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As many of you have known, the investigations of biological functions of genes and proteins and their relationship with the pathogenesis of human diseases requires appropriate and relevant animal models. Although mice (wild and genetically modified) offer many possibilities, other non-murine species are required in many studies. Rabbit is one of these species. Rabbit is genetically and physiologically close to humans and has been extensively used in many aspects of biological studies and medical researches. During the last decade, many progresses in rabbit biotechnology including rabbit cloning, rabbit ES cells and rabbit genome have been achieved, and a specific biotechnology of the rabbit is emerging.

The 3rd International Meeting on Rabbit Biotechnology was held in the famous ancient capital, Xi'an, China, June 4-5, 2009. This international forum was initiated by Prof. Fan J. in Tsukuba, Japan (2005) and the 2nd international symposium was successfully held in Jouy en Josas, France (2007) by Prof. Houdebine L.M. The two-day meeting provided opportunities of discussing and exchanging the state-of-the art information obtained from academic and industrial scientists in the world.

CURRENT STATUS OF RABBIT EMBRYONIC STEM CELLS: WHERE WE ARE AND WHERE WE GO?

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Although embryonic stem (ES) cell lines derived from mice and primates are now used extensively, the development of such lines from other mammals has been extremely difficult. However, stem cell research has been accelerated greatly by Yamanaka's derivation of induced pluripotent stem cells. It has become clear that some gene and chemical treatments can induce an undifferentiated status in several types of cells, and two groups have now succeeded in deriving germline-competent rat ES cells using chemical treatments. Rabbit ES cell studies are also progressing towards direct applications, and our analysis here is based on several groups active in the field. It appears that research on rabbit ES cells should aim at two goals. One is gene targeting using homologous recombination as a model system for elucidating gene functions. This would complement the current extensive knowledge on mouse genomics. The other goal is to assess the safety and effectiveness of *in vitro* differentiation of rabbit ES cells for applications in human regenerative medicine. We have analyzed the various methods used for establishing rabbit ES cells and the mechanisms by which they can be maintained in an undifferentiated state. When we tried to establish rabbit ES cell lines from blastocysts by the same methods used for the mouse, we found that the chemicals for zona pellucida removal -such as acid Tyrode's solution and pronase- caused differentiation of the inner cell mass. Moreover, we found that the feeder cell density determined the fate of the ES cells. Thus, maximum proliferation potential was obtained when the cells were cultured on a feeder cell density of one-sixth of the density at confluency. Under optimized conditions, rabbit ES cells could be passaged more than 50 times, after which they still showed undifferentiated markers. We then investigated the mechanisms for maintaining the undifferentiated nature of rabbit ES cells. We found that bFGF and Activin/Nodal signaling through Smad2/3 activation were necessary to maintain pluripotency. We further showed that, despite the expression of STAT3, LIF was dispensable for maintenance of an undifferentiated status. These findings suggest that the molecular mechanisms underlying rabbit ES cell self-renewal and pluripotency are similar to those of primate ES cells.

RABBIT CLONING: CURRENT STATUS AND FUTURE PERSPECTIVES

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In the last decade, rapid progress has been made to improve the somatic cell nuclear transfer (SCNT) technology which makes people to get animals from single cells. It has been demonstrated that in bovines, pigs and ferret, the cultured cells could be genetically modified and used as nuclear donors to produce human disease model animals with specific genetic mutant traits. Theoretically, it should then also be feasible to produce gene modified rabbits for special use. The development of SCNT technology in rabbits was slowly. Only after the female rabbits were successfully cloned from freshly isolated cumulus cells using modified oocyte activation and embryo transfer protocols, new progress were got gradually in the sequenced studies and the transgenic cloned rabbit were reported recently by different protocols. There are many factors effecting on the development of the cloned embryos from SCNT, such as activation protocols, donor cells prepare, cytoplasm status, embryo activation, and embryo transfer. Here we reviewed the progress and status of the cloned rabbit with cumulus cells, fibroblast cells, Mesenhcymal stem cells and ES like cells; the cytoplasm condition and different enucleation methods used in successfully cloning rabbits; the mechanism of activation in reconstructed embryos and the suitable activation ways in rabbits; and the special details of embryo culture and embryo transfer of cloned embryos. Finally, the future usage of this technique will be to elaborate in production animal models, reprogramming research, and genome preservation and so on. The SCNT technique in rabbit has been improved by many groups and it is possible to produce gene targeted rabbits now. More gene targeