

The European Pharmacopoeia and humane endpoints

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Summary

The European Pharmacopoeia (EP) has conducted a programme of work over the last 12 years (since the adoption of the European Convention on animal protection) directed at the application of the Three Rs. Much progress has been made in replacing and reducing the use of animals, but animal tests are still prescribed in a number of monographs, mainly those for vaccines. Attention has therefore turned increasingly to the refinement of animal tests, including the use of humane endpoints. It has been possible to include a number of such endpoints in monographs. For many others, while the use of a humane endpoint seems feasible, research and validation work, often extensive, is needed to allow the monographs in question to be revised. The EP will be devoting resources to this kind of work and would like to encourage others also to do so.

The European Pharmacopoeia has an interest in animal welfare for two quite different reasons: the European Convention on animal welfare was prepared within the Council of Europe (Council of Europe 1986), which is the parent organization of the EP; and secondly in a number of monographs, which constitute the official standards for medicines in member countries (Council of Europe 1997), animals are required for testing, and we are of course obliged to apply the principles of the European Convention when establishing the standards.

In the 12 years since the Convention was opened for signature, the EP has carried out an extensive programme for the replacement, reduction and refinement of animal use. The successes are very clear:

1. Bioassays for hormones such as insulin, growth hormone, oxytocin and lypressin have been replaced by chromatographic methods.
2. The abnormal toxicity test has been eliminated or reduced to the status of a development test in the monographs on blood products, vaccines, hormones, enzymes and antibiotics.
3. The potency assay of tetanus immunoglobulin in mice has been replaced by an immunochemical method.
4. Immunochemical potency assays have been introduced for hepatitis A and hepatitis B vaccines.
5. The Limulus test for bacterial endotoxins has been introduced in many monographs and is allowed wherever it can be validated for a given product as an alternative to the rabbit pyrogen test.
6. A number of monographs on vaccines now require no animal tests for the routine release of batches, and for some others the only animal test is the rabbit pyrogen test.
7. A potency assay with a humane endpoint has been introduced as one of the methods for diphtheria vaccine although lethal challenge is also allowed.
8. In monographs on live viral canine and feline vaccines, the test for rabies virus by intracerebral injection into mice has been phased out because the 'upstream' tests on the seed lot and viral harvest in cell cultures are considered more sensitive.

This is the good news: nevertheless many animal tests still need to be published in monographs, mainly those describing vaccines. The resources for carrying out the necessary work for improvements in animal welfare are limited and this was one of the reasons why the introduction of humane endpoints has formed part of the second wave of change. Where it has been impossible to replace animal tests, attention has turned to reducing the number of animals used to a minimum and to the use of humane endpoints.

The animal tests included in monographs can be either development tests carried out on one or only a few occasions, or routine tests carried out for each batch manufactured. The priority must be given to routine tests since this is where the largest numbers of animals are used. For routine testing, the principal animal tests are assays and, to a lesser extent, safety tests for inactivated and toxoid vaccines. These tests will probably be a part of the official standards for the foreseeable future; alternative testing schemes combining physicochemical and immunochemical characterization have been proposed and these could be used within the rules governing compliance with pharmacopoeial standards, if properly validated, but in practice they have not been adopted widely, if at all. If the animal tests are to remain, then refinement clearly has to be considered.

Before looking in detail at some monographs and the possibilities for further refinement of animal tests by the inclusion of humane endpoints, it is worth mentioning that the concept of refinement is not always well understood and even when understood it is not always considered to be a very important goal. This is a conclusion resulting from many discussions over the last 10 years. While it is clear what is meant by the replacement of animal tests and the reduction in animal usage, not everyone will agree that a given change leads to refinement. Is it worthwhile practising the humane killing of animals rather than using lethal endpoints? Is a skin reaction, which may involve necrosis, more humane than a lethal endpoint?

