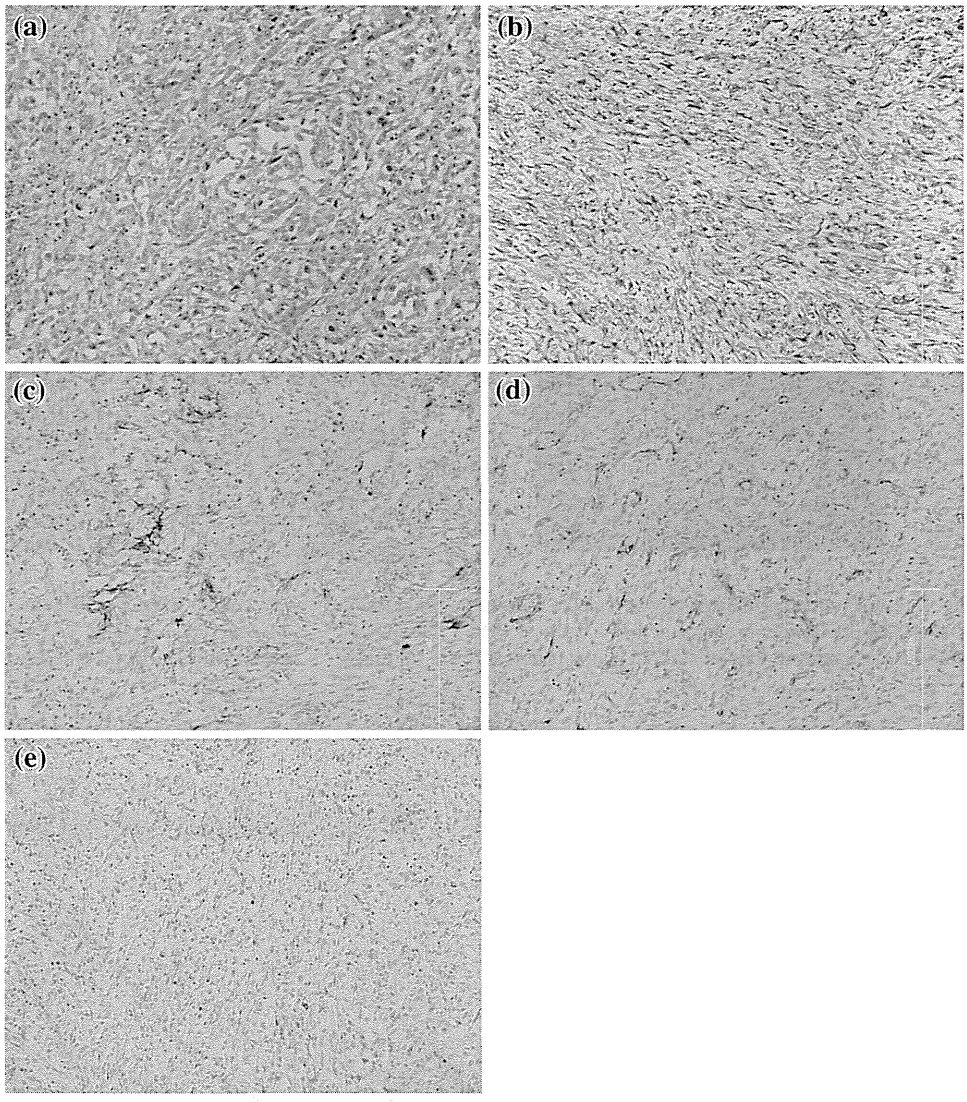

Fig. 2 **a** Histopathological findings showed spindle-shape tumor cells that formed vessel-like structures (hematoxylin and eosin staining, ×200). Immunohistochemical staining was positive for vimentin and weakly positive for both factor VIII-related antigen and CD31 in tumor cells (**b** vimentin; **c** factor VIII-related antigen; **d** CD31, ×200). **e** Immunohistochemical staining was positive for G-CSF in tumor cells (G-CSF, ×200)

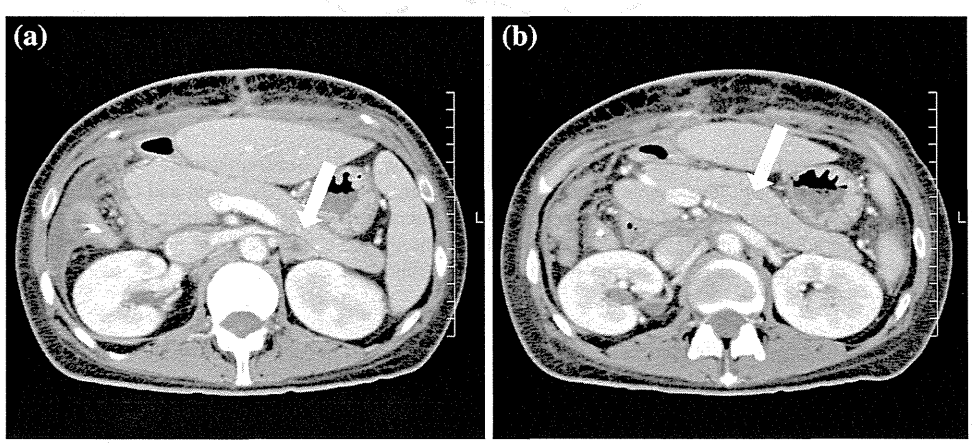

Fig. 3 **a**, **b** Computed tomography revealed a non-enhanced tumor in the left adrenal gland and a small low-density area in the pancreatic body, indicating the recurrence of angiosarcoma

beneficial, and long-term survival is occasionally expected [8–11]. However, lesser than 20 % patients can undergo surgery [10, 12], and primary hepatic angiosarcoma is almost far advanced and unrespectable at diagnosis because of its rapid progression, low prevalence, and difficult diagnosis. Therefore, most patients receive systemic

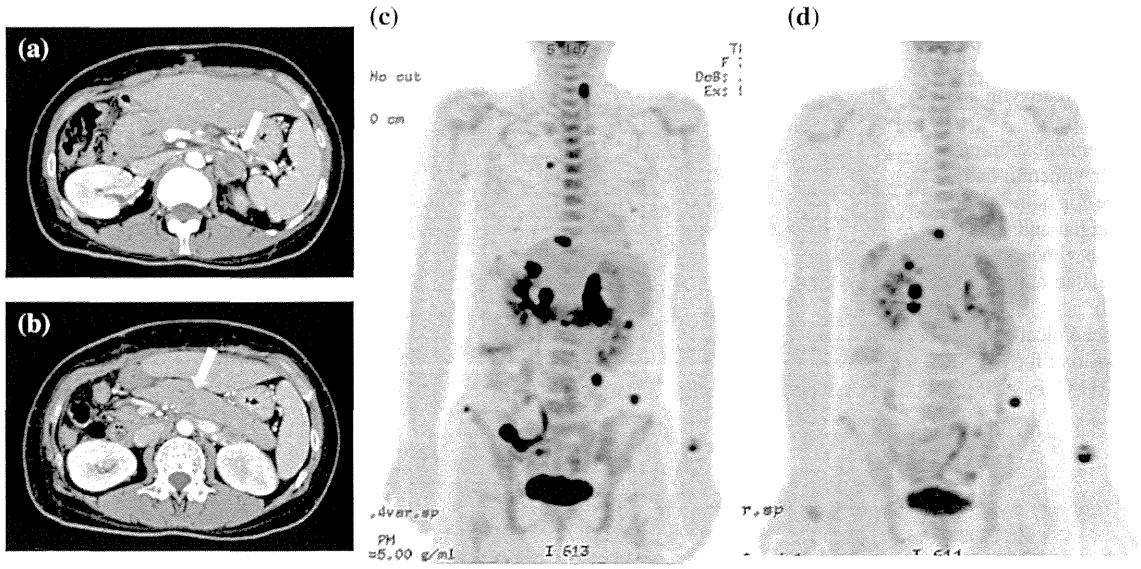

Fig. 4 **a**, **b** Computed tomography revealed a metastatic progression in the left adrenal gland and pancreatic body. **c** FDG-PET showed multiple metastases in the left adrenal gland, pancreas, bilateral lungs, left breast, liver, peritoneum, left thyroid lobe, and left wrist joint. **d** After one course of weekly paclitaxel, FDG-PET revealed decreased FDG uptake in most metastatic lesions

