Current progress in the development of a therapy for clinical rabies

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Writer's Comment: *This paper was originally written as an assignment for UWP 104F, Writing for Health Sciences. The goal of the paper was to research a topic, then evaluate journal articles to write a literature review in the style of a health journal. I chose to write about rabies because it is an infamous disease whose frightening symptoms captivate the imagination. Despite its long history, recent studies, such as those outlined in this paper, highlight how much is still unknown. I thoroughly enjoyed this assignment, and I hope that readers will find this topic as fascinating as I did. I'd like to thank Dr. Amy Clarke for all of her help and for pushing me to improve my writing.*

Instructor's Comment: *Jamie Leung's literature review on treatment options for rabies is surprisingly gripping. In a cultural context this is understandable enough; the story of Atticus Finch shooting the rabid dog in* To Kill a Mockingbird *speaks to our deep-seated fear of a disease that is fast acting and fatal, but that first turns the afflicted into a raving maniac. Nonetheless, harnessing this inherent interest to brief medical professionals on rabies treatment is a real feat. Jamie succeeds because she follows the drama inherent in all medical literature, and of course because she researched the field deeply. But the article's true strength lies in Jamie's well argued stance against uncritically adopting this costly and mostly unsuccessful "miracle cure." As inspiring as the few success stories are – and one example even originates from UC Davis Medical Center – the literature does not support the protocol in its current state. It's hard to deny a feel-good ending, but Jamie is entirely convincing that our best protection against rabies is still prevention.*

—Amy Clarke, University Writing Program

Abstract

Rabies has no proven therapy once clinical symptoms have appeared and is almost always fatal in humans. An emerging therapy is the Milwaukee Protocol (MP), which includes a combination antiviral drug therapy and a drug-induced coma. Much of the support for the MP comes from a few case studies wherein patients survived with minimal neurological impairments. However, the application of the MP has resulted in a markedly low success rate. Here we review several case studies in which post-mortem examinations detected extensive neurological damage and rabies virus (RABV) in the central nervous system of MP patients. These findings challenge the use of an induced coma for neural protection. Furthermore, we show that the few survivals attributed to the MP may actually have been due to the individuals' immune response and viral strain, rather than the protocol. Our findings suggest that the MP is an ineffective therapy for clinical rabies and that further research should focus on alternative therapies and prevention.

Introduction

Rabies is a nearly 100% fatal encephalomyelitis caused by zoonotic viruses of the *Lyssavirus* genus[1]. This makes rabies one of the most deadly diseases by case with an estimated 55,000 cases per year worldwide[2]. Human rabies occurs rarely in the developed world, and there are only 2 to 3 cases per year in the United States[2]. This low rate is largely due to effective pre- and post-exposure prophylaxis and vaccination of pets, which has largely eliminated domestic dogs as a reservoir for the disease. Still, rabies continues to be problematic in resource poor areas of the world, where canine rabies is endemic and the rabies immune globulin and full vaccine series needed for prophylaxis may be less accessible[3].

Rabies has 5 clinical stages: incubation, prodrome, acute neurological signs, coma, and death[1]. Despite the high success of prophylaxis, its use is limited once the incubation period has passed, which occurs on average weeks to months after exposure[3]. After clinical symptoms appear, death usually follows within 5-11 days[1]. This timeline may be extended up to a month or more with intensive care[1]. Care for clinical rabies is often only palliative, and no therapy has been proven to cure the disease. One emerging treatment for clinical rabies is the Milwaukee Protocol (MP), which includes a combination antiviral drug therapy and a therapeutic coma. Since the first application of the MP to a rabies survivor in 2005,

subsequent attempts have shown limited success. In addition, survivors had several commonalities, which indicates their survival may have been due to factors of their disease, rather than the therapy itself. Examination of recent literature reveals minimal scientific basis to continue the use of therapeutic coma, which carries a high risk of complications and adverse effects. Further research is needed to understand the pathophysiology of rabies and to explore alternative therapies for its treatment.

