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Preclinical Evaluation of Prototype Products

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Abstract

Preclinical evaluation of medical devices (prototype products) offers the opportunity to investigate and study the intended use of device materials. Preclinical evaluation programs are designed to determine the efficacy, safety, and biocompatibility of biomaterials, prostheses, and medical devices. The purpose of safety testing is to determine if a material presents potential harm to the human; it evaluates the interaction of the material with the *in vivo* environment and determines the effect of the host on the implant. Preclinical evaluation is the determination of the ability of the prototype product to perform with appropriate host response in a specific application, considered from the perspective of human clinical use. Therefore, preclinical data should include materials science and engineering, biology, biochemistry, medicine, host reactions and their evaluation, the testing of biomaterials, and the degradation of materials in a biological environment.

Key Words: Preclinical evaluation, medical device, biomaterial, safety, biocompatibility

INTRODUCTION

This document is intended to inform the device manufacturer or investigator of the preclinical testing that should be performed to generate data that will provide reasonable assurance of the safety and effectiveness of medical devices for their intended use. The preclinical data should include a comprehensive description of the device. The device manufacturer should clearly list the device components and materials and state whether or not they have been used previously for human implantation, and, if so, to list these components and/or materials. For frequently-used materials, several examples of previous use will suffice. If the material has not been used for human implantation, but has industrial uses, then these uses and any adverse data concerning the effect on animals or the environment should be stated.

The requirements for preclinical testing will be influenced by the type of material, the type of prosthesis, and previous use of the material in humans. For example, processed products of biological origin will require extensive immunological testing. If a material degrades, the fate of the material in the body or joint must be determined.

A comprehensive summary of all preclinical testing should be included in addition to specific detailed test descriptions. For each test, the device manufacturer should detail the test procedures including equipment, protocol, measurement techniques, and test parameters. Test descriptions should clearly state what component of the device is being tested. The consequences of test results should be discussed in terms of the expected *in vivo* performance of the device in human tissue.

PHYSICAL AND CHEMICAL ANALYSES

These procedures are intended to supplement biological testing and are required for all device types. The first objective of physical and chemical analyses is to identify and characterize the device in its entirety. If the device is thought to be reasonably comparable to devices described in the literature, these tests can be used to demonstrate that data from the literature can be extrapolated in support of the investigational device safety.

The objective of these analyses is also to identify leachable materials per unit weight of finished device material under exhaustive extraction conditions. At present, it is suggested that at least two solvents (one polar, one non-polar) be used for extraction at

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elevated temperatures (37°C for 5 days) in a ratio of 1 g of synthetic polymer (shredded, if possible, to maximize surface/wt) per 5 ml of extraction media, according to ASTM F619.[1] It is suggested that the extracts should then be re-extracted with a compatible solvent, such as methylene chloride or tetrahydrofuran, to a minimum possible volume in order to achieve maximum sensitivity of the analytic technique. When possible, and where a potentially leachable substance is known, calibration standards should be prepared and the concentration of the substance in the extract should be calculated using suitable analytical techniques (GC, HPLC, etc.). For processed materials of biological origin, the extraction process may be tailored to identify the extraneous processing agents in an optimal fashion, e.g., cross-linking chemicals. Identification of the extracted material should be performed on extracts concentrated to a convenient volume. The Center for Devices and Radiological Health (CDRH) recommends that a sensitive procedure such as gas chromatography be used in conjunction with mass spectroscopic analysis for identification of separated peaks. However, other validated, sensitive analytic methods may also be used.

BIOLOGICAL TESTING

The objective of preclinical biologic testing is to establish that the material and processing used to fabricate the device do not present adverse toxicological effects. The ultimate goal of these tests is to ensure that the final device does not impose undue risk to the patient. If the material has a prior history of clinical usage, many conclusions regarding device safety can be made by reviewing such data. Similarly, toxicological information, particularly component toxicology and pharmacokinetic information, can often be obtained from a careful literature search. It should be noted that in order to use data taken from the literature, the device manufacturer must establish that the chemical and physical characteristics of the investigational device, including the process residuals, are reasonably comparable to those of the device found in the literature.

The following tests describe methods of worst-case determinations used to identify toxic substances. The results of these tests, so-called "hazard identification information", should be provided. It should be realized that "hazard parameters" are generally utilized in accordance with basic tenets of toxicology and consist of three distinct phases: identifying the hazard, extrapolating from the dose given to obtain a risk estimate, and evaluating the risk compared to the benefit of using the substance. All testing procedures must conform to acceptable toxicological principles such as exaggerated dose/response criteria and statistical validity of data.

