SOFIA: An Assay Platform for Ultrasensitive Detection of PrP^{Sc} in Brain and Blood

SUNY Downstate Medical Center

Richard Rubenstein[a], Binggong Chang[a], Perry Gray[b], Martin Piltch[b], Marie S. Bulgin[c], Sharon Sorensen-Melson[c], Michael W. Miller[d]

[a]Departments of Neurology and Physiology/Pharmacology, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA, [b]Los Alamos National Laboratories, Los Alamos, NM 87545, USA, [c]University of Idaho, Caine Veterinary Teaching and Research Center, 1020 E. Homedale Road, Caldwell, ID 83607, USA, [d]Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, CO 80526-2097, USA

Abstract

SOFIA is a **s**urround **o**ptical **f**iber **i**mmuno**a**ssay which is comprised of a set of specific monoclonal antibodies and comprehensive capture of high energy fluorescence emission. In its current format, this system is capable of detecting less than 10 attogram (ag) of hamster, sheep and deer recombinant PrP. Approximately 10 ag of PrP^{Sc} from 263K-infected hamster brains can be detected with similar lower limits of PrP^{Sc} detection from the brains of scrapie-infected sheep and deer infected with CWD. These detection limits allow protease treated and untreated material to be diluted beyond the point where PrP^{C}, non-specific proteins or other extraneous material may interfere with PrP^{Sc} signal detection and/or specificity. We further demonstrate that a moderate amount of sPMCA coupled to SOFIA can be used to detect PrP^{Sc} in protease-untreated plasma from preclinical and clinical scrapie sheep, and white-tailed deer with chronic wasting disease, following natural and experimental infection. The sensitivity of SOFIA requires limited sPMCA thus avoiding the possibility of PMCA-induced spontaneous conversion of PrP^{C}, i.e. false positives. We did not find any enhancement of sPMCA with the addition of poly(A) nor was it necessary to match the genotypes of the PrP^{C} and PrP^{Sc} sources for efficient amplification. SOFIA is a sensitive platform for detecting specific proteins and other biomarkers involved in disease pathogenesis and/or diagnosis that extends beyond the scope of the transmissible spongiform encephalopathies.

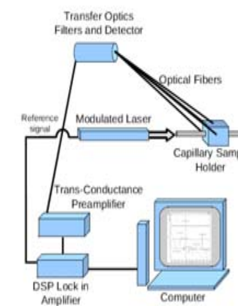

Figure 1. Diagram of SOFIA Instrumentation. Time modulated (chopper not shown) laser illuminates the micro-capillary in the sample holder. The light collected from the sample is directed to transfer optics by optical fibers. The light is subsequently optically filtered for detection, which is performed as a current measurement and amplified against noise by a digital signal processing (DSP) lock-in amplifier (employing the reference signal from the modulated laser). Results are displayed and stored on computer software designed for data acquisition.

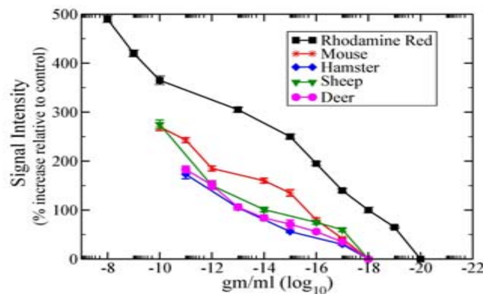

Figure 2. Dynamic Range of SOFIA using recPrP. Dilutions of Rhodamine Red (■) in water were added to 100 µl micro-capillary tubes, and surround optical fiber fluorescent signal emission was recorded. The relative signal intensities were calculated based on the fluorescence signal emission of water alone. In the case of the rPrP from mouse (*), hamster (♦), sheep (▼) and deer (●), the rPrP was diluted in 1% PrP-/- brain homogenate and subjected to SOFIA. The relative fluorescent signal intensities were calculated based on similar assays performed with rPrP diluent (1% PrP-/- brain homogenate) alone. Triplicate assays at a preamplifier setting of 1 nA/V were performed for each rPrP concentration and the data was plotted as the mean of the signal intensities (% increase compared to control) ± SD.

