1. Introduction

Rabies is the most severe acute viral infection of humans, with a case fatality rate of almost 100%. Although the prompt administration of rabies vaccine and rabies immune globulin after a dog bite or other recognized exposure can reliably prevent the disease, no effective measures have been identified to rescue a patient who has developed signs of illness. The past decade has seen intense interest in the treatment of rabies, in large part because of the survival of a young patient who was treated with a combination of drugs, including the induction of “therapeutic coma” (Willoughby et al., 2005). Unfortunately, numerous subsequent applications of this approach have failed to achieve success. This paper reviews the current status of rabies therapy and identifies promising directions for future research.

2. Rabies virus and the disease

Rabies is usually caused by infection with rabies virus, a single-stranded, negative-sense RNA virus in the genus Lyssavirus, family Rhabdoviridae; only very rarely is rabies caused by other non-rabies virus lyssaviruses (e.g., Duvenhage virus). Rabies is an acute viral infection of the central nervous system (CNS) that is transmitted by biting animals. Worldwide, most cases of human rabies occur in Africa and Asia as a result of exposure to dogs in rabies-endemic areas. In contrast, most cases in North America are caused by bat rabies virus variants, even though in many cases no bat exposure is recognized.

The incubation period of rabies may last 20–90 days or longer. During most of this period, there is a delay in progression of infection from the site of inoculation (Fig. 1). The virus subsequently spreads in peripheral nerves to the CNS and then within the CNS by fast axonal transport along neuroanatomical connections. After the development of CNS infection, the virus spreads centrifugally along sensory and autonomic nerves to multiple organs.

The prodromal symptoms of rabies are non-specific. Early localized symptoms include paresthesias, pruritus and pain at the site of entry, which are thought to result from infection and inflammation in local sensory ganglia. Eighty percent of patients then progress to encephalitic rabies, which is characterized by episodes of generalized arousal or hyperexcitability separated by lucid periods, autonomic dysfunction, and hydrophobia. The remainder develop paralytic rabies, with quadriparesis and sphincter dysfunction. Both forms of rabies are virtually always fatal. Patients who are
managed aggressively in critical care units frequently develop cardio pulmonary and other complications, including multiple organ failure.

3. How does postexposure prophylaxis prevent rabies?

Rabies can be effectively prevented after a recognized exposure through postexposure prophylaxis (PEP), providing current recommendations are followed closely (Manning et al., 2008; World Health Organization, 2005). PEP consists of immediate wound cleansing, active immunization with multiple doses of rabies vaccine, and passive immunization with human rabies immune globulin, injected into and around the wound and intramuscularly. The objective of PEP is to prevent rabies virus from gaining access to the nervous system. It is of no proven value after clinical signs of rabies develop.

Infection with rabies virus induces a neutralizing antibody response, but patients may die before antibodies become detectable in the serum or cerebrospinal fluid (CSF). Individuals have occasionally been found to have anti-rabies antibodies in their serum, without a history of neurological illness (Black and Wiktor, 1986; Gilbert et al., 2012; Orr et al., 1988; Ruegsegger et al., 1961). These cases are thought to represent unrecognized natural exposures to rabies virus, leading to immunization without CNS involvement.

