Phospholipase A₂ Inhibitors or Platelet-activating Factor Antagonists Prevent Prion Replication*

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A key feature of prion diseases is the conversion of the cellular prion protein (PrPC) into disease-related isoforms (PrPSc), the deposition of which is thought to lead to neurodegeneration. In this study a pharmacological approach was used to determine the metabolic pathways involved in the formation of protease-resistant PrP (PrPRES) in three prion-infected cell lines (ScN2a, SMB, and ScGT1 cells). Daily treatment of these cells with phospholipase A₂ (PLA₂) inhibitors for 7 days prevented the accumulation of PrPRES. Glucocorticoids with anti-PLA₂ activity also prevented the formation of PrPRES and reduced the infectivity of SMB cells. Treatment with platelet-activating factor (PAF) antagonists also reduced the PrPRES content of cells, while the addition of PAF reversed the inhibitory effect of PLA₂ inhibitors on PrPRES formation. ScGT1 cells treated with PLA₂ inhibitors or PAF antagonists for 7 days remained clear of detectable PrPRES when grown in control medium for a further 12 weeks. Treatment of non-infected cells with PLA₂ inhibitors or PAF antagonists reduced PrP⁺ levels suggesting that limiting cellular PrP⁺ may restrict prion formation in infected cells. These data indicate a pivotal role for PLA₂ and PAF in controlling PrPRES formation and identify them as potential therapeutic agents.

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders that include Kuru, Creutzfeldt-Jakob disease (CJD), and Gerstman-Sträussler-Scheinker (GSS) disease in man. Central to the pathogenesis of TSEs is the conversion of the host-encoded cellular prion protein (PrP⁺) into β-sheet-rich disease-related isoforms (PrPSc) (1). The formation of PrPSc is accompanied by changes in biological and biochemical properties such as an increased resistance to proteases (2), the protease-resistant core of PrPSc designated PrPRES. This PrPRES self-aggregates and forms amyloidogenic fibrils and, in most prion diseases, aggregates of PrPSc are detected in the diseased brain before neuronal loss is observed (3).

The development of current therapeutic strategies is largely based on the belief that the deposition of amyloidogenic PrPSc fibrils leads to neurodegeneration and the clinical symptoms of prion diseases. Many compounds that interact directly with PrP to prevent PrPSc formation and/or disrupt preformed PrPSc aggregates have now been identified; these include large, flat multicyclic compounds and synthetic peptides specifically designed to disrupt the β-sheets in PrPSc (4–6). However, recent studies demonstrated that the propagation of PrPSc within prion-infected cells could be reduced following re-routing the trafficking of PrPSc following treatment with suramin (7). Other studies have also shown that restricting the supply, or alterations in the trafficking, of PrP⁺C can prevent the formation of PrPSc (8–11). In the present study we tested the hypothesis that the trafficking of PrP⁺C within cells, that is vital to PrPSc formation, is controlled by activation of specific signaling pathways. Previous studies have variously reported that PrP⁺C is associated with activation of the tyrosine kinases Fyn (12), with the cyclic AMP/protein kinase A pathway (13), or with the phospholipase A₂ (PLA₂)/cyclo-oxygenase (COX) pathway (14). Thus, in this study, a pharmacological approach was used to investigate the role of signal transduction mechanisms on levels of PrP⁺C in non-infected cells, and PrPSc in scrapie-infected neuroblastoma cell lines (ScN2a, ScGT1, or SMB cells). These studies indicate that activation of PLA₂ and the production of platelet-activating factor (PAF) (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine), a bioactive phospholipid that is not stored in a preformed state (15) but rapidly synthesized in neurons in response to cell specific stimuli via the remodeling pathway (16), are essential factors in the production of PrPSc.