Chang, B., et al. 2009. Surround Optical Fiber Immunoassay (SOFIA): More than an UltraSensitive Assay for PrP Detection. J. Virol. Methods 159: 15-22.
Rubenstein, R. et al. 2010. A Novel Method for Preclinical Detection of PrP^{Sc} in Blood. J.Gen. Virol. 91: 1883-1892.

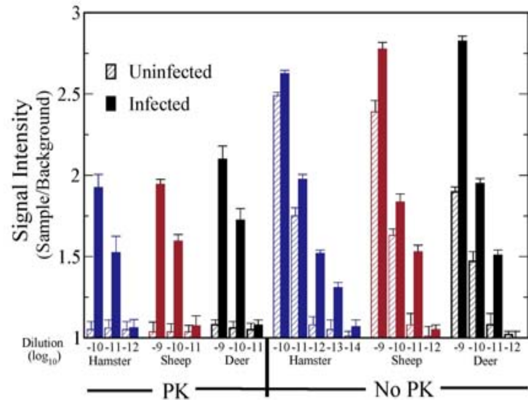

Figure 3. PrP Detection by SOFIA in PK-treated and Untreated Normal and Infected Brain. Brain homogenates from normal (open bars) and infected (solid bars) hamsters, sheep and deer were prepared and either untreated or PK treated. The homogenates were serially diluted and PrP was measured by SOFIA. Values were expressed as a ratio of signal from the samples' Rhodamine Red fluorescence emission (S) vs. background baseline signal derived from fluorescent emission of the diluent (1% $PrP^{-/-}$ brain homogenate or homogenizing buffer) alone (B). The data represents the mean ± SD from three independent experiments, each performed in triplicate at a preamplifier setting of 1 nA/V, for each brain homogenate dilution.

Table 1. Description and Timing of Sheep Scrapie Disease Progression and Blood Collection.*

Genotype	Infection†	IHC (dpi‡)	Blood Collection (dpi)	Clinical Signs (dpi)	Death (dpi)
Group 1: Clinical +, IHC -					
ARQ/ARR	E	Neg.	2019	1936	2290
ARQ/ARQ	E	Neg.	1565	1145	1885
ARQ/ARQ	E	Neg.	1565	1413	1885
ARQ/VRR	E	Neg.	783	604	1100
Group 2: Clinical +, IHC +					
ARQ/VRQ	N	800	1303	883	1320
ARQ/VRQ	N	801	1304	884	1321
ARQ/VRQ	N	792	1295	875	1574
ARQ/VRQ	N	779	931	925	1249
ARQ/VRQ	N	402	926	926	1085
Group 3: Clinical -§, IHC +					
ARQ/VRQ	N	732	934	> 934	1080
ARQ/VRQ	N	778	930	> 930	1250
ARQ/VRQ	E	631	783	> 783	1243
ARQ/VRQ	E	259	783	> 783	1243
ARQ/VRQ	E	581	783	> 783	945
ARQ/VRQ	N	393	917	> 917	1076
Group 4: Uninfected					
ARQ/ARQ					
ARQ/VRQ					
ARQ/VRQ					
ARQ/VRQ					
ARQ/VRQ					
ARQ/VRQ					
ARQ/VRR					

*Animals are grouped based on clinical signs and third eyelid lymphoid follicle immunohistochemistry (IHC) at the time of blood collection.
†E - experimental (oral) infection, N - natural infection assumed to occur at birth.
‡days post-infection
§All animals were clinically negative at the time of blood collection but all displayed clinical symptoms at time of death.

