and chronic health evaluation (APACHE) II scoring system and the sequential organ failure assessment (SOFA) scoring system were used to evaluate the severity of the patient's condition.<sup>28,29</sup> Routine preoperative evaluation included a complete blood count, serum C-reactive protein (CRP) concentrations, electrocardiography, spirometry, creatinine clearance (Ccr), activated partial thromboplastin time, prothrombin time, and an indocyanine green test retention rate. No patients had anemia preoperatively. Preoperative white blood cell counts and platelet counts of all patients were within normal ranges. Electrocardiography studies were all normal. None of the patients had obstructive or restrictive respiratory dysfunction, according to the spirometry data. The Ccr values of all patients were greater than 60 mL/min. No patient had coagulopathy. The liver function of all patients was classified as Child's class A. These preoperative data showed that none of the patients had high risk factors for surgery under general anesthesia. All the patients were routinely admitted to the intensive care unit (ICU) on mechanical ventilation at least overnight after undergoing transthoracic esophagectomy. The timing of weaning from mechanical ventilation was determined based on the following criteria: mode of spontaneous respiration, fraction of inspired oxygen (FiO<sub>2</sub>) < 0.4, respiratory rate < 25/min and arterial oxygen pressure (PaO<sub>2</sub>) > 100 torr. On the day after extubation, the patients were discharged from the ICU if their condition remained stable.

Table 1 Patients' background data, showing median and inter-quartile ranges

		Sivelestat-treated group ( <i>n</i> = 18)	Control group ( <i>n</i> = 25)	<i>P</i> value
Age	Median	60	56	0.306
	Inter-quartile range	55-65	52-66	
Gender (male : female)		15/3	20/5	0.782
UICC stage (I : II <sub>A</sub> : II <sub>B</sub> : III : IV <sub>A</sub> )		4 : 5 : 1 : 7 : 1	9 : 3 : 2 : 8 : 3	0.848
Intraoperative blood loss (mL)	Median	361	520	0.730
	Inter-quartile range	218-682	216-700	
Operation time (min)	Median	458	626	0.002
	Inter-quartile range	373-545	541-700	
APACHE II score	Median	11	7.5	< 0.001
	Inter-quartile range	9-13	6-10	
Preoperative CRP (mg/dL)	Median	0.18	0.13	0.712
	Inter-quartile range	0.06-0.68	0.07-0.33	

APACHE, acute physiology and chronic health evaluation; CRP, C-reactive protein; UICC, International Union against Cancer Classification.

From 1998, we successfully obtained informed consent to perform a clinical study about surgical stress by using patients' clinical data and preserved sera from all the patients who underwent trans-thoracic esophagectomy without preoperative treatment. In addition, from 2003, we simultaneously obtained informed consent to administer Sivelestat for a week after esophagectomy in order to investigate whether Sivelestat could treat postoperative SIRS. As a result, we were able to enrol 25 consecutive controls from 1998 to 2003 and 18 consecutive Sivelestat-treated patients from 2003 to 2004 to the present study. This protocol was approved by the Institutional Review Board of Keio University Hospital.

#### Dosage and administration of Sivelestat

In the Sivelestat-treated group, 0.2 mg/kg/h Sivelestat diluted with 0.9% sodium chloride was continuously intravenously administered immediately after surgery until postoperative day (POD) 6.

#### Measurements

Blood samples were collected, centrifuged and stored at  $-80^{\circ}\text{C}$ . Serum tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin 1 $\beta$  (IL-1 $\beta$ ), interleukin 6 (IL-6) and high mobility group box chromosomal protein 1 (HMGB1) levels were measured at 11 time points (preoperatively, immediately postoperatively, and on PODs 1-7, POD 10 and POD 14) by enzyme-linked immunosorbent assay (Research and Diagnostics Systems, Minneapolis, MN for TNF $\alpha$ , IL-1 $\beta$  and IL-6, and Central Institute, Shino-Test Corporation, Kanagawa, Japan for HMGB1).<sup>20</sup> NE activities in each plasma sample were spectrophotometrically determined at the same time points using Meo-Suc-Ala-Ala-Pro-Val pNA as a substrate. A mixture of 0.1 M Tris-HCl buffer (pH 8.0) containing the substrate at a final concentration of 1 mM and 0.5 M sodium chloride pentose nucleic acid was incubated with plasma at  $37^{\circ}\text{C}$  for 24 h and the amount of pNA liberated was determined at 405 nm and considered to be plasma NE activity.<sup>22,24</sup>

#### Statistical study

First, the patients' background factors, including their APACHE II scores, intraoperative blood loss, operation time, age and pathologic cancer stage, were compared between the two groups using a Mann-Whitney *U*-test and their gender was compared using the  $\chi^2$ -test.

