



Toxicological requirements for sclerosing agents or other chemicals for female sterilization

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Abstract

There are no guidelines regulating the technologies available for Fallopian tube occlusion. Generally accepted regulatory requirements cannot be applied directly to the safety assessment of these technologies. The more appropriate guidelines are those regulating medical devices. Each method has to be evaluated on its own merits taking into consideration the duration of contact with tissue and the chemical and physical composition of the occlusive agents.

Keywords: Female sterilization; Tubal occlusion; Sclerosing agents; Toxicological tests

1. Introduction

The techniques available for Fallopian tube occlusion have been developed either to sclerose the tubular lumen or to serve as occlusive agents. There are chemical sclerosing agents, notably quinacrine [1], but in the main, the technologies are best classified as devices, for they do not rely on their pharmacological action to achieve the intended effect [2]. Medical devices are produced from biomaterials. The study of biomaterials is usually a study of mixtures rather than of a single chemical entity.

The generally accepted guidelines and regulatory requirements apply to chemicals but are not directly applicable to the safety assessment of biomaterials. As the chemical composition of these agents and their modes of use and application differ widely, it is not possible to establish a standard package of safety tests: each agent has to

be evaluated on its own merits, on the basis of its intended clinical use, chemical composition, duration of contact and possible metabolism or degradation. Priorities for safety testing can be established and a set of safety evaluations can be devised to support applications for registration. The testing of chemicals, biomaterials and medical devices is directed to biosafety and biocompatibility.

2. Safety tests

2.1. Biosafety

Chemical and physical properties of the biomaterial or device. The biomaterial should be chemically defined and the impurities should be known. Impurities are often starting materials, or are process intermediates or degradation products. With some compounds, the potential for toxicity can be identified before testing in vivo is started.

Some preformed devices may have to be sterilized; maximal allowable concentrations of ethylene oxide (5 ppm), ethylene chlorohydrin (10 ppm) and ethylene glycol (10 ppm) have been proposed for intrauterine devices by the US Food and Drug Administration (FDA) [3].

The physical properties of the biomaterial give indications of its potential to cause damage. Interaction with water and proteins, ion exchange, oxidation and biodegradation are parameters that should be considered.

3. Toxicity tests

3.1. *Safety/compatibility tests*

The United States Pharmacopoeia (USP XXI) and National Formulary (NF XVI) has produced guidelines for Biological Tests for Plastic Containers, Transfusion and Infusion Assemblies, elastomeric closures, and containers for ophthalmics [4]. These compendial tests have been adapted for evaluating the safety of a wide variety of biomaterials and medical devices. Evaluation of medical devices for biological hazards is now covered by guidelines laid down by organizations such as the American Society for Testing Materials (ASTM) and the British Standards Institute (BSI). Tests in the UK are carried out according to British Standard 5736 [5]. The British Standard 5736 addresses the evaluation of medical devices by categorization based on the end use of the device (long-term implant, long-term or repeated contact with a mucosal surface or ocular tissue, short-term use within the body or contact with a mucosal surface or skin). The tests rely on simple procedures in vivo in laboratory animals in which the test materials, or extracts of the materials, are administered.

The standard models for toxicological evaluation of biomaterials are systemic and local toxicity models with variable periods of duration [6]. In the systemic models, an extract of the material is the test article. The British Standard 5736 requires that extracts of the test material be prepared using polar (0.9% saline) or non-polar (cotton seed oil USP) solvents. The extraction procedures are defined. For the USP XXI tests, extracts are also required in alcohol/saline and polyethylene glycol

400. The extracts are then tested in animals using either an intravenous or an intraperitoneal route of administration.

Toxicity testing or any statistical exercise based on toxicological data cannot demonstrate an absence of toxicity, but one can, by defining toxic events, deduce doses that cause "no effect". The no-effect level is an important concept for regulatory agencies, for it is the basis for assessing safety and establishing acceptable daily intakes. In these tests for systemic biosafety, the limitations of the model do not permit demonstration of toxicity. Low levels of potentially toxic substances are found in the extracts obtained from biomaterials but the volume that can be administered intravenously is limited by the vascular volume of the rodent model. Using the intravenous route of administration, exposure is maximized by giving a bolus injection of an extract. The intraperitoneal route of administration appears to minimize the rate of absorption.

The local toxicity models for biomaterials are intracutaneous injection, subcutaneous implantation, muscle implantation or direct application to the skin or to mucous membranes. The local toxicity models have been shown to be more sensitive than the systemic toxicity models. There is also a high correlation between cytotoxicity in vitro and implantation tests in vivo. The US Pharmacopoeia proposes reductions in the use of animals in compendial testing.