4. Why is the prognosis so poor in human rabies?

In contrast to rabies, acute encephalomyelitis caused by West Nile virus, Japanese encephalitis virus and other arboviruses has a lower case fatality rate, though survivors often have severe neurological sequelae (Jackson, 2013b). Because viral clearance from the CNS is essential for recovery, immunocompromised patients tend to develop more severe disease. Neutralizing anti-rabies virus antibodies are thought to be the critical mediator of the immune response in rabies, and there is evidence that antibodies can actually help clear rabies virus infection from infected neurons (Dietzschold et al., 1992). The poor prognosis in rabies may reflect the fact that infection induces immune unresponsiveness, characterized by impaired T-cell function, with altered cytokine patterns, inhibition of T-cell proliferation, and the destruction of immune cells (Lafon, 2013). Recent studies in laboratory animals infected with wild-type ("street") rabies virus indicate that even...
Table 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Age of patient</th>
<th>Source of infection</th>
<th>Immunization prior to onset</th>
<th>Neurologic sequelae</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>1970</td>
<td>6</td>
<td>Bat bite</td>
<td>Duck embryo vaccine</td>
<td>None</td>
<td>Hattwick et al. (1972)</td>
</tr>
<tr>
<td>Argentina</td>
<td>1972</td>
<td>45</td>
<td>Dog bites</td>
<td>Suckling mouse brain vaccine</td>
<td>Mild</td>
<td>Porras et al. (1976)</td>
</tr>
<tr>
<td>United States</td>
<td>1977</td>
<td>32</td>
<td>Laboratory (vaccine strain)</td>
<td>Pre-exposure vaccination (combination)</td>
<td>Mild</td>
<td>Tillotson et al. (1977a,b)</td>
</tr>
<tr>
<td>Mexico</td>
<td>1992</td>
<td>9</td>
<td>Dog bites</td>
<td>Postexposure vaccination (combination)</td>
<td>Severe</td>
<td>Alvarez et al. (1994)</td>
</tr>
<tr>
<td>India</td>
<td>2000</td>
<td>6</td>
<td>Dog bites</td>
<td>Postexposure vaccination (combination)</td>
<td>Severe</td>
<td>Madhusudana et al. (2002)</td>
</tr>
<tr>
<td>United States</td>
<td>2004</td>
<td>15</td>
<td>Bat bite</td>
<td>None</td>
<td>Mild</td>
<td>Hu et al. (2007); Willoughby et al. (2005)</td>
</tr>
<tr>
<td>Brazil</td>
<td>2008</td>
<td>15</td>
<td>Vampire bat bite</td>
<td>Postexposure vaccination</td>
<td>Severe</td>
<td>Ministerio da Saude in Brazil (2008)</td>
</tr>
</tbody>
</table>

a Patient died less than four years after developing rabies with marked neurological sequelae (Dr. L. Alvarez, personal communication).
b Patient died about two years after developing rabies with marked neurological sequelae (Dr. S. Madhusudana, personal communication).

In situations in which a robust immune response develops in the periphery, immune effectors are unable to penetrate the blood-brain barrier and clear CNS infection (Roy et al., 2007).

A review of the literature identifies only seven well-documented cases in which humans have survived rabies (Table 1). This excludes two cases which were reported as rabies, but in which the individuals were probably not infected with the virus, since neither developed neutralizing anti-rabies virus antibodies, and one had a highly atypical clinical course and did not require intensive care (Holzmann-Pazgal et al., 2010; Wiedeman et al., 2012). Six of the seven survivors were given rabies vaccine before the onset of illness, suggesting that vaccination played a role in reducing disease severity. Interestingly, the two patients who survived with few or no neurologic sequelae, a 15-year-old girl from Wisconsin (Hu et al., 2007; Willoughby et al., 2005) and a 6-year-old boy from Ohio (Hattwick et al., 1972), were infected with bat rabies viruses. This suggests the possibility that rabies virus variants that circulate in bats may be less virulent for humans than those transmitted by dogs (Lafor, 2005), especially in light of the fact that the number of cases caused by canine rabies virus variants has been many orders of magnitude larger than those due to bat rabies virus variants. Further comparative studies should be performed to confirm if this is really true.

5. Approaches to the therapy of rabies: the “Milwaukee protocol”

In 2003, a group of physicians and researchers with expertise in rabies published an article describing a variety of potential therapies, including rabies vaccination, rabies immune globulin, ribavirin, interferon-α and ketamine (Jackson et al., 2003). Because combination therapies have shown success in the treatment of cancer and a variety of infectious diseases, including human immunodeficiency virus infection and chronic hepatitis C, the authors suggested a similar approach to rabies. The inclusion of ketamine as part of combination therapy was based on animal studies performed nearly two decades earlier at the Institut Pasteur (Lockhart et al., 1991).

In the following year, a combination approach was used to treat a 15-year-old girl in Wisconsin, who had been bitten by a bat on her left hand about a month before admission, and had not received PEP (Willoughby et al., 2005). Neutralizing anti-rabies virus antibodies were demonstrated in her serum and CSF shortly after presentation. She was treated with ketamine (48 mg/kg/day as a continuous intravenous infusion) and given antiviral therapy with intravenous ribavirin and amantadine (200 mg/day given enterally). She also underwent induced therapeutic coma with intravenous midazolam and supplemental phenobarbital, to maintain a burst-suppression pattern on her electroencephalogram. This therapeutic approach has subsequently been dubbed the “Milwaukee Protocol.”