Pyrogenicity testing

The goal of pyrogenicity testing is to determine the presence of fever-producing substances. For most devices, it may be appropriate to conduct a USP rabbit test on a saline extract of the device to demonstrate preclinical device safety. An *in vitro* limulus amebocyte lysate (LAL) assay, for bacterial endotoxin detection, should be conducted as an end-product test for quality assurance. However, for biological materials, both USP rabbit tests[2] and LAL assays[3,4] should be conducted and reported as part of the preclinical safety testing.

The pyrogen test and the LAL assay should be performed with sterilized device saline extract. The test extract should be prepared at elevated temperatures (37−40°C) using a high surface area to solution ratio. Additionally, other methods such as sonication may be used. For the LAL assay, appropriate sensitivity and inhibition/enhancement tests should also be performed concurrently, and all results should be expressed in standardized units (nanograms or standard units of endotoxin per unit weight of the device).

Hemolysis testing

Contact tests or saline extract tests should be used for determining the hemolytic potential of the device or material. Any standard protocol which uses spectrophotometric analysis for hemoglobin may be used. CDRH recommends using the "Standard Practice for Assessment of Hemolytic Properties of Materials", ASTM F756.[5]

Acute toxicity and irritation testing

Acute toxicity and irritation tests should be con-

ducted using extracts prepared according to the United States Pharmacopeia (USP). One polar and one non-polar solvent, such as water or saline and cottonseed or sesame oil should be evaluated. Irritation studies assess the short-term and generally-localized hazards of medical devices in the immediate region of their applications. Topical local tolerance effects are almost entirely limited to irritation. Though this usually means dermal irritation, it can also be vaginal, rectal, nasal, or occular. Two tests should be performed: a USP systemic injection test, and a USP irritation test.[6]

Cytotoxicity testing

An appropriate cell line such as L929 mouse fibroblasts should be exposed to the device material and to both the polar and non-polar USP extracts of the intact device. It may be appropriate to expose the cell lines to a DMSO extract in addition to an aqueous extract. It should be noted that DMSO should be used at concentrations below 5 percent to prevent toxicity to the cell culture. The basic purpose of these tests are to detect soluble leachate (primarily low-molecular weight chemicals) during early investigations.

Agar diffusion test[7]: An *in vitro* assay that measures the toxic response of the device in L929 mouse fibroblasts. The assay is designed to detect toxic water-soluble and diffusive entities in the product. In addition to agar diffusion tests, the device manufacturer should attempt to conduct direct contact and/or water or minimal essential medium (MEM) elution tests.

Direct testing for cytotoxicity: CDRH recommends that the USP extracts be tested for cytolethality by comparing colony-forming ability (colony suppression assay) and growth pattern changes at low cellular plating densities. These are simple, inexpensive tests in which the cell division time parameters and the ability of individual cells to establish colonies are measured in both control and treated groups.

Genetic toxicity testing

It is recommended that the battery of tests listed below be performed on a minimum of two extracts, one polar solvent and one non-polar solvent. When evaluating data from this test battery, equal weight is assigned to each system without preferential weight given to any particular system. Substitutions of other accepted genetic toxicity tests may be made for those listed below. The device manufacturer should give justification for any variation in the tests performed.

Ames/Salmonella assay[8]: This assay should be performed with and without metabolic activation in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100.

Mammalian mutagenesis assay[9,10]: Two mammalian mutagenesis systems are recommended. These are the L5178Y/TK+/− assay and the CHO/HGPRT assay. Both systems utilize mammalian cells in culture and are believed to detect forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells or the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovary cells (CHO). Both systems have been demonstrated to identify both base pair substitution type and frame shift type mutagens.

Mammalian cell transformation assay[11]: This is the only *in vitro* assay that may detect a carcinogenic response, i.e., transformation of a normal cell to a malignant cell. Two systems are recommended, C3H/10T1/2 assay and Balb/C3T3.

Unscheduled DNA synthesis in primary rat hepatocytes (UDS assay)[12]: This is an assay system that can detect damage produced to molecular DNA in cultures of primary rat hepatocytes. A positive response indicates potential mutagenic or carcinogenic properties of the test material since the damage detected is to the genetic material of the cell.

Immunological potential testing

The biomaterial used for the fabrication of the ligament should be evaluated for delayed-type contact sensitization potential by a suitable method.[13-15]

Immunologic studies other than contact sensitization studies are not required for synthetic polymers. However, if the ligament is fabricated from materials of biological origin (e.g., processed heterograft) extensive preclinical testing should be performed in suitable models, such as the rabbit and guinea pig. These studies should be directed to establish the quantitative biologic response toward the device material. A sensitive test procedure for circulating antibody response (e.g., competition radioimmune assay

or ELISA assay) and for cell-mediated immune response should be utilized. Careful documentation must also be made for histological studies in device implantation studies in terms of immune response. CDRH recommends that special staining techniques be used in addition to standard histological staining.