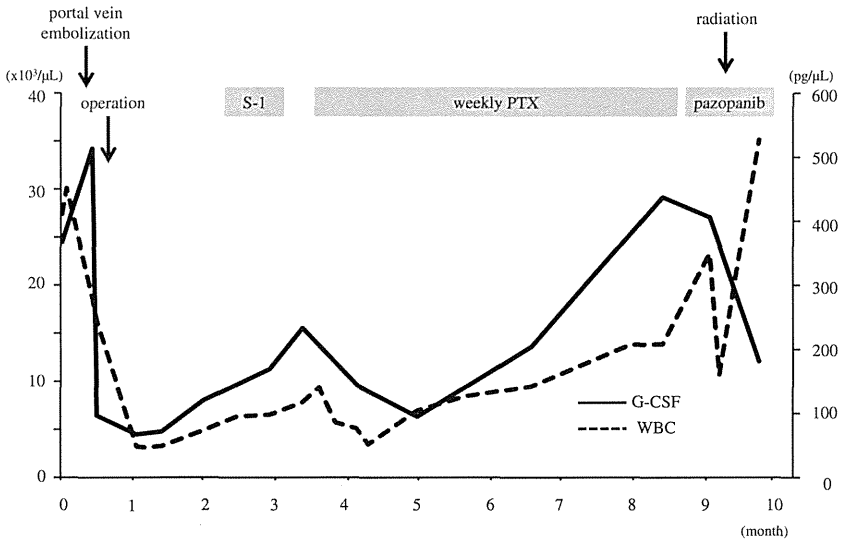

Fig. 5 Revealed the patient's clinical course. After hepatectomy, her WBC count and G-CSF level rapidly decreased; however, after 1 month, the WBC count and G-CSF level gradually increased. Despite S-1 therapy (100 mg/day), the G-CSF level increased, and weekly PTX (130 mg/week) was administered as the second-line chemotherapy. The G-CSF level temporarily decreased, but both the WBC count and G-CSF level increased after three courses of weekly PTX. Therapy with pazopanib (800 mg/day) resulted in a rapid decrease in the patient's G-CSF level

chemotherapy, but an effective chemotherapy for this sarcoma has not been established. Conventional first-line chemotherapy for unresectable soft tissue sarcoma involves an anthracycline-based chemotherapy [13]; however, the efficacy of this chemotherapy is low. We attempted S-1 administration as an orally administered systemic chemotherapy for convenience, but it showed no efficacy. A recent in vitro study suggested that PTX has a strong angiogenic activity [14], and a phase-II trial of weekly PTX for unresectable angiosarcoma demonstrated its efficacy and tolerable toxicity [6]. In this case, weekly PTX as the second-line chemotherapy temporarily decreased FDG uptake in the metastatic lesions, which indicated the efficacy of weekly PTX. A phase-III trial of pazopanib, an inhibitor of VEGFR and PDGFR, has recently revealed that it significantly improved progression-free survival

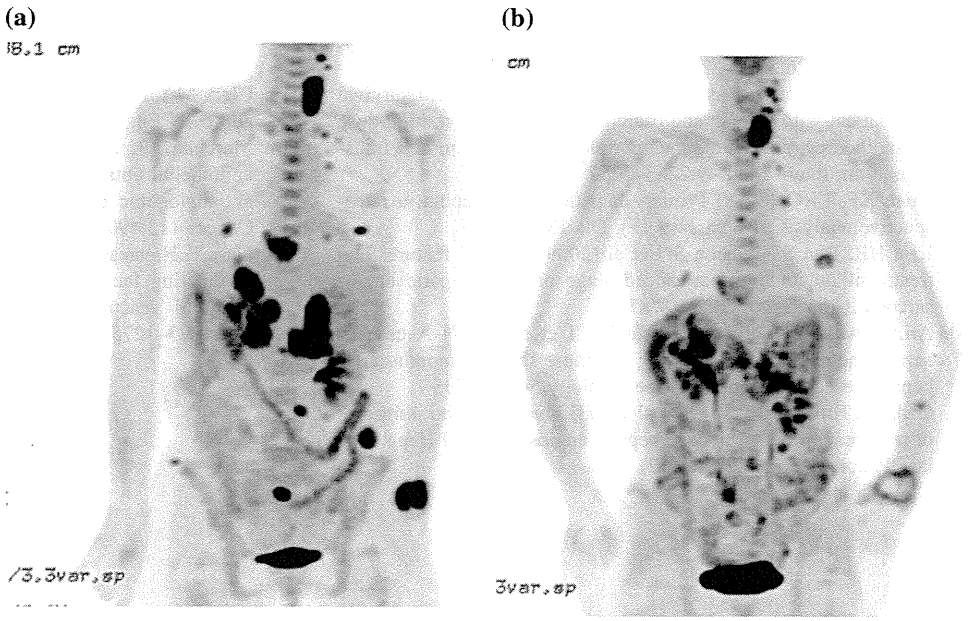

Fig. 6 **a** FDG-PET revealed the progression of angiosarcoma after three courses of weekly PTX. **b** One month after pazopanib administration, FDG-PET showed decreased FDG uptake in the metastatic lesions

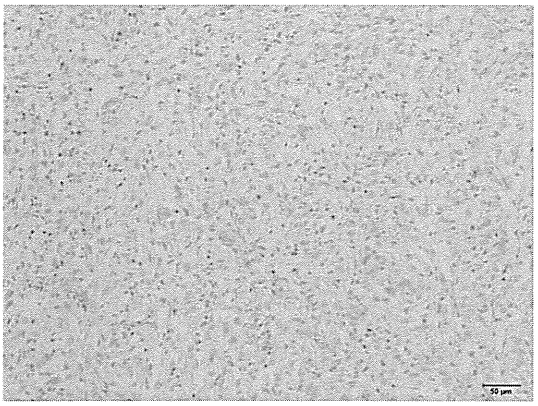

Fig. 7 Immunohistochemical staining was positive for PDGFR in tumor cells (×200)

compared with a placebo in patients with metastatic soft tissue sarcoma after the failure of standard chemotherapy [4]. This phase-III trial included most soft tissue sarcoma subtypes; however, leiomyosarcoma and synovial sarcoma were the major subtypes, and only three cases were soft tissue angiosarcoma. Therefore, it remains unknown whether pazopanib is effective for treating hepatic angiosarcoma. Because most angiosarcomas express VEGFR and PDGFR [5], it may be reasonable to treat angiosarcoma with pazopanib, which inhibits both VEGFR and PDGFR. After administration of pazopanib as the third-line chemotherapy, FDG-PET revealed decreased FDG uptake in all metastatic lesions. Laboratory data revealed a decreased G-CSF level, which indicated the efficacy of pazopanib administration for treating hepatic angiosarcoma.

No tumor marker has been established for the diagnosis and evaluation of angiosarcoma. There is a case report of a G-CSF-producing cutaneous angiosarcoma in which the patient's laboratory data revealed a high serum G-CSF level, and immunochemical staining for G-CSF demonstrated a positive reaction in tumor cells [15]. In the present case, an immunohistochemical evaluation of G-CSF in tumor cells was positive. The serum G-CSF level was high and parallel to the clinical course, which suggested G-CSF-producing hepatic angiosarcoma.

In summary, we present the first case of G-CSF-producing primary hepatic angiosarcoma that rapidly recurred after hepatectomy. Pazopanib was effective as the third-line chemotherapy, and G-CSF was useful as a tumor marker. Our case suggests that an early pazopanib therapy as an adjuvant chemotherapy can improve the prognosis of hepatic angiosarcoma.

Conflict of interest The authors declare that they have no conflict of interest.

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別刷

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腹水・浮腫を伴う非代償性肝硬変症例に対するトルバプタンの効果
―尿浸透圧による有効性の評価―

Effect of tolvaptan in decompensated liver cirrhosis patients with ascites or hepatic edema ; evaluation of efficacy by urine osmolality

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Summary

非代償性肝硬変症例の難治性腹水・浮腫に対する治療として，選択的バソプレシンV_2受容体拮抗薬のトルバプタン（TLV）が使用可能となった。今回，TLVによる治療を試みた非代償性肝硬変症例に関して治療効果と効果予測因子を検討し，さらにTLV投与後の尿浸透圧変化と治療効果との関連につき検討した。TLVにより体重減少効果がみられた有効率は83%（10/12），投与7日後に3kg以上の体重減少がみられた例は64%（7/11）であり，従来の利尿薬治療で効果が不十分な症例における治療効果は十分に期待できる結果であった。副作用は重篤なものは認めず，Na値の異常上昇も観察された範囲ではみられなかった。なお，TLV投与前データによる治療効果予測は困難であった。投与後6時間での尿浸透圧は投与症例の多くで低下しており，その低下の程度は投与7日後の体重減少量と相関していた。相関から外れていたのは肝不全進行中の例・電解質異常例・腎機能低下例であり，このような要因をもつ症例では治療に注意が必要と考えられた。

Keywords

トルバプタン　難治性腹水　肝性胸水　尿浸透圧

はじめに

非代償性肝硬変症例において，腹水・胸水・浮腫は患者のQOLを大きく損なう病態であり，通常は塩分制限や利尿薬投与で治療されるが，従来の利尿薬などの治療に反応しない難治例もしばしば経験する。このような症例に対して選択的バソプレシンV_2受容体拮抗薬トルバプタン（TLV）が使われるようになった。TLVは腎の集合管における水利尿を促す薬剤であり，血清K値に影響を及ぼすことなく利尿をつけることができるという特