The young patient survived with mild neurological deficits (Hu et al., 2007), but as stated in an editorial accompanying the case report (Jackson, 2005), it is unclear why she survived. Good medical treatment in a critical care unit likely played an important role in the favorable outcome, but there is much less certainty about the benefit of any specific therapy. In particular, therapeutic coma was the most dubious and controversial component of the protocol, and the one most likely to cause harm (Jackson, 2005). Therapeutic coma is effective for status epilepticus (Claassen et al., 2012), but there is no clear scientific rationale or other evidence supporting its use for rabies or other CNS infections. The further evaluation of ketamine, including in vitro studies of virus-infected primary neurons and experimental studies in mice, has also cast doubt on its therapeutic value (Weli et al., 2006).

Since the “Milwaukee Protocol” was first used in 2004, there have been at least 26 reports of the failure of similar approaches to therapy (Table 2) (Jackson, 2013a), and there have likely been additional instances of treatment failure that have not been published. Notably, the online clinical reference UpToDate does not recommend use of the Milwaukee Protocol, pending further data (Rupprecht, 2012). Important potential adverse effects of the Milwaukee Protocol include immunosuppression from barbiturates (particularly the short-acting barbiturate thiopental) (Neuwelt et al., 1982), midazolam (Freire-Garabal et al., 1992), ketamine (Wilson et al., 1971), and ribavirin (Powers et al., 1982), and cessation of the therapy may even potentially lead to the immune reconstitution inflammatory syndrome (Reinke et al., 2013). Continued repetition of the Milwaukee Protocol has made it more difficult to move forward with the development of new therapies. In particular, assessment of the protocol’s true efficacy has been obscured by claims of survival in two cases in Colombia and Peru that were actually fatal and by the inclusion of a patient who received rabies vaccine before the onset of illness (Ministerio da Saude in Brazil, 2008) and of a young patient in California who never developed neutralizing anti-rabies virus antibodies in the serum or CSF and recovered quickly from the illness (Wiedeman et al., 2012), and likely did not have rabies. Unfortunately, reviews of the protocol’s efficacy have not provided literature citations or basic information about the ages, dates and geographical locations of...
Antiviral drugs, which aim to inhibit viral replication and spread, are a logical component of combination therapy for rabies. However, ribavirin and interferon-α are the main currently available agents with known activity against rabies virus, and studies of their efficacy have been very limited (Jackson et al., 2003). Ribavirin inhibited rabies virus in vitro (Busseseau et al., 1983; Busseseau and Ermine, 1983), but it was not effective in laboratory animals (Busseseau et al., 1988), and a patient given intrathecal and intravenous ribavirin did not survive (Warrell et al., 1989).

In contrast, interferon-α was effective in rabies virus-infected monkeys (Weinmann et al., 1979), but no beneficial effect was seen in three patients given high doses of intrathecal and intravenous interferon-α at an early stage of clinical rabies (Warrell et al., 1989). Because penetration of the blood–brain barrier is essential for therapeutic efficacy in CNS infections without resorting to intrathecal administration, this is a potential limitation for both of these antiviral agents. An experimental study in rats showed that intranasal therapy with ribavirin could bypass the blood–brain barrier (Colombo et al., 2011). Molecular strategies to inhibit the replication of RNA viruses and the associated challenges have recently been reviewed (Bray, 2008; Leyssen et al., 2008; Viral enzymes, particularly polymerases, are potential targets of antiviral drugs (Oberg, 2006). New broad-spectrum RNA polymerase inhibitors, such as favipiravir (T-705) (Furuta et al., 2009), which has shown efficacy in a mouse model of western equine encephalitis (Julanter et al., 2009), appears to avoid the toxicity of ribavirin and may be useful in rabies. Oligonucleotide antiviral therapeutics will also be a future area for development (Spurgers et al., 2008).

There is little evidence supporting therapy of rabies with amantadine, apart from one in vitro study (Superti et al., 1985). Ketamine was reported to inhibit the replication of rabies virus in cell culture at high concentrations (1–2 mM), by inhibiting genome transcription (Lockhart et al., 1992). After stereotaxic inoculation of a strain of fixed rabies virus into the neostriatum of rats, high-dose ketamine (60 mg/kg given intraperitonally every 12 h) led to reduced infection in multiple brain regions, including the hippocampus, cerebral cortex, and thalamus (Lockhart et al., 1991). However, more recent evidence from studies in primary neuron cultures and in mice does not support this approach (Well et al., 2006). Hence, there is no basis for the continued use of ketamine for the treatment of human rabies.

