The incurable wound revisited: progress in human rabies prevention?

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Abstract

Rabies is the most important viral zoonosis from a global perspective. Modern human postexposure prophylaxis consists of potent vaccines and local infiltration of rabies immune globulins (RIGs), but the latter biologicals are not widely available or affordable. Monoclonal antibodies (Mabs) offer several theoretical advantages over RIGs. To this end, several human and equine RIGS, alone or in combination with vaccine, were investigated for postexposure efficacy in a Syrian hamster model, compared with a single neutralizing murine Mab. Preliminary results suggest that: (1) animal models continue to provide utility as human surrogates in the demonstration of product efficacy against rabies; (2) RIG preparations differ substantially in experimental effectiveness and clearance; and (3) relevant alternatives, such as Mabs, should be pursued for future improvements to human rabies prevention. Published by Elsevier Science Ltd.

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1. Introduction

Rabies is an acute viral encephalomyelitis caused by etiologic agents in the family *Rhabdoviridae*, genus *Lyssavirus*. Considering the global distribution, incidence, human and veterinary health costs, and severe case-fatality ratio associated with the disease, rabies remains the most important viral zoonosis recognized today, despite its historical occurrence for millennia [1–5]. Undeniably, considerable progress has taken place during the past century, particularly related to animal rabies control, diagnosis, and human rabies prevention [6–10]. This communication focuses on the latter topic.

Multiple technological improvements have occurred since Pasteur's 19th century use of live rabies virus in prophylaxis [11,12]. Modern human rabies prevention after exposure hinges upon prompt first aid and proper wound care, local infiltration with rabies immune globulin of homologous or heterologous origin, and administration of multiple doses of potent cell culture vaccines [13]. Vaccination without rabies immune globulin may not prevent rabies, particularly in severe exposures, such as with multiple bites [14–23]. Unfortunately, human rabies immune globulin is comparatively expensive, especially in developing countries, which results in a continuing market for heterologous biologicals. Prior problems of low potency, serum sickness, and anaphylaxis associated with crude horse serum have been overcome with better primary vaccines and immunization regimens, and advanced protein purification methods [24–26]. However, global supplies of polyclonal immune globulins are often restricted [27], related in part to: (1) a limited capacity to predict demand; (2) difficulties in recruitment of high-producing donors; and (3) extended production times from the period of initial blood harvesting to release of final product including meticulous screening for adventitious agents.

Owing to their defined antigenic specificity and expected yields in bulk culture, monoclonal antibodies [28–35] have been touted as one possible solution for reducing problems associated with current rabies immune globulins. Originally, this research entailed an applied investigation into the potential utility of monoclonal antibodies in experimental rabies prophylaxis

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with direct comparison to various commercial biologicals as the current gold standards. However, initial findings raised questions about the in vivo effectiveness of current products, and no recent literature was obtained for which related comparisons had been conducted. Thus, the overall objective of the current preliminary study was to compare the efficacy of human and equine rabies immune globulins, with and without vaccine, in a Syrian hamster model [36].

2. Materials and methods

2.1. Animals

Animals consisted of $\sim$ 2-month-old (100 g) female Syrian hamsters (Harlan, Sprague, Dawley) [Note: The listing of specific commercial entities throughout this document is for identification only and does not imply endorsement by the US].

2.2. Challenge virus

Challenge virus was prepared from the homogenate of the salivary gland tissue of a naturally infected rabid coyote in Texas (# 323R). This virus is characteristic of a canine rabies virus variant of public health significance, in circulation at the United States–Mexico border. A 2% equine serum in sterile distilled water solution was added to form a 10% weight/volume mixture. Virus was titered by intracranial mouse inoculation. In each case, for standard hamster inoculation, 0.05 ml of a 1:1000 ($10^{6.8}$ $MICLD_{50}$/ml) dilution was selected to produce $> 80\%$ mortality from intramuscular inoculation. After rabies virus administration, animals were observed daily and were euthanized by CO_2 intoxication when clinical signs of rabies were demonstrable. Routine rabies virus diagnosis was confirmed by the standard fluorescent antibody test [37], performed on brain impressions from suspected rabid animals.

