

P-glycoprotein is localized in caveolae in resistant cells and in brain capillaries

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Abstract A significant proportion of P-glycoprotein (P-gp) and caveolin was co-localized in caveolae isolated from resistant (CH^RC5) cells overexpressing P-gp and from drug-sensitive Chinese hamster ovary cells (AuxB1). The proportion of P-gp and caveolin associated with caveolar microdomains was higher in CH^RC5 cells grown in the presence of P-gp substrates (cyclosporin A or colchicine) than in untreated CH^RC5 cells. Co-immunoprecipitation of P-gp and caveolin from CH^RC5 lysates suggests that there is a physical interaction between them. Furthermore, co-localization of P-gp and caveolin was found in caveolae from brain capillaries, indicating that this association also takes place *in vivo*.

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Key words: Caveola; Caveolin; P-glycoprotein; Cyclosporin A; Multidrug resistance; Brain capillary

1. Introduction

Multidrug resistance phenotype in cancer cells has been associated with P-glycoprotein (P-gp) expression [1,2]. P-gp is a member of the ABC (ATP binding cassette) superfamily of transporters, which excludes from cells a wide variety of anticancer drugs and hydrophobic molecules [3]. This capacity of P-gp to interact with a large panoply of hydrophobic substrates [2,4–6], either endogenous (hydrophobic peptides and steroids) or exogenous (cyclosporin A (CsA), verapamil, calmodulin antagonists, etc.), suggests an important role for P-gp in drug metabolism and disposal. Its importance as a pharmacological target for drugs has far exceeded the role initially found for it as an export protein in multidrug resistance [7–9]. Previous studies have also proposed that P-gp may be involved in the intracellular processing of cholesterol and in lipid transport across membranes [10–15].

In various MDR cells changes in membrane lipid composition have been reported (elevated levels of cholesterol, glyco-

sphingolipids and sphingolipids) [16]. High amounts of cholesterol, sphingomyelin and glycosphingolipids are found in invaginated microdomains of the plasma membrane called caveolae [17,18]. These microdomains are present in a wide variety of cell types and are dynamic structures involved in transcytosis, potocytosis and signal transduction [19,20]. In addition to their distinct lipid composition, caveolar domains include signaling molecules such as tyrosine kinases [21], protein kinase C [22], growth factor receptors [23,24], glycerol-phosphatidylinositol-linked receptors, and GTPases such as Ras [25], Rap [26], RhoA [27] and subunits of heterotrimeric G proteins [28,29]. Recent studies have also shown that a portion of P-gp is localized in low density detergent-insoluble membrane fractions derived from a human colon adenocarcinoma cell line (HT-29-MDR) and from an MCF-7 cell line stably transfected with P-gp [30].

Caveolin, a 22 kDa integral membrane protein, is a marker of caveolae which binds cholesterol, interacts with other proteins and may play an important structural role in the formation of caveolae [19,20,31,32]. In the present study, co-localization of P-gp and caveolin was observed in sensitive cells (AuxB1) and in resistant cells (CH^RC5) overexpressing P-gp. Experiments were performed to determine whether this co-localization of P-gp is altered in resistant cells exposed to P-gp substrates (colchicine and CsA). Co-immunoprecipitation of both proteins was also carried out to evaluate whether P-gp and caveolin could be physically associated. Furthermore, using brain capillaries where P-gp expression is very highly expressed [33], we examine the possibility that P-gp is also localized in caveolae-enriched microdomains *in vivo*.

2. Materials and methods

2.1. Chemicals

Sensitive (AuxB1) and resistant (CH^RC5) cell lines were grown in alpha-MEM from Gibco BRL (Burlington, Ont., Canada) and 10% serum from HyClone Laboratories (Logan, UT, USA). Electrophoresis reagents were purchased from Bio-Rad (Mississauga, Ont., Canada). MAbs C219 and Ab-2, directed against P-gp, were from ID Labs (London, Ont., Canada) and from NeoMarkers (Union City, CA, USA), respectively. PABs against caveolin and paxillin were obtained from Transduction Laboratories (Lexington, KY, USA). The pAb directed against caveolin-1 linked to agarose beads and the pAb against focal adhesion kinase (FAK) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-mouse and anti-rabbit IgG horseradish peroxidase-linked whole antibody were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA) and enhanced chemiluminescence (ECL) reagents were from Amersham Pharmacia Biotech (Baie d'Urfée, Que., Canada). All other reagents were from Sigma-Aldrich Canada (Oakville, Ont., Canada).