徴をもち，うっ血性心不全に対して認可された後，肝性浮腫・腹水に対する効能も追加された。難治性の肝性浮腫・腹水に対するTLVの効果が期待されているが，利尿効果があまり得られない症例もみられ，その効果予測に関してはまだ確実な方法はないとされている。われわれは，TLV投与後の尿浸透圧変化を測定し，その変化と治療効果との関連につき検討した。

方　法

対象は，スピロノラクトンおよびフロセミドによる治療効果が不十分で腹水貯留や浮腫を伴う非代償性肝硬変14例である（表1）。平均年齢は68.7歳で，男性12例，女性2例であった。肝硬変の原因はB型肝炎ウイルス1例，C型肝炎ウイルス8例，非B非C型（NBNC）5例で，肝細胞癌（HCC）合併例は6例であった。Child-Pugh（CP）スコアは10点以上の症例が9例と，進行した肝硬変症例が多く含まれていた。これら14例のうち当院でTLVを導入した12例を治療効果予測因子の検討対象とした。TLVは3.75mg，7.5mg（一部症例は15mg）で投与を開始しており，投与開始後は毎日体重と尿量を測定し，各種血液検査は投与3日後・7日後に施行した。また，投与開始前および初回投与の6時間後・1日後・3日後に尿浸透圧の測定を行った。経過中飲水制限は行わず，食事での塩分摂取は6 g/日とした。

一部の症例はうっ血性心不全を合併し，その投与量に準じた15mg投与とした。

表1．対象症例の臨床的背景

年齢（歳（mean ± SD））	68.7 ± 9.4
性別（男性：女性）	12：2
肝炎ウイルス（B：C：NBNC）	1：8：5
大量飲酒歴（有：無）	7：7
HCC合併（有：治療後：無）	3：3：8
CPスコア（9点以下：10点以上）	5：9
フロセミド平均用量（mg）	52.9
スピロノラクトン平均用量（mg）	46.4
カンレノ酸カリウム平均用量（mg）	57.1
TLV開始量（15mg：7.5mg：3.75mg）	6：7：1

結　果

当院でTLVを導入した12例中10例で2～11kgの体重減少がみられたが，残る2例では投与開始後1週間を経過しても体重減少がみられなかった（図1）。血液検査データでは，血清Na値は投与3日後にわずかに上昇する例が多かったが正常範囲内の変動で，7日後には低下していた。血清Cr値は，TLV投与後に特別な異常変動はみられず，投与前から2 mg/dLを超える腎機能低下症例でもそれ以上の悪化はみられなかった（図2）。尿浸透圧の変化をみると，測定しえた9例中7例で投与6時間後に明らかな低下が認められたが，2例では変化はみられなかった（図3）。また，6時間後に尿浸透圧の低下がみられた症例でも投与3日後には尿浸透圧はやや上昇する例が多かった。

効果予測因子について検討するため，投与開始後1週間での体重減少が3 kg以上であった症例を有効群として有効群と効果不十分群に分け，臨床的背景を比較した（表2）。有効群で年齢が有意に高かった以外は，CPスコアや肝硬変の成因，飲酒歴，治療開始時の体重などの臨床的背景には有意差を認めなかった。治療前の血液データや投与後の尿浸透圧変化を比較した結果でも，有効群と効果不十分群の間に有意差を認めなかった。

TLV投与後の尿浸透圧低下量と体重減少量との相関

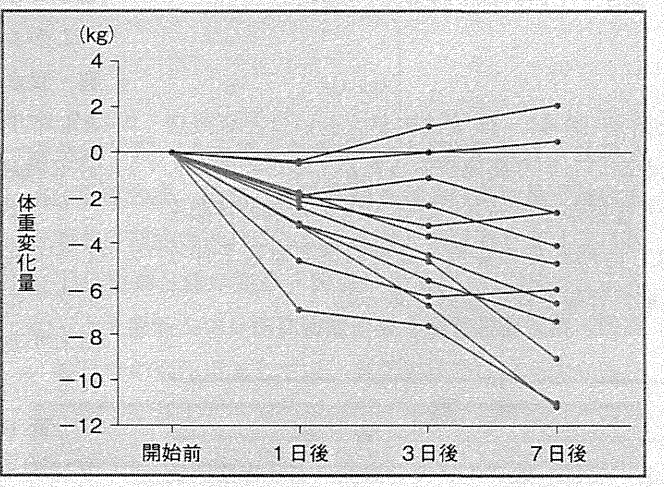

図1．TLV投与時の体重変化

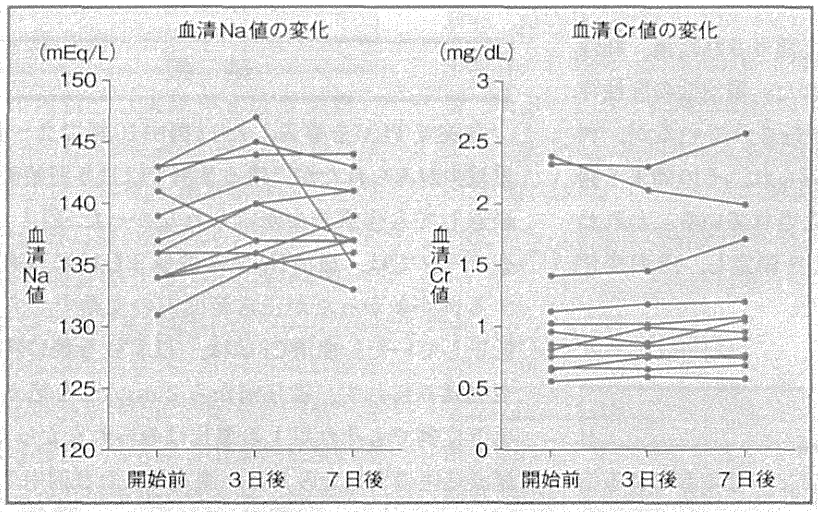

図2．TLV投与時の血清Na値および血清Cr値の変化

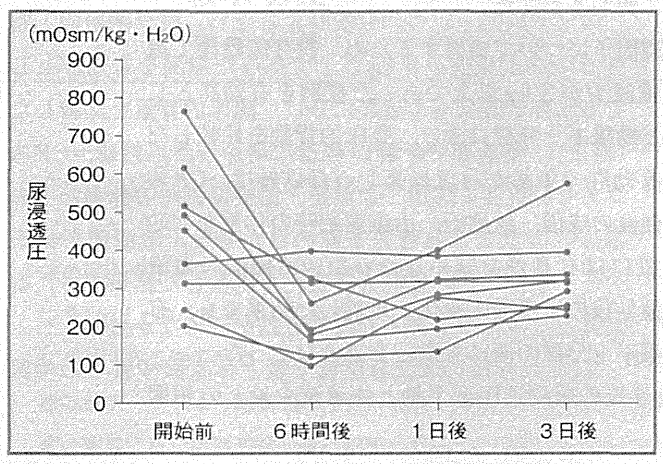

図3．TLV投与時の尿浸透圧の変化

を検討した結果（図4），体重減少が大きいほど尿浸透圧の低下量が高いという関連があったが，その相関から外れてしまう症例が3例あった。これらの症例の特徴をみると，HCC治療直後の症例・高度の低K血症を伴う症例・糖尿病性腎症を伴う症例であった。

考　察

肝硬変においては，低アルブミン血症による血漿膠質浸透圧の低下・門脈圧亢進・全身の末梢毛細血管の拡張などが複雑に関与しあい，腹水・浮腫などの水貯留症状が出現するとされている[1]。このような病態は肝性浮腫・腹水と呼ばれており，安静・塩分制限・利尿薬投与などによる治療が一般的である[2]。難治性の場合には腹水穿刺排液や腹水濾過濃縮再静注，また腹腔－上大静脈シャントの造設で対応することもある[2]。腹水に対する利尿薬治療としては，ループ利尿薬であるフロセミドや抗アルドステロン薬のスピロノラクトンが一般的であるが，これらの薬剤を多用すると電解質異常や腎機能障害などの副作用がしばしば発生し，その使用制限が必要となることが多い。

このような，従来の利尿薬で効果不十分である肝性浮腫・腹水に対して，新たなメカニズムで利尿を促す薬剤としてTLVが使用可能となった[3)-5)]。TLVは腎の集合管主細胞の血管側に分布するV_2受容体をブロックすることにより，抗利尿ホルモン（ADH；バソプレシン）によって起こる水の再吸収を抑制する。このADH作用はアクアポリンを介して起こるものであり，Na^+やK^+の出入りを伴わずに純粋な水利尿として利尿効果が発揮される。