2.3. Biologicals

Human and equine rabies immune globulin preparations were obtained from the manufacturers. Commercial human rabies immune products consisted of different anti-rabies immune globulin preparations, each concentrated by cold ethanol fractionation from the plasma of hyperimmunized human donors. The equine anti-rabies products were obtained from pooled plasma of horses hyperimmunized with rabies virus vaccine. The following products were used: HRIG, a commercial lot of human rabies immune globulin (BayRab®); HRIG HT, a pre-release commercial lot of heat-treated (60°C for 10 h) human rabies immune

globulin (Imogam® Rabies HT); ERIG, a commercial equine rabies immune serum product (Rabies Immunoserum Behring); pERIG, a pre-release lot of a commercial purified equine rabies immune F(ab′)2 fragment product (Serum Antirabies Pasteur); pERIG HT, an experimental preparation of a purified, heat-treated, equine rabies immune globulin F(ab′)2 fragment under development [38]; and 1112, an experimental murine neutralizing monoclonal antibody directed against the rabies virus glycoprotein [39]. The rabies virus neutralizing antibody content in international units (IU) per ml was evaluated from each preparation by the rapid fluorescent focus inhibition test (RFFIT)[40]. To avoid potential dilution errors, rabies immune globulin preparations, normally packaged at 150–200 IU/ml, were used neat and were stored at 4°C prior to use. Rabies vaccine consisted of a commercial inactivated purified Vero cell rabies virus vaccine (PVRV) reconstituted with the manufacturer's diluent (0.9% NaCl; 0.5 ml) and diluted 1:2 with sterile phosphate-buffered saline.

2.4. Use of rabies immune globulin or antibody administration alone

The effect of rabies immune globulin or antibody alone after rabies virus exposure was studied. At 24 h after inoculation of the coyote rabies virus isolate, experimental prophylaxis was initiated. Each rabies immune globulin or monoclonal antibody was administered in 0.05 ml at the same site (right gastrocnemius muscle) as virus inoculation. Treatment groups consisted of: HRIG (141 IU/kg), HRIG HT (120 IU/kg), pERIG (117 IU/kg), pERIG HT (144 IU/kg), and monoclonal antibody 1112 (183 IU/kg). Rabies immune globulin groups consisted of 18 animals each. Both the monoclonal antibody group (1112) and the control group (untreated) consisted of nine animals each.

2.5. Use of combined rabies immune globulin and vaccine administration

The combined effect of immune globulin and vaccine after rabies virus exposure was investigated. At 24 h after inoculation of the coyote rabies virus isolate in the right gastrocnemius muscle, prophylaxis was initiated in four different groups of nine animals each. Each immune globulin preparation was administered once at the site of virus inoculation. Treatment groups consisted of HRIG HT (147 IU/kg), ERIG (169 IU/kg), pERIG (115 IU/kg), and pERIG HT (155 IU/kg). Rabies vaccine was administered in the opposite (left) gastrocnemius muscle on days 0 (the first day of treatment), 3, 7, 14 and 28. The control group consisted of nine untreated animals.

2.6. Rabies immune globulin clearance

The serological decay of the different immune globulin preparations was compared. Hamsters were divided into five groups of three animals each. Each group was inoculated in the right gastrocnemius muscle with 0.05 ml of HRIG (141 IU/kg), HRIG HT (147 IU/kg), ERIG (169 IU/ml), pERIG (115 IU/kg), or pERIG HT (155 IU/kg). Hamsters were sedated with an intramuscular inoculation of ketamine hydrochloride (50 mg/kg) and were bled at 12, 18, 24, 36, 48, 96, and 144 h following immune globulin administration. Rabies virus neutralizing antibody levels were determined using the RFFIT. Titers were standardized to International Units/ml using human reference sera. The titers were log transformed prior to the calculation of the geometric mean titers (GMT) and standard deviations.