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Abbreviations: CsA, cyclosporin A; FAK, focal adhesion kinase; mAb, monoclonal antibody; MDR, multidrug resistance; Mrp, multidrug resistance associated protein; P-gp, P-glycoprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2.2. Isolation of caveolae-enriched membrane domains

Low-density caveolae-enriched domains were isolated by a carbonate-based fractionation method as described previously [25]. Briefly, confluent CH^RC5 or AuxB1 cells cultured in 100 mm dishes (containing about 10⁷ cells) were scraped into 2 ml of 0.5 M sodium carbonate (pH 11) and homogenized extensively using a Dounce homogenizer (10 strokes), a Polytron tissue grinder (three 10 s bursts at medium speed) and a sonicator (three 20 s bursts at 50% maximal power). The resulting homogenate was brought to 45% sucrose by the addition of 2 ml of 90% sucrose in Mes-buffered saline (MBS; 25 mM Mes, pH 6.5, 150 mM NaCl) and overlaid with two layers of 35% and 5% sucrose in MBS containing 0.25 M carbonate (4 ml each). The gradient was then centrifuged at 200 000×*g* for 18 h using a Beckman SW41Ti rotor. For the analysis of the resulting gradient, 1 ml fractions were collected from the top to the bottom of the gradient. 10 or 20 µl from each fraction was subjected to SDS-PAGE for the detection of P-gp or caveolin, respectively.

2.3. Detection of P-gp, caveolin, FAK and paxillin

P-gp, caveolin, FAK and paxillin were detected by Western blot analysis. Proteins from CH^RC5 or AuxB1 cells were resolved by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Samples were resuspended in sample buffer and loaded on 7.5% acrylamide-bisacrylamide (29.2:0.8) gels for P-gp and FAK or 12.5% acrylamide-bisacrylamide gels for caveolin and paxillin. P-gp was detected (without prior heating) using mAb C219 as described previously [33] while caveolin was evaluated using a pAb [27]. Gels were subjected to SDS-PAGE and electroblotted onto PVDF membranes using standard procedures. Blots were blocked overnight in Tris-buffered saline (TBS; 147 mM NaCl, 20 mM Tris-HCl, pH 7.5 and 0.3% Tween 20) containing 5% non-fat dry milk, followed by a 1 h 30 min incubation with the primary antibody. Blots were washed three times with TBS containing 0.3% Tween 20 followed by a 1 h incubation with horseradish peroxidase-conjugated antibodies directed against mouse and rabbit IgGs. Blots were again washed three times with TBS and 0.3% Tween 20, and detection was performed with ECL reagents (Amersham) according to the manufacturer's instructions.

2.4. Immunoprecipitation procedures

Confluent CH^RC5 or AuxB1 cells cultured in 100 mm dishes were washed with PBS (150 mM NaCl, 2.7 mM KCl, 1.3 mM KH₂PO₄, 8.1 mM Na₂HPO₄·7H₂O, pH 7.4), lysed in ice-cold lysis buffer containing 1% Triton X-100, 0.5% Nonidet P-40, 60 mM *N*-octylglucoside, 1 mM EDTA, 1 mM EGTA, 10 mM Tris-HCl (pH 7.4) and insoluble material was removed by centrifugation at 10 000×*g* for 10 min. The lysate (100 µg) was precleared by incubation for 1 h with Protein G-Sepharose beads and incubated overnight with 1 µg of monoclonal anti-P-gp antibodies (C219 or Ab-2) or 1 µg of anti-caveolin antibody linked to agarose beads. The immune complexes were collected following a 2 h incubation with protein G-Sepharose beads and washed extensively (three times) with the lysis buffer. The beads were resuspended in 20 µl of two-fold concentrated Laemmli electrophoresis buffer, separated by SDS-PAGE and electroblotted onto PVDF membranes for the detection of caveolin or P-gp.