今回TLVによる治療を行った症例のなかでは，12例中10例で7日後までに体重減少が認められ，利尿効果

表2．有効群と効果不十分群の比較

		有効群（n＝7）	効果不十分群（n＝4）	p値
臨床的背景	年齢	73 ± 6	64 ± 14	0.038
	性別（男性：女性）	6 ： 1	3 ： 1	NS
	体重	68.3 ± 7.7	63.8 ± 8.1	NS
	CPスコア	10.1 ± 1.5	9.8 ± 1.7	NS
	成因（B：C：NBNC）	1 ： 4 ： 2	0 ： 3 ： 1	NS
	飲酒歴（有：無）	3 ： 4	2 ： 2	NS
治療前データ	Alb	2.4 ± 0.3	2.3 ± 0.5	NS
	BUN	24.5（12.1～52.2）	16.1（11.8～59.0）	NS
	Cr	1.03（0.6～2.4）	0.79（0.7～2.3）	NS
	Na	138 ± 3	138 ± 5	NS
	K	3.9 ± 0.3	4.0 ± 0.8	NS
	血漿浸透圧	294 ± 6	278 ± 14	NS
	尿浸透圧	518 ± 172	421 ± 173	NS
治療後データ	尿浸透圧低下（6時間後）	228 ± 94	219 ± 94	NS

mean ± SD
有効群：1週目の体重減少が3 kg以上の症例

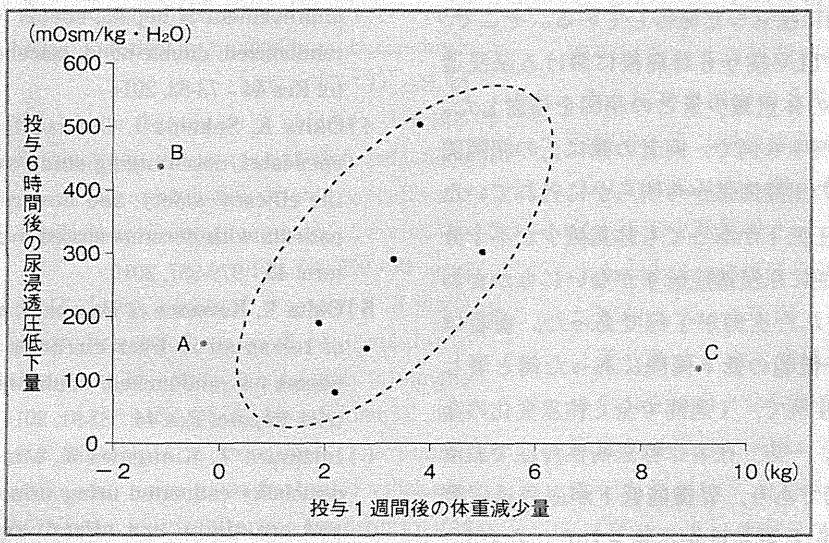

図4．TLV投与後の尿浸透圧低下量と体重減少量の関係
A：HCC治療直後の症例，B：電解質異常症例，C：腎障害合併症例

はきわめて良好であった。全く体重減少効果がみられなかった2例のうち1例は低K血症の電解質異常を伴った症例で，K補正後に体重減少効果がみられた。他の1例はHCCに対するinterventional radiology（IVR）治療直後で肝予備能の低下傾向にある症例であり，その後腹腔－上大静脈シャントによる治療を要した。

投与中の副作用としては，自覚症状では口渇感や倦怠感の訴えがあり，一部の症例では頻尿による不眠がみられていた。水利尿に伴って血清Na値の上昇が起こりうることが指摘されているが，今回の症例のなかでは臨床

的に問題となるような高Na血症を呈した症例は認められなかった。また，投与中の血清Cr値の変動もほとんどみられず，従来の利尿薬でしばしば指摘された腎機能障害はTLV使用においては少ないことが確認された。

TLVの効果予測の可能性を検討するために，体重減少の程度により有効群と効果不十分群に分けてその臨床所見を比較したが，両群の間に有意差を呈する項目は認められなかった。今回の経験症例数はまだ少なく，統計学的な有意差を論じるのは適切ではないが，少なくとも有効群と効果不十分群の間の開始前背景に明らかな差があるとは考えにくい。このことから，TLV投与前データからその効果を予測することは困難であろうと考えられる。

TLVの作用機序が水の再吸収抑制による水利尿であることから，TLV投与後には尿浸透圧が低下することは容易に了解しうる。Imamuraら[6]は，うっ血性心不全に対する治療でTLVを使用した際の尿浸透圧変化の程度がTLV効果予測に役立つと報告している。そこで，今回の症例に対してTLV投与6時間後における尿浸透圧低下量と1週間後の体重減少量との相関を検討した。データの得られた9例中6例で，両者の間に正の相関関係が認められた。この相関関係から明らかに外れていたのは3例で，尿浸透圧低下があっても体重減少が不十分であった例が2例，逆に尿浸透圧低下がないにもかかわらず体重減少がみられた症例が1例であった。前者はHCC治療直後で肝予備能の低下傾向にあった例と著しい低K血症を伴った症例で，1週間で全く体重変化のなかった例と一致した。一方，後者は糖尿病性腎症で血清Cr値の高値を伴う例であり，腎機能低下が尿浸透圧変化に影響を及ぼしている可能性が示唆された。ただし，尿浸透圧の低下がみられなかったこの症例で体重減少がみられたメカニズムに関しては不明である。治療効果予測の可能性に関しては，今後さらに検討を重ねていく必要があると思われる。

まとめ

腹水・胸水・浮腫を伴う非代償性肝硬変症例に対してTLV投与を行った結果，12例中10例（83%）の症例で体重減少効果が得られた一方，TLVによる重篤な副作用は認めなかった。また，TLV投与前データからの効果予測は困難であった。投与後6時間での尿浸透圧低下量は多くの例で体重減少量と相関していたが，肝不全進行中の例・電解質異常例・腎機能低下例はその相関から外れていた。

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強力ネオミノファーゲンC（SNMC）の効果

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アークメディア

強力ネオミノファーゲンC（SNMC）の効果

長谷部　千登美*

索引用語：薬物性肝障害，強力ネオミノファーゲンC（SNMC），グリチルリチン

1 はじめに

薬物性肝障害は多種多様の薬物が原因となって発生するが，その多くはアレルギー的なメカニズムによるといわれている．病態として，肝細胞障害型と胆汁うっ滞型・そして両者の混合型に分類され，治療を考えるうえでその病態を考慮することも必要になる．薬物性肝障害に対する治療の原則は，原因薬剤を直ちに止めることであり，多くの場合はそれだけで肝障害の改善をみる．しかし臨床の場においては，肝障害の改善を目的として，強力ネオミノファーゲンC(SNMC)などの肝庇護剤もしばしば使用されている．

本稿では，薬物性肝障害に対する治療としてSNMCの適応ならびにその効果につき，文献的考察を加えて概説する．

2 薬物性肝障害に対する一般的治療

薬物性肝障害は，薬物自体の毒性による肝障害とアレルギー機序による肝障害に大別されるが，その多くが後者によるものといわれている．これは，投与された薬物自体，あるいはその代謝産物に対して生体がなんらかの過敏反応を起こして肝障害が出現するものであり，同一の薬物でも肝障害を呈する症例と呈さない症例が存在する．肝障害のパターンとしては肝細胞障害型と胆汁うっ滞型，そしてその両者混合型に，臨床所見から分類されている．

薬物性肝障害が疑われた場合にまず重要なことは，原因として疑わしい薬物を休薬することである．肝障害の程度が軽い場合には，原因薬物の休薬のみで改善することが多い．

薬物性肝障害に関する全国調査の報告によると，このように原因薬物の休薬で治療された症例が98％を占めていたとされている[1]．

治療方法としてしばしば用いられているのが，SNMCの静脈注射による治療である．これは上記全国調査の報告では，29％の症例で用いられたと報告されている[1]．そのほかの治療方法としてしばしば用いられているのが，UDCAの経口投与であり，重症例に対してはステロイド剤投与も行われることがあ

Chitomi HASEBE: Effect of Stronger Neo-Minophagen C on drug-induced hepatitis
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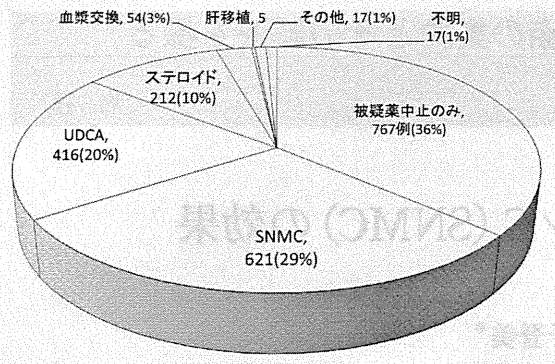

図1　薬剤性肝障害に対する治療法の現状（文献1のデータをもとにグラフ化）

り，さらに血漿交換療法・肝移植などの肝不全に対する治療法が必要となる症例も報告されている（図1）．

3　強力ネオミノファーゲンC（SNMC）に関して

SNMCはグリチルリチン（0.2%），システイン（0.3%），グリシン（2.0%）からなる配合薬で，その主要な成分はグリチルリチンである[2]．グリチルリチンは生薬として古くから用いられてきた甘草の一有効成分で，2分子のグルクロン酸とトリテルペノイド系サポニンの一種であるグリチルレチン酸の抱合物である．本剤の歴史は古く，本邦においてはその抗アレルギー作用および解毒作用を利用して，アレルギー性疾患および薬物中毒の治療として皮膚科領域で広く用いられてきた．