Second, to identify the effect of the Sivelestat treatment on morbidity and postoperative clinical course we compared the morbidity rate, duration of postoperative SIRS, mechanical ventilation and ICU stay, and the sum of the SOFA scores at all

time points after the operation, between those of the Sivelestat-treated group and the control group. The duration of assessment of postoperative complications was 30 days. Both groups were monitored over identical time intervals. The comparisons between the data were made with the  $\chi^2$ -test and the Mantel-Haenzel test.

Third, to identify the effect of the Sivelestat treatment, even in patients without complications, on the postoperative clinical course and respiratory function, we compared the data of all patients without complications between the two groups regarding the duration of postoperative SIRS, mechanical ventilation, and ICU stay, the sum of the SOFA scores at all time points after the operation and the PaO<sub>2</sub>/FiO<sub>2</sub> ratio at each time point. The comparisons between the data were made with the Mantel-Haenzel test.

Fourth, to identify the effects of Sivelestat treatment on concentrations of serum mediators in patients without complications, the serum NE activity and serum levels of TNF $\alpha$ , IL-1 $\beta$ , IL-6 and HMGB1 at each time point were compared between the two groups. The comparisons between the data were made with the Mantel-Haenzel test. *P*-values of less than 0.05 were considered to indicate a statistically significant difference. Statistical analyses were performed with the STATVIEW 5.0 and SAS statistical analysis package (SAS Institute, Cary, NC).

## RESULTS

#### Patients' background factors

There were no significant differences between the groups in age, gender, pathologic cancer stage, intraoperative blood loss or preoperative serum CRP concentrations. APACHE II scores of the Sivelestat-treated group were significantly higher than those of the control group ( $P < 0.001$ ). The average operation time of the Sivelestat-treated group was significantly shorter than that of the control group ( $P = 0.002$ ) (Table 1).

#### Effect of Sivelestat on morbidity and postoperative clinical course

In the Sivelestat-treated group only one of the 18 patients had postoperative complications (sepsis), while, in the control group, three of the 25 patients had postoperative complications: two had sepsis, and one had ALI. The morbidity rates were not significantly different in the two groups ( $P = 0.470$ ).

In the Sivelestat-treated group, the duration of postoperative SIRS, mechanical ventilation and ICU stay were significantly shorter than those in the control group ( $P = 0.026$ ,  $0.008$  and  $0.018$ , respectively). The sum of the SOFA scores at all

**Table 2** Postoperative clinical courses of the patients. Median and interquartile ranges are shown. The duration of SIRS, sum of SOFA scores, duration of mechanical ventilation, and duration of ICU stay are compared between Sivelestat-treated group and control group by the Mantel-Haenzel test

		Sivelestat-treated group (n = 18)	Control group (n = 25)	P value
Duration of SIRS (d)	Median	3.5	5	0.026
	Inter-quartile range	2-5.8	3.8-10.3	
Sum of SOFA scores	Median	31	37	0.29
	Inter-quartile range	26-45	29-44	
Duration of ventilator dependence (d)	Median	1	2	0.008
	Inter-quartile range	1-1.5	1-2.5	
ICU stay (d)	Median	1.5	2.5	0.018
	Inter-quartile range	1.5-1.9	1.5-3.5	

ICU, intensive care unit; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment.

**Table 3** Postoperative clinical courses and respiratory function of the patients without complications. Median and inter-quartile ranges are shown. The duration of SIRS, sum of SOFA scores, duration of mechanical ventilation, duration of intensive care unit (ICU) stay, and the PaO<sub>2</sub>/FiO<sub>2</sub> ratio at POD 1 are compared between the Sivelestat-treated group and control group by the Mantel-Haenzel test

		Sivelestat-treated group (n = 17)	Control group (n = 22)	P value
Duration of SIRS (d)	Median	3	5	0.067
	Inter-quartile range	2-5	3-7	
Sum of SOFA scores	Median	30	37	0.760
	Inter-quartile range	25-43	27-42	
Duration of ventilator dependence (d)	Median	1	1.5	0.041
	Inter-quartile range	1-1.5	1-2	
ICU stay (d)	Median	1.5	2.5	0.041
	Inter-quartile range	1.5-1.5	1.5-2.5	
PaO <sub>2</sub> /FiO <sub>2</sub> ratio at POD1	Median	378	250	< 0.001
	Inter-quartile range	338-404	233-286	

FiO<sub>2</sub>, fraction of inspired oxygen; PaO<sub>2</sub>, arterial oxygen pressure; POD, post-operative day; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment.

time points after the operation was not significantly different between the two groups ( $P = 0.290$ ) (Table 2).