STERILIZATION AND STABILITY

Sterility information for devices and their packaging must be included in the description of manufacturing. In addition, devices of biological origin should be tested preclinically to validate the sterilization process and to demonstrate that the process does not have a deleterious effect on the biological or mechanical properties of the device.

For devices of biological origin, the method and details of the sterilization process and validation and bioburden level data must be submitted. Validation data should include mechanical testing performed on the sterilized device.

Products sterilized by ethylene oxide gas must be analyzed to determine residual ethylene oxide levels. The shelf-life of the sterilized device should also be stated. Data should be submitted which demonstrate that device properties are not compromised by prolonged storage. For products not marketed sterile, labeling must be provided recommending the method and details of the sterilization process. Data must be submitted to assure that the process will reasonably achieve the desired sterility levels.

MECHANICAL TESTING

The determination of mechanical properties is indispensable to the practical design and understanding of load-bearing structures. It is important that at every point mechanical stresses are safely below the yield strength of the material. The following mechanical tests[16-18] should be conducted to characterize the biomechanical properties of implantable devices and components.

(1) Tensile test; elastic modulus, yield strength, ultimate tensile strength, ductility, toughness

(2) Fatigue test: fatigue strength

(3) Fracture toughness test: critical stress intensity factor

LONG-TERM ANIMAL STUDIES

Device implantation

Preclinical *in vivo* testing should include chronic (1 year or more) device implantation in animal tissue in a loaded configuration to characterize the type and time course of the post-implantation biological and mechanical events.[19,20]

Therefore, *in vivo* test data will be relied on heavily as evidence of:

(1) The histological reaction to the device and device particulate;
(2) The immunological reaction to the device;
(3) Device material degradation leading to a loss of desired properties;
(4) Device abrasion and/or damage;
(5) The migration of particulate matter;
(6) The strength of fixation;

The testing should include the same device and, preferably, the same fixation system intended for human application, although a different size may be necessary. Any difference between the device or fixation system used for human implantation versus that used for animal studies should be clarified by the device manufacturer. Interim animal sacrifices should be scheduled to reflect the histological and mechanical response at acute and subchronic time points.

CDRH recommends that evaluations be conducted at a minimum 3-months and 12-months post-implantation. Careful consideration should be placed on the type of device and the purpose of the animal study particular to that device type. For example, investigators of devices intended to achieve tissue ingrowth must demonstrate the nature of ingrowth with animal studies and must demonstrate whether or not device strength is increasing with time due to ingrowth.

Pathology studies

At sacrifice, each implanted tissue should be examined and described in detail and *in situ* photographs of the prosthesis and surrounding tissues should be taken, whether the tissue components will be used for mechanical or histologic studies.[21,22] For the pathology study using animals, the gross and microscopic pathology of the tissue surrounding the device, the amount of fibrous ingrowth into the

device should be reported. Abraded particles should be evaluated for size distribution, quantity, and type of reaction elicited. Gross necropsy examinations should be conducted on all animals and conventional histologic studies of major organs (e.g., liver, kidney, lungs, spleen) should be performed. Lymph nodes, particularly regional lymph nodes, should be examined histologically in detail for migrated particulates. Raw histological data in addition to summarized data should be submitted.

Mechanical testing

At sacrifice, the device itself should be examined, while the gross and, if possible, the microscopic findings should be described. The amount of fibrous ingrowth and any abraded or damaged material should be reported. The mechanical tests should be conducted to characterize the biomechanical properties of implanted devices and components.

Particulate migration studies

The purpose of particulate migration studies is to obtain worst-case information for possible future corroboration with clinical results. For devices in which abrasion may cause the release of material particles, preclinical *in vivo* testing should include a study of the migration of particulate matter. Detailed justification should be provided for the doses and size/geometry of the particles. Some animals should be kept for a minimum of 1 year to estimate the long-term effects. The type of histologic reaction elicited by abraded particles, and the effect on intrastructures should be documented with gross and microscopic pathology. Regional lymph nodes of the animals should be examined for migration of particulate matter.

Carcinogenesis bioassay

If the device manufacturer cannot demonstrate that the device material(s) has been previously used for human implantation for a significant period of time, it should considered as a new biomaterial. For all new implant materials, the carcinogenic risk to humans must be addressed. For a new biomaterial, it is required that a lifetime (2 year) implant bioassay be performed.

The bioassay should be performed as follows.[23-25] The maximum implantable dose (MID) of the device should be implanted in the paravertebral muscle of rats. The MID should be expressed as a multiple of the actual "worst case" exposure with detailed justification of the calculation given. The MID may be introduced in either a solid or a ground/shredded form, again, with justification given for the chosen method. Rats with a reasonable natural background occurrence of tumors, such as a Fischers rat, should be chosen. There should be 100 animals (50 male and 50 female) receiving a suitable negative control material and 100 animals (50 male and 50 female) receiving the investigated implant material. Animals should be examined regularly. Detailed gross pathology and microscopy must be presented for animals that die during the interim. Complete accounting and post-mortal examination, with microscopic pathology, must be performed on all animals.