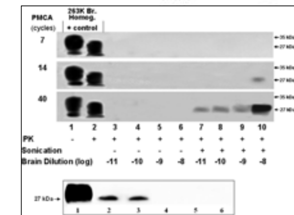

Figure 4. Western Blot of PrP^{Sc} Following PMCA.
Top. Dilutions (10^{-8}-10^{-11}) of infected hamster brain homogenates were subjected to 7, 14 and 40 cycles of PMCA (lanes 10-7) while identical samples of 10^{-8}-10^{-11} were processed similarly without sonication (lanes 6-3). Ten ul aliquots of each sample was analyzed by WB with Mab 11F12. Controls samples were 10^{-2} dilutions of 263K-infected hamster brain homogenates without (lane 1) and with (lane 2) PK digestion prior to WB.

Bottom. Immunoblotting (Mab 11F12) of PK-digested PMCA products following 28 cycles of a 10^{-8} dilution of brain from 263K-infected hamsters (lane 1), scrapie sheep (lane 2) and CWD deer (lane 3). As a control, PK-treated PMCA products from normal hamster (lane 4), sheep (lane 5) and deer (lane 6) brain were also immunoblotted.

Table 2. Description and Timing of Blood Collection, Disease Progression and Clinical Presentation for White-Tailed Deer CWD.

ID #	IHC* (dpi)	Blood Collection dpi	Blood Collection animal status	Death dpi	Death cause
BC04	253	891	clinical CWD	891	clinical CWD
W1004	253	891	subtle changes	981	hemorrhagic disease (preclinical)
N204	253	891	subtle changes	1012	hemorrhagic disease (preclinical)
I304	343	891	preclinical	1182	clinical CWD
K304	253	891	preclinical	1774	clinical CWD

*PrP^{Sc} immunohistochemistry of tonsil biopsy.

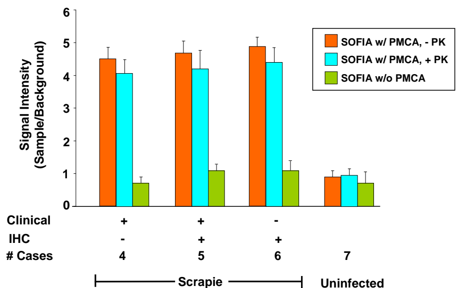

Figure 5. PrP^{Sc} Detection in Sheep Scrapie Blood Samples Using PrP^{Sc} SOFIA With and Without PMCA. Plasma samples were divided into 3 groups according to the appearance of clinical signs and immunohistochemistry (IHC) associated with sheep scrapie. Each plasma sample was subjected to $sPMCA_{40}$ or incubated without PMCA. Each sPMCA product was either untreated or PK digested followed by immunoprecipitation with Mab 8E9 and analyzed for PrP^{Sc} by SOFIA. Plasma samples from each of the 3 groups were assayed in triplicate and the data for all the samples in each group combined and expressed as mean ± SD.

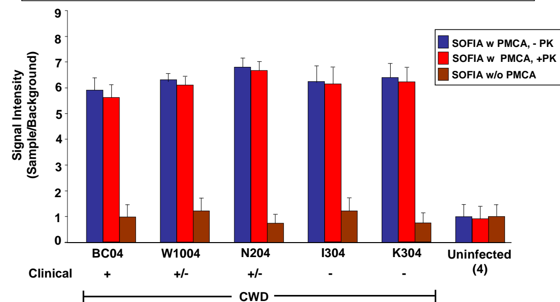

Figure 6. PrP^{Sc} Detection in CWD Blood Samples Using SOFIA With and Without PMCA. Each of the plasma samples from the five CWD cases was first subjected to $sPMCA_{40}$ or maintained in the absence of sPMCA. All sPMCA products were either undigested or PK treated followed by immunoprecipitation with Mab 8E9 and SOFIA. Values represent the mean of triplicate assays ± SD. In the case of the 4 uninfected deer plasma samples, each of the 4 samples was analyzed in triplicate and the combined results of the 4 samples are expressed as the mean ± SD.

