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ESTIV questionnaire on the acquisition and use of primary human cells and tissue in toxicology

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Abstract

The ability to use human cells and tissues in toxicology research and testing has the benefit that it obviates the need to undertake species extrapolation when assessing human hazard. However, obtaining and using human cells and tissues is logistically difficult, ethically complex and is a potential source of infections to those coming into contact with human cell material. The issue is also controversial, with the recent EU legislation draft on tissue engineering, and also due to some instances of human material being obtained and used without informed consent. There are also varying regulations and attitudes relating to the use of human cells and tissues throughout Member States of the EU, and there is a need for harmonisation.

The European Society of Toxicology in Vitro (ESTIV) Executive Board and the European Network of Human Research Tissue Banks (ENRTB) have conducted a survey to ascertain the extent to which human cells and tissues are used by its members, how these are obtained, what local regulations are in force, how the material is used, and the advantages and disadvantages experienced by members in using such material, as opposed to cell lines. The results obtained have been compared with the results from a previous survey conducted in 2000. It is hoped that this information will help to facilitate the process of acquiring and using human cells and tissues in a safe and effective way to promote the use of non-animal approaches for investigating the mechanisms of toxicity, and for predicting the toxic hazard of substances.

Keywords: Toxicology; Human cells; Human tissue; Cell banking; Ethics; Legislation; Cooperation; Alternative methods

1. Introduction

As human cells and tissues represent a very attractive alternative to animal-derived material it is crucial to secure legitimate, practical and ethical sources of human material for research purposes. Up to now there has been no common legal system in the EU, which regulates this very important issue. Taking into account that tests developed for regulatory purposes must comply with the legal system in all EU member states, the above is one of the limiting elements for development, validation and acceptation of methods based on human material. Despite the limitations there are already two tests based on human material which have been successfully validated in Europe, the human skin model for phototoxicity and corosivity testing (Fentem et al.,

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1998; Directive 2000/70/EC, 2000; Fentem and Botham, 2002), while the haematotoxicity and pyrogenicity (Hartung et al., 2001) tests are at the final evaluation stage.

The problem of the acquisition and use of human material for safety evaluation and regulatory testing has been addressed in several publications. For example "Human cells in in vitro pharmaco-toxicology in Europe" (Rogiers et al., 1993) was one of the first major publications in this area. This publication covers methodologies based on human cells and tissues in pharmaco-toxicology (skin models at that time were just being developed, but are now well-established constituents of validated tests), however, the aspect of acquiring human material was not fully addressed. The summary of the meeting held in Stirling in the same year also addressed similar issues (Fentem, 1994). An ECVAM report (Anderson et al., 1998) was the first publication which focused on the acquisition, storage and distribution of human material for research. This report was followed by a further ECVAM report (Anderson et al., 2001), based on discussions of an expert group in 2001, following the establishment of the first specialised human tissue bank for research purposes. Several key elements from the report (e.g. anonymisation of samples, traceability) were adopted by the EU in its draft Directive covering human cells and tissues for tissue engineering (DG Enterprise, 1994). During the second ECVAM workshop the establishment of a European Network of Research Tissue Banks was proposed. It was suggested that such a network would enable the effective distribution of human material within and between Member States, to promote high safety and ethical standards.

Existing and proposed EU regulations on human cells and tissues are focused on clinical applications and do not cover the use of human material for research and regulatory purposes. It should, however, be noted that the process of legislation covering tissue banking, tissue engineering and transplantation has not yet been fully-regulated by the European Directives. Moreover, the legislative proposals do not fully cover new developments in biotechnology and medicine. Thus, only blood and blood-derived products are covered in sufficient detail, and the corresponding directives have been adopted during the last four years (Directive 2000/70/EC, 2000; Directive 2001/83/EC, 2001; Directive 2002/98/EC, 2002). Since several new toxicity assays are based on the use of human blood (e.g. pyrogenicity and immunotoxicity tests) these Directives are also relevant for regulatory toxicity testing.

In March 2004, the European Parliament adopted a Directive which regulates the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells intended for human applications (Directive 2004/23/EC, 2004). This Directive does not cover directly the problems related to the use of human cells and tissues for research and toxicity testing, although the part regulating the use of human material for medical products with regards to its donation, procurement and storage is also applicable to research and regulatory toxicity testing.

The most relevant set of recommendations applicable to the use of human derived material for research purposes was published in the interim statement of the UK Department of Health Clinical Ethics and Human Tissue Branch (Department of Health Clinical Ethics and Human Tissue Branch, 2003).