The Milwaukee Protocol

The MP was developed by Willoughby et al. for the treatment of a 15-year-old female rabies patient infected through a bite wound from a bat. No medical attention was sought until symptoms appeared one month later, and she did not receive post-exposure prophylaxis[4]. Previous research had suggested death from rabies was the result of autonomic dysfunction and, if given enough time, the immune system would mount a sufficient response to the rabies virus (RABV)[5]. Thus, the focus of treatment was to prevent secondary complications. The patient was treated with ketamine, midazolam, benzodiazepines, and barbiturates to induce a coma and reduce the risk of excitotoxicity[4]. The antiviral drugs ribavirin and amantadine were also administered. Ribavirin has shown limited success against rabies in animal models but was administered due to evidence of increased permeability of the patient's blood-brain barrier and to prevent myocarditis. Post-exposure prophylaxis, which consists of the rabies vaccine and human anti-rabies immunoglobulin (HRIG), was not used and is contraindicated in the MP, largely due to concerns of interference with the patient's immune system. The patient was declared clear of RABV on hospital day 31 and was discharged on day 76. She had minimal neurologic impairment and was the first known person to survive rabies without receiving post-exposure prophylaxis.

Failure of the Milwaukee Protocol

According to the rabies registry of the Medical College of Wisconsin, the protocol has been attempted 43 additional times and is currently on its fourth version[6]. Of these attempts, only five patients survived, and autopsy results indicate the MP was ineffective. For example, in 2006 an 8-year-old male patient presented with symptoms of encephalitic rabies[7]. RABV antigen was found in corneal impressions and molecular testing detected RABV RNA in saliva samples. The specific viral strain was determined to be Philippine dog rabies. The patient was treated with

ketamine and midazolam to induce coma, along with a combination treatment of ribavirin, amantadine, tetrahydrobiopterin, coenzyme Q, and ascorbic acid. This was a modified version of the MP, and all changes were made with consultation of the authors of the MP. Despite successful implementation of the modified MP, support was stopped on hospital day 27 after isoelectric EEG, cerebral edema, and signs of encephalitis were exhibited. Through autopsy, widespread neural damage, negri bodies, and RABV particles were found in the brain.

Similarly, a female patient infected with Duvenhage virus from a bat was treated with pentobarbital, midazolam, and ketamine to induce coma[8]. She was also given the antiviral drugs amantadine and ribavirin. Viral presence was confirmed through nuchal skin biopsy. However, unlike the original MP, this patient also received rabies vaccine and human anti-rabies immunoglobulin (HRIG). After low EEG activity, abnormal MRI, lack of response to stimuli after ketamine withdrawal, and the loss of brainstem, deep tendon, and cough reflexes, respiratory support was withdrawn on hospital day 20. Autopsy revealed neural cell damage in the hippocampus, midbrain, pons, medulla oblongata, and cerebellum. Rabies virus antigen was detected in the frontal and temporal cortex, hippocampus, and entorhinal cortex.

These two cases represent recent, well-documented, and complete attempts at the MP. The use of an induced coma in the original MP was based on previous post-mortem examinations, which suggested that rabies causes relatively little brain loss or inflammation and that the immune system is eventually able to clear RABV[5]. However, the presence of RABV and extensive neural cell loss in postmortem examinations of these patients reveals this is not the case. More recent research by Scott et al. has shown that in transgenic YFP mice infected with a challenge virus standard (CVS), the resultant axonal beading and location of structural changes in infected neurons is atypical of excitotoxicty[9]. In addition, Scott et al. found morphological changes in neurons and their intracellular organelles, which may explain the fatal outcome of clinical rabies.

Limited Success

Despite its many failures, the MP has been declared successful in a few cases. In 2011, an 8-year old female patient treated with the MP became the third known patient in the US to survive without receiving the rabies vaccine[10]. She presented with sore throat, dysphagia, and

abdominal and neck pain that had been ongoing for one week prior. Several days later, weakness developed in her lower extremities. Her serum and CSF were tested with indirect fluorescent antibody (IFA) tests, and IgG and IgM rabies virus-specific antibodies were detected in both. Rabies virus-neutralizing antibodies (rVNA), RABV antigens, and RABV RNA were not detected. A combination therapy of ketamine, midazolam, amantadine, nimodipine, and fludrocortisone was used for treatment. This followed the original MP but without ribavirin, which has been contraindicated in recent versions of the protocol, because of its potentially toxic effects in large doses[4]. The patient was extubated after hospital day 15 and discharged on day 37. No cognitive impairment was apparent upon discharge.

While such cases seem to defend the use of the MP, a review by Jackson proposes the patient's unusually quick recovery and lack of rVNA suggest the patient did not have rabies[11]. Even if this is not the case, the few survivals of rabies with application of the MP were likely due to confounding variables. In both this case and in the index survivor, RABV antibodies were detected but neither RABV antigen nor RABV RNA were isolated in any samples[4,10]. Consequently, the survival of these patients may have been due to a rapid innate and adaptive immune response that was sufficient to clear the virus.