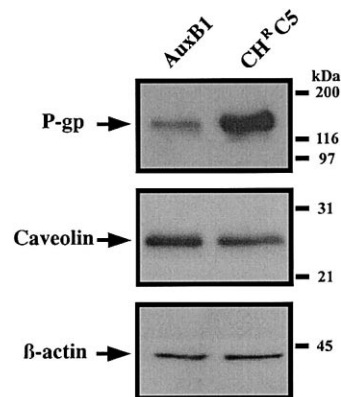


Fig. 1. Immunodetection of P-gp and caveolin in CH^RC5 and AuxB1 cells. P-gp was detected in lysates from CH^RC5 cells (2 µg of protein) or AuxB1 cells (20 µg of protein). Caveolin and β-actin were also detected in lysates (20 µg of protein) from both cell lines by Western blot as described under Section 2.

3. Results and discussion

3.1. Detection of P-gp and caveolin

In the present study, we first investigated the levels of P-gp and caveolin in resistant (CH^RC5) and sensitive (AuxB1) cells (Fig. 1). P-gp levels detected by Western blots using mAb C219 were much higher in CH^RC5 cells (around 75-fold higher) than in AuxB1 cells. The levels of caveolin were similar in both types of cells. This is in contrast to a previous study that reported an up-regulation of caveolin-1 in multidrug-resistant HT-29 human colon adenocarcinoma cells and in Taxol-resistant A549 cells compared to the corresponding sensitive cells [34]. As a control, β-actin was also evaluated in both cell lines. Its level was identical in the cell lines, indicating that equal amounts of proteins were loaded onto the Western blots.

3.2. Co-sedimentation of P-gp with caveolar membrane proteins

Caveolae-enriched membrane microdomains were isolated both from resistant (CH^RC5) cells grown in the presence of colchicine and from sensitive (AuxB1) cells (Fig. 2). Both cells samples were fractionated by a carbonate-based fractionation method, in the absence of detergent, for the isolation of caveolae. Caveolin-1, a caveolae marker [19,20], as well as P-gp, paxillin and FAK were detected by Western blots in fractions

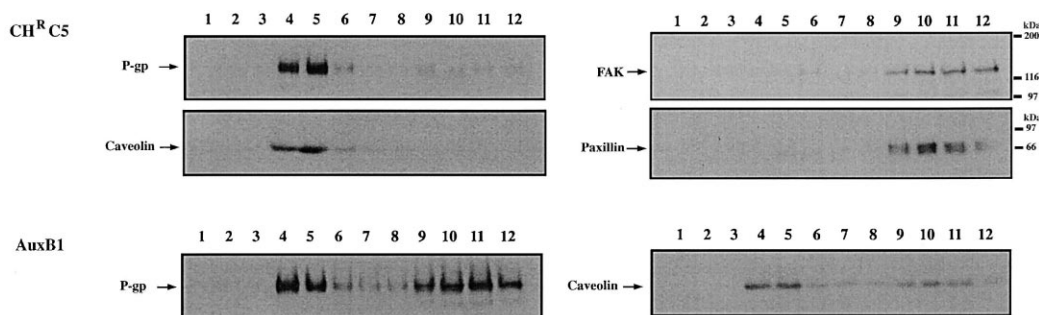


Fig. 2. Distribution of caveolin and P-gp following fractionation of CH^RC5 and AuxB1 cells. CH^RC5 cells, grown in the presence of colchicine, and AuxB1 cells were subjected to subcellular fractionation after homogenization in buffer containing 0.25 M sodium carbonate, as described previously under Section 2. Gradients were fractionated by collecting 1 ml fractions from the top and 10 µl or 20 µl of each fraction were loaded onto acrylamide gels for the detection of caveolin, P-gp, FAK and paxillin. For AuxB1 fractions, longer film exposure was required for the detection of P-gp compared to the time needed for the detection of P-gp in the CH^RC5 fractions. One representative experiment is shown. (*n* = 3)