#### Effect of Sivelestat in patients without complications on postoperative clinical course and respiratory function

In patients without complications, the duration of mechanical ventilation and ICU stay in the Sivelestat-treated group were significantly shorter than those in the control group ( $P = 0.041$ , and  $0.041$ , respectively). The duration of postoperative SIRS was shorter, but not significantly so, in the Sivelestat-treated group ( $P = 0.067$ ). The sum of the SOFA scores at all time points after the operation was not significantly different between the two groups ( $P = 0.760$ ). The PaO<sub>2</sub>/FiO<sub>2</sub> ratio at POD 1 was significantly higher in the Sivelestat-treated group than that in the control group ( $P < 0.001$ ) (Table 3). The Mantel-Haenzel test was performed because in the patients' background factors, the operation time was significantly shorter in the Sivelestat-treated group, which might be related to their improved postoperative clinical courses.

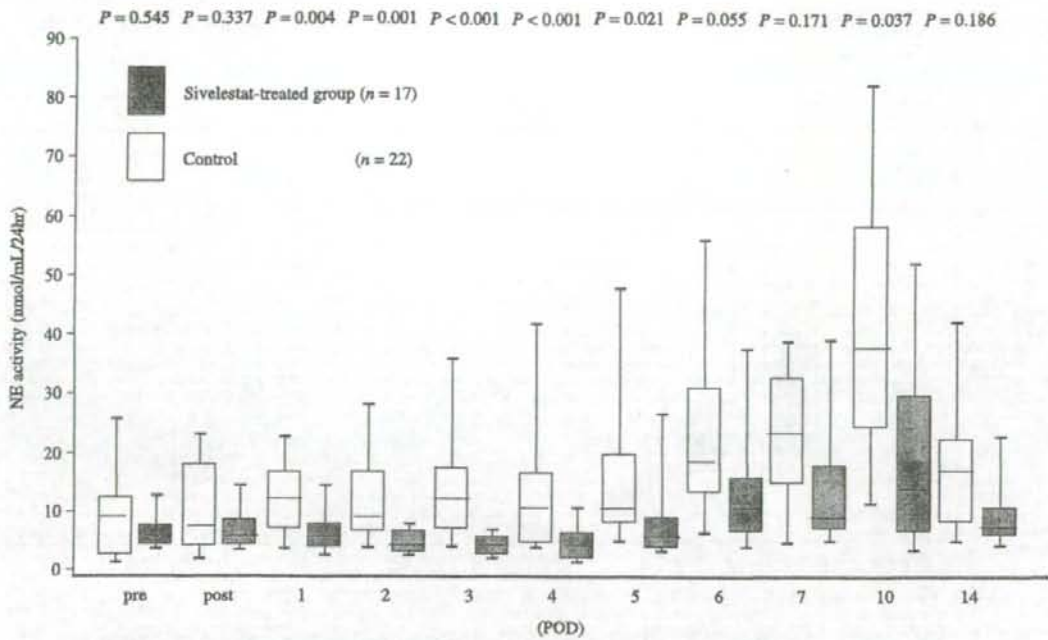
#### Effect of Sivelestat in the patients without complications on concentrations of serum mediators

In the Sivelestat-treated group, serum NE activities and concentrations of IL-1 $\beta$  were significantly suppressed in comparison with the control group from POD 1 through POD 6 ( $P < 0.05-0.001$ ) (Figs 1 and 2, respectively). Serum concentrations of IL-6 in the Sivelestat-treated group were significantly suppressed in comparison with the control group at POD 1 ( $P = 0.030$ ) (Fig. 3). Serum concentrations of HMGB1 in the Sivelestat-treated group were also significantly suppressed in comparison with the control group at POD 5 ( $P = 0.015$ ) (Fig. 4). Serum TNF $\alpha$  concentrations at each time point were less than 2 pg/mL in both groups.