EXPERIMENTAL PROCEDURES

**PrPRES Production—Scrapie-infected neuroblastoma cells (ScN2a cells; gift from Dr. M. Rogers, University College, Dublin, Ireland) that produce PrP⁺ and infectious agent, were grown in Hams F12 medium containing 2 mM glutamine, standard antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) and 2% fetal calf serum. SMB cells (TSE Resource Centre, Institute for Animal Health, Compton, UK), which also produce PrP⁺ and infectious agent, were grown in RPMI 1640 medium containing standard antibiotics, 2 mM glutamine and 2% fetal calf serum. ScGT1 cells (supplied by Dr. Sylvain Lehmann, CNRES-IGH, Montpellier, France), an immortalized murine hypothalamic neuron cell line infected by the scrapie Chandler isolate and that persistently expresses PrP⁺, were grown in Optimem supplemented with 2 mM glutamine, 5% fetal calf serum, and standard antibiotics. To measure the effect of drugs on PrPRES formation, cells were plated at 1 × 10⁶ cells/well in 6-well microtiter plates in the presence of drugs. Cells were then grown with daily changes of media and PrP⁺ production was evaluated after 7 days. Non-infected N2a cells or SMB cells

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that had been "cured" of infectivity by serial passages in the presence of pentosan polysulphate (PS cells) (17) were used as controls. For time courses experiments, ScN2a cells were plated at 5 × 10⁶ cells/well in 6-well plates. Cells were grown for 7 days, with daily changes of medium, in the presence or absence of the drugs shown. The levels of protease-resistant PrP in cellular lysates were then determined using an ELISA. Values shown are the mean PrP⁺⁺⁺ pg/1 × 10⁶ cells ± S.D. of triplicate experiments repeated three times (9 observations).

**Table I**

| Enzyme        | Drug                  | ScN2a       | SMB        | ScGT1       |
|---------------|-----------------------|-------------|------------|-------------|
| Control       |                       | 1047 ± 202  | 2193 ± 249 | 6118 ± 332  |
| Phospholipase C| Neomycin sulfate (10 μM) | 955 ± 57    | 2245 ± 188 | 6238 ± 348  |
|               | U-73122 (5 μM)         | 1032 ± 93   | 2303 ± 204 | 6407 ± 404  |
| Phospholipase A₂| Ethyl-18-OCH₃ (10 μM) | 1052 ± 115  | 2150 ± 138 | 6082 ± 429  |
|               | CDP (1 μM)             | <50°        | <50°       | <50°        |
|               | BEL (1 μM)             | <50°        | <50°       | <50°        |
|               | Aristolochic acid (0.5 μg/ml) | <50°     | <50°       | <50°        |
|               | AACOCF₃ (1 μg/ml)      | <50°        | <50°       | <50°        |

* PrP⁺⁺⁺ content of cells significantly less (p < 0.05) than that of untreated cells.

**RESULTS**

**PLA₂ inhibitors reduce the PrP⁺⁺⁺ content of prion-infected cell lines**

To measure the effect of drugs on PrP⁺⁺⁺ formation, ScN2a, SMB, or ScGT1 cells were plated at 1 × 10⁶ cells/well in 6-well plates. Cells were grown for 7 days, with daily changes of medium, in the presence or absence of the drugs shown. The levels of protease-resistant PrP in cellular lysates were then determined using an ELISA. Values shown are the mean PrP⁺⁺⁺ pg/1 × 10⁶ cells ± S.D. of triplicate experiments repeated three times (9 observations).

**Figure 1.** PLA₂ inhibitors reduce the PrP⁺⁺⁺ content of ScGT1 cells. ScGT1 cells were grown for 7 days in the presence of control medium (lane 1), 1 μg/ml AACOCF₃ (lane 2), 0.5 μg/ml aristolochic acid (lane 3), 1 μM CDP (lane 4), or 10 μm ethyl-18-OCH₃ (lane 5). Cellular lysates were digested with 10 μg/ml protease K for 1 h at 37 °C and protease-resistant PrP was visualized by immunoblot with mAb SAF83 using enhanced chemiluminescence.

1-O-alkyl-2-acyetyl-sn-glycerol-3-phospho-(N,N,N-trimethyl)-hexanolamine (hexa-PAF) was obtained from Novabiochem (Nottingham, UK). C-PAF, CV-6209, U73122, and ethyl-18-OCH₃ were obtained from Biomol (Exeter, UK).

**Prostaglandin (PG)E₂ Assay—**Analysis of cellular PGE₂ levels was determined in cells by using an enzyme immunoassay kit (Amersham Biosciences) according to the manufacturer's instructions. This assay is based on competition between unlabeled PGE₂ in the sample and a fixed amount of labeled PGE₂ for a PGE₂-specific antibody. The detection limit of this assay is 20 pg/ml.

