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Commentary

WHO guidelines dealing with immunoglobulin use impede rabies prevention

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Official Guidelines from an authoritative body are a double-edged sword. On the one hand they serve as a useful template to help raise standards of medical practice, while on the other they are often regarded as gospel truth, and if flawed, may do more harm than good. Regarding current recommendations made by the WHO Expert Committee on Rabies Prevention [1], much is excellent, but there is one dangerous flaw. I believe that the amount of Rabies Immune Globulin (RIG) to be injected locally in and around the wound(s) was not calculated on a scientific basis. Furthermore I suggest that the residue injected intramuscularly at sites other than bite wounds is wasted. The ambiguity and ambivalence in instructions is contributing to the gross under-use of RIG.

The rabies problem

My experience of rabies derives largely from having recently made two educational films on dog bites and rabies prevention [2, 3]. The disease is still a big killer, especially in poorer countries such as India. Human rabies deaths, data on which is certainly underreported, are officially estimated at 55,000 worldwide. India alone may have as many as 30,000, and rabies deaths in China are increasing and may be close to 5,000 annually. Close to 50% of worldwide rabies deaths are in children [4]. With prompt and effective post-exposure treatment, most of these deaths can be prevented.

Rabies virus is inoculated into the bite victim's soft tissues from the saliva of a rabid animal, usually a dog. The virus does not immediately enter the peripheral nerves, but is believed to replicate for a variable time in muscle cells, before entering local nerve endings [5]. The virus then travels via axonal flow to the spinal cord and thence to the brain stem, where it replicates and eventually destroys vital centers. Once the virus is in peripheral nerves it is out of range of the immune system and death is believed to be inevitable. The incubation period is usually between 2 and 3 months, but may be as short as 2 weeks especially for face wounds, or as long as 6 years [1].

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Post-exposure rabies prophylaxis and WHO guidelines

Effective post-exposure prophylaxis demands three complementary sets of measures. **First,** immediately to wash away and/or chemically inactivate as much virus from the wound as possible; **second** to immobilize remaining virus by local inoculation of rabies immune globulin (RIG) into the bite site; and **third** to institute an effective programme of active immunization, thereby raising circulating antibody to effective levels. Each step is complementary to the other two, and all three are important.

For step 1, washing and inactivation, speed and thoroughness are of the essence, and the focus is clearly on more effective public education. Up to 40% of deaths may be prevented by this step alone [6]. A recent discussion with WHO rabies experts revealed that this step is virtually completely neglected in several canine rabies endemic countries. The WHO Expert Committee's Guidelines seem eminently appropriate here.

My criticisms relate to step 2, relating to the use of RIG. In the individual not previously immunized, there are at least ten critical days after starting active immunization before blood and tissue antibody levels are sufficient to inactivate residual virus in the wound. It is to cover this window of vulnerability that the injection of rabies immune globulin (RIG) into the wound is so important. Yet worldwide less than 3 % of at risk dog bite cases receive RIG and it is often still not injected into wounds [7].

For step 3, active immunization, I believe the Committee's recommendations are also appropriate. Much effort has focused on replacing outdated nerve

tissue vaccines (NTV) by modern tissue culture products. The WHO recognizes that the latter are expensive, and has strongly supported the introduction of more cost-effective intradermal schedules to large dog bite clinics. The reduction to small volumes (0.1 ml per site) means that in the most practical such regimen, called the Modified Thai Red Cross schedule, the cost of the vaccine is reduced to 20 %, with no loss in immune response.

WHO Expert Committee Guidelines regarding RIG

There is no doubt that the vast majority of animal bite victims exposed to rabies will survive with vaccine alone. However, there is no way in which the patient who absolutely required passive immunization (RIG) can be consistently predicted. The reasons for the low use of RIG relate to availability, cost and inconvenience. RIG comes from two hyper-immune sources; humans and horses. Human RIG (HRIG) is for practical purposes unaffordable in most poor countries. Equine RIG (ERIG) carries the theoretical risk of allergic reactions which, however, is very low with current highly purified preparations where serum sickness-like reactions are seen in only 1-3 % of recipients and the risk of anaphylaxis is very much lower, less than that with penicillin [8, 9]. In any case skin testing does not accurately predict those at risk of anaphylaxis [1, 8, 9].

The principal reason for the low use of ERIG is the cost, which is obviously a direct function of the dose (volume) needed. It is estimated that following the current official protocol the cost for a 50 Kg patient is US$ 400.- for HRIG and US$ 38.- for ERIG (4). This is compared to $10.- for intradermal tissue culture vaccine. If use of RIG is to increase, as is clearly needed, it is surely incumbent on the WHO Expert Committee to recommend the lowest dose of RIG that is likely to be effective. I present evidence here that the recommended dose is excessive and has been calculated irrationally. The core problem behind this irrationality is that the dose of RIG is still calculated as if the injection is given at a distal site in order to neutralize the virus at the site of virus inoculation. Yet this is no longer the case when the RIG is to be injected into the wound. To understand how such confused thinking came about we need to review the history of the use of RIG.