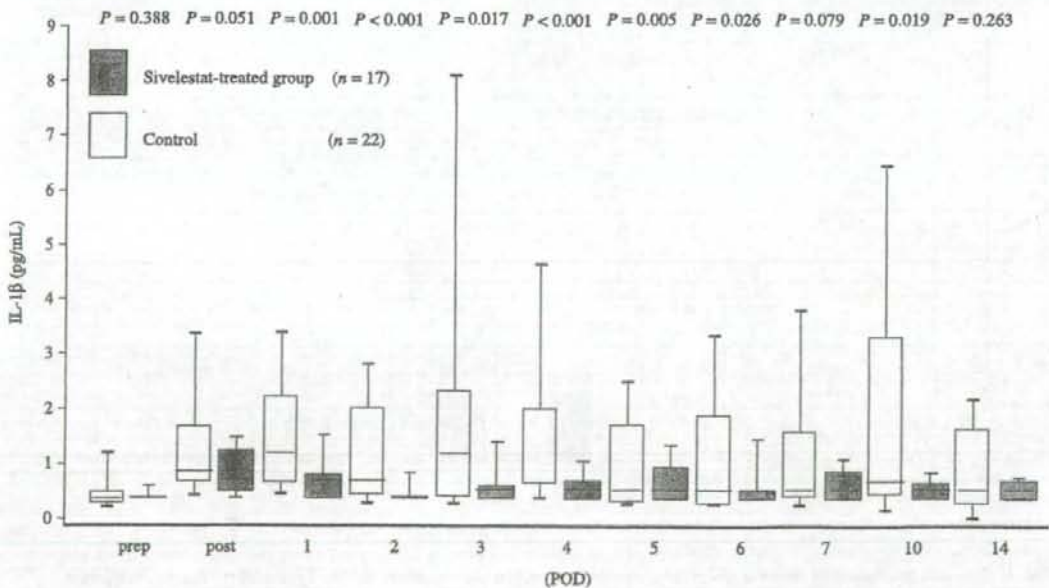
#### DISCUSSION

Our study yielded two major findings. First, postoperative Sivelestat treatment after transthoracic esophagectomy improved postoperative respiratory function and clinical courses, even in patients without complications, although the monitoring period was





**Fig. 1** Pre- and postoperative serum levels of neutrophil elastase (NE) activity. The median serum level of NE activity at each time point is indicated by horizontal bars. The vertical bars indicate the range, and the horizontal boundaries of the boxes represent the first and third quartiles.



**Fig. 2** Pre- and postoperative serum levels of interleukin 1 $\beta$  (IL-1 $\beta$ ). The median serum IL-1 $\beta$  concentration at each time point is indicated by horizontal bars. The vertical bars indicate the range, and the horizontal boundaries of the boxes represent the first and third quartiles.