2.7. Statistical analysis

Survivorship results were compared for statistical significance using the Mantel-Haenszel test [41].

3. Results

3.1. Survivorship against rabies without vaccine (Fig. 1)

In this postexposure evaluation with street rabies virus using only anti-rabies immune globulins as a treatment, at day 30, 89% of control animals (not treated) succumbed to rabies, as did 22, 82, and 100% of the animals treated with monoclonal antibody 1112, pERIG, and pERIG HT, respectively; however, no deaths were noted for HRIG and HRIG HT. At day 75, 33, 72, 94, and 100% of the animals treated with monoclonal antibody 1112, HRIG HT, pERIG, or pERIG HT, respectively, succumbed. In contrast, 100% of hamsters treated with HRIG were protected. Most of the cases in the control and the pERIG and pERIG HT groups occurred within 2–4 weeks after rabies virus challenge, whereas most fatalities in the HRIG HT group occurred 1–2 months after exposure. Differences in survivorship observed between the HRIG-treated animals and all other rabies immune globulin treatment groups were highly significant ($P < 0.001$).

3.2. Survivorship against rabies with immune globulin and vaccine

In this postexposure evaluation using anti-rabies immune globulins and vaccine, all nine controls (not treated) succumbed to rabies within 30 days after rabies virus inoculation, whereas 100, 100, 67, and 22% of hamsters treated with HRIG HT, ERIG, pERIG, or pERIG HT, respectively, and vaccine, were protected (Fig. 2). All fatalities in treatment groups occurred within 2–4 weeks of rabies virus infection. Differences in survivorship observed between the HRIG HT and ERIG groups, when combined with vaccine, and the
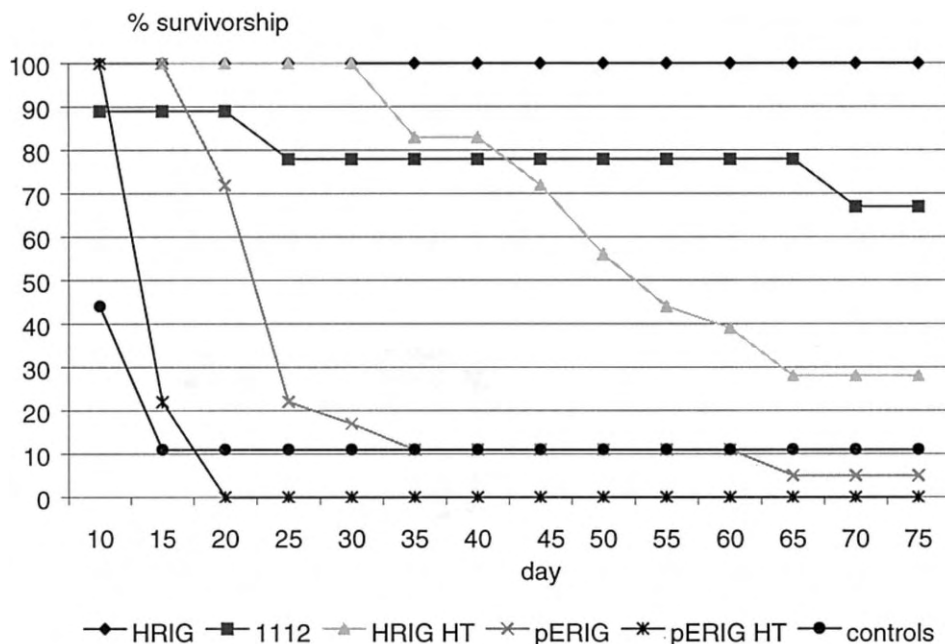

Fig. 1. Post-exposure comparison of rabies immune globulin or antibody, without vaccine, in Syrian hamsters. At 24 h after inoculation with street rabies virus, animals were treated at the site of inoculation with a single dose of monoclonal antibody or rabies immune globulin. Animals were observed daily and were euthanized when signs of rabies appeared. Each group contained 18 animals, with the exception of the monoclonal antibody and control groups, which consisted of nine animals.
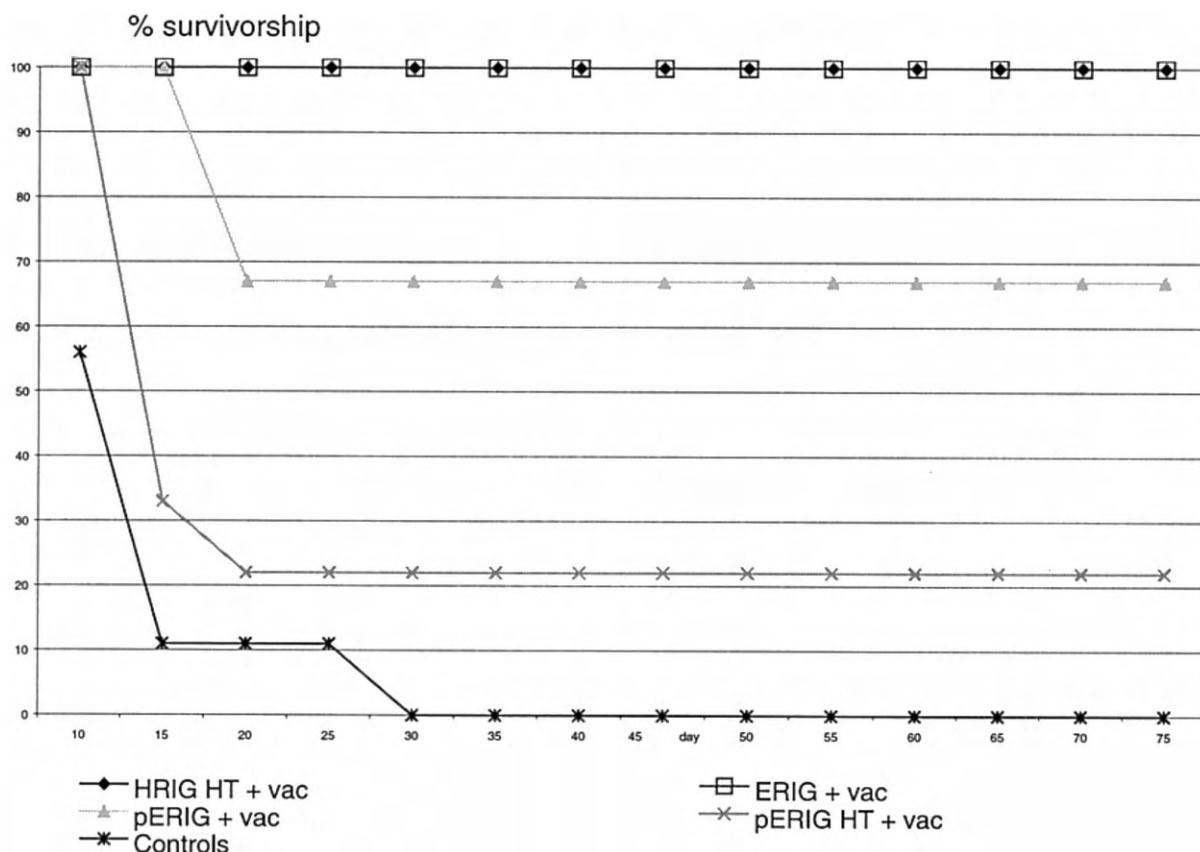
Fig. 2. Comparison of post-exposure prophylaxis with rabies immune globulins in combination with vaccine in Syrian hamsters. At 24 h after inoculation with street rabies virus, nine animals per group were treated at the site of inoculation with a single dose of rabies immune globulin, and the first of five doses of human rabies vaccine. Animals were observed daily and were euthanized when signs of rabies appeared.

pERIG HT and vaccine group, were significant ($P <$ 0.01).