Results

- Rhodamine Red was detectable to a concentration of 0.1 attograms (ag) (Fig. 2).
- Using serial 10-fold dilutions (prepared in 1% $PrP^{-/-}$ brain homogenate) of full- length recombinant PrP (rPrP) from deer, hamster, mouse and sheep, the limits of detectability were ≥ 10 ag rPrP (Fig. 2).
- Following PK treatment all samples from normal brain tissues had sample/background (S/B) ratios of less than 1.1 indicating the absence of PrP^{C} (Fig. 3).
- PK-treated PrP^{Sc} from infected hamster brain homogenates was detectable to a dilution of 10^{-11} and from sheep and deer to 10^{-10} as demonstrated by S/B ratios above 1.1 (Fig. 3).
- PrP^{C} was detectable in non-PK treated normal brain homogenates to a dilution of 10^{-11} for hamsters and 10^{-10} for deer and sheep (with peak detection at 10^{-6} – 10^{-7} dilutions) after which the S/B ratios fell below 1.1 (Fig. 3).
- Non-PK treated brain tissue of 263K infected hamsters, scrapie-infected sheep and CWD-infected deer showed S/B values greater than 1.1 to a dilution of 10^{-11} for sheep and deer and 10^{-13} for hamsters (Fig. 3).
- Non PK-treated PrP^{Sc} could be detected by SOFIA on the immunoprecipitated $sPMCA_{40}$ products (Fig. 5).
- The range of SOFIA signal intensities for all three groups of scrapie sheep were similar to each other regardless of their clinical manifestations, and significantly greater than both the pre-PMCA values as well as the uninfected samples (Fig. 5).
- PrP^{Sc} amplification was also independent of genotype compatibility since there was no difference in the amplification when normal brain homogenates from either ARQ/ARQ or ARQ/VRQ sheep were used with any of the infected sheep plasma samples (Table 1, Fig. 5).
- The need for PK digestion to distinguish PrP^{C} from PrP^{Sc} was unnecessary since the results of SOFIA were the same regardless of whether the $sPMCA_{40}$ products were untreated or PK-treated prior to IP and immunoassay analysis (Fig. 5).
- Following IP of the $sPMCA_{40}$ products, and regardless of whether the immunoprecipitants were PK digested or not, PrP^{Sc} was detectable by SOFIA from all preclinical and clinical CWD plasma (Table 2, Fig. 6).
- Detection of CWD PrP^{Sc} by SOFIA was dependent on the samples originating from infected animals but confirmation of disease by SOFIA was independent of the clinical status of the diseased animal (Fig. 6).

Conclusions

- Approximately 10 ag of PrP^{Sc} can be detected from 263K infected hamster brains at clinical disease [with similar (10-100 ag) results calculated from infected sheep and deer brain at clinical disease assuming at least 10-100 fold more PrP^{Sc} in hamster brains than in sheep and deer brain material on a gram equivalent basis]. This translates to the detection of approximately 200 PrP molecules or less than 10^{-4} infectious units [this assumes an association of 1-10 femtograms of PrP^{Sc} from infected hamster brains with 1 LD_{50} by endpoint dilution assays [Atarashi et al. Nature Meth. 4(2007) 645-650].

- Our studies demonstrate the detection of PK untreated PrP^{Sc} in plasma from naturally and experimentally infected clinical and preclinical scrapie sheep and CWD white-tailed deer. SOFIA values, which measure and are proportional to the levels of $sPMCA_{40}$-amplified PrP^{Sc}, were similar for both preclinical and clinical cases when compared within a given species. Therefore, it appears that the level of PrP^{Sc} in plasma does not correlate with the extent of clinical disease. Based on the limits of detection for scrapie sheep and CWD deer brain PrP^{Sc} and the amplification of PrP^{Sc} in the infected sheep and deer plasma, there is less than 1 ag PrP^{Sc}/ml plasma in sheep and deer.