These recommendations, together with the elements of published Directives should be used to formulate a separate new Directive focused on the use of human material for research and testing purposes. It seems to be a very important issue from the European perspective when US initiatives in this field are also taken into account (National Bioethics Advisory Commission, 1999).

2. Objectives

The Executive Board of the European Society of Toxicology in Vitro (ESTIV) and ENRTB (European Network of Human Research Tissue Banks) have decided to conduct a survey to ascertain the extent to which human cells and tissues are used by its members, how they are obtained, what local regulations are in force and how the material is used, and the advantages and disadvantages experienced by members in using such material, as opposed to using cell lines and primary animal cells.

3. Methods

The survey has been conducted by the use of questionnaires. A similar method has been used by the authors of the report on human tissue-engineered products, which was recently published (DG Enterprise, 2004). The same questionnaire (Fig. 1) was circulated among the members of the ESTIV and ENRTB. The survey included questions that were aimed at determination of the end-user background, needs and knowledge concerning the use of human cells and tissues for research purposes. The obtained results were compared with those arising from a similar survey performed in 2000 (40 responders, results unpublished).

4. Results and discussion

The total number of valid responses was 46 (response rate 23%), which was similar to the number of responses in the survey on human tissue-engineered products. The responders represent almost all European countries. The

- Country
- Affiliation
- Main research areas
- Routine use of human material
- Type of tissues or organs used (ie. skin, liver etc)
- Culture systems used in the studies
- Main sources of the human material
- Type of information supplied with human biological material
- Country specific legislation for use of the human tissue in research (sources)
- Obtaining ethical or formal approval to human use cell, tissues or organs from living donor
- Obtaining ethical or formal approval to human use cell, tissues or organs from cadaveric donor
- Information about donor cells/organs use, research goals and results
- Main problems associated with the use of human tissues and/or organs
- Comments about the use of human material in research now and in the future.

Fig. 1. Key topics of the ESTIV questionnaire on the acquisition and use of primary human cells and tissue in toxicology.

highest number of responses was from the UK (12 responses). This is consistent with the fact that the UK has relatively well-established legislation covering this aspect. The level of response from researchers from other countries was an average of 4 per country. Twenty eight responders were from universities and research institutes. Thirteen responses were obtained from commercial laboratories, 2 responses from government institutions, and 3 from health services. Seventy eight percent of the responders used human cells and tissues in their laboratories on a daily basis.

4.1. Demand

The most important use of human cells by commercial laboratories and universities is associated with general toxicity, topical toxicity and metabolic studies (Fig. 2), implying that methods based on human-derived material are more likely to be performed for those specific areas than for other toxicity testing methods. Skin and liver were the most demanded types of human materials. This information is consistent with the high level of interest in metabolism and topical toxicity studies. A comparison of the results obtained in 2000 and 2004 shows that in the earlier year there was a large interest in stem cell technology. However, in 2004 researchers are interested in the development of inhalation toxicity models, resulting in a decline in the application of stem cells from 41% in 2000 to 8% in 2004 (Figs. 3 and 4). The

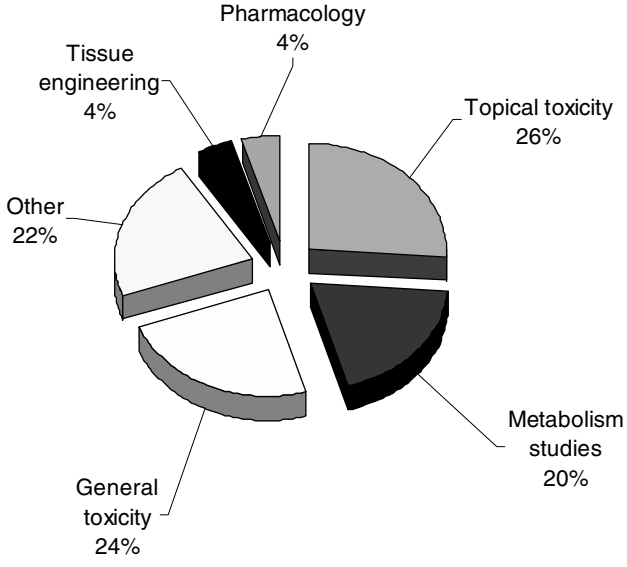

Fig. 2. The use of human-derived material in research (2004).