**Statistical Analysis—**Comparison of treatment effects were carried out using one and two way analysis of variance techniques as appropriate. Post-hoc comparisons of means were performed as necessary. For all statistical tests, significance was set at the 5% level.

**FIG. 1.** PLA₂ inhibitors reduce the PrP⁺⁺⁺ content of ScGT1 cells. ScGT1 cells were grown for 7 days in the presence of control medium (lane 1), 1 μg/ml AACOCF₃ (lane 2), 0.5 μg/ml aristolochic acid (lane 3), 1 μM CDP (lane 4), or 10 μM ethyl-18-OCH₃ (lane 5). Cellular lysates were digested with 10 μg/ml protease K for 1 h at 37 °C and protease-resistant PrP was visualized by immunoblot with mAb SAF83 using enhanced chemiluminescence.

**PLA₂ Inhibitors Reduce the PrP⁺⁺⁺ Content of Three Prion-infected Cell Lines—**In an initial screening experiment, the effects of drugs that inhibit some of the common signal transduction pathways were investigated for their effects on the PrP⁺⁺⁺ content of ScN2a cells. ScN2a cells treated daily for 7 days with one of four different PLA₂ inhibitors (1 μM CDP, 1 μg/ml aristolochic acid, 1 μM BEL, or 1 μg/ml AACOCF₃) contained significantly less PrP⁺⁺⁺ than did untreated cells. In contrast, the levels of PrP⁺⁺⁺ in ScGT1 cells were not significantly affected by treatment with three inhibitors of phospholipase C (Table I and Fig. 1). To confirm the effects of PLA₂ inhibitors on PrP⁺⁺⁺ production, two other prion-infected neuroblastoma cell lines (SMB and ScGT1 cells) were also treated with these drugs. The PrP⁺⁺⁺ content of SMB or ScGT1 cells, treated with CDP, aristolochic acid, BEL, or AACOCF₃ was also greatly reduced. Even at concentrations 10 times higher than those used in these experiments, the drugs used did not alter cell survival or cell growth (data not shown).
Glucocorticoids reduce the PrP\textsuperscript{res} content of prion-infected cell lines

The effects of glucocorticoids on PrP\textsuperscript{res} formation were determined by plating ScN2a, SMB or ScGT1 cells at 1 x 10\textsuperscript{5} cells/well in 6 well plates. Cells were grown for 7 days in the presence of the 1 \mu M glucocorticoids as shown. The levels of protease-resistant PrP in cellular lysates were then determined using an ELISA. Values shown are the mean PrP\textsuperscript{res} pg/1 x 10\textsuperscript{7} cells ± S.D. of quadruplicate experiments repeated three times (12 observations).

| Drug          | ScN2a                | SMB               | ScGT1                |
|---------------|----------------------|-------------------|----------------------|
| None          | 1104 ± 148           | 2004 ± 261        | 5848 ± 435           |
| Dexamethasone | <50\(^{\circ}\)       | <50\(^{\circ}\)   | <50\(^{\circ}\)      |
| Hydrocortisone| <50\(^{\circ}\)       | <50\(^{\circ}\)   | <50\(^{\circ}\)      |
| Prednisolone  | <50\(^{\circ}\)       | <50\(^{\circ}\)   | <50\(^{\circ}\)      |
| Prednisone    | 1142 ± 98            | 1983 ± 224        | 5985 ± 389           |

\textsuperscript{a} PrP\textsuperscript{res} content of cells significantly less (p < 0.05) than that of untreated cells.

Corticosteroids Reduce the PrP\textsuperscript{res} Content of Prion-infected Cell Lines—In the present study, ScN2a, SMB, or ScGT1 cells treated with 1 \mu M dexamethasone, 1 \mu M hydrocortisone, or 1 \mu M prednisolone contained undetectable amounts of PrP\textsuperscript{res}, whereas cells treated with 1 \mu M prednisolone, an inactive pro-drug that is converted to active prednisolone in the liver, did not affect PrP\textsuperscript{res} levels (Table II). In further studies, the inhibitory effects of dexamethasone on PrP\textsuperscript{res} content of ScN2a cells was shown to be dose-dependent (Fig. 2). SMB and ScGT1 cells treated with dexamethasone also demonstrated a dose-dependent reduction in PrP\textsuperscript{res} (data not shown).