In addition to supportive critical care, antiviral and neuroprotective approaches should be important components of therapy. There remains uncertainty whether rabies vaccine and/or rabies immune globulin should be included in the therapy (Jackson et al., 2003), but there is no clear evidence that administration of rabies vaccine to a patient with rabies leads to an unfavorable outcome or ‘early death’ phenomenon.

### Table 2

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Year of death</th>
<th>Age and sex of patient</th>
<th>Source of infection</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2005</td>
<td>47 Male</td>
<td>Kidney and pancreas transplant (dog)</td>
<td>Germany</td>
<td>Maier et al. (2010)</td>
</tr>
<tr>
<td>2</td>
<td>2005</td>
<td>46 Female</td>
<td>Lung transplant (dog)</td>
<td>Germany</td>
<td>Maier et al. (2010)</td>
</tr>
<tr>
<td>3</td>
<td>2005</td>
<td>72 Male</td>
<td>Kidney transplant (dog)</td>
<td>Germany</td>
<td>Maier et al. (2010)</td>
</tr>
<tr>
<td>4</td>
<td>2005</td>
<td>Unknown</td>
<td>Dog</td>
<td>India</td>
<td>Bagchi (2005)</td>
</tr>
<tr>
<td>5</td>
<td>2005</td>
<td>7 Male</td>
<td>Vampire bat</td>
<td>Brazil</td>
<td>Brazil</td>
</tr>
<tr>
<td>6</td>
<td>2005</td>
<td>20–30 Female</td>
<td>Vampire bat</td>
<td>Thailand</td>
<td>Hemachudha et al. (2006)</td>
</tr>
<tr>
<td>7</td>
<td>2006</td>
<td>33 Male</td>
<td>Dog</td>
<td>USA (Texas)</td>
<td>Houston Chronicle (2006)</td>
</tr>
<tr>
<td>8</td>
<td>2006</td>
<td>16 Male</td>
<td>Bat</td>
<td>USA (Indiana)</td>
<td>Christensen et al. (2007)</td>
</tr>
<tr>
<td>9</td>
<td>2006</td>
<td>10 Female</td>
<td>Bat</td>
<td>USA (California)</td>
<td>Aramburo et al. (2011); Christensen et al. (2007)</td>
</tr>
<tr>
<td>11</td>
<td>2007</td>
<td>73 Male</td>
<td>Bat</td>
<td>Germany</td>
<td>Drosten (2007)</td>
</tr>
<tr>
<td>13</td>
<td>2007</td>
<td>34 Female</td>
<td>Bat (Kenya)</td>
<td>Equatorial Guinea</td>
<td>Rubin et al. (2009)</td>
</tr>
<tr>
<td>14</td>
<td>2008</td>
<td>5 Male</td>
<td>Dog</td>
<td>USA (Missouri)</td>
<td>Pue et al. (2009); Turabelidze et al. (2009)</td>
</tr>
<tr>
<td>16</td>
<td>2008</td>
<td>8 Female</td>
<td>Cat</td>
<td>Colombia</td>
<td>Badillo et al. (2009)</td>
</tr>
<tr>
<td>17</td>
<td>2008</td>
<td>15 Male</td>
<td>Vampire bat</td>
<td>Northern Ireland</td>
<td>Hunter et al. (2010)</td>
</tr>
<tr>
<td>18</td>
<td>2009</td>
<td>37 Female</td>
<td>Dog (South Africa)</td>
<td>USA (Virginia)</td>
<td>Blanton et al. (2010)</td>
</tr>
<tr>
<td>19</td>
<td>2009</td>
<td>42 Male</td>
<td>Dog (India)</td>
<td>Romania</td>
<td>Luminos et al. (2011)</td>
</tr>
<tr>
<td>20</td>
<td>2010</td>
<td>11 Female</td>
<td>Cat</td>
<td>Portugal</td>
<td>Santos et al. (2012)</td>
</tr>
<tr>
<td>21</td>
<td>2011</td>
<td>41 Female</td>
<td>Dog (Guinea-Bissau)</td>
<td>USA (Massachusetts)</td>
<td>Javad et al. (2012)</td>
</tr>
<tr>
<td>22</td>
<td>2011</td>
<td>25 Male</td>
<td>Dog (Afghanistan)</td>
<td>USA (Massachusetts)</td>
<td>Greer et al. (2013)</td>
</tr>
<tr>
<td>23</td>
<td>2012</td>
<td>63 Male</td>
<td>Brown bat</td>
<td>NE (10)</td>
<td>2012</td>
</tr>
<tr>
<td>24</td>
<td>2012</td>
<td>9 Male</td>
<td>Marmoset</td>
<td>Brazil</td>
<td>Brazil</td>
</tr>
<tr>
<td>25</td>
<td>2012</td>
<td>41 Male</td>
<td>Dog (Dominican Republic)</td>
<td>Canada (Ontario)</td>
<td>Branswell (2012)</td>
</tr>
</tbody>
</table>