collected from the resulting sucrose gradient. In CH^RC5 fractions, the major proportions of caveolin and P-gp co-sedimented at the low-density sucrose interface (fractions 4–6). Paxillin and Fak, which are negative markers of caveolae domains in endothelial cells, were only detected in fractions 9–12 [27]. The absence of paxillin and Fak in the low-density sucrose fractions, which correspond to caveolae, indicates that the co-localization of P-gp and caveolin is not caused by the presence of other types of membrane domains. In fractions from sensitive cells (AuxB1), caveolin was detected in the low-density sucrose (fractions 4–6) and a considerable amount of caveolin was also detected in the high-density sucrose interface (fractions 9–12) of the gradient. A longer film exposure was required for ECL-detection of P-gp in the fractions isolated from AuxB1 cells than for those from CH^RC5 cells. In AuxB1 cells, P-gp was present in fractions 4–6 as well as in fractions 9–12 suggesting that P-gp co-sedimented with caveolin in both resistant and sensitive cells.

A previous study detected functional intracellular P-gp in CH^RC5 cytoplasmic vesicles [35]. The origin and nature of these vesicles containing P-gp is unknown and they are not associated with pinocytotic vesicles. Our results indicate that a major proportion of P-gp in CH^RC5 is co-localized with caveolin, suggesting association with caveolar-like microdomains. Up-regulation of caveolin in caveolar-like structures by 12 and five-fold was observed in resistant human colon adenocarcinoma cells (HT-29-MDR cells) and in adriamycin-resistant MCF-7 human breast carcinoma transfected with P-gp, compared to their corresponding sensitive parental cells [16,30]. A portion of P-gp (40%) was reported to be present in low-density detergent-insoluble membranes derived from either of these cell lines, suggesting the presence of P-gp in caveolar domains. In addition, caveolin was shown to be variably expressed in different MDR-cell lines and was not expressed in the corresponding parental drug-sensitive cells [30,34]. In contrast to these results, the levels of caveolin (on an equal protein basis) were very similar in resistant CH^RC5 cells and in sensitive AuxB1 cells. Thus the endogenous level of caveolin in AuxB1 cells is not up-regulated during the acquisition of resistance by CH^RC5 cells, suggesting that augmentation of the levels of caveolin in these cells is not required for the development of the MDR-phenotype. This is in contrast to a previous study reporting that up-regulation of caveolin-1 in Taxol-resistant A549 cells may play a role in the development of Taxol resistance but occurs independently of P-gp expression [34].

3.3. Modulation of the localization of P-gp in caveolae by drug treatment

We further investigated the effects of drugs such as colchicine and CsA on P-gp localization in caveolar-like microdomains (Fig. 3). Colchicine was used since CH^RC5 cells were selected for their resistance to this drug whereas CsA is known as a reversing agent. CH^RC5 cells were grown in the presence or absence of the reversing agent CsA (10 μ M) or colchicine (4 μ g/ml). First, P-gp and caveolin were immunodetected in CH^RC5 cell lysates following CsA and colchicine treatments (Fig. 3A). Neither CsA nor colchicine affected P-gp or caveolin expression in CH^RC5 cells. Second, the same number of cells for each treatment was fractionated by the carbonate method for isolation of the caveolar-like microdomains (Fig. 3B). When CH^RC5 cells were grown in the absence of drugs

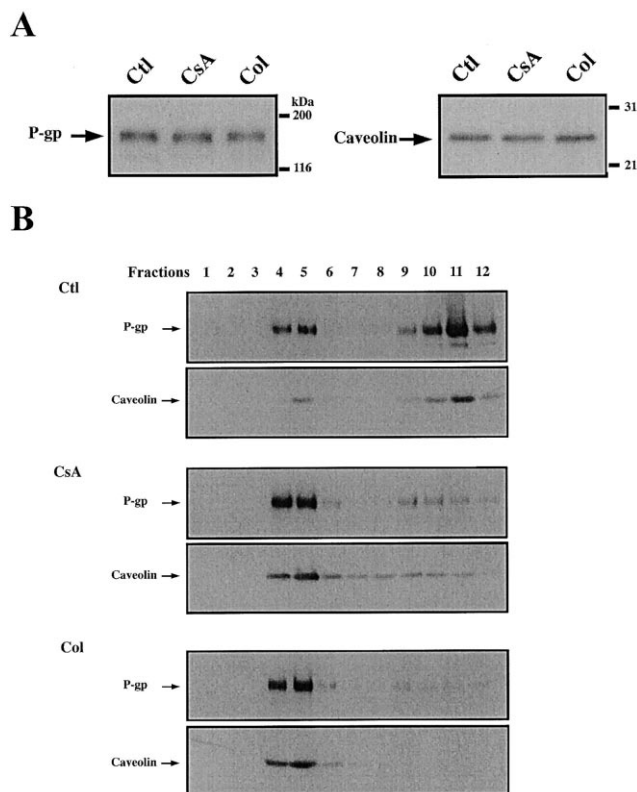


Fig. 3. Effect of drug treatments on P-gp localization in caveolae-enriched domains. CH^RC5 were grown to confluence in the absence of any drug (Ctl) or in the presence of cyclosporin A (CsA, 10 μ M) or colchicine (Col, 4 μ g/ml). A: P-gp and caveolin were detected in samples from lysed CH^RC5 cells grown without drugs (Ctl) or in the presence of CsA (CsA) or colchicine (Col) by Western blot analysis. For P-gp detection 2 μ g of protein were loaded onto the gel whereas for caveolin detection 10 μ g of protein were used. B: Fractionation of these cells was carried out using the carbonate-based procedure. Distributions of P-gp and caveolin in fractions collected from the gradients was evaluated by Western blot analysis as described under Section 2. One representative experiment is shown. ($n=3$)

(CsA or colchicine), two populations of P-gp and caveolin were immunodetected. One population was associated with fractions 4–6 and the other was detected in fractions 9–12. The profile of distribution of both proteins in these fractions was modified when cells were grown in the presence of CsA or colchicine. P-gp became more strongly detected in fractions 4–6, suggesting a subcellular redistribution of the protein in CH^RC5 cells caused by both drugs. In sensitive cells (AuxB1), the levels of P-gp and caveolin as well as their distribution in sucrose fractions were unaffected by CsA (data not shown). Caveolin was also detected in fractions 4–6 rather than in fractions 9–12 when resistant cells were grown in the presence of both drugs, suggesting a subcellular redistribution of caveolin.

P-gp and caveolin were also evaluated using equal amounts of proteins from caveolar fractions (4–6) and non-caveolar fractions (9–12) (Fig. 4A). In the absence of drugs, the ratio of the levels for both P-gp and caveolin in the caveolar and non-caveolar fractions clearly indicate that on an equal protein basis P-gp and caveolin are equally distributed in these fractions (Fig. 4B). This ratio for both proteins (P-gp or caveolin) increased to 10–12 and 20–22 when CH^RC5 cells were

grown in the presence of colchicine or CsA, respectively, suggesting a strong enrichment of both proteins in the caveolar fractions. These results indicate that drug treatments can modify the subcellular localization of P-gp and caveolin and that CsA or colchicine may promote an increase in the formation of caveolar-like microdomains. It is unclear whether this phenomenon is related to the interaction of both drugs with P-gp or to other cellular events. For example, since colchicine is known to interfere with the formation of microtubules, the presence of this drug might interfere with membrane vesicle trafficking and lead to a redistribution of P-gp and caveolin. In the case of CsA, this reversing agent may also affect intracellular vesicle trafficking since it was previously shown, in some human cancer cells, to interfere with the metabolism of sphingolipid, one of the major constituents of caveolae [36,37].

