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Ⅱ．委託業務成果報告（業務項目）

厚生労働科学研究委託費(難治性疾患実用化研究事業)
委託業務成果報告(業務項目)

ブレオマイシン誘発強皮症モデルマウスの線維化と炎症・免疫異常、血管内皮特異的Fli1欠失マウスの血管障害に対してRhoキナーゼ阻害薬ファスジルが及ぼす影響についての検討

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研究要旨

ファスジルはくも膜下出血術後の脳血管攣縮およびそれに伴う脳虚血症状の改善薬として承認されているRhoキナーゼ阻害薬である。近年ファスジルは動物実験モデルで線維化、血管異常、炎症反応を調節する作用があると報告されている。今回我々はファスジルの持つこれらの作用が全身性強皮症の病態に及ぼす影響を、BLM誘発強皮症モデルマウス、*Fli1*$^{+/-}$マウス、血管内皮細胞特異的*Fli1*欠失(*Fli1* ECKO)マウスを用いて検討した。ファスジルは、BLM誘発強皮症モデルマウスにおいて皮膚肥厚を有意に抑制し、TGF-β1で刺激した正常皮膚線維芽細胞および強皮症皮膚線維芽細胞において*COL1A2*遺伝子の発現を抑制し、*MMP1*遺伝子の発現を亢進させた。また、ファスジルはCD4陽性細胞とCD8陽性細胞におけるIL-4、IL-6、IL-17Aの産生を抑制し、CD19陽性細胞におけるIL-6の産生を抑制した。一方、*Fli1*$^{+/-}$マウスおよび*Fli1* ECKOマウスでは皮膚において血管透過性の亢進と血管の構造異常が見られるが、ファスジル投与によりこれらの異常は改善した。さらに、*Fli1*$^{+/-}$マウスおよび*Fli1* ECKOマウスでは皮膚微小血管においてα-SMA、VE-Cadherin、PECAM-1の発現低下が見られるが、ファスジル投与でこれらの発現が回復した。以上より、ファスジルは強皮症モデルマウスにおいて皮膚線維芽細胞に直接作用して抗線維化作用を示すほか、炎症抑制作用や血管異常を改善する作用を

有することが明らかとなり、同薬は全身性強皮症の疾患修飾薬となりうる可能性が示唆された。

A. 研究目的

全身性強皮症は血管障害と線維化を特徴とする原因不明の自己免疫疾患である。強皮症患者の病変部皮膚では、炎症や血管障害により線維芽細胞が恒常的に活性化され、細胞外マトリックスの産生亢進と分解遅延を引き起こし、結果的に細胞外マトリックスが過剰に沈着する [1]。その病態はいまだ不明な点も多く、現時点では本症の病態に立脚した確立された治療法は存在しない。

ファスジルは、くも膜下出血術後の脳血管攣縮およびそれに伴う脳虚血症状の改善薬として承認されている Rho キナーゼ(ROCK)阻害薬である。ROCK は脱リン酸化酵素である MLCP をリン酸化して抑制することでミオシン軽鎖(MLC)をリン酸化し、アクチンとミオシンの相互作用を強め、血管収縮を亢進させる。ファスジルはこの系を阻害するにより、血管収縮に抑制的に作用する。近年、ファスジルは動物実験モデルで線維化、血管異常、炎症反応を調節する作用があると報告されている。例えば、ループスモデルマウスではファスジル投与により、生存期間の延長と蛋白尿の軽快が得られ [2]、高脂血症ラットではファスジルが抗炎症作用を発揮する [3]。血管障害に対しては、モノクロタリン誘導肺高血圧症モデルラットにおいて、ファスジル投与が肺血管のリモデリングを抑制する [4]。また複数のモデルマウスにおいて、ファスジルの心筋線維化抑制作用が示されている [5] [6]。

今回我々はファスジルの持つこれらの作用が強皮症の病態形成を妨げ、新規治療法の候補となる可能性を仮説として提唱し、BLM 誘発強皮症モデルマウス、強皮症血管障害モデルマウス(*Fli1*$^{+/-}$マウス、血管内皮細胞特異的 *Fli1* 欠失[*Fli1* ECKO]マウス)を用いて検討を行った。

B. 研究方法

1) マウス

本研究では 6 週齢(C57BL/6)のマウスを用いて BLM 誘発強皮症モデルマウスを作成した。BLM 誘発強皮症モデルマウスはブレオマイシン(BLM) (Nippon Kayaku Co. Ltd)をリン酸緩衝液(phosphate-buffered saline; PBS)に 1mg/ml となるように溶解し、フィルター濾過後、剃毛したそれぞれのマウスの背部皮膚へ 4 週間にわたり 27 ゲージの注射針で 200μg を連日皮下投与することで作成した。コントロールとして PBS のみを BLM と同様の方法でそれぞれマウスに投与した。解析にはそれぞれ 5-8 匹のマウスを用いた。また、*Fli1*$^{+/-}$マウスおよび *Fli1* ECKO マウス(*Fli1*$^{flox/flox}$; *Tie2*-Cre)は Boston University of Medicine, Arthritis Center の Maria Trojanowska 氏より供与を受けた。いずれの研究も東京大学動物実験規則を遵守して行った。

2) ファスジルの投与

ファスジル(Asahi Kasei Pharma, Tokyo, Japan)は PBS に 2 mg/ml となるように溶解し、フィルター濾過後、20 mg/kg/day となるように連日腹腔内投与を行った。コントロール群では PBS を投与した。

3) 病理組織学的検討

4 週間のファスジルの投与が終了した BLM 誘