The effects of 1 \mu M dexamethasone on ScN2a cells were not immediate as levels of PrP\textsuperscript{res} in treated cells were not significantly different from control cells after 24 h (1009 pg/ml ± 116 versus 1122 pg/ml ± 97 in untreated cells, n = 8 independent observations, mean PrP\textsuperscript{res} ± S.D.) but they were significantly reduced after 48 h (561 pg/ml ± 116 versus 1129 pg/ml ± 76, n = 8, p < 0.05) and further reduced after 72 h (66 pg/ml ± 55 versus 1076 pg/ml ± 77, n = 8, p < 0.05). We were unable to detect PrP\textsuperscript{res} in cells that had been treated for 4 days or more with 1 \mu M dexamethasone. When ScN2a cells that had been treated with 1 \mu M dexamethasone for 7 days were then grown for a further 12 weeks in drug-free medium these cells remained clear of detectable amounts of PrP\textsuperscript{res} (<50 pg/ml).

Similarly, SMB or ScGT1 that had been treated with 1 \mu M dexamethasone for 7 days remained clear of detectable amounts of PrP\textsuperscript{res} when grown in drug-free medium for 12 weeks.

PLA\textsubscript{2} Inhibitors Reduce PGE\textsubscript{2} Production in Prion-infected Cells—PrP peptides increase PLA\textsubscript{2} activity resulting in the production of PGE\textsubscript{2} (14). In the present study the levels of PGE\textsubscript{2} were significantly raised in prion-infected cells when compared with their non-infected counterparts, which suggests that prion infection activates PLA\textsubscript{2} pathways in neurons. Prion-infected cells treated with 1 \mu M CDP, 1 \mu g/ml AACOCF\textsubscript{3}, or 1 \mu M dexamethasone produced significantly less PGE\textsubscript{2} than did untreated cells showing that drug treatment did indeed inhibit PLA\textsubscript{2} (Fig. 3).

PAF Antagonists Block PrP\textsuperscript{res} Formation—The effects of downstream pathways following PLA\textsubscript{2} activation on the formation of PrP\textsuperscript{res} were investigated. Arachidonic acid, released from membrane phospholipids by PLA\textsubscript{2}, is converted to leukotrienes and prostaglandins by the lipoxygenase (LOX) and COX enzymes respectively. Because the PrP\textsuperscript{res} content of ScN2a, SMB or ScGT1 cells was not affected by treatment with the LOX or COX inhibitors, other factors produced following PLA\textsubscript{2} activation were therefore examined. PAF is generated in neurons by the remodeling pathway following PLA\textsubscript{2} activation (16) and the PrP\textsuperscript{res} content of cells was reduced following treatment with the PAF antagonists hexa-PAF, CV-6209, ginkgolide A, or ginkgolide B (Table III). These PAF antagonists did not affect the survival, or growth rates of prion-infected cells. All four PAF antagonists caused a dose-dependent reduction in the PrP\textsuperscript{res} content of ScN2a cells (Fig. 4), and there was a highly significant relationship between the level of PrP\textsuperscript{res} and the concentration of PAF antagonist (p < 0.05) for all 4 drugs. We noted that at each concentration, PrP\textsuperscript{res} levels were greater in cells treated with ginkgolide A compared with ginkgolide B (p < 0.05). Furthermore, the PrP\textsuperscript{res} content of ScN2a cells was reduced to below detectable levels following 7 days of treatment with 2 \mu M PAF antagonists (<50pg/1 x 10\textsuperscript{7} cells). Treatment with the PAF antagonists also caused a dose-dependent reduction in the PrP\textsuperscript{res} content of SMB and ScGT1 cells. Furthermore, ScN2a, SMB, or ScGT1 cells treated with 2 \mu M hexa-PAF, or with 2 \mu M ginkgolide B, for 7 days remained free of detectable PrP\textsuperscript{res} when grown in drug-free medium for a further 12 weeks (data not shown).