Previous studies have also described the accumulation of drugs such as doxorubicin or daunorubicin into cytoplasmic structures and intracellular vesicles in MDR-resistant cells and that P-gp and the multidrug-associated protein (Mrp) may be associated with these vesicles and contribute to the sequestration of the drugs [35,38,39]. Our results suggest that this previous cytoplasmic localization of P-gp and drugs in intracellular vesicles may be associated with caveolae-like microdomains. Further studies are required to characterize the presence of P-gp in caveolar-like domains in order to determine whether an intracellular pool of P-gp may affect the efficacy of anti-cancer drugs or reversing agents.

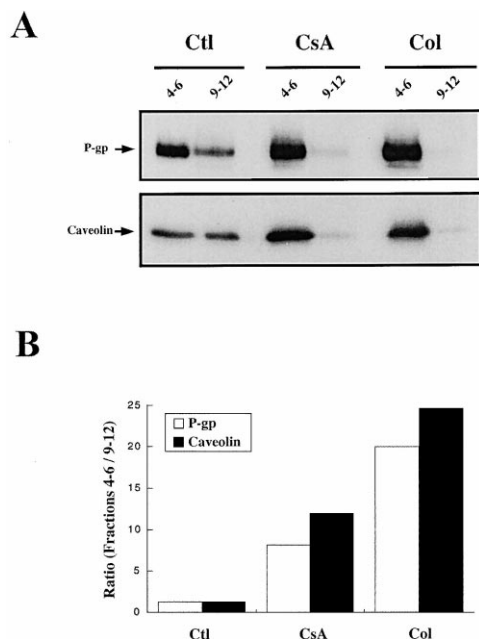


Fig. 4. Detection of P-gp and caveolin in caveolar and non-caveolar fractions. A: Fractions 4–6 (caveolar fractions) and 9–12 (non-caveolar fractions) were pooled, diluted in Tris-HCl 10 mM, pH 7.5, and centrifuged at $100\,000\times g$ for 1 h 30 min. Equal amounts of proteins (2 μ g) or 10 μ g of caveolar and non-caveolar fractions were used to detect P-gp and caveolin, respectively. B: The bands corresponding to both proteins were analyzed by laser densitometry. The results were plotted as the ratios between relative band densities of P-gp (open bars) and caveolin (closed bars) in caveolar fractions and in non-caveolar fractions. One representative experiment is shown. ($n = 3$)