REFERENCES

1. ASTM F619. Standard practice for extraction of medical plastics. 1991.
2. U.S. Pharmacopeia. Pyrogen test. In: United States Pharmacopeial Convention, editors. United States Pharmacopeia. 22nd ed. Rockville (MD): Mack Publishing; 1990. p.1515.
3. Cooper JF. Principles and applications of the limulus test for pyrogen in parenteral drugs. Bull Parenter Drug Assoc 1975;3:122-30.
4. Weary M, Baker B. Utilization of the limulus amebocyte lysate test for pyrogen testing of large volume parenterals, administration sets and medical devices. Bull Parenter Drug Assoc 1977;31:1127-33.
5. ASTM F756. Standard practice for assessment of hemolytic properties of materials. 1993.
6. Shayne CG. Irritation and pyrogenicity. In: Safety evaluation of medical devices. New York: Marcel Dekker; 1997. p.97-116.
7. Autian J. Toxicological evaluation of biomaterials: Primary acute toxicity screening programme. Artif Organs 1977; 1:53-60.
8. Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test. Mutat Res 1975;31: 237-364.
9. Clive D, Spector JFS. Laboratory procedure for assessing specific locus mutations at the TK locus in cultures of L5178Y mouse lymphoma cells. Mutat Res 1975;31: 17-29.
10. Hsie AW, Couch DB, O'Neill JP. Utilization of a quantitative mammalian cell mutation system, CHO/HGPRT,

in experimental mutagenesis and genetic Toxicology. In: Butterworth BE, editor. Strategies for Short-Term Testing for Mutagens/Carcinogens. 2nd ed. West Palm Beach Florida: CRC Press; 1979. p.39-54.
11. Heidelberger C, Freeman AE, Pienta RJ, Sivak A, Bertram JS, Casto BC, et al. Cell transformation by chemical agents - a review and analysis of the literature: A report of the U.S. Environmental protection agency gene-tox program. Mutat Res 1983;114:283-385.
12. Mitchell AD, Casciano DA, Meltz ML, Robinson DE, San RH, Williams GM, et al. Unscheduled DNA synthesis tests: A export of the U.S. environmental protection agency gene-tox program. Mutat Res 1983;123:363-410.
13. Anderson JM. Inflammatory response to implants. Trans Am Soc Artif Intern Organs 1988;34:101-7.
14. Bick PH. The immune system: organization and function. In: Dean JH, Luster MI, Munson AE, Amos H, editors. Immunotoxicology and Immunopharmacology. 2nd ed. New York: Raven Press; 1985. p.1-10.
15. Luster MI, Pait DG, Portier C, Rosenthal GJ, Germolec DR, Comment CE, et al. Qualitative and quantitative experimental models to aid in risk assessment for immunotoxicology. Toxicol Lett 1992;64-5 Spec No:71-8.
16. Budinski K. Engineering materials, properties and selection. 5th ed. Old Tappan (NJ): Prentice-Hall; 1995.
17. John VB. Introduction to engineering materials. 2nd ed. New York: MacMillan; 1983.
18. Levinson IJ. Statics and strength of materials. Englewood Cliffs (NJ): Prentice-Hall; 1971.
19. Kaminski EJ, Oglesby RJ, Wood NK, Sandrik J. The behavior of biological materials at different sites of implantation. J Biomed Mater Res 1968;2:81-8.
20. Leininger RI, Mirkovitch V, Peters A, Hawks WA. Change in properties of plastics during implantation. Trans Am Soc Artif Intern Organs 1964;10:320-2.
21. Pizzoferrato A, Ciapetti G, Savarino L, Stea S, Tarabusi C. Results of histological grading on 100 cases of hip prosthesis failure. Biomaterials 1988;9:314-8.
22. Turner JE, Lawrence WH, Autian J. Subacute toxicity testing of biomaterials using histopathologic evaluation of rabbit muscle tissue. J Biomed Mater Res 1973;7:39-58.
23. Ecanow B, Gold BH, Sadove M. The role of inert foreign bodies in the pathogenesis of cancer. Br J Cancer 1977; 36:397.
24. Farrar DB, Crump KS. Exact statistical tests for any carcinogenic effect in animal bioassays. Fundam Appl Toxicol 1988;11:652-63.
25. Hajian G. Statistical issues in the design and analysis of carcinogenicity bioassays. Toxicol Pathol 1983;11:83-9.