3.3. Antibody decay

Measurement of the various rabies immune globulins in hamster sera showed measurable differences in peak virus neutralization activity and longevity during the 1-week period after intramuscular inoculation, regardless of the initial concentration (Table 1). The two human immune globulins persisted longer than the three equine preparations. In general, peak antibody detection occurred in these two groups within 12–24 h of inoculation, with declines by day 6 but with GMTs still well above 0.5 IU/ml. The HRIG HT preparation resulted in stable serologic GMT values from 0.74 to 1.4 IU/ml at 12 h through day 6. The ERIG preparation resulted in GMTs above 0.5 IU/ml for up to 24 h, with subsequent declines to low but still detectable levels on day 6. All GMT values for the pERIG preparation were less than 0.2 IU/ml, and detectable up until day 4, but were below detectable levels by day 6. Similarly, all antibody levels in the pERIG HT were less than 0.2 IU/ml, and no detectable rabies virus neutralizing activity was associated with the prepara-

tion from 36 h and beyond, even though the inoculated formulation was comparable in concentration to the other immune globulins of either equine or human origin, when analyzed by the RFFIT.

4. Discussion

Initially, this study began as a preliminary assessment of a neutralizing anti-rabies glycoprotein murine monoclonal antibody that had been used successfully in prior studies either alone or as a cocktail with other monoclonal antibodies [28,39]. Our intention was to compare the murine monoclonal antibody with commercial products and pre-release or experimental lots of biologicals formulated for human rabies postexposure prophylaxis. Although this single monoclonal antibody resulted in nearly 70% protection of animals when used alone, and which approximated results with a commercial human rabies immune globulin, two separate purified equine rabies immune globulin products were associated with more than 90% mortality, when used without vaccine. The simultaneous administration of immune globulin with a commercial human rabies vaccine substantially abrogated this trend in nearly all

groups, but major overt mortality was still observed in the animals administered pERIG HT, a purified, heat-treated equine rabies immune globulin product. The complete absence of detection of this latter purified and pasteurized biological 36 h after administration suggested significant differences in potential clearance mechanisms, kinetics, or bioequivalence of $F(ab')_2$ fragments, particularly following additional chromatographic purification and heat treatment, but this observation requires further investigation. If differences in clearance rates occur, higher absolute concentrations may be needed than with an intact molecule to achieve maximal effectiveness [28]. Prior work with pERIG and pERIG HT showed similar pharmacokinetic parameters and had not detected any significant differences in human safety or with vaccine interference with these products, when volunteers were inoculated in simulated postexposure studies [38]. As the action of rabies virus-specific antibodies may extend well beyond mere extracellular neutralization to complement-mediated lysis of virus-infected cells, as well as antibody-dependent cellular cytotoxicity, the Fc portion of such an immunoglobulin molecule may be a critical feature in immuno-protection [42]. In addition to human clinical trials evaluating safety and pharmacokinetics, these current experimental data emphasize the continued utility of animal models in demonstrating the efficacy of various biologicals in experimental rabies postexposure prophylaxis. Furthermore, these data confirm the critical necessity for immune globulin infiltration in combination with vaccine for ultimate protective benefit and raise additional questions about the comparative effectiveness of homologous or heterologous products produced by different methods of purification or heat treatment for regulatory safety considerations.

Obviously, there are a number of caveats to this preliminary research. Hamsters are not *Homo sapiens*, even though similar trends and generalities can be observed in this particular animal model [36] in comparison to those seen under field conditions with human rabies postexposure prophylaxis. Other animal models should be used to investigate the basic proof of principle provided herein. In addition, rabies exposure rarely results in 100% human fatality [43], but rather, rabies-associated mortality is related to a number of variables such as virus dose, locality of the bite, and severity of the exposure. This animal model employed a severe exposure scenario to generate $> 90\%$ of mortality in untreated groups as a worst-case situation. Moreover, sample sizes were somewhat limited, particularly in experiments with the combination of vaccine and immune globulin. Although starting concentrations were not equivalent, they were all between 115 and 170 IU/ml. The doses exceeded the recommended 20 IU/kg for homologous or 40 IU/kg for heterologous preparations by $\sim$3-fold and 1.5- to 2-fold, respectively. No local wound care was performed and such treatment may result in a substantial reduction in human mortality by itself. Only a single rabies virus variant was used for experimental challenge. All interventions were performed within 24 h after rabies virus exposure, whereas delays of up to 1 week or more may not be uncommon in developing countries. These are all variables meriting further investigation.