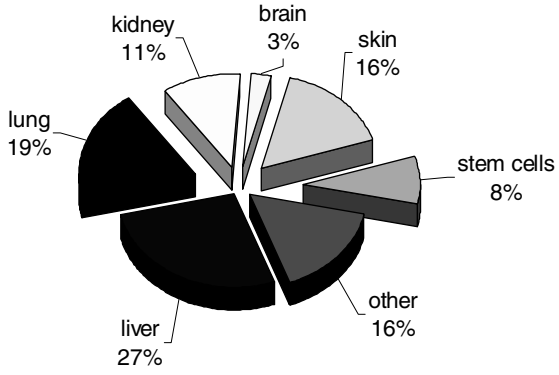

Fig. 3. Tissue types used by responders (survey 2004).

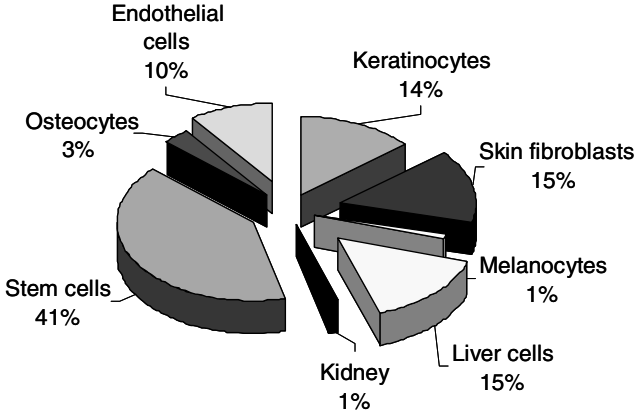

Fig. 4. Tissue types used by responders (survey 2000).

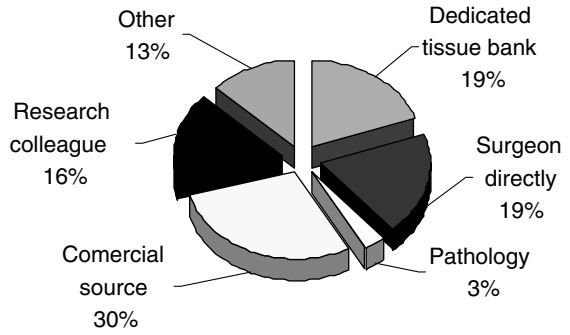

Fig. 5. Sources of human material for research (survey 2004).

initial high interest in stem cells coincides with the development of the methodologies for using them.

4.2. Source

Human cells and tissues for research are primarily derived from commercial sources (30% of responders to the questionnaire), and, secondly, from dedicated tissue banks and direct arrangements with surgeons have 19% (Fig. 5). The results of the survey in 2004 indicate that more material was obtained from commercial sources than in 2000 (20% in 2000 vs. 30% in 2004). The importance of private arrangements with surgeons in the process of material acquisition decreased (30% in 2000 vs. 19% in 2004). Researchers from commercial laboratories indicated that donor consent is a prerequisite for obtaining human cell material for research. Scientists at universities and research institutes mostly used already processed (primary cultures and tissue slices) material.

4.3. Problems

A local ethical committee was the most common way in which experiments with human cells and tissues were assessed. It is striking that almost 13% of responders were not aware of any regulations concerning the use of human material in research, which increases the probability that a large proportion might be performed without suitable safety and ethical control.

Lastly, responders noted that unclear legislation (17%), technical problems (26%) and lack of information on availability (19%) were indicated as their main concern with costs being not a major issue 17% (Fig. 6).

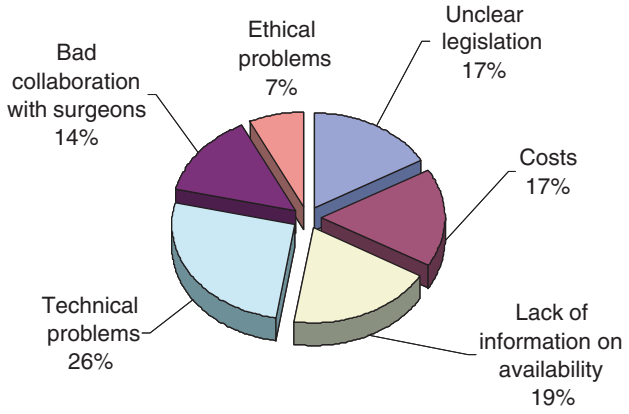

Fig. 6. The main problems associated with the use of human cells and tissues (survey 2004).

5. Conclusions

The number of human based systems in research increases, creating demand for human tissue suppliers.

There is an urgent need for the European Directive regulating the issue of human cells and tissues used for research and regulatory testing procedures to replace the existing patchwork of different national regulations with uniform rules across the European Union.

There is a need for the uniform system of information on and distribution of human material ensuring high ethical and technical quality of research conducted.

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