PAF Increases PrP\textsuperscript{res} Formation—To compliment the PAF antagonist studies, prion-infected cells were grown in medium containing PAF agonists. The PrP\textsuperscript{res} content of cells treated with PAF agonists (2 \mu M PAF or 2 \mu M C-PAF) were significantly higher than untreated cells (Table IV and Fig. 5). The PAF
TABLE III
PAF antagonists prevent PrP\textsuperscript{res} formation in prion-infected cells

| Enzyme          | Drug         | ScN2a | SMB  | ScGT1 |
|-----------------|--------------|-------|------|-------|
| Control         |               | 1047 ± 202 | 2193 ± 249 | 6118 ± 388 |
| COX             | Aspirin      | 955 ± 57  | 2245 ± 188 | 6238 ± 348 |
| LOX             | Ibuprofen    | 1032 ± 93 | 2303 ± 204 | 6407 ± 404 |
| LOX             | NDGA         | 1052 ± 115| 2150 ± 138 | 6082 ± 429 |
| PAF antagonists | Hexa-PAF     | 985 ± 148 | 1894 ± 268 | 5958 ± 482 |
| PAF antagonists | CV-6209      | <50\textsuperscript{a} | <50\textsuperscript{a} | <50\textsuperscript{a} |
|                 | Ginkgolide A | 55 ± 25\textsuperscript{a} | 100 ± 78\textsuperscript{a} | 167 ± 60\textsuperscript{a} |
|                 | Ginkgolide B | <50\textsuperscript{a} | <50\textsuperscript{a} | <50\textsuperscript{a} |

\textsuperscript{a} PrP\textsuperscript{res} content of cells significantly less (p < 0.05) than that of untreated cells.

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**FIG. 4.** PAF antagonists cause a dose-dependent reduction in the PrP\textsuperscript{res} content of prion-infected cells. ScN2a cells were grown in the presence of different concentrations of PAF antagonists: hexa-PAF (open circles), CV-6209 (closed circles), ginkgolide A (open squares), or ginkgolide B (closed squares) for 7 days. The levels of protease-resistant PrP were subsequently determined in an ELISA. Values shown are the mean PrP\textsuperscript{res} pg/10\textsuperscript{7} cells ± S.D. of triplicate experiments repeated three times (9 observations).

**TABLE IV**
PAF agonists increase the PrP\textsuperscript{res} prion-infected cells

| Conc (nM) | ScN2a | SMB | ScGT1 |
|-----------|-------|-----|-------|
| 1         | 1047 ± 202 | 2193 ± 249 | 6079 ± 542 |
| 2         | 1371 ± 90  | 3584 ± 332 | 18528 ± 3325 |
| 5         | 1320 ± 64  | 3169 ± 404 | 12484 ± 1842 |

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PrP\textsuperscript{res} content of untreated N2a cells (33.2 ± 2.9 ng/10\textsuperscript{7} cells) was significantly higher than that of cells treated with PLA\textsubscript{2} inhibitors (1 μM CDP: 12.8 ± 3.2, n = 9, p < 0.05; 1 μM AAOCF\textsubscript{3}: 2.5 ± 2.1, n = 9, p < 0.05; 1 μM dexamethasone: 8.4 ± 2.4, n = 9, p < 0.05), or with the PAF antagonists 2 μM hexa-PAF (1.1 ± 1.2, n = 9, p < 0.05), 1 μM CV-6209 (6.8 ± 1.4, n = 9, p < 0.05), 1 μM ginkgolide A (1.5 ± 1.7, n = 9, p < 0.05), or 1 μM dexamethasone (Fig. 5).

**FIG. 5.** PAF increases the PrP\textsuperscript{res} content of ScN2a and ScGT1 cells. ScN2a or ScGT1 cells were grown for 7 days in the presence of control medium (lanes 3 and 4), 2 μM C-PAF (lanes 2 and 5), or 2 μM PAF (lanes 1 and 6). Protease-resistant PrP was demonstrated by immunoblot with mAb SAF83.