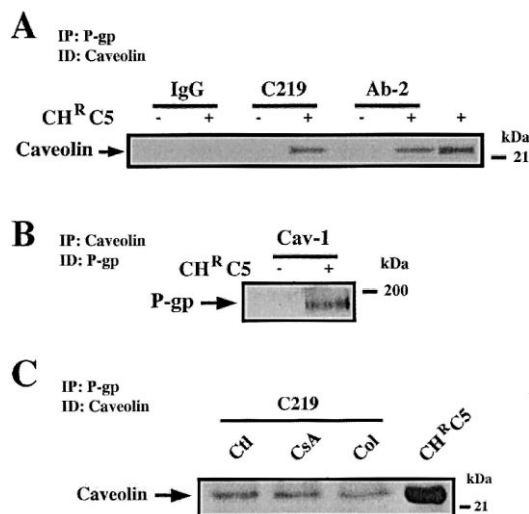


Fig. 5. Co-immunoprecipitation of caveolin with P-gp in $CH^R C5$ cells. A: P-gp was immunoprecipitated (IP) from $CH^R C5$ cell lysates, followed by immunodetection (ID) of caveolin, as follows. Lysed $CH^R C5$ cells (100 μ g) were precleared with protein G-Sepharose beads. Overnight incubations with (+) or without (–) cell lysates were performed in the presence of 1 μ g of non-specific mouse IgGs or 1 μ g of mAbs C219 or Ab-2. The immune complexes were collected by the addition of Protein G-Sepharose beads and analyzed by SDS-PAGE using 12.5% polyacrylamide gels; caveolin was immunodetected. B: Caveolin in $CH^R C5$ lysates (100 μ g) was immunoprecipitated using an anti-caveolin antibody linked to agarose beads, as follows. Lysed $CH^R C5$ cells (100 μ g) were precleared with protein G-Sepharose beads. The anti-caveolin antibody (2 μ g) linked to agarose beads was incubated overnight in the absence (–) or in the presence (+) of $CH^R C5$ lysates. The immune complexes were collected and analyzed by SDS-PAGE using 6.5% polyacrylamide gels and P-gp was immunodetected with mAb C219. C: $CH^R C5$ cells grown in the absence of drugs (Ctl) or in the presence of CsA (10 μ M) or colchicine (4 μ g/ml) were lysed and P-gp was immunoprecipitated (100 μ g) with mAb C219 as described under Section 2. Caveolin was immunodetected using 12.5% polyacrylamide gels. 10 μ g of lysed $CH^R C5$ cells were used as a positive control.

3.4. Coimmunoprecipitation of caveolin and P-gp

To further investigate the association of P-gp with caveolae-enriched membrane domains, immunoprecipitation using specific mAbs against P-gp were performed (Fig. 4). P-gp was immunoprecipitated with both mAbs C219 and Ab-2, which recognize two different P-gp epitopes [40,41], and Western blots of immunoprecipitates were carried out using a pAb directed against caveolin (Fig. 5A). Controls with non-specific mouse IgGs and with protein G-Sepharose beads incubated with or without cell lysates were also performed. In $CH^R C5$ lysates, caveolin was detected at 23 kDa. When P-gp from these cell lysates was immunoprecipitated by both C219 and Ab-2 mAbs, caveolin could be immunodetected in the immune complexes but not immunodetected when $CH^R C5$ lysate was incubated with non-specific mouse IgGs. Furthermore, caveolin was completely absent when $CH^R C5$ cell lysates were omitted during the immunoprecipitation procedures, clearly indicating that the band corresponding to caveolin is not related to the presence of the light antibody chains of C219 or Ab-2 detected at 23–25 kDa. In addition, P-gp was detected when caveolin in $CH^R C5$ lysates was immunoprecipitated using an anti-caveolin antibody linked to agarose beads. Both of these co-immunoprecipitation experiments indicate that there is a physical interaction between P-gp and caveolin in resistant