But attitudes to this are changing: at the beginning of the 1970s, at the start of the EP, when the monograph on the tetanus vaccine was introduced, a standard assay method involved the vaccination of mice or guinea-pigs and a subsequent lethal challenge with tetanus toxin. Some laboratories used tetanic paralysis as the endpoint and the animals were humanely destroyed when this point was reached; other laboratories used death from tetanus as the endpoint. A collaborative trial was carried out which demonstrated the equivalence of the two methods. The conclusion at the time was that both methods should be described in the *Pharmacopoeia*; the principal method being that with lethal challenge, and the paralysis endpoint being mentioned as an alternative. However, by the time of the 3rd edition (1997), the main method had become the paralysis endpoint but the lethal endpoint was still mentioned. If the paralysis endpoint is considered to be a refinement then a strict application of the European Convention (Council of Europe 1986) would seem to require its use.

A similar series of events occurred with the diphtheria vaccine, the choice in this case being between lethal challenge and intradermal challenge with erythema as the response. The position is less clear-cut than for tetanus vaccine since the intradermal challenge method requires the introduction of new techniques which can be difficult to master. However, if it were clearly recognized that the intradermal challenge method was a refinement of animal use, then the necessary effort to adopt it would be made. There are different perceptions of the nature of refinement in force with this test. Also, the provisions of the European Convention are probably not well known to all those involved in animal testing nor are they foremost in their minds when designing tests.

Tests involving the following are the areas where there is most need for humane endpoints in EP tests:

1. Pertussis vaccine
2. Rabies vaccine
3. Tetanus vaccine
4. Diphtheria vaccine
5. Leptospira vaccine

6. Clostridial vaccines
7. Target animal potency tests for veterinary vaccines

For some of these, humane endpoints will be introduced within the foreseeable future. For others, more work is required. The extent of this work should not be underestimated.

Pertussis vaccine

The assay in mice uses a virulent challenge by the intracerebral route, with death as the outcome measured. It is clear that a more humane endpoint is needed. The test has been in use for several decades in exactly the same form, and there are a number of reasons for this lack of change. First of all the test is widely considered to be a good indicator of the potency of the vaccine; this reputation was established in trials in the 1940s and 1950s and has been reinforced since then. When pertussis vaccination was introduced 50 years ago there was an opportunity to assess the control methods on the basis of the correlation with the protective effect of the vaccines. The reduction in the incidence of the disease and the high quality of the available vaccines mean that this is no longer possible, and this has hampered the introduction of new control methods that would be preferable from the point of view of animal welfare.

More recently, interest for pertussis has turned to the acellular vaccine where a serological model rather than challenge is used for the assay. Although acellular vaccines can be expected to replace whole-cell vaccines in many countries, the latter will still be widely used for many years, especially since they are cheaper and at least as effective as acellular vaccines. The replacement of the present assay by one with a humane endpoint would require an extensive research programme and cooperation from manufacturers. There are no plans at present for such work at the EP. There is a lack of finance and ideas to make such a project feasible.

If the intracerebral challenge protocol is kept, then one way of decreasing the degree of suffering is to allow moribund animals to be killed and for them to be considered as

having died from challenge. This possibility was considered for the EP when the monograph was last revised, but since it is really a question of training the technicians who carry out the test to recognize moribund animals it was decided not to modify the monograph. This does not exclude the use of this method for scoring the test, but it puts the onus on the analyst to demonstrate its equivalence.

Rabies vaccine

The assay for the rabies vaccine is another example of a test where a humane endpoint would lead to a considerable decrease in the degree of suffering (see Cussler *et al.* 1999). The intracerebral challenge of mice with the rabies virus is used, and most of the controls, and some of the vaccinates, subsequently die of rabies. This method is well-established and ensures vaccine potency. Since antibodies are known to correlate with protection for rabies vaccination, a serological model should be possible. This would need a research programme and would be worthwhile in terms of animal welfare, although it would probably not lead to a better 'standard control' method. There are no plans to work on this at the EP at present, but if any group is planning to do such research then we would be interested in hearing from them. Current interest in the potency testing of the rabies vaccine is directed more to *in vitro* antigen determination, since glycoprotein content shows good correlation with efficacy. This seems a more promising avenue than the use of a humane endpoint.

