SUMMARY

CONTRIBUTION TO SAFETY ASSURANCE IN THE CRYOPRESERVATION OF CELLS AND TISSUES FOR CLINICAL TRANSPLANTATION

Aim of the study: The aim of the study was to analyse and to meet in practice current requirements on the safety and quality assurance in collection, processing and cryopreservation of cells and tissues. The new high requirements were set by recent changes in the national legislation of the Czech Republic (Act No. 285/2002) and especially by coming into force of the new legislation of the European Union (Directive 2004/23/EC). It is required, now that a system of safety and quality assurance known from pharmaceutical industry be established in the tissue banks and that the tissue banks be licensed or accredited by a national authority. The study was limited to safety assurance in manufacturing cryopreserved, ie viable cell and tissues grafts that cannot be terminaly sterilized.

Methods: The cryopreservation methodology used in the Tissue Bank of the University Hospital Hradec Králové was elaborated by the author and his co-workers in the 80s and 90s and regularly upgraded. The controlled-rate freezing in presence of a mixture of penetrating and non-penetrating cryoprotectants is followed by storage at liquid nitrogen temperatures or in mechanical freezers operating at temperature of –85°C. The Transplantation Act (Act No. 285/2002) regulates cell, tissue and organ transplantations and defines the duties of tissue banks. The safety aspects covered by this act are oriented only to defining the contraindications of the cell, tissue and organ donation and assuring the traceability. For this reason the issue of the possibility of infection transmission by transplanted tissue was divided into 2 major groups of tasks. The first one deals with the donor suitability including the serological testing of the donor and long-term archivation of the donor serum as well as with decontamination of the collected tissue. There have been already many publications dealing with this issue and criteria of the suitability of the donor were settled by the Decree of the Ministry of Health of the Czech Republic No 437/2002 Sb.The references dealing with the second issue, the safety asurance in tissue processing preservation and storage, are sparse. As there was not any specific national regulation of these issues the author used the principles of Good Manufacturing Practice of sterile drugs published by the State Institute of Drug Control in Prague in 1998 to elaborate specifications of cell and tissue grafts, to establish or change existing standard operation procedures for processing and cryopreservation,storage and releasing the grafts for clinical application as well as to select appropriate control methods. A special attention was

paid to elaboration special rules for storage at liquid nitrogen temperatures as the cases of cross contamination by hepatitis B virus were described by Tedder in storage of red blood cells in the liquid phase of nitrogen in 1995.

Results: Examples of specifications of collected tissues and manufactured cryopreserved cell and solid tissue grafts are included in the appendix of this thesis. Examples of standard operating procedures for donation, processing and preservation of cells and tissues collected both in living and deceased donors are included as well. The appendix includes also template forms of labels designed by author and used for traceability assurance. These labels are used exclusively for labeling grafts released for clinical application that are stored separately from grafts that did not undergo the output control. The labels contain a unique graft identification number consisting of the code of the producer, the type of tissue preserved, the tissue split number, the donor identification number and the year of manufacturing.

The system of prevention of secondary contamination is based on using clean-room technology for processing of viable cells and tissues before cryopreservation. Processing of cell concentrates or solid tissue samples is performed in the environment of the grade A with the backgroud B, regularly validated by an authorized company.The system of cross contamination prevention during long-term storage of cells is based on meticulous evaluation of the results of serological testing of the donor and/or patient and decision on the optimal storage conditions according to the results of this evaluation. The cell or tissue samples originating from donors or patients free of any markers of active infection can be stored in the liquid phase of liquid nitrogen, the samples originating from donors or patients with markers of active severe infection, such as hepatitis, are stored in the mechanical freezers exclusively. The rest of samples is stored in the vapour phase of liquid nitrogen. The analysis of stability of physical parameters during storage confirmed that temperature stability is achieved best in storage in the liquid phase of the liquid nitrogen. With regard to the possibility of cross contamination prevention described above this way of long-term storage can be used in more than 50% of stored concentrates in the investigated group of persons. In solid tissue grafts with regular findings of bacterial contamination, such as skin, storage in mechanical freezer is preferred.

Discussion and conclusions: The Transplantation Act that was approved by the Parliament and Senate of the Czech Republic after a long-time lasting disscussions in 2002 implements into the national legislation high standards of protection of the autonomy of the cell, tissue or organ donor set by the The European Convention on Human Rights and Biomedicine. The author contributed to covering both tissue and organ transplantation by a single act by his publications, active participation in committees of experts in the field of cell and tissue banking and transplantation as well as during preparation of drafts of the Act. The Act contains an important safety

requirement, traceability of the way from the donor to the host of the transplanted tissue and back together with strict keeping of anonymity of the donor. The labeling system used by author since 1998 meets this requirement. The safety aspects of cell and tissue banking in general are expected to be a subject of the future special national legal norm. Application of methods of prevention of secondary and cross contamination was possible because of acceptance of the author's proposal to design and to build a new cell and tissue establishment as a combination of cryogenic and clean-room technology. The proposal was submitted in 1998 and the facility was built in the years 2000–2002 with the financial support of the Ministry of Health of the Czech Republic and University Hospital Hradec Králové.

The aim of the study was achieved and the results were published; full texts of publications are attached. The practical result was receiving a licence of the Ministry of Health of Czech Republic to operate a multifunctional cell and tissue establishment in the University Hospital Hradec Králové.