3.2. *General toxicology*

If the sclerosing agent is a single chemical or a mixture of chemicals, then the regulations relating to proprietary medicinal products apply. Repeat-dose toxicity tests are required to reveal any physiological and/or pathological changes induced by repeat administration of the active substance or combination of active substances under examination, with due regard to the route of administration (intrauterine) and the pharmacokinetic properties of the test compound(s). The duration of the study is defined by the proposed frequency and duration of clinical use, the route of application to humans, the reproductive physiology of the test animal species and the pharmacokinetic characteristics of the compound(s).

3.3. **Reproductive studies**

Despite the generally low predictive value of these studies, chemicals will have to be investigated to determine if there are embryotoxic or teratogenic effects of in utero exposure. If the method is not 100% efficient in blocking the Fallopian tubes, a fertility and general reproductive study may be required. There are technical problems in conducting reproductive studies with devices and biomaterials.

3.4. **Genotoxicity**

The EEC regulations [7] require that chemicals used for medical purposes are subjected to tests to define their mutagenic potential. These tests are directed to investigating gene mutations in bacteria and mammalian cells and cytogenetics in vivo (usually a micronucleus test monitoring gross chromosomal damage in rodent bone marrow) and in vitro (usually a metaphase analysis in human lymphocytes or CHO cells).

3.5. **Carcinogenicity**

If a chemical is retained in tissue or if a device or biomaterial is to be left in situ over a long period it may be necessary to test the potential for carcinogenic hazard. This can be done only by lifetime studies in rodent models.

3.6. **Immunogenicity**

Foreign substances may be capable of activating the immune system by functioning as antigens. Low-molecular weight chemicals are not antigenic in themselves but may become immunogenic after binding with tissue or body fluid macromolecules. Hypersensitivity reactions occur as a consequence of antigenic stimulation and tissue damage may result.

3.7. **Hemolytic and thrombogenic effects**

When blood comes into contact with an artificial surface, a complex series of reactions occurs. These reactions are controlled by the physico-chemical nature and structure of the surface of the material, blood composition and non-physiological blood interfaces and blood flow rate. Tests are available to measure blood interaction and the potential for thrombogenesis. Most of the systems reported are in vitro.

3.8. **Biocompatibility**

Biocompatibility is related to the safety in use of medical devices and is defined as the ability of a material to perform with an appropriate host response in a specific application. The emphasis is on the active role of the biomaterial, although there is a relationship with both the safety and efficacy of the device.

Test procedures are required that allow biocompatibility to be characterized. Biocompatibility is concerned with the behavior of the host tissue and of the host in response to contact with the material or its released substances, and with the behavior of the material implanted in the host tissue. It is difficult to study these parameters together. Tests are often reduced to an assessment in vitro of the interface of a human cell system with the biomaterial. Two protocols are usually followed, the first investigating the direct cytocompatibility with the biomaterial, the second investigating by means of material extracts prepared from the test biomaterial, the indirect cytotoxicity of products released from the biomaterial. These studies can be directed to investigating basal cytocompatibility, specific biocompatibility and assessments using prolonged contact times. For basal cytocompatibility the parameters that can be measured include viability, proliferation, cell cycle, cell protein and plasma membrane lysis. For specific biocompatibility, tissue morphology, and concentrations of collagens, non-collagenous proteins or specific enzymes can be assessed. Colonization of the material by cells can be assessed using prolonged contact times; the indices that can be monitored include cell attachment, spreading, mobility, cell and interface morphology, as well as the material surface modification and/or biodegradation.

To test for foreign body reactions, mechanical and chemical stress effects, toxicity and integration into the implant site, appropriate studies in vivo may have to be considered.

4. **Product evaluation**

These tests must address the adverse effects of biomaterial applied in a manner similar to that proposed for use in humans. The application or

implantation of the material should be considered, together with the performance, resistance to wear and tear, and tissue compatibility.

5. Discussion

The regulatory viewpoint on the safety of biomaterials is confused. Drug regulations are found in most countries, whereas medical devices are regulated only in a limited number. Some countries where medical devices and biomaterials are regulated have assigned devices to different regulatory classes. Devices pose different types of risk: the risk of a device used in a life-threatening situation is very different to the risk of a device used for sterilizing healthy humans. If a device is of a similar type or principle as one that has been marketed previously, then in use experience gives good guidance.