**FIG. 6.** PAF reverses the inhibition of PrP\textsuperscript{res} formation by PLA\textsubscript{2} inhibitors. ScN2a cells were grown in control medium (Con), in 1 μM dexamethasone (DYM), 1 μM CDP, or 1 μg/ml aristolochic acid (AA) in the absence (shaded bars) or presence of 1 μM PAF (open bars). Values shown are the mean PrP\textsuperscript{res} pg/10\textsuperscript{7} cells ± S.D. of triplicate experiments repeated four times (n = 12).

PAF Antagonists Reduce PrP\textsubscript{IC} Levels in Non-infected Cells—Since the production of PrP\textsubscript{IC} is dependent on the presence of PrP\textsuperscript{C}, the effect of PLA\textsubscript{2} inhibitors, PAF antagonists or PAF on PrP\textsubscript{IC} levels in non-infected cells was investigated. The PrP\textsubscript{IC} content of untreated N2a cells (33.2 ± 2.9 ng/10\textsuperscript{7} cells) was significantly higher than that of cells treated with PLA\textsubscript{2} inhibitors (1 μM CDP: 12.8 ± 3.2, n = 9, p < 0.05; 1 μM AAOCF\textsubscript{3}: 2.5 ± 2.1, n = 9, p < 0.05; 1 μM dexamethasone: 8.4 ± 2.4, n = 9, p < 0.05), or with the PAF antagonists 2 μM hexa-PAF (1.1 ± 1.2, n = 9, p < 0.05), 1 μM CV-6209 (6.8 ± 1.4, n = 9, p < 0.05), 1 μM ginkgolide A (1.5 ± 1.7, n = 9, p < 0.05), or 1 μM dexamethasone (Fig. 5).
ginkgolide B (0.4 ± 0.8, n = 9, p < 0.05). All PAF antagonists used caused a dose-dependent reduction in the levels of PrP<sub>C</sub> in N2a cells, and there was a highly significant relationship between PAF antagonist concentration and PrP<sub>C</sub> levels (p < 0.05). In addition, the type of ginkgolide used had a significant effect on PrP<sub>C</sub> levels (p < 0.05) with cells treated with ginkgolide A containing more PrP<sub>C</sub> than cells treated with the same concentration of ginkgolide B (Fig. 7). Time course studies showed that PrP<sub>C</sub> levels were reduced within 24 h and remained low in the presence of any of the PAF antagonists for up to 7 days. However, even after prolonged treatment (7 days), removal of the PAF antagonists resulted in PrP<sub>C</sub> levels returning to normal within 24 h. Conversely, the PrP<sub>C</sub> content of N2a cells treated with PAF agonists, 2 μM PAF (74.6 ± 4.8, n = 9, p < 0.05), or 2 μM C-PAF (58.9 ± 3.8, n = 9, p < 0.05) was significantly higher than that of untreated N2a cells. The PrP<sub>C</sub> formed in N2a cells treated with PAF agonists remained sensitive to digestion with proteinase K.

Dexamethasone Reduces the Infectivity of SMB Cells—Dexamethasone is a glucocorticoid widely used in medical practice. It has various mechanisms of action, including inhibition of PLA<sub>2</sub>. To determine if dexamethasone-treated SMB cells retained infectivity, C57/BL mice were inoculated via the intracerebral route with homogenates from untreated SMB cells or SMB cells treated for 7 days with 200 nM dexamethasone. The mean incubation period in mice inoculated with lysates from dexamethasone-treated cells (206 ± 8 days) (incubation period ± S.D.) was significantly longer than in mice inoculated with lysates from untreated SMB cells (179 ± 6 days; n = 8 mice in each group, p < 0.05).