肝炎に対しては，1958年に，血清トランスアミナーゼ値の改善効果が示され[3]，以後慢性肝炎や肝硬変に対して血清トランスアミナーゼ値の改善効果やそれに伴う発癌抑制効果も報告され[4,5]，インターフェロンなどの抗ウイルス治療が無効あるいは不適応の症例に対して広く用いられている．

4　SNMCの効果・作用機序

SNMCの主成分であるグリチルリチンは，グリチルレチン酸の3位に2分子のグルクロン酸が結合した化合物であり（図2），抗酸化作用・抗炎症作用・抗ウイルス作用・抗アレルギー作用やT細胞の活性化作用などの多彩な生理作用が報告されている（表1）．

抗炎症作用の機序としては，phospholipaseA2活性の阻害[6]，prostaglandinE2産生抑制[7]などが報告されてきた．炎症による免疫系細胞や肝細胞の障害に関与する機能性因子（Functional protein）の中には，グリチルリチン結合サイトを有する因子（Glycyrrhizin binding functional protein: GBFP）が多く，グリチルリチンがこれらのGBFPに直接結合して生理機能を抑制することにより，抗炎症作用を発揮すると理解されている[8]．

また，グリチルリチンは抗原提示細胞に対するT細胞の細胞障害性を抑制し，TNF-αによって誘導される細胞障害性も抑制することが示されている．これはグルココルチコイド類似作用であり，肝障害における効果発現のメカニズムにも関与している可能性が考えられる[9]．

5　薬物性肝障害におけるSNMC治療の現状

薬物性肝障害の多くはアレルギー機序によるものであり，SNMCは抗炎症作用・抗アレルギー作用を有すると証明されていることから，その治療手段としてSNMCがたびたび用いられるのは理にかなったことと考えられる．実際に約30%の症例で本剤が使用されているという現状[1]もあり，治療効果が得られる可能性が高いものと考えられる．

1例報告で，薬物性肝障害に対してSNMC

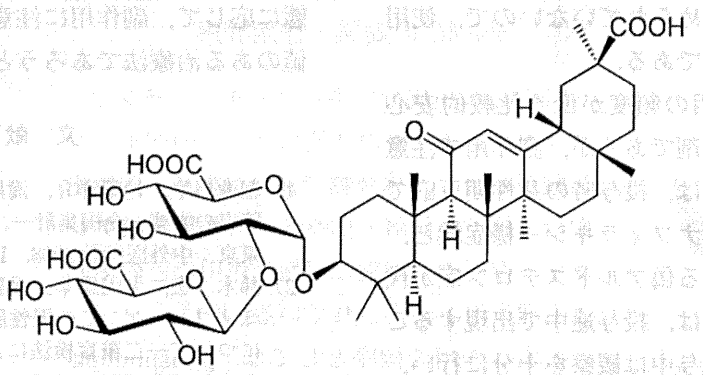

図2　グリチルリチンの化学構造

表1　SNMC各成分の薬効

グリチルリチン酸，グリチルレチン酸	
抗炎症作用	①抗アレルギー作用…ウサギにおけるアルツス反応抑制・シュワルツマン反応抑制，コルチゾンの作用に対しストレス反応抑制作用を増強 ②アラキドン酸代謝系酵素の阻害作用…ホスホリパーゼA2などの酵素のリン酸化を介する活性化を選択的に阻害
免疫調節作用	T細胞活性化調節作用，インターフェロン-γ誘起作用，NK細胞活性化作用，胸腺外Tリンパ球分化増強作用
実験的肝細胞障害抑制作用	四塩化炭素による肝細胞障害を抑制（ラットの初代培養肝細胞の系）
肝細胞増殖促進作用	肝細胞増殖を促進（ラットの初代培養肝細胞の系）
ウイルス増殖抑制・不活化作用	マウス肝炎ウイルス感染実験で，生存日数の延長 ヘルペスウイルスなどの増殖抑制・不活化作用
グリシン，L-システイン塩酸塩	
副作用軽減	グリチルリチン酸の大量長期投与による電解質異常に基づく偽アルドステロン症の発症を抑制ないし予防

が有効であったとする報告は，過去に散見される[10～12]．しかし，SNMCの薬物性肝障害に対する効果を，非投与例と比較するなどして厳密に証明した報告はないようである．一方，SNMC投与群と非投与群で，血清ALT値の改善に要した期間に差はなかったとする報告[13]もある．

SNMCの肝障害に対する効果が証明されているのは，慢性肝炎・肝硬変に対する使用であり，40 mL～100 mLの静注または点滴静注という方法で大変頻繁に使われている[5]．このような使用法で血清ALT値の改善が明らかにみられることが多く，急性肝炎や薬物性肝障害における血清ALT上昇に対しても，同様の効果が期待されて頻用されているものと考えられる．

SNMCは従来から，抗アレルギー作用により皮膚科領域での適応が広く認められており，保険適応ともなっている．しかし，薬物性肝障害を初めとした急性肝障害に対して

は，保険適応は認められていないので，使用に際し注意が必要である．

SNMCは副作用の頻度が低く比較的安心して使用可能な薬剤であるが，副作用で注意を要する点としては，投与時の急性期反応であるショック・アナフィラキシー様症状と，長期投与時に起こる偽アルドステロン症が代表的である．前者は，投与途中で出現することが多いので，投与中は観察を十分に行い，異常が認められた場合には直ちに投与を中止し，適切な処置を行わなければならない．偽アルドステロン症は，増量または長期連用により低カリウム血症・血圧上昇・ナトリウム貯留・浮腫・体重増加などの症状が現れるものである[5]．臨床的にSNMCと同時に使用されることの多い抗潰瘍薬や健胃消化剤・鎮咳去痰剤・下剤などの中には甘草成分を含有するものがあるため，これらの併用には特に注意を要する．また，肝硬変症例ではフロセミドなど低カリウム血症を助長しやすい薬剤の併用を要することも多く，慎重な経過観察が重要である．

6 まとめ

薬物性肝障害に対するSNMC治療の現状ならびにその作用機序について，文献的考察を加えて概説した．SNMCは抗炎症作用・抗アレルギー作用などをもち，古くから広く使用されている薬剤であるが，薬物性肝障害に対する効果のevidenceは確立されていない現状である．しかし，その作用機序からみて肝障害改善効果が発揮される可能性は高く，実際の臨床においても高頻度に使用されている．薬物性肝障害の治療としては，原因薬剤の中止が基本となるが，SNMCは肝障害の病態に応じて，副作用に注意しながら試みる価値のある治療法であろうと考えられる．

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Review

Actin directly interacts with different membrane channel proteins and influences channel activities: AQP2 as a model ☆

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ABSTRACT

The interplay between actin and 10 membrane channel proteins that have been shown to directly bind to actin are reviewed. The 10 membrane channel proteins covered in this review are aquaporin 2 (AQP2), cystic fibrosis transmembrane conductance regulator (CFTR), ClC2, short form of ClC3 (sClC3), chloride intracellular channel 1 (CLIC1), chloride intracellular channel 5 (CLIC5), epithelial sodium channel (ENaC), large-conductance calcium-activated potassium channel (Maxi-K), transient receptor potential vanilloid 4 (TRPV4), and voltage-dependent anion channel (VDAC), with particular attention to AQP2. In regard to AQP2, most reciprocal interactions between actin and AQP2 occur during intracellular trafficking, which are largely mediated through indirect binding. Actin and the actin cytoskeleton work as cables, barriers, stabilizers, and force generators for motility. However, as with ENaC, the effects of actin cytoskeleton on channel gating should be investigated further. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

Contents

☆ This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

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1. Introduction

Actin is a major cytoskeleton protein found ubiquitously within the cells that is involved in almost all biological events, particularly events related to motility [1,2]. The production of membrane channel proteins involves the following sequential events: proteins are first produced in the endoplasmic reticulum (ER); then they are incorporated into the membrane of small vesicles, transported through the Golgi apparatus and trans-Golgi network, transferred to the sub-plasma membrane region of designated domains, introduced into a dense actin mesh beneath the plasma membrane, and lastly fused with the plasma membrane such that they appear on the surface. The membrane channel activities can be directly modulated while they are located at the surface, then the channels get endocytosed and pinched off from the plasma membrane, and finally transferred to a pool of recycling vesicles or lysosomes for degradation. In each of these sequential events, rapidly growing evidence indicates that actin and actin-based cytoskeleton complexes are involved. Molecular and proteomic studies have identified a large number of actin binding proteins, and expanded our understanding of functional interplay between membrane channel proteins and actin. The interplay can be by direct binding or indirect binding via actin binding proteins [1,3,4]. In this review, 10 membrane channel proteins that have been previously shown to directly bind to actin are selected and their binding characteristics are summarized (Table 1). We searched the PubMed database with the key words "actin and channel" and evaluated each report whether or not it concerned "direct binding". Direct binding was judged by the presence of experiments with co-sedimentation, co-immunoprecipitation, gel overlay, surface plasmon resonance, fluorescence cross-correlation spectroscopy, FRET, and atomic force microscopy. Special attention is paid to AQP2, which serves as a model for direct and indirect binding of actin and membrane channel proteins.