Diphtheria vaccine

The potency assay uses lethal or intradermal challenge. The best option for refinement at present is to move away from the lethal challenge either to intradermal challenge or to a serological model using the Vero cell assay for antibodies. For the latter, the EP has carried out a collaborative trial on a number of vaccines. The results were somewhat equivocal in that for some vaccines it proved difficult to obtain valid results (compared

with the animal tests) and there were doubts about the equivalence with the established methods. Work will be resumed on this in the future when current studies on the tetanus vaccine are completed.

Tetanus vaccine (human and veterinary)

For the assay of tetanus vaccine, work is currently being carried out, sponsored by the EP and ECVAM, to introduce a humane endpoint in the form of a serological model. The aim is to use a single-dose test in guinea-pigs for the routine release of batches. This will lead to a reduction in the number of animals and in the degree of suffering. Reference sera necessary for this test are being established as part of the project. If the potency assays for diphtheria and tetanus vaccines using the serological model in guinea-pigs can be validated so that the same animals can be used for both products then this will be a very attractive option for manufacturers since these vaccines are often combined in the same vial.

Leptospirosis vaccine (veterinary)

This vaccine is assayed in hamsters using a lethal challenge. This test is a clear candidate for the introduction of either a humane endpoint or an alternative method. Despite work over many years and the unsatisfactory nature of the present test, there is still no practical alternative. The EP would like to bring together specialists in this field to encourage a pooling of resources that may bring an improvement for this vaccine. The alternative to the challenge assay may be an animal test with a humane endpoint or an *in vitro* determination of the antigen. (A meeting was held with support from ECVAM on 5 March 1999 in Strasbourg to discuss this subject.)

Clostridial vaccines

These veterinary vaccines are assayed by the vaccination of rabbits and the determination

of antitoxin concentrations in the rabbits by a neutralization test in mice. This procedure causes a high degree of suffering. In each test about half of the animals suffer from the effects of the challenge toxin. The mouse test can now be replaced by an immunochemical determination of antibodies. The monographs have been revised and will probably become official in about a year. In the meantime a rabbit reference serum has to be established.

Target animal safety tests for veterinary vaccines

Most monographs on veterinary vaccines have a potency test in the target animal. This test is not for routine use but is carried out once or only a few times for a given vaccine to establish a baseline for the routine batch potency test. Since challenge tests will continue to be a part of the regulatory requirements, we have begun to look at the use of humane endpoints. The tests use a virulent challenge so that the control animals and possibly some of the vaccinates suffer the effects of the disease. One approach to humane endpoints for these tests is to define a 'positive' animal in such a way that suffering is reduced while the discriminatory value of the test for vaccine potency is not compromised. Once an animal is recorded as 'positive' it can be given suitable treatment or humanely killed. Wherever possible such a provision is now added to monographs requiring the use of humane endpoints (see Johannes *et al.* 1999).

Another approach concerns vaccines intended for the protection of progeny by the vaccination of the parent. So far, vaccination and challenge tests have been included. More recently the possibility of using antibody level determination in colostrum or measuring transfer of maternal antibody have been identified. This approach is being considered for the bovine rotavirus and coronavirus vaccines. It requires the establishment of the correlation between antibody level and protection. This is not the kind of

work that we are able to do on our own and we would like to encourage others to help us.

References

- Council of Europe (1986) European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. *European Treaty Series* No. 123
- Council of Europe (1997) *European Pharmacopoeia*, 3rd edition
- Cussler K, Morton DB, Hendriksen CFM (1999) Humane endpoints in vaccine research and quality control. In: *Humane Endpoints in Animal Experiments for Biomedical Research* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 95–101
- Johannes S, Roskopf-Streicher U, Hausleithner D, Gyra H, Cussler K (1999) Use of clinical signs in efficacy testing of erysipelas vaccines. In: *Humane Endpoints in Animal Experiments for Biomedical Research* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 102–5