**DISCUSSION**

In the present study we utilized a pharmacological approach to determine the metabolic pathways that underlie the formation of PrP<sup>res</sup> in three prion-infected neuroblastoma cell lines (ScN2a, ScGT1, and SMB cells). In a broad screen of compounds we found that 4 different drugs that inhibit PLA<sub>2</sub> (aristolochic acid, AACOCF<sub>3</sub>, BEL, and CDP) reduced the PrP<sup>res</sup> content of prion-infected cells. The concentrations of the PLA<sub>2</sub> inhibitors used were at least 10 times less than the concentration of these drugs that had a toxic effect and treatment with PLA<sub>2</sub> inhibitors did not affect total cellular protein levels.<sup>2</sup> We confirmed that the drugs used inhibited PLA<sub>2</sub> by measuring levels of PGE<sub>2</sub> (a marker of PLA<sub>2</sub> activity). In the present study prion-infected cells treated with CDP, aristolochic acid or AACOCF<sub>3</sub> produced significantly less PGE<sub>2</sub> than untreated cells. It is of interest to note that none of the drugs completely inhibited PLA<sub>2</sub> activity, possibly because there exist several distinct enzymes with PLA<sub>2</sub> activity including cytosolic (cPLA<sub>2</sub>) and secretory (sPLA<sub>2</sub>) isozymes (19). Although aristolochic acid and CDP inhibit both cPLA<sub>2</sub> and sPLA<sub>2</sub>, low concentrations of AACOCF<sub>3</sub> or BEL, which are reported to selectively inhibit cPLA<sub>2</sub> (20), inhibited PrP<sup>res</sup> formation (Table I) indicating that cPLA<sub>2</sub> may be the isozyme of interest.

PLA<sub>2</sub> can also be inhibited by the lipocortins, a family of proteins that are produced in response to the glucocorticoids (21). In the present study cells treated with the active glucocorticoids: dexamethasone, hydrocortisone, and prednisolone showed a reduced PrP<sup>res</sup> content, whereas the inactive precursor prednisone had no effect. The effect of dexamethasone was dose-dependent, and PrP<sup>res</sup> was reduced to below detectable levels at nanomolar concentrations of dexamethasone. A significant effect on PrP<sup>res</sup> content was not seen until 2 days after the commencement of treatment with dexamethasone, and cells were not clear of PrP<sup>res</sup> until 4 days after treatment. Nevertheless, ScN2a cells that had been treated with 1 μM dexamethasone for 7 days remained free of detectable PrP<sup>res</sup> when grown in drug-free medium for a further 12 weeks. Our in vivo observations showed that SMB cells treated with 200 nM dexamethasone for 7 days contained reduced levels of infectivity. Such observations are consistent with previous reports that transient steroid administration immediately postinfection reduced the susceptibility of mice to scrapie after peripheral challenge (22). However, the use of glucocorticoids in prion diseases should be treated with caution due to the observation that chronic administration of glucocorticoids can itself lead to neuronal atrophy (23).

Since PLA<sub>2</sub> and many of its metabolites play important roles in signal transduction, it is possible that altered levels of second messengers could cause the decrease in the PrP<sup>res</sup> content of cells indirectly. Although the activation of PLA<sub>2</sub> is functionally associated with the production of prostaglandins the PrP<sup>res</sup>...
content of cells was not affected by treatment with inhibitors of either COX or LOX. The activation of PLA₂ also leads to the synthesis of the bioactive phospholipid PAF in neurons via the remodeling pathway (16). PAF is not stored in a preformed state, but rather is rapidly synthesized in response to cell-specific stimuli (15) and in this study four different PAF antagonists all reduced the PrP楼市 content of Scnta2a, ScGT1, or SMB cells. The effects of PAF antagonists were dose-dependent with an IC₅₀ ~ 50 nM, and at a concentration of 2 μM two PAF antagonists (hexa-PAF and ginkgolide B) were able to reduce PrP楼市 to below detectable levels. The finding in the present study that ginkgolide B had a greater effect on PrP楼市 formation than ginkgolide A is consistent with previous reports that ginkgolide B a more potent PAF antagonist than ginkgolide A (24). The role of PAF in prion replication was supported by two further complementary studies. Firstly, the addition of PAF agonists (PAF or C-PAF) increased the production of PrP楼市 in all 3 prion-infected cell lines without affecting total cellular protein concentrations. The magnitude of the effects of the PAF agonists were cell type-dependent, with a greater increase in PrP楼市 content seen in ScGT1 cells than in SMB and cells both showing greater effects than the Scnta2a cells. Secondly, the addition of PAF restored PAF楼市 production in dexamethasone or CDP-treated Scnta2a cells. Collectively, these results suggest that the effect of dexamethasone or the PLAb inhibitors on PrP楼市 formation is mediated via a reduction in PAF formation.