Table 1
Membrane channel proteins directly bound to actin.

Channels	F- or G-actin	Detection methods	Physiological roles	References
AQP2	G-actin	Co-immunoprecipitation, co-immunoprecipitation, surface plasmon resonance, fluorescence cross-correlation spectroscopy	Exocytosis	Noda et al. [20,22], Moeller et al. [30]
CFTR	F-actin	Patch clump, atomic force microscopy	Channel activity	Cantiello et al. [42], Chasan et al. [43]
ClC2	F-actin	Gel overlay, co-sedimentation	Channel activity	Ahmed et al. [45]
sClC3	F-actin	Co-sedimentation	Channel activity?	McCloskey et al. [47]
CLIC1, CLIC5		In vitro reconstitution	Channel activity	Singh et al. [49]
ENaC	F-actin	In vitro reconstitution, co-immunoprecipitation, gel overlay, co-sedimentation	Channel activity	Berdiev et al. [57], Jovov et al. [58], Copeland et al. [59], Mazzochi et al. [60]
Maxi-K	F-actin?	Co-immunoprecipitation	Channel activity	Brainard et al. [64]
TRPV4	F-actin?	FRET, co-immunoprecipitation, co-sedimentation	Channel activity	Ramadass et al. [67], Goswami et al. [68], Shin et al. [69]
VDAC	G-actin	In vitro reconstitution, surface plasmon resonance	Channel activity	Xu et al. [70], Roman et al. [72]

F- or G-actin indicates the kind of binding actin; F for filamentatous and G for globular actin.

2. Actin and actin binding proteins

Actin is indispensably involved in cell shape and movement in all eukaryotic cells [5]. An actin monomer is a globular protein (G-actin) that contains one molecule of ATP or ADP. Its physiological function largely depends on the ability to polymerize and to form filamentous F-actin. F-actin consists of two strands characterized by a left-handed double helix. F-actin is polar in structure with a fast growing plus- or barbed-end and a minus- or pointed-end. The continuous occurrence of plus-end polymerization and minus-end depolymerization is called treadmilling. Actin binds a large number of actin-binding proteins, including proteins that initiate the nucleation of new actin filaments (Arp2/3 complex, WASP, formins); sequester monomeric actin (β-thymosins, profiling, cofilin); stabilize F-actin (tropomyosins); sever and cap F-actin (gelsolin, severin); crosslink F-actin (α-actinin, fimbrin), myosin filaments and motor proteins; and many others [1].

3. Direct or indirect binding

Membrane channel proteins can bind to actin directly or indirectly via actin-binding proteins. Given that the nature of actin is dynamic due to its continuous remodeling, the binding of actin to membrane channel proteins may not be static. This results in difficulty in examining the precise interaction between actin and membrane proteins. Nevertheless, many strategies have been employed to examine the binding of actin to membrane channel proteins, including co-sedimentation, co-immunoprecipitation, gel overlay, surface plasmon resonance, fluorescence cross-correlation spectroscopy, FRET, and atomic force microscopy. When the functional effects of bindings are considered, it is not a major issue whether binding of actin is direct or indirect via actin binding proteins. However, it is important when considering the precise mechanisms and the future development of drugs that interact the binding. The methodologies used to determine the direct binding of actin to each channel protein are summarized in Table 1. It is desirable that several methods are used to demonstrate direct binding, and indeed in most cases this has been done (Table 1).

4. AQP2: Intracellular trafficking is the main mechanism of regulation

AQP2 is a vasopressin-regulated water channel predominantly expressed in kidney collecting duct principal cells [6,7]. Upon vasopressin stimulation, water permeability of the collecting duct is dramatically increased, allowing significant water reabsorption and urine concentration. Gene mutations in AQP2 cause human nephrogenic diabetes insipidus, a disease characterized by the inability to concentrate urine, demonstrating the indispensable role of AQP2 in urine concentration [7]. Circulating vasopressin binds to vasopressin V2 receptor expressed on the basolateral membrane of collecting duct cells, stimulates adenylate cyclase, and increases intracellular cAMP levels. Increased cAMP then activates protein kinase A, which phosphorylates the AQP2 protein itself, and this phosphorylation in turn induces accumulation of AQP2 on the luminal surface via a balance between exocytosis and endocytosis [8,9]. Thus, the main regulatory mechanism of AQP2 is intracellular trafficking, where both actin and actin binding proteins are involved.

4.1. Transport to the subapical region of the luminal membrane

Newly produced AQP2 is transported from the ER to the subapical region of the apical membrane. AQP2, like other proteins, carries signals embedded within its structure, which specify its subcellular destinations and micro-domains [10]. AQP2 was thought to be delivered directly to the apical membrane; however, Yui et al. [11] recently showed that a considerable amount of AQP2 first travels to the basolateral membrane and then back to the apical membrane via a

process known as "transcytosis". The physiological significance of this needs to be further clarified.

Intracellular trafficking of AQP2 vesicles requires cables, motor proteins and other cargo associated (adapter) proteins. Findings from proteomic analysis of intracellular AQP2 vesicles provide useful information [12]. Immunoisolated AQP2-bearing intracellular vesicles from rat inner medullary collecting ducts show the presence of a large variety of proteins, including actin cytoskeletal proteins (i.e., β- and γ-actin, ARP2/3, destrin, gelsolin, β-spectrin-3, α-tropomyosin), myosin isoforms, tubulin, Rab GTPases, and SNARE proteins. The identified myosin isoforms include myosin regulatory light chain and conventional non-muscle myosin IIA and IIB as well as unconventional myosin 1C, VI, and IXB. All of these proteins together with actin fibers may participate in vesicle trafficking. It is important to note that the origin of vesicles could be heterogeneous, including vesicles during all stages of trafficking, and all these interacting proteins do not necessarily co-exist at the same time.

4.2. Exocytosis

Transported AQP2 vesicles are stored beneath the apical membrane and are characterized by Rab11 staining [13]. While these storage vesicles undergo exocytosis and fuse with the apical membrane after vasopressin stimulation, exocytosis occurs constitutively to a certain extent. The actin cytoskeleton has been thought of as a barrier for AQP2 exocytosis. Vasopressin-induced depolymerization of apical F-actin in rat inner medullary collecting duct supports this theory [14]. Tamma et al. [15] demonstrated that cytochalasin D, an F-actin depolymerizing agent, induces AQP2 plasma membrane accumulation in cultured collecting duct cells. RhoA and its downstream effecter Rho kinase are known to stimulate actin polymerization and indeed, the inhibition of this cascade by *Clostridium difficile* toxin B or by Y-27632 results in AQP2 accumulation in the plasma membrane [15,16], confirming the barrier function of F-actin during exocytosis.

Recent observations from experiments performed in adrenomedullary chromaffin cells (reviewed by Gutierrez [17]) seem highly relevant in examining the interplay between AQP2 exocytosis and actin. Advancements in microscopic technologies (i.e., use of high numerical aperture objectives in transmitted light scanning microscopy and three-dimensional reconstruction, and total internal reflection fluorescence microscopy) allow for the analysis of fine structures of living cells. Studies using novel techniques revealed that the cell cortex consists of a dense network of F-actin that blocks the access of vesicles to their secretory sites. After stimulation, dynamic changes occur in these dense cytoskeletal structures with the formation cortical disruption of F-actin mesh and channel-like structures, which allow vesicles to access their docking sites [18]. Thus, depolymerization of F-actin is necessary to clear the route toward the cell surface; however, positive participation of actin in the movement of secretory vesicles toward the cell surface is necessary. Actin forces vesicles to move toward the cell surface by the force generating mechanisms: 1) polymerization and creating new actin fibers, and 2) interactions with myosin motor proteins [19].

Similar mechanisms are expected to take place in the exocytosis of AQP2 vesicles. To clarify the molecular mechanisms involved in AQP2 trafficking, Noda et al. [20,21] isolated proteins that directly bind to AQP2. Isolation via immunoaffinity chromatography covalently coupled with anti-AQP2 antibody and subsequent analysis by MALDI-TOF MS identified 13 new AQP2-binding proteins; namely, actin, SPA-1, tropomyosin 5b (later classified as an isoform of tropomyosin 1), ionized calcium binding adapter molecule 2, myosin regulatory light chain smooth muscle isoforms 2-A and 2-B, annexins A2 and A6, scinderin, gelsolin, alpha-actinin 4, alpha-II spectrin, and myosin heavy chain nonmuscle type A. The region to which actin binds was determined to be at the C-terminus [20]. Since most identified proteins have actin binding ability, it was speculated that a part of these proteins may indirectly associate with AQP2 via actin, and that these proteins create AQP2-binding protein complexes. Thus, dynamic interactions of these proteins are expected to regulate AQP2 trafficking [10,19].

