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Reduced Accumulation of the Prion Pathogenic Protein (PrP^{sc}) in Mice Spleen Post Inoculation with a Mixture of the Scrapie C506M3 Strain and Streptomycin

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Abstract

The hydrogen bond transfer between the two guanidine groups present on streptomycin and the different amino acids on the PrP^{sc} peptides provoked changes of the surface electric charges and even probably affected the stability and induced a drop in the PrP^{sc} infectivity. These can explain the reduction to even absence of accumulated prion infectivity marker PrP^{sc} observed at 45 days post intraperitonial inoculation of a mixture of streptomycin and infected mouse brain homogenate of the scrapie strain C506M3 compared to controls.

Introduction

Transmissible spongiform encephalopathies (TSEs) are Protein misfolding diseases with fetal neurodegenerative disorders including scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, chronic wasting disease in deer and Creutzfeldt-Jakob disease (CJD) in humans. This group of disorders is also called prion diseases because they are caused by an infectious protein (PrP^{sc}) induced by conformational changes in the membrane bound cellular glycoprotein (PrP^{c}) and is mainly composed of a detergent-insoluble aggregates and is proteinase K resistant [1,2].

The supposed mechanism of interactions between streptomycin and the prion proteins in vitro is described elsewhere [3] and suggesting that it occurs through an hydrogen bond transfer between the guanidine groups present on the streptomycin molecules and amino acids of one or several prion proteins thus forming multimolecular aggregates.

The transmission of the C506M3 scrapie strain to the C57BL6 mice is remarkably stable among passages and offered quite reproducible incubation periods. After intraperitonial injection in mice the formation of insoluble protein aggregates was first detected in the spleen by the 5^{th} to 7^{th} days post inoculation and progressively accumulated in the mesenteric lymph nodes, the payer's patches and maxillary lymph nodes [4]. The PrP^{sc} load is generally thought to increase progressively after infection, in lymphoid and peripheral nervous tissue and reach the brain where its aggregation determines the fetal neuropathological issue the TSEs [4,5].

The results presented here concern the effect of inoculating a mixture of streptomycin and C506M3 infected mice brain homogenate intraperitonially in mice on the accumulation of the PrP^{sc} in the spleen 45 days post injection.

Materials and Methods

2% Brain suspension of the mouse adapted strain C506M3 diluted in 5% glucose was used to prepare each of the following inoculums:

a) 800 µl brain suspension and 200 µl glucose at 5%.

b) 800 µl brain suspension, 50 µl streptomycin 0.6 M and 150 µl glucose at 5%.

c) 800 µl brain suspension and 200 µl streptomycin 0.6 M.

3 groups composed each of 10 female C57BL/6 mice, 4 weeks old, were inoculated intraperitonially (i/p) by 100 µl from either one of the prepared inoculums. 45 days post inoculation (p/i) the mice were scarified and the spleen were collected individually.

Each spleen was homogenized individually at 10% (weight/volume) in glucose 5%. To 100 µl of spleen suspension was added 10 µl of proteinase K (at 200 µg per ml) then incubated at 37°C for one hour. Streptomycin at 0.14M was added, vortexed, incubated for a second hour at 37°C, centrifugation at 12000 g for 5 min and discard the supernatant. Add 50 µl of 50% vol.: vol. 8 M urea and Laemmli denaturing buffer per tube. After vigorous vortexing, heat at 100°C for 5 min. then centrifuge 5 min. at 12000 g. To 5 µl of each supernatant was added 5 µl Laemmli buffer, heated 5 min. at 100°C, loaded on 15% SDS-polyacrylamide gel, transferred onto nitrocellulose membranes and immuno-blotted using SAF 84 monoclonal antibodies [3].

Results and Discussion

Where 9 spleen out of 10 from the control mice group inoculated with the C506M3 brain homogenate without streptomycin showed the accumulation of PrP^{sc} Figure 1a. Three (7,8 and 9) spleen were weekly positive in the group inoculated with the suspension containing 20 µl streptomycin per mouse and the infectious homogenate Figure 1b. The mice group inoculated with the infectious material and 5 µl streptomycin per mouse showed no accumulated PrP^{sc} in their spleen Figure 1c.

The propagation pathway of the C506M3 in mice following inoculation was in the spleen, where from the 5^{th} day's p/i the pathogenic PrP^{sc} can be detected and progressively accumulated in this organ suggesting a strong affinity of the infectious agent for the lymphoid compartments [4,6].

In a previous paper we reported on the mechanism of interaction

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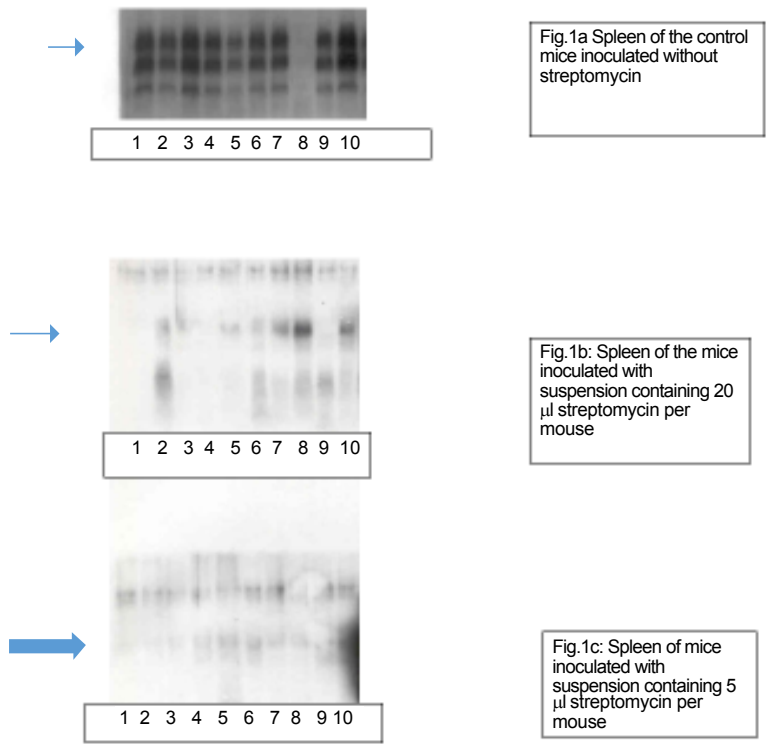

Figure 1: Accumulation of PrP^{sc}.

of streptomycin, as well as the other chemicals, possessing two guanidine groups with the prion proteins [3]. This is most likely to occur via hydrogen bond transfer between each of these chemical groups and the different amino acids from the same or from different PrP molecules. The addition of low concentrations of streptomycin to a PrP^{sc} suspension result in attachment of the antibiotic to the 3 peptides isoforms leading to the increase of their molecular weight. Reticulation is expected when increasing streptomycin concentration was added due to cross-linking by such a proportionally small streptomycin molecule and the different PrP^{sc} isoforms or fragments thus leading to formation of flocculated aggregates in liquid solutions [3]. Therefore streptomycin interaction with the PrP^{sc} had probably induced electric charge changes on the protein surface leading to slight structural changes affecting the infectivity.

As PrP^{sc} is used as a marker of infectivity thus the observed decrease of the PrP^{sc} accumulated in the mice spleen 45 days post inoculation compared to controls can be considered as a drop in infectivity of the infectious agent. Longer observation of inoculated mice beyond the incubation period and comparing PrP^{sc} accumulated in the spleen and brains with those recorded here at 45 days p/i will confirm our obtained results.

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