The observation that PrP楼市 is essential for the development of prion diseases (25) suggests that the density and cellular localization of PrP楼市 may influence PrP楼市 production. Both the PLAb inhibitors and the PAF antagonists reduced cellular PrP楼市 levels indicating that these drugs may prevent the formation of PrP楼市 by limiting the supply of the PrP楼市 substrate. Ginkgolide B, a more potent PAF antagonist than ginkgolide A (24), had a greater effect on PrP楼市 levels in N2a cells than ginkgolide A. In contrast, PAF agonists increased cellular PrP楼市 levels, further indicating the importance of PAF in controlling PrP楼市 expression. The PrP楼市 in PAF treated cells remained sensitive to proteinase K digestion, unlike PrP楼市 species induced in N2a cells treated with proteasome inhibitors (26). The regulation of PrP楼市 expression is poorly understood, previous studies have shown that in neuronal cells PrP楼市 expression was increased after treatment with insulin, nerve growth factor, epidermal growth factor, or tumor necrosis factor α (27, 28).

There are a number of possible mechanisms for the exact manner by which PAF antagonists could affect PrP楼市 formation. PrP楼市 is found in lipid rafts or caveolae (29), specialized membrane compartments that contain high levels of cholesterol and sphingomyelin (30). Since the formation of these lipid rafts is cholesterol-dependent (31), and drugs that affect cholesterol levels influence the formation of PrP楼市 (8, 18), it is possible that PAF may regulate the composition and hence the function of lipid rafts. In this respect it should be noted that PAF induces sphingomyelinase which itself has been shown to increase the formation of PrP楼市 in Scnta2a cells (32). PAF has been demonstrated to increase sterol synthesis (34) and to inhibit cholesterol esterification (33), while PAF antagonists inhibit cholesterol biosynthesis from lanosterol (35). Collectively, these data suggest that PAF may be involved in the maintenance of cholesterol-dependent lipid rafts.

The conversion of PrP楼市 to PrP楼市 is thought to occur after PrP楼市 has reached the plasma membrane and subsequently been re-internalized for degradation (36–38). These observations raise the possibility that the activation of PLAb seen in prion infected cells and the production of PAF may encourage the formation of PrP楼市 by enhancing proipotaxis and sorting pathways. In some cell lines PAF antagonists prevent endocytosis (39), while in other studies, cPLA₂ inhibitors (AA-COCP or BEL) prevent the maintenance of the Golgi network (40), endosome fusion, and endocytosis (41), and modulate the intracellular trafficking of some proteins (42). Together with the observation that the Golgi and the endosomal compartments are involved in the trafficking of a GFP-tagged PrP楼市 (43), these observations suggest that treatment of neurons with PLAb inhibitors or PAF antagonists may inhibit PrP楼市 formation by altering the intracellular trafficking of PrP楼市.

Currently, the development of therapeutic strategies to combat prion disease is largely based on the identification of drugs that bind to and disrupt aggregated PrP楼市. This strategy is based on the belief that PrP楼市 is a major, if not the only, component of the infectious agent (44), and that the formation of fibrillar aggregates of PrP楼市 leads to neurodegeneration. Thus, it is thought that inhibiting PrP楼市 formation, or disrupting pre-formed PrP楼市, will prevent the establishment of disease. The data presented here support the view that PLAb and PAF regulate the formation of PrP楼市 and thus presumably the propagation of infectious prions since dexamethasone-treated SMB cells showed reduced levels of infectivity. The effects of the PAF antagonists were dose-dependent and caused a 50% reduction in PrP楼市 content at nanomolar concentrations. Both PLAb inhibitors and PAF antagonists caused a rapid reduction in the PrP楼市 content of N2a cells. Thus, the effects of PLAb inhibitors and PAF antagonists on PrP楼市 formation may result from reducing the supply of PrP楼市 to sites conducive to conversion of PrP楼市 to PrP楼市. While PrP楼市 formation is undoubtedly a complex process, these observations provide insight into the signaling processes that initiate the formation of PrP楼市 and presumably prions. We therefore propose that PAF antagonists may have a role in preventing neurodegeneration in prion diseases when used in combination with drugs targeted at the structure of PrP楼市 itself.

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Signal Transduction and Prion Replication