Studies have shown that both α- and γ-actin bind to the C-terminus of AQP2, and furthermore, that G-actin binds to AQP2, whereas F-actin does not [20]. Further studies by Noda et al. [22] showed that phosphorylation of AQP2 via cAMP dissociates G-actin from AQP2, and that phosphorylated AQP2 increases its affinity to tropomyosin 5b, resulting in reduction of tropomyosin 5b bound to F-actin, which subsequently induces F-actin destabilization and depolymerization. These findings indicate that the transport membrane protein, AQP2, per se works to reorganize its surrounding actin cytoskeleton to open up its way to the cell surface. Consistent with this idea, the presence of AQP2 is necessary for vasopressin-mediated burst of exocytosis and cortical F-actin depolymerization in cultured renal epithelial cells [23,24].

Another study performed by Zwang et al. [25] isolated AQP2-binding proteins via proteomic analysis of pulled-down samples that were bound to a synthesized AQP2 C-terminus. Consequently, seven proteins were identified: heat shock protein 70 (isoforms 1, 2, 5, 8), annexin II, protein phosphatase 1 catalytic subunit (PP1c), GDP dissociation inhibitor 2 (GDI-2), and Ras-related nuclear protein (RAN). Although these proteins do not overlap with those found by Noda et al. [21], the difference may be explained by the use of different methodologies. Nonetheless, the important message from these proteomic analyses of AQP2-binding proteins is that membrane channel proteins have a large number of partners that directly or indirectly bind to them and are involved in their trafficking.

4.3. Endocytosis and recycling

The abundance of AQP2 at the surface of the plasma membrane depends on the balance between continuing endocytosis and exocytosis. These two opposing mechanisms occur constitutively at a significant rate even without vasopressin stimulation [26]. With respect to endocytosis, AQP2 accumulates in clathrin-coated pits and is internalized via a clathrin-mediated process. Indeed, accumulation of AQP2 is observed when actin-mediated endocytosis is inhibited by a GTPase-deficient dominant negative dynamin mutant [27] and a cholesterol-depleting agent, methyl-beta-cyclodextrin [28]. The heat shock protein, hsc70, an actin-binding protein that is important for uncoating clathrin-coated vesicles, has been shown to bind to the C-terminus of non-phosphorylated AQP2 and contribute to AQP2 endocytosis [29].

In cultured MDCK cells, Moeller et al. [30] demonstrated that internalization of a mutant AQP2, which mimics phosphorylated-AQP2 is slower than that of a wild-type AQP2. Further a co-immunoprecipitation study showed that the slower internalization corresponds to a reduced interaction of the mutant AQP2 with several proteins involved in endocytosis, namely Hsp70, Hsc70, dynamin, and clathrin heavy chain, indicating that these proteins participate in endocytosis of AQP2. Interestingly, in the same study, a strong binding of γ-actin to the non-phosphorylated mimicking AQP2 peptide was observed, whereas binding to the phosphorylated mimicking AQP2 was weak, indicating that γ-actin is released from AQP2 after it is phosphorylated [30]. This observation is consistent with the view of Noda et al. [22] described above in which the binding of actin works as stabilizers of AQP2 to keep AQP2 away from exocytic process.

Following endocytosis, AQP2 is retrieved to EEA1-positive early endosomes through a phosphatidylinositol-3-kinase-dependent mechanism and then is transferred to Rab11-positive storage vesicles [13]. This transfer is believed to be mediated by actin filaments because the disruption of actin filaments with cytochalasin D or latrunculin B induces the accumulation of AQP2 in EEA1-positive early endosomes [31]. Rab11-positive storage vesicles are specific to AQP2 and may exclude other membrane transport proteins. Furthermore, AQP2 in the storage vesicles reappears on the apical membrane upon vasopressin stimulation [13]. Ubiquitination is known to promote endocytosis and subsequent degradation by multivesicular bodies or proteasomes. Ubiquitination at

lysine 270 of AQP2 is important for AQP2 endocytosis and degradation [32].

4.4. Regulation of channel function

There is controversy concerning whether phosphorylation of AQP2 per se regulates the water permeability of the individual AQP2 molecule. An earlier study in a *Xenopus* oocyte expression system showed that phosphorylation of AQP2 increases the osmotic water permeability (Pf) of oocytes by 1.6-fold without a significant increase in AQP2 protein on the oocyte surface [33]. However, this finding was not supported by subsequent studies by other groups using a similar method [34,35]. Consistent with the negative results, Lande et al. [36] showed no significant differences in Pf values of phosphorylated and dephosphorylated AQP2-rich endosomes prepared from rat inner medullary collecting ducts. However, in a recent study, Eto et al. [37] reconstituted recombinant human AQP2 in proteoliposomes, and showed that the Pf of proteoliposomes was enhanced approximately 2-fold by phosphorylation at serine 256, indicating that AQP2 water channel activity (possibly open probability) is directly regulated by its phosphorylation. There is increasing evidence that aquaporins are gated [38]. X-ray crystal structure analysis of the spinach aquaporin SoPIP2;1 suggests that in the closed conformation, a cytosolic loop connecting α helices caps the pore of the channel from the cytoplasm, preventing water passage, and in the open conformation, the loop is displaced [39]. Interestingly, the presence of a layer of densities sandwiched between single-layered crystal sheets of AQP2 has been observed and has been interpreted to be the N- and/or C-termini of AQP2, suggesting the possibility that the N- and/or C-termini of AQP2 function as the gate of the channel [40]. Given that the C-terminus of AQP2 binds to many proteins, including actin as described above, regulation of the channel activity (the open probability) of AQP2 together with interacting proteins should be examined further.

As seen above, there are many mutual influences between AQP2 and the actin cytoskeleton through direct and indirect bindings. Most influences occur in the trafficking process, which may also apply to other membrane channel proteins.

5. Other membrane channel proteins directly bound to actin

Most interactions of channels with actin cytoskeleton are mediated by indirect bindings through acting-binding proteins including scaffolding proteins. However, several proteins in addition to AQP2 have been reported to directly bind to actin. Table 1 summarizes these proteins. Below is a brief description of how their bindings were determined and of their functional roles.

5.1. Cystic fibrosis transmembrane conductance regulator (CFTR)

CFTR (cystic fibrosis transmembrane conductance regulator) is localized in the apical plasma membrane of many epithelia, including the airway and sweat gland, and functions as a regulated chloride channel. Extensive studies have been performed to decipher its regulatory mechanisms, as genetic mutations of CFTR result in the most common channelopathy, cystic fibrosis [41]. Cantiello [42] showed that when actin is added to the cytoplasmic side of excised inside-out patches obtained from a CFTR-expressing cell line, the CFTR current is activated. In contrast, the same treatment on the patches from the parental cell line does not induce a chloride current. Additionally, when cells are exposed to cytochalasin D, protein kinase A-activated CFTR current does not occur; however, it can be reversed by the addition of actin [42].

Chasan et al. [43] used atomic force microscopy (AFM) to visualize a direct interaction between actin and CFTR. Purified functional CFTR that had been reconstituted into phospholipid vesicles was placed on freshly cleaved mica and observed by AFM. After adding actin to the sample, AFM images showed the formation of filamentous actin associating with the CFTR molecules, indicating that there is a direct interaction between F-actin and CFTR. Taken together, the results of electrophysiological studies (patch clump) and AFM studies suggest that direct binding of CFTR with actin modulates the channel activity of CFTR; however, two reservations remain: 1) the above studies used native cell-derived materials, and thus, the co-segregation of actin binding proteins cannot be denied; and 2) biochemical data supporting a direct interaction between the two proteins, such as co-immunoprecipitation assays, are lacking.

5.2. ClC2 and sClC3

Both ClC2 and sClC3 (short isoform of ClC3) belong to the family of voltage-regulated chloride channels and show characteristics of hypotonic cell swelling-sensitive channels. Activation of these channels has been shown to contribute to the maintenance of physiological cell volume and fluid secretion by airway and gastric mucosa epithelia [44]. These channels have different biophysical properties, namely ClC2 exhibits inward rectification, whereas sClC3 exhibits outward rectification. Remodeling of the actin cytoskeleton has been shown to modulate the activation of ClC2 in *Xenopus* oocytes [45] and sClC3 during hypotonic stress of pulmonary artery smooth muscle cells [46] by using actin-disrupting agents.

Ahmed et al. [45] showed that a GST fusion protein containing the N-terminus of ClC2, which is known as an inhibitory domain, is capable of binding F-actin in overlay and co-sedimentation assays. The binding of actin to this synthetic peptide is expected to be mediated through electrostatic interactions because binding is inhibited at high concentrations of NaCl. This work suggests that actin directly and electrostatically binds to the N-terminus of ClC-2 and contributes to the regulation of this channel [45].