Use of rabies immune globulin (RIG) for post-exposure prophylaxis (PEP)

Passive immunization with rabies antiserum dates back to 1889 [10], four years after Louis Pasteur and following successful experiments in dogs [11], created history by inoculating bite victim Joseph Meister with attenuated virus. Experiments with RIG at first were largely uncontrolled, but in 1934 Proca et al [12, 13] injected rabies virus into the footpad of guinea-pigs and found that survival was considerably enhanced by an injection of anti-rabies sheep serum into the same footpad. In a systematic study of rabies immune serum in mice, guinea pigs and monkeys, Habel [14] found that serum prophylaxis alone gave better protection than vaccine alone and that the two together were better still. The first dramatic human study of efficacy was made in 1954 when a rabid wolf in Iran bit 29 people, many severely on head and face [15]. All 29 received a course of NTV, 17 with and 12 without one or more systemic injections of Rabies Immune Serum. More of the former were severely injured yet only one of the 17 died of rabies, compared to three of the 12 who received vaccine without antiserum. Rabies virus neutralizing antibody was not detectable before day 19 in vaccine alone group but could be detected as early as the first day in the serum plus vaccine group.

It has been suggested that RIG given systemically, presumably by binding immunogen, may reduce the active immune response. In 1957 Atanasiu et al [16] showed that human anti-rabies serum greatly inhibited the active immune response in man to three intradermal inoculations of high egg passage Flury strain vaccine given five days apart, and this also removed the later anamnestic response. At about that time, it was reasoned that RIG might be more effective if given locally to achieve a high concentration into and around the bite site. In 1958 Gallardo et al [17] inoculated guinea pigs with rabies virus into the thigh, comparing concurrent local infiltration with antiserum to cauterization with nitric acid or flushing with a detergent. Antiserum was significantly superior and protected all animals, even when this treatment was delayed for 24 hours. It was also effective when administered in the opposite leg, and protection was enhanced as the dose of antiserum was increased. In 1962 Soloviev and Kobrinski [18] rubbed dried equine antiserum onto guinea pig skin scarified with rabies virus. Optimum protection was obtained when rapid application of antiserum was followed by vaccine

administration every other day for 6 days. In 1963 Kaplan and Paccaud [19] compared the efficacy of equine antirabies serum with that of equine IgG, both given after infection and into either the same or the opposite footpad. Treatment in the same pad with serum one hour after infection was very effective, and IgG less so. Dean et al [20] found excellent protection by deep flushing and infiltration of equine antiserum around the rabies inoculated wound, but little or no protection when antiserum was given into the opposite leg. Studies in 2000 by Saesow et al [21] on the local intramuscular retention of radiolabelled HRIG in rabbits showed significant retention at the injection site for at least 24 hours.

The balance of evidence, as well as current expert opinion, therefore favours local injection of RIG into the infection site; compared to systemic injection of RIG. Lower doses should also achieve the desired high levels at the infection site where binding to the virus is needed. Lower circulating levels of RIG must also mean less risk of inhibiting the patient's active immune response to the simultaneously administered vaccine.

Although the evidence for local injection takes us back nearly half a Century, recommended practice from the WHO Expert Committees has lagged considerably. In 1966, the fifth WHO Technical Report on Rabies Prophylaxis [22] recommended the combined inoculation of Rabies Vaccine, and hyperimmune serum, the latter to be injected into a distal site different from that of vaccine injection. The aim was to achieve inhibitory circulating levels of antibody. Subsequently hyperimmune serum was replaced by purified rabies immunoglobulin (RIG). In 1969 Winkler et al [23] prepared four lots of human rabies immunoglobulin (HRIG), pooled from previously vaccinated veterinarians, and compared it with high potency equine rabies immunoglobulin (ERIG). The protective effect in mice was the same for both, although the three lots of HRIG contained considerably lower amounts of antibody.

In 1971 Cabasso et al [24] prepared two lots of high potency fractionated HRIG from hyperimmune individuals and conducted a detailed clinical trial to determine the most appropriate dose for post-exposure prophylaxis (PEP), using circulating antibody titers as an end-point. Forty-one volunteers were divided into 5 groups, given respectively none, 10, 20 and 40 IU/kg HRIG IM with vaccine, and the highest dose without vaccine. The circulating half-life of HRIG was found to be 21 days. 24 hours after administration of the two higher doses of HRIG, detectable levels of anti-rabies antibody in excess of 0.5 IU/ml were found in all subjects from Day 1. However, a dose of 10 IU/kg did not produce early antibodies and the antibodies rose slowly in this group. From the profiles, the authors conclude that 10 IU/kg HRIG systemically is insufficient for early protection, that HRIG at all these doses interfered with active immunization, and that 20 IU/kg resulted in minimal interference. This dose was therefore selected for intramuscular inoculation at a site away from the wound, with twice this dose of ERIG being given because its circulating half life is shorter than HRIG. Cabasso (1974) [25] was also the first to standardize packaging of HRIG at 150 IU/ml, dispensed in vials of 2 and 10 ml, 'sufficient for a 15 kg child and a 75 kg adult respectively'. These findings determined the dose recommended in the next WHO reports [26].