The importance of defining the chemical and physical characteristics of a biomaterial before testing starts in vivo cannot be overemphasized, for many potential hazards can be identified. For example, acrylate copolymers may contain residual amounts of acrylamide monomer. Silicone elastomers came under suspicion because they contained stannous octoate, hydrolysis of which yields 2-ethylhexanoic acid, and questions of safety have been raised with respect to the latter. Cyanoacrylates were widely used as tissue adhesives, as they polymerize when placed on tissue. Recently, these compounds have fallen into disfavor because of their irritant properties and their rapid degradation to products including formaldehyde and cyanoacetate, which were considered to pose a potential toxic hazard.

It is possible to design materials with surfaces that reduce immunological bioincompatibility. Complement activation induced by artificial materials requires the presence of OH and/or NH 'acceptor sites that allow the covalent binding of C3b molecules to the surface.

The., tests for biocompatibility are largely a search for biologically active extractables. A battery of tests is used because of the variety of extractables that may be present in a material and the various mechanisms by which they exert their biological effects. The test procedures are compro-

mises, for quantification and qualification of any components released from the device are nearly always impossible. It is usually possible only to demonstrate that no adverse effects are produced in tests that are undertaken currently.

All biomaterials induce host reactions. The more biocompatible materials may induce host reactions that can be beneficial: for example, mice in which silicone rubber is introduced subcutaneously show increased resistance against *Listeria monocytogenes* infection and against the Lewis carcinoma [8].

If the method of obstructing Fallopian tubes is not 100% effective, then the possibility of teratogenic risk is real. Animal studies are of value in assessing the risk of embryotoxicity and teratogenic effects. However, the risk of ectopic pregnancy can only be assessed in careful clinical trial.

Materials used for sclerosing or obstructing the Fallopian tube do not present a significant risk with respect to immunotoxic potential and their contact with blood is limited.

Mutagenicity tests give rise to a number of false-positive and false-negative results. A positive mutagenic test is a matter of concern to the regulatory authorities: for example, residues such as acrylamide have a potential to cause clastogenic damage. Similarly, the anti-malarial drug quinacrine has been suggested as a potential sclerosing agent; however, quinacrine is a mutagen in bacterial test systems, it is a DNA intercalator and there is evidence that it inhibits DNA excision repair [9]. Such positive data will have to be put into a biological context before quinacrine can be developed for international markets. Genotoxicity data provide a basis for support of a carcinogenic hazard; heritable mutation as a separate end point is an academic concept, for no chemical with such properties has been identified and there is no way of obtaining human evidence of such an event. The genotoxic risk of biomaterials should not be overemphasized.

Some authorities insist that biomaterials and devices to be used in the uterus or Fallopian tubes must be implanted in rodents to demonstrate the absence of carcinogenic risk. These studies are nonsensical and are a profane use of animals. Neoplastic responses to intraluminal foreign ma-

terials in rats are well documented. Steel coils, polyethylene rods, cotton string with paraffin-vinyl copolymer mixtures and silk sutures implanted into the uteri of rats, have all been shown to induce squamous cell carcinomas [10-12]; indeed, isolated foci of squamous metaplasia can be induced with silk sutures in uteri of ovariectomized rats within 10-14 days. In rats, a decidual reaction is caused by the foreign body, which reaction can progress through to tumors in these animals.

It may be difficult to evaluate a prototype device or a miniature of the device using animal models; devices used in humans cannot always be administered or implanted into laboratory animals. For example, in macaque monkeys the tortuous configuration of the endocervical canal necessitates surgical insertion of intrauterine devices by hysterotomy. Some of the materials used for occlusion are injected in a viscous state and form in place. If a material is to be introduced into the Fallopian tube, the possibility of spillage into the peritoneal cavity must be considered as a secondary hazard. However, the long-term implantation of materials and devices in animal models does give some indication of performance and wear and tear of the product, together with indications of tissue compatibility.

Chemical sclerosing agents pose particular problems, for if these materials are to be used then they must be delivered to the appropriate site in precise quantities. The technology to carry out such a procedure as a routine is not available. The use of quinacrine is controversial and many safety issues remain unresolved. Tissue promoters and the new collagens may be potential new materials, but their use is dependent on developing a simple, reliable, non-incisional transcervical approach of delivery.

On the basis of knowledge of the clinical use of chemicals and biomaterials, the site of application, the duration of contact with human tissue

and the chemical and physical composition, a list of priorities for safety testing for each product can be established. One must hope that any future regulation will focus on real issues of safety to humans, rather than on organizational or economic issues, and will take international aspects into consideration.

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