Survival without the Milwaukee Protocol

Human rabies may possess a lower mortality rate than expected. Abortive rabies occurs readily in animals[12–14], so it follows that abortive cases may occur in humans as well. The first and only case of abortive human rabies to date was reported in 2009. Rabies-specific antibodies, IgG and IgM, were detected in the serum and CSF of a female patient with a history of bat exposure[15]. She had not received post-exposure prophylaxis, but neither RABV antigens nor RABV RNA were found. The patient was given one dose of rabies vaccine and 1,500 IU of HRIG, but the complete series was halted to prevent a potentiated immune response. Following partial vaccination, rVNA were detected in the patient's serum, but not in her CSF. The patient never received intensive care and was discharged within a month of hospitalization after symptoms resolved unaided.

Furthermore, in 2010 two Peruvian Amazon communities were studied by Gilbert et al. because of the high incidence of vampire bat-

linked rabies in these areas[16]. After collecting serum samples, researchers used rapid fluorescent focus inhibition tests to determine complete neutralization of RABV at a 1:5 dilution, which was considered positive for rVNA. RABV IgG and IgM antibodies were detected through IFA at 1:4 to 1:128 dilutions. rVNA were detected in 11%, and RABV IgG were detected in 5% of sera. Of those who were seropositive for IgG, 67% were also positive for rVNA. Gilbert et al. concluded these cases were examples of nonfatal human rabies exposure, because 86% of rVNA positive respondents had not received vaccination and all reported prior physical contact with bats. However, it should be noted that these cases are not definitive proof of abortive human rabies, because, without CSF samples, it is uncertain if the RABV replicated and entered the CNS.

Still, these cases do suggest that bats carry an attenuated strain of RABV. All known survivors of rabies were likely infected by bats or by animals likely carrying the bat strain of the virus[4,10,15,16]. While bats are the leading source of human infection in the US, canine rabies is the most prevalent worldwide, and it seems to be the most virulent of the viruses that cause rabies[2].

Future directions and challenges

Rabies is difficult to diagnose and study due to its infrequency in the developed world, and examination of the recent literature reveals that much is still unknown about this ancient disease. One promising area of research is exploring the role the blood brain barrier (BBB) plays in limiting the ability of the immune system to clear RABV. Roy and Hooper found that a failure to increase permeability of the BBB after infection with RABV increases the probability of death in mice[17]. Mice exposed to attenuated neuroinvasive RABV strains could clear the virus if there were an increase in BBB permeability, which allowed immune effectors to cross into the CNS[17]. Further research should consider the practicality of temporarily increasing BBB permeability as a possible treatment for clinical rabies.

Alternatively, further research using animal models is needed to identify antiviral agents that exhibit greater efficacy against RABV. The BBB is often impermeable to antiviral drugs, so the development of drugs that can enter the CSF, especially without increasing permeability, is critical.

Conclusions

The medical community seems to have prematurely embraced the Milwaukee Protocol (MP) on the basis of a few case studies. Despite its low success rate, high cost, and questionable scientific basis, the MP has been repeatedly chosen for the treatment of clinical rabies. However, postmortem examination does not support the belief that induction of coma provides neural protection in rabies patients[7,8]. In addition, recent research has revealed that morphological changes in infected neurons may be the cause of fatal outcome, rather than excitotoxicity[9]. These findings challenge the basis of the MP, which was developed under the assumption that secondary complications and excitotoxicity are the main causes of death[4]. In spite of this, the survival of a few patients has been attributed to the MP. However, evidence of abortive and multiple nonfatal human rabies exposures suggests the success of these patients was actually due to a rapid innate and adaptive immune response to a less pathogenic strain of the virus[4,10,16].

Recent research has provided evidence for increasing the permeability of the blood brain barrier for treatment[17]. Further research into this therapy would also have implications in the treatment of other brain infections and disorders. Additional research is also needed to identify effective rabies-specific antiviral agents. Nonetheless, prevention is the most efficient and cost effective method of reducing human death from rabies. Therapies such as the MP carry a high risk of complications and, as a result, require a team of specialists and around-the-clock care. Such resource-intensive methods are cost prohibitive in the areas most heavily afflicted by rabies. Before an effective therapy for clinical rabies can be developed, additional research using *in vitro* and *in vivo* models is clearly needed to improve current knowledge about the pathophysiology of the virus.

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