Unfortunately a careful later study, using more sophisticated technology, did not confirm this. Lang et al [27, 28] found circulating levels after intramuscular injection of WHO recommended doses of purified heat-treated pepsin digested (split IgG) ERIG or whole IgG HRIG well below the recommended 0.5 IU/ml. It has been argued that the inefficiency of distal site inoculation has contributed to post-exposure failures [29, 30].

In 1992, the WHO Expert Committee's recommendation [31] was changed from intraqmuscular administration to one where 'as much as possible' of the calculated dose of RIG was to be injected into and around the wound. The dose was, and remains, calculated on the basis of body weight, suggesting that these recommendations have been trapped in their own irrational history. Recognising that dog bites vary in number, size and location, these recommendations allow RIG to be diluted in saline to ensure there is sufficient volume to inject into all wounds. Curiously, where the wound is too small to take the whole dose, it is still stated that the residue is to be inoculated into a distal site, even though it is accepted that this will not result in a significant circulation antibody level!

Attention diverted by over-concentrating on new rabies vaccines?

These issues have been largely ignored and all efforts have gone into improving the safety and efficacy of the rabies vaccines. The very low use of

RIG means it is hardly worth the costs of commercial production and there is no other authority than WHO to address this problem. To calculate the dose of RIG according to the weight of the patient, is without rational basis now that we all agree that it is that RIG which is injected into and around the wound that is important. Why do we require 5 times as much RIG for a 100 kg man (4,000 Units of ERIG) than for his 20 kg son (800 Units), with equal size of wounds?

Conclusions and Recommendations

1. Numerous experimental studies point to the efficacy of locally as well as systemically injected preparations of RIG of either equine or human origin. For obvious reasons, the only controlled studies of RIG for the prevention of rabies have been done in animals, although the Tehran wolf bite experience strongly supports its use in man. The remaining conclusions in man have inevitably been surrogate studies, looking at circulating levels of antibody.

2. Despite its undoubted importance, Rabies Immune Globulin is almost never given to victims of rabid dog bites in poor parts of the world where rabies is still a major public health problem. Until this is rectified, it is unlikely that there will be a significant reduction in the number of global rabies deaths. The WHO and other relevant public health authorities have a responsibility to address this problem, which relates both to cost and availability.

3. Where resources are scarce, it makes sense for the **minimum** effective dose to be given in the **optimal** way to the **maximum** number of victims at risk. This is the basis for the local administration of RIG into wounds. If we can reduce this dose safely, this will also reduce cost, which is a major factor in why it is so rarely being used.

4. Studies to determine the dosage of RIG, were carried out nearly 35 years ago. They resulted in the recommendation for systemic administration intramuscularly into a site distal to the wound [22, 24-26]. They aimed to achieve circulating antibody levels. The dosage was crudely calculated based on the body weight of the patient. It has since been recognized that RIG should be given locally into and around the wound where it is needed to neutralize virus prior to it entering nerve endings [1, 20, 31]. Furthermore, later studies cast doubt on the method of the earlier dose calculations [27, 28].

5. HRIG has a longer half-life than ERIG and this was the basis for doubling the dose of the latter. This may not be relevant when injections are given into the wound.

6. It is well documented that vaccine alone will save the majority of animal bite patients. We can, however, not reliably predict which patient will succumb to rabies if wounds are not injected with rapidly virus neutralizing RIG. Subjects with severe facial, head and hand bites, areas with a large supply of superficial nerves, are particularly prone to a short incubation period and treatment failures when no RIG is used [1]. Once a virus has entered a peripheral nerve and has started the ascent to the CNS, it may well be in an immune protected environment [1]. By the time vaccine has induced endogenous neutralizing antibodies which takes approximately 10 days, infection may be well established in the axons where the virus is protected from endogenous antibodies. This is the very patient who will die if RIG is not instilled locally.

7. The 2005 WHO revised post exposure treatment guidelines (1) may impede the proper treatment by encouraging the wasting of costly RIG which in many cases becomes unaffordable to a 50 Kg patient who would do well if his finger wound is injected with 2 mL and the remaining 8 mL are not used at all.

Suggested new WHO Guidelines to increase the use of RIG

An important first step would be to drop all mention of intramuscular administration of any RIG residue after wound injection. Rabies endemic countries must also take a much more active role in helping with production, standardization and distribution of RIG.

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