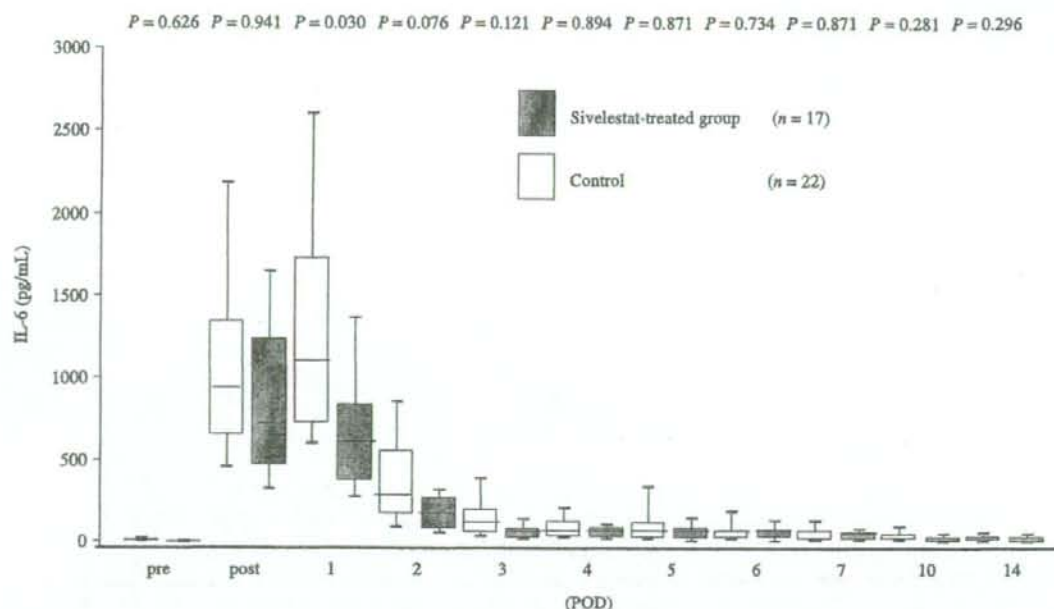


Fig. 3 Pre- and postoperative serum levels of interleukin-6 (IL-6). The median serum IL-6 concentration at each time point is indicated by horizontal bars. The vertical bars indicate the range and the horizontal boundaries of the boxes represent the first and third quartiles.

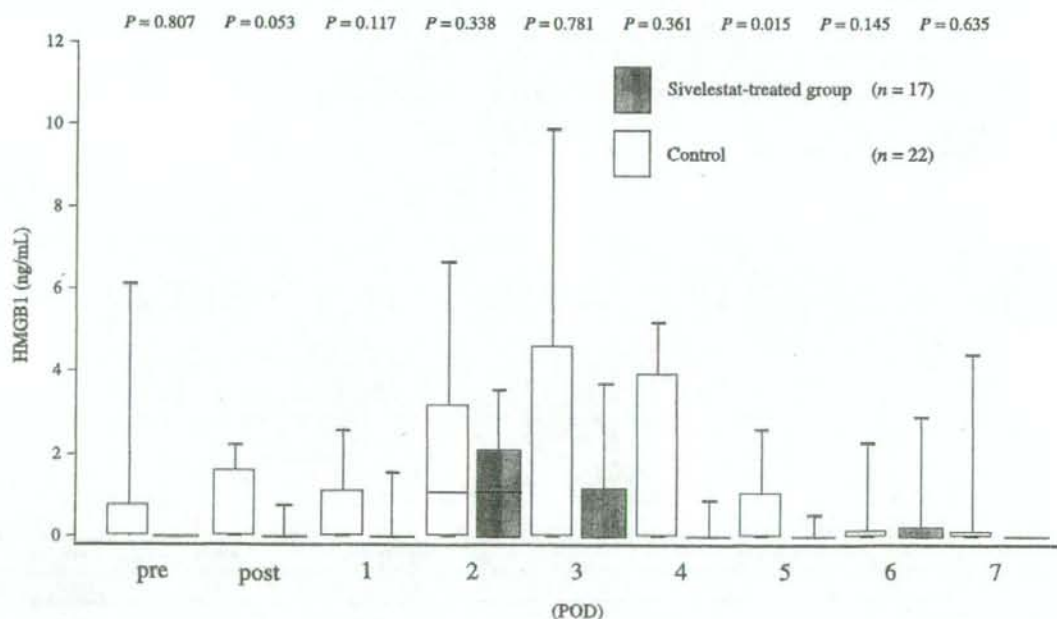


Fig. 4 Pre- and postoperative serum levels of high mobility group box chromosomal protein 1 (HMGB1). The median serum HMGB-1 concentration at each time point is indicated by horizontal bars. The vertical bars indicate the range and the horizontal boundaries of the boxes represent the first and third quartiles.

so short that the present study lacked information on long-term complications after esophagectomy and the late effect of the drug. Second, the treatment significantly suppressed NE activities and concentra-

tions of IL-1 $\beta$ , IL-6 and HMGB1 in serum and improved the SIRS condition.

As reported by Zeiher *et al.*, Sivelestat has been effective in multiple animal models of ALI.<sup>21,31</sup> A

phase III study conducted in Japan demonstrated that Sivelestat improved investigator assessment of pulmonary function improvement and significantly reduced the duration of the patient's ICU stay.<sup>21,25</sup> Sivelestat has been approved and is commercially available in Japan as a treatment for ALI associated with SIRS.<sup>21</sup> All the patients in this study met the standards of SIRS in both groups, at least from immediately after surgery to POD 1, and all but two patients in the control group met those of ALI at POD 1. Therefore, administration of Sivelestat immediately after transthoracic esophagectomy was warranted. In the Sivelestat-treated group, PaO<sub>2</sub>/FiO<sub>2</sub> ratios on POD 1 were significantly improved compared to the control group, and this was considered to be related to the significantly shorter duration of mechanical ventilation and ICU stay.