Human rabies is nearly completely preventable either by avoiding exposure to rabid animals or via the application of prompt and proper modern prophylaxis. Most human rabies cases occur because of one or more of the following reasons: (1) no postexposure prophylaxis of any kind is used; (2) rabies immune globulin is unavailable or unaffordable; (3) local vaccines are substandard; (4) treatment is significantly delayed or inappropriate; or (5) acute illness, malnutrition, or other underlying conditions compromise appropriate immune responses. Documented failures of human rabies postexposure prophylaxis are uncommon [18–21], but surveillance for such events is, in many cases, inadequate. Considering the above complexities, issues surrounding modern biologicals themselves are rarely questioned, and it is unlikely they would be implicated

Table 1
Serologic decay of neutralizing antibody after administration of rabies immune globulins[a]

GMT ($\pm$ SD, IU/ml)

Group	12 h	18 h	24 h	36 h	48 h	96 h	144 h
HRIG	2.81 (2.56–3.07)	1.87 (0.89–3.66)	2.07 (1.30–3.21)	1.57 (0.96–2.50)	1.70 (0.93–3.1)	0.92 (0.87–0.95)	0.81 (0.80–0.83)
HRIG HT	0.84 (0.71–1.01)	0.92 (0.70–1.20)	1.42 (0.65–2.92)	0.89 (0.83–0.96)	0.83 (0.72–0.96)	0.77 (0.73–0.81)	0.74 (0.64–0.84)
ERIG	0.73 (0.62–0.83)	0.61 (0.39–0.90)	0.58 (0.44–0.77)	0.27 (0.15–0.43)	0.18 (0.16–0.21)	0.16 (0.15–0.16)	0.09 (0–0.15)
pERIG	0.16	0.16	0.16	0.16	0.15 (0.07–0.30)	0.09 (< –0.13)	<0.01
pERIG HT	0.16 (0.13–0.19)	0.12 (0.09–0.14)	0.08 (0.05–0.10)	<0.01	<0.01	<0.01	<0.01

[a] Hamsters were sedated, given a single inoculation of rabies immune globulin, and were bled at 12, 18, 24, 36, 48, 96, and 144 h. Rabies virus neutralizing antibodies are expressed in IU/ml.

or assessed independently from other variables. In actual severe rabies exposure situations, homologous products may be used preferentially as a matter of policy (e.g. such as at the Thai Red Cross Treatment Center in Bangkok, Thailand), even in situations where availability is limited and the patient is not able to pay for treatment. It is unknown how many severe rabies exposures throughout the world are treated with heterologous products.

Modern technical methods that are intended to maximize immune globulin safety through purification by the removal of extraneous substances and complexes, or the inactivation of contaminants, may have an untoward effect in vivo, even when thorough testing shows little or no effect upon absolute antibody concentration, in vitro virus neutralization, nor adverse health effects when administered to healthy human volunteers. With the continued maintenance of endemic dog rabies, discovery of new lyssaviruses, and recurrent mass exposure events [44–47], public health demands will continue to challenge the immune globulin market. Given the issues raised by this study, additional introspection is necessary to address these basic concerns and to further develop pure, potent, safe, and efficacious alternatives to current rabies immune globulins, so that they may be made more widely available and economically realistic. Such pre-requisites remain a formidable research challenge in further progress against this zoonosis during the next century [48].

Acknowledgements

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