McCloskey et al. [47] also used a GST-tagged C-terminus of sClC3 to co-sediment actin. These assays demonstrated strong binding between the C-terminus of sClC3 to F-actin, but not to G-actin. Using co-sedimentation assays, F-actin binding region was determined to be located between amino acids 690 and 760 of sClC3. Furthermore, synthetic peptides corresponding to the actin-binding region prevented the binding of F-actin in vitro and greatly reduced the maximal current density of hypotonic stress-induced currents. The authors interpreted these findings to indicate that sClC3 is the major source of the hypotonic stress-induced, volume-sensitive outwardly rectifying anion currents, and the channel is regulated by direct interaction with F-actin [47].

5.3. CLIC1 and CLIC5

The chloride intracellular channel (CLIC) proteins are anion channels located intracellularly that do not fit the characteristics of classical ion channel proteins, as they have soluble and integral membrane forms with ion channel function. While CLIC proteins are highly conserved in vertebrates and there are six distinct isoforms (CLIC1–CLIC6), their physiological functions are largely unknown and knockout mice of these isoforms do not produce apparent defects [48].

Singh et al. [49] purified recombinant mammalian CLIC1, CLIC4 and CLIC5, and incorporated them into planar lipid bilayers. CLIC-induced membrane conductance was observed after the incorporation of CLIC proteins. However, F-actin added to the cytosolic side reversibly inhibited the membrane conductance in CLIC1 and CLIC5 membranes, whereas there was no effect on conductance in the CLIC4 membrane. This inhibitory effect could be reversed by cytochalasins, indicating the involvement of F-actin. These results suggest that the membrane form of CLICs modifies solute transport through interaction with actin and contributes to many cellular activities, including swelling or division of the cell, endocytosis and exocytosis, intracellular vesicle fusion, and apoptosis [49].

5.4. ENaC

ENaC is an amiloride-sensitive epithelial sodium channel consisting of a heteromultimer composed of three homologous but distinct subunits (α, β, and γ) that are encoded by different genes [50]. ENaC is expressed in the epithelia of the distal nephron, distal colon, airway, and other tissues where aldosterone stimulates sodium reabsorption. Interestingly, in the distal nephron, ENaC is co-localized with AQP2 except for the inner medullary collecting duct, where ENaC is absent [51]. Aldosterone-regulated sodium reabsorption in the kidney plays a central role in the maintenance of body fluid and blood pressure. It is also known that vasopressin stimulates ENaC-mediated sodium reabsorption significantly, but to a lesser extent, in humans [52].

There are several mechanisms responsible for the hormone-induced stimulation of ENaC. Available evidence suggests that most of the effects are due to an increased abundance of ENaC proteins at the apical surface of distal nephrons, which are produced by both the stimulated transcription of the gene and dysregulated trafficking of ENaC to and from the apical membrane [53]. Regulation via channel gating (changing open probability) may not be dominant in the regulation of ENaC activity, although some controversy regarding this remains [54]. Endocytosis of ENaC determines the apical surface expression of ENaC, and this process is accelerated by binding to Nedd4-2, which results in ubiquitin-dependent internalization (clathrin-mediated endocytosis) and degradation. Thus, both aldosterone and vasopressin phosphorylate Nedd4-2, which in turn impairs the binding of ENaC to Nedd4-2, thereby increasing the protein abundance of ENaC and sodium reabsorption [53].

Similar to other channel proteins, ENaC proteins travel within the cell from its site of production to final degradation, and during this process, interact with actin (mostly indirect) [55,56]. However, there are several lines of evidence that ENaC also interacts directly with actin. Berdiev et al. [57] demonstrated that short F-actin causes a two-fold decrease in unitary conductance and a two-fold increase in the open probability of recombinant αβγ-ENaC subunits reconstituted into planar lipid bilayers. The same group further defined the region of the C-terminus participating in actin-mediated regulation of α-ENaC, which is a 14-amino acid residue region between E631 and F644 [58,59].

To confirm these electrophysiological results, Mazzochi et al. [60] provided biochemical evidence for a direct interaction between F-actin and the C-terminus of α-ENaC. F-actin was found to co-immunoprecipitate with α-ENaC from whole cell lysate of MDCK cells stably transfected with three isoforms of ENaC. Gel overlay assays demonstrated that F-actin specifically binds to the C-terminus of α-ENaC. A direct interaction between F-actin and the C-terminus of α-ENaC was further corroborated by GST-tagged F-actin co-sedimentation studies. These data indicate that F-actin directly and specifically binds to ENaC and changes its channel activity, presumably the gating. Recently, even actin-binding proteins such as filamin and cortactin were shown to modulate the gating of ENaC [61,62]. Therefore, the importance of actin cytoskeleton binding either directly or indirectly in the channel gating of ENaC should be investigated further.

5.5. Maxi-K

The maxi-K channel, also called the big potassium (BK) channel or slo1, is large-conductance, voltage- and calcium-sensitive potassium channels. Maxi-K channels are formed by 2 subunits: the pore-forming α-subunit (KCNMA1) and the modulatory β-subunit. Intracellular calcium regulates the physical association between the α- and β-subunits. This channel creates a strong repolarizing current to counter-balance cell excitation in response to stimuli, and plays an essential role in the regulation of several key physiological processes, including smooth muscle tone and neuronal excitability [63].

Brainard et al. [64] found large amount of maxi-K channel proteins in the detergent-resistant membrane fractions (lipid raft microdomains) from human myometrium. Direct binding of actin to maxi-K was demonstrated by co-immunoprecipitation studies; both α- and γ-actin bind to it. Immunocytochemical and immunoelectron microscopy showed the proximity of both actin and the maxi-K channel within the same cell surface caveolar structures. Functionally, disruption of the actin cytoskeleton in cultured human myocardium smooth muscle cells by cytochalasins greatly increased the open-state probability of the channel, while stabilization of the actin cytoskeleton with jasplakinolide abolished this effect. These data indicate that direct interaction with actin cytoskeleton is important in the regulation of maxi-K channel function [64].

5.6. TRPV4

The transient receptor potential vanilloid 4 (TRPV4) cation channel, a member of the TRP vanilloid subfamily, is expressed in a wide range of tissues, where it contributes to the generation of a calcium signal and the depolarization of the membrane potential. The channel is activated by hypotonic cell swelling and other physical stimuli (shear stress and high temperature), and by many endogenous and synthetic ligands (arachidonic acids and phorbol esters) [65]. The importance of a functional interaction between TRPV4 and actin for the sensing of hypotonicity and the onset of regulatory volume decrease has been suggested in kidney collecting ducts where TRPV4 is located and may contribute to the concentration of hypotonic prourine [66].

Several proteins have been proposed to modulate TRPV4 subcellular localization and/or function. Ramadass et al. [67] used Forster resonance energy transfer (FRET) to examine the interaction of TRPV4 with actin. FRET is a nonradiative phenomenon between two fluorochromes requiring the emission spectra of the donor molecule overlapping with the absorption spectrum of the acceptor molecule. FRET only occurs when the interacting molecules are at a distance of 1–10 nm in living cells. In cultured CHO cells expressing both TRPV4-CFP and actin-YFP, excited donor molecules (CFP) exhibit an emission peak at 527 nm and a decreased lifetime between 460 and 490 nm, which corresponds to the resonance energy transfer to YFP. The observed difference in FRET efficiency indicates that the two fluorescent proteins are close in the range of 4 nm [67]. Thus, this study shows that TRPV4 and actin intimately associate with each other in living cells.

Goswami et al. [68] provided biological evidence for the direct binding of these two proteins by showing co-immunoprecipitation of TRPV4 and actin from cell lysate of CHO-TRPV4 stable cell line. They further showed that the C-terminus is the domain for actin binding. Similarly, using co-immunoprecipitation and co-sedimentation assays, Shin et al. [69] compared the bindings of actin with TRPV4 mutants and showed that phosphorylation on the Ser 824 residue of TRPV4 is required for its interaction with F-actin, which promotes channel activity, Ca^{2+} influx, and protein stability.

5.7. VDAC

The mitochondrial outer membrane is the interface between the mitochondria and the cytosol, and voltage-dependent anion channel (VDAC) is abundantly present in this membrane. There are three isoforms, specifically VDAC1–3, and almost all tissues express all three isoforms. VDAC is involved in the main pathways for ATP, ADP, and other mitochondrial metabolic substrates, and it performs multiple functions such as metabolite and energy exchange and apoptosis. Recent findings suggest that VDAC is regulated by cytosolic proteins, including actin [70,71].

Xu et al. [70] reconstituted purified yeast VDAC into membranes made with phosphatidylcholine. The addition of a very low concentration of actin (50 nM) to the bathing solution was enough to abolish most of the VDAC-mediated membrane conductance. The addition of α-actin had a stronger effect than β- and γ-actin had and the form of G-actin was effective, whereas F-actin was ineffective. Roman et al. [72] adopted another technology, surface plasmon resonance to examine