Although a STRIVE study, another multicenter prospective trial, showed that continuously infused Sivelestat had no effect on the primary end points of 28-day all-cause mortality or ventilator-free days in a heterogeneous ALI patient population managed with low-tidal volume mechanical ventilation, Sivelestat was effective, but without statistical significance, for ALI patients who had a relatively high PaO<sub>2</sub>/FiO<sub>2</sub> ratio (100–300) and non-infectious etiology, according to the analyses of the subgroups.<sup>21</sup> Sivelestat can thus be effective when administered as early as any insults that may induce ALI. Because transthoracic esophagectomy is non-infectious, the PaO<sub>2</sub>/FiO<sub>2</sub> ratio of the patients is relatively high (median of the lowest PaO<sub>2</sub>/FiO<sub>2</sub> ratio of the patients without complications in the control group [PaO<sub>2</sub>/FiO<sub>2</sub> ratio at POD 1]; 250 [233–286]), and moreover, initiation of the insult, that is, at the start of the operation, is clear. Transthoracic esophagectomy is considered to be a good indication for Sivelestat treatment, even in patients before they develop complications.

In the present study, serum NE activities were significantly suppressed in the Sivelestat-treated group while Sivelestat was being administered. This result meant that the dosage, (0.2 mg/kg/h) was enough to suppress NE in the serum and the administered Sivelestat was systemically effective during that period. Under the effect of Sivelestat, the serum concentrations of pro-inflammatory cytokines, such as IL-1 $\beta$  and IL-6, were significantly suppressed, even in patients without complications. In cases of esophageal cancer, marked elevation of cytokine levels is observed during and shortly after operation, and hypercytokinemia produces excessive stress and may trigger postoperative complications.<sup>1</sup> Several reports on the relationships between the severity of sepsis, trauma and burns and SIRS have recently been published.<sup>1,32</sup> SIRS is thought to be induced by hypercytokinemia, and the longer the duration of SIRS, the more likely

it is to progress to multiple organ dysfunction syndrome.<sup>1,33–35</sup> Considering these results, the reason why Sivelestat improved postoperative clinical courses, even in patients without complications, was that Sivelestat decreased hypercytokinemia, leading to an improvement of the state of SIRS. Further investigations are warranted in order to clarify how Sivelestat decreased hypercytokinemia on the molecular basis.

HMGB1 is a nuclear DNA-binding protein acting as a proinflammatory cytokine when released in the extracellular space from necrotic cells, activated macrophages and dendritic cells.<sup>36–38</sup> In a previous study, we have demonstrated that surgical stress of transthoracic esophagectomy itself induced an increase in HMGB1 in serum, even in patients without complications, and higher levels of HMGB1 in the immediate postoperative period correlated with elevated preoperative serum HMGB1 concentrations and a complicated clinical course, indicating that elevations in HMGB1 might play a contributory role in the development of postoperative organ system dysfunction.<sup>39</sup> Furthermore, HMGB1 is one of the key mediators of ALI/ARDS.<sup>40</sup> In the present study, serum HMGB1 concentrations were significantly suppressed in the Sivelestat-treated group. Although the actual mechanism of the suppression of serum HMGB1 by Sivelestat treatment was unclear, the suppression of serum HMGB1 concentrations might contribute to the improvement of postoperative clinical courses and respiratory function in the Sivelestat-treated group.

Regarding cytokine modulation in esophagectomy, only preoperative administrations of corticosteroid, ulinastatin, and gabexate mesilate have been reported to be effective.<sup>1,2,14</sup> However, although Sivelestat could be more effective when administered as early as insults occur as described before, the postoperative administration of Sivelestat was still effective in this study. It is possible to speculate that Sivelestat modulates downstream of the inflammatory cascade, while corticosteroid does so upstream of it.<sup>2,36,38</sup> Development of cytokine modulators can modulate the downstream inflammatory cascade and may offer a clinically relevant prophylaxis and treatment to surgical stress-induced complications. From the preliminary data of the present study, we can speculate that an early phase administration of Sivelestat after major surgical insults may reduce the risk of serious complications in patients at risk. To investigate the preventive effect of the Sivelestat treatment for postoperative complication in patients with radical esophagectomy, a large-scale prospective randomized trial would be required.

In the present study, improved surgical techniques arising from greater experience and more practice, and not Sivelestat alone, may account for the more favorable clinical courses after transthoracic

esophagectomy in the treatment group, since the control group was earlier and the treatment group was later. A randomized double-blinded trial would be warranted to prove this efficacy in a future study.

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