* Personal communication from Dr. Rita Medeiros, University of Para, Belem, Brazil.
8. The role of neuroprotective therapies

Treatments are needed to prevent neuronal damage in human rabies, but effective therapies to reduce neuronal injury for acute neurological diseases are currently very limited. A “trial and error” approach to finding an effective treatment is unlikely to succeed. In the case of acute stroke, numerous clinical trials have shown a lack of efficacy of candidate neuroprotective drugs, despite promising studies in animal models (Sutherland et al., 2012).

One approach that has proven effective in trials in Australia and Europe of patients who remained unconscious after witnessed cardiac arrest due to ventricular fibrillation is therapeutic hypothermia, in which body cooling is used to prevent neuronal injury and improve clinical outcomes (Bernard et al., 2002) (The Hypothermia After Cardiac Arrest Study Group, 2002). There is also interest in using hypothermia for traumatic brain injury (Christian et al., 2008) and for acute ischemic stroke (Watson, 2012), but its efficacy has not yet been established in clinical trials. Hypothermia reduces cerebral metabolism, production of reactive oxygen species, lipid peroxidation and inflammatory responses, at least partially explaining its benefit. There is evidence that similar effects may be helpful in rabies, based on recent insights into the role of oxidative stress in its pathogenesis obtained from studies in cultured neurons and laboratory animals (Jackson et al., 2010; Scott et al., 2008). Mitochondrial free radical production is thought to be an important target mechanism for therapeutic hypothermia in ischemia/reperfusion injury (Lampe and Becker, 2011).

In addition to the induction of generalized hypothermia, regional methods can be applied to the head and neck using a cooling helmet (Wang et al., 2010) or by intranasal administration of an iced coolant that rapidly evaporates after contact with the nasopharynx (Busch et al., 2010; Castren et al., 2010). Regional cooling is associated with less adverse systemic effects, and would be expected to produce less impairment of natural or vaccine-induced systemic immune responses, which are essential for viral clearance. Cooling could be maintained for a period of 24 to 72 h, which would provide some time for the development of a systemic immune response in addition to the desired neuroprotective effect.

Although rabies virus replication is fairly efficient at lower-than-normal body temperatures (e.g., 34 °C), particularly for bat rabies virus variants (Morimoto et al., 1996), hypothermia might be expected to reduce viral spread due to the inhibition of fast axonal transport (Bisby and Jones, 1978) and trans-synaptic spread. Ideally, new therapeutic approaches should first be evaluated in good animal models of rabies before being used to treat patients.

9. Challenges of studying rabies therapy in laboratory animals and humans

The evaluation of potential therapies for human rabies in laboratory animals is expected to be very challenging. Even the best animal model cannot replicate the management of critically ill patients, which require a variety of resources, including the expertise of multiple specialists, readily available diagnostic investigations, therapies for a wide range of potential systemic complications, and around-the-clock care. A veterinary critical care setting would be the most appropriate setting for this approach, despite the difficult challenges involved.

Trials of experimental therapies in rabies patients are not appropriate at this time, because no known approach has a reasonable chance of demonstrating efficacy. Should such a therapy be developed, its evaluation in patients will be very challenging, because testing will have to be performed at sites with the necessary resources for critical care management, while recognizing that the financial costs will be high and the chance of success low. Most cases of human rabies occur in resource-poor and resource-limited areas of Africa and Asia, where canine rabies is endemic and appropriate facilities are often not available for intensive medical care. No government or non-governmental funding agency is likely to invest in a trial without a high probability of demonstrating efficacy. The potential market for anti-rabies therapeutics, which is mostly located in countries with limited resources, also would not justify significant investment by the pharmaceutical industry. Funding for the prevention of human rabies in developing countries, through canine vaccination and the rapid and reliable provision of PEP after recognized exposures, would provide a much better return on investment.

10. Conclusion

New approaches are needed for the treatment of rabies, which may combine hypothermia, antiviral drugs, and other therapeutic agents. Much work is needed to identify new therapies, which will require a better understanding of basic mechanisms involved in the pathogenesis of rabies.

References