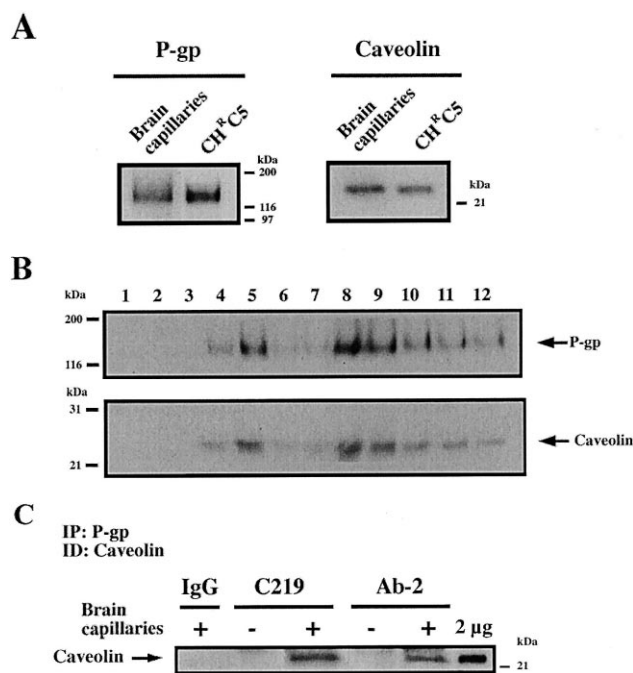


Fig. 6. Distribution of caveolin and P-gp in brain capillaries. **A**: P-gp and caveolin were immunodetected in brain capillaries (10 µg of protein) and in CH^RC5 cells (2 µg of protein). **B**: Brain capillaries isolated from rat brain, as described previously [33], were subjected to subcellular fractionation after homogenization in buffer containing 0.25 M sodium carbonate. Gradients were fractionated by collecting 1 ml fractions from the top and 10 µl or 20 µl of each fraction were loaded onto acrylamide gels for the detection of caveolin and P-gp. **C**: P-gp from brain capillaries was immunoprecipitated (IP) and caveolin was immunodetected (ID). Brain capillary lysates (500 µg) were precleared with protein G-Sepharose beads, followed by an overnight incubation in the presence of 1 µg of either non-specific mouse IgGs, mAb C219 or mAb Ab-2. The immune complexes were collected by the addition of Protein G-Sepharose beads and analyzed by SDS-PAGE using 12.5% polyacrylamide gels; caveolin was immunodetected. 2 µg of lysed brain capillaries were used as a positive control for caveolin. One representative experiment is shown. ($n = 3$)

CH^RC5 cells. Proteins that have been shown to interact with caveolin have a consensus caveolin-binding motif (Φ XXXX Φ XX Φ XX Φ , where Φ is an aromatic residue and X is any amino acid) [42]. We found that this caveolin-binding motif is present in P-gp between amino acid residues 36 and 44 (FTMFRYAGW). This raises the possibility that caveolin interacts with the cytosolic N-terminal region of P-gp.

Since colchicine and CsA modulated the distribution of P-gp and caveolin in CH^RC5 cells, the association of caveolin with P-gp was also evaluated in cells grown in the presence of each drug (Fig. 5B). The levels of caveolin coimmunoprecipitated with P-gp by mAb C219 were similar, indicating that the physical interaction of P-gp and caveolin was unaffected when CH^RC5 cells were exposed to colchicine or CsA. These results suggest that the redistribution of both P-gp and caveolin in CH^RC5 cells by both drugs was not caused by a modulation in the number of caveolin molecules bound to P-gp.

3.5. Co-localization of caveolin and P-gp in brain capillaries

To establish whether P-gp could be associated with caveolar microdomains in vivo, the distribution of P-gp and caveolin following fractionation of brain capillaries was determined (Fig. 6). Brain capillaries were used since this tissue has one

of the highest levels of P-gp expression in vivo [33]. P-gp and caveolin levels were determined in isolated brain capillaries (10 µg of protein) and in CH^RC5 cells (2 µg of protein) (Fig. 6A). P-gp levels were approximately six-fold lower than in CH^RC5 cells whereas caveolin levels were eight-fold higher in brain capillaries than in CH^RC5 cells. As shown in Fig. 6B, a fraction of P-gp co-localized with caveolin following fractionation of isolated brain capillaries, suggesting that a population of P-gp molecules in this tissue is associated with these microdomains. Since P-gp levels were low in brain capillaries, immunoprecipitation experiments using brain capillaries were performed with 500 µg of protein instead of the 100 µg of protein previously used for CH^RC5 cells. When P-gp from brain capillaries was immunoprecipitated with mAbs C219 or Ab-2, caveolin was co-immunoprecipitated (Fig. 6C). These results indicate that co-localization of P-gp and caveolin as well as their physical association is not restricted to cells selected in vitro for their resistance.

Further studies are needed to establish the exact implication of the physical interaction between P-gp and caveolin. The interaction of a number of proteins such as Ras, Src, protein kinase C and the epidermal growth factor receptor with the specific scaffolding domain of caveolin caused a strong inhibition of their enzymatic activities suggesting that the interaction of caveolin with P-gp may also negatively control its activity [19]. Our results suggest that caveolae-like structures containing P-gp may also be involved in the intracellular accumulation of anticancer drugs previously reported [35]. The identification of the physical interaction of P-gp with caveolin in caveolae-enriched membrane domains may provide new insights in the understanding of the role and the regulation of P-gp in caveolae-like domains. Furthermore, we have shown that the association of P-gp with caveolae and the physical interaction between P-gp and caveolin also takes place in brain capillaries.

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