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ERYTHROPOIETIN, RECOMBINANT DNA-DERIVED

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Proposed 2nd International Standard

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Summary

Stocks of the International Standard for Recombinant DNA-derived Erythropoietin (IS) are exhausted. Another preparation of recombinant DNA-derived erythropoietin (rEPO) in ampoules coded 88/574 (EPO 88/574), prepared from similar bulk rEPO as the IS, is available to replace the IS. EPO 88/574 was assessed and calibrated in the international collaborative study of the IS by in-vivo bioassay in terms of the Second International Reference Preparation of Human Urinary EPO, for Bioassay. Recent longer-term accelerated thermal degradation studies of EPO 88/574 confirmed its apparent stability. It is proposed that EPO 88/574 be established as the 2nd International Standard for rEPO Recombinant DNA-derived Erythropoietin, and that each ampoule of EPO 88/574 be assigned a value of 120 international units of rEPO activity.

Introduction

The International Standard for Recombinant DNA-derived Erythropoietin (IS; in ampoules coded 87/684) was established by the WHO Expert Committee on Biological Standardization (WHO ECBS) in 1990 (WHO Expert Committee on Biological Standardization 1991). This Standard has been widely used for the calibration of assays to control the quality and potency of recombinant DNA-derived EPO (rEPO) used in the treatment of anaemias associated with a wide range of clinical conditions, and has also been used for the calibration of some immunoassay systems for erythropoietin (EPO) used in clinical diagnosis. Stocks of the IS are now exhausted, and it needs to be replaced.

International collaborative study of the IS and of EPO 88/574

Prior to its establishment by WHO, an international collaborative study had been undertaken of the IS and of three other candidate ISs of rEPO by 26 laboratories in

11 countries, using a wide variety of in-vivo and in-vitro bioassays and immunoassays (Storring & Gaines Das 1992). The bulk rEPO used to prepare the IS and one of the other candidate ISs in ampoules coded 88/574 (EPO 88/574) had been synthesized in Chinese hamster ovary cell lines, although by different manufacturers, and those used to prepare the other two candidate ISs had been synthesized in baby hamster kidney and mouse C127 fibroblast cell lines, respectively. The physicochemical properties of these rEPOs were compared by electrophoresis and isoelectric focusing, as documented in BS/90.1650 and in Storring & Gaines Das (1992). Ampoules of the IS and of the other three candidate ISs were all prepared in the same way.

On the basis of the results of the study, the participants in the collaborative study agreed to recommend to the WHO ECBS that the preparation in ampoules coded 87/684 be established as the International Standard for rEPO, and that the other three candidate ISs for rEPO, including EPO 88/574, were also suitable to serve as international standards. Furthermore, the participants agreed to recommend that the potency assigned to the IS and to the other candidate ISs should be based on their calibration in the collaborative study by in-vivo bioassay in terms of the in terms of the Second International Reference Preparation of Human Urinary EPO, for Bioassay (2nd IRP)(Annable *et al.* 1972), namely 86iu/ampoule for the IS and 120iu/ampoule for EPO 88/574.

At its 41st meeting in October 1990, the WHO ECBS established the preparation in ampoules coded 87/684 as the International Standard for rEPO, and, on the basis of the results of the collaborative study and with the agreement of the participants, assigned an activity of 86 international units of rEPO to the contents of each ampoule (WHO Expert Committee on Biological Standardization 1991). The Committee also noted the differences shown in the study between human urinary EPO and rEPO synthesized in different cell lines and recommended that the WHO keep under consideration the establishment of separate standards for naturally occurring EPO and for rEPO produced in different cell lines. The Committee further noted that the three other preparations of rEPO included in the collaborative study may be useful as international reference materials in the future.

Further studies of the stability of EPO 88/574

In the collaborative study the EPO content of ampoules of EPO 88/574 kept at +20°C and +37°C for 286 days had been estimated in terms of that of EPO 88/574 kept at -20°C by in-vivo and in-vitro bioassays, and by immunoassays(Storring & Gaines Das 1992). The overall mean activity for each sample did not differ significantly from that of the material kept at -20°C.

During 2002 estimates of the EPO activity of ampoules of EPO 88/574 kept at +4°C, +20°C and +37°C for 13.7 years were carried out using the normocythaemic mouse assay (2002). The mean estimates of activity as % of that in ampoules kept at -20°C (with 95% confidence limits) were 100 (77.5-130)% from two assays of ampoules kept at +4°C, 102 (84.5-124)% from two assays of ampoules kept at +20°C and 96.7 (76.1-123)% from two assays of EPO 88/574 kept at +37°C.

Degradation rates were considered using the methods of Jerne & Perry (Jerne & Perry 1956) and Kirkwood (Kirkwood 1977). However, in the absence of any detectable loss in activity, no reliable prediction of degradation rates was possible. Nevertheless, these data indicated that EPO 88/574 appeared to be adequately stable when stored under normal conditions at -20°C in the dark.

Proposal for the establishment of EPO 88/574 as the second International Standard for Recombinant DNA-derived Erythropoietin

Now that stocks of the IS are exhausted, it is proposed that EPO 88/574 be established as the 2nd International Standard for rEPO, and that it be assigned a potency of 120 international units of rEPO activity per ampoule. This proposal is based on the fact that:

1. The suitability of EPO 88/574 to serve as an international standard was demonstrated in its collaborative study, and this was agreed by the participants in the study, and noted by the WHO ECBS.
2. Further accelerated thermal degradation studies have confirmed the stability of EPO 88/574 when stored under normal conditions, at -20°C in the dark.
3. The bulk rEPO used to prepare EPO 88/574 resembled that of the IS in having been synthesized in a Chinese hamster ovary cell line, although by different manufacturers.
4. The participants in the collaborative study had agreed to recommend to the WHO ECBS that the potency assigned to EPO 88/574 should be based on its calibration in the collaborative study by in-vivo bioassay in terms of the in terms of the 2nd IRP, namely 120 international units of rEPO activity per ampoule.

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Plasma fibrinogen

The Committee was informed of the need for an international reference preparation for use in the standardization of assays of fibrinogen in plasma, which are of increasing importance in epidemiological studies, for example of coronary heart disease. It was also informed that a collaborative study of plasma fibrinogen assays had been completed and that the results suggested that further collaborative studies were necessary before an international reference material could be considered.

Endocrinological and related substances

Recombinant-DNA-derived erythropoietin

The Committee noted that, in accordance with the request made in its thirty-sixth report (WHO Technical Report Series, No. 745, 1987, p. 23), the National Institute for Biological Standards and Control, Potters Bar, had obtained candidate materials to serve as an international standard for erythropoietin derived from recombinant DNA (rDNA), and that the collaborative study in 26 laboratories in 11 countries referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 21) had been completed (BS/90.1650).

The Committee established one of the materials studied, in ampoules coded 87/684, as the International Standard for Erythropoietin, rDNA-Derived, and, on the basis of the results of the collaborative study and with the agreement of the participants, assigned an activity of 86 International Units of Erythropoietin, rDNA-Derived, to the contents of each ampoule.

The Committee noted the differences shown in this study between human urinary erythropoietin and rDNA-derived erythropoietin synthesized in different cell lines, and recommended that WHO keep under consideration the possibility of establishing separate standards for naturally occurring erythropoietin and for rDNA-derived erythropoietin produced in different cell lines.

The Committee further noted that the three other preparations of rDNA-derived erythropoietin included in the collaborative study might be useful as international reference materials in the future.

Porcine inhibin

The Committee noted that the collaborative study of the proposed international reference material for porcine inhibin referred to in its thirty-ninth report (WHO Technical Report Series, No. 786, 1988, p. 27) had been completed (BS/90.1648). It also noted that a preparation of porcine inhibin partially purified from ovarian follicular fluid had been included in the study, together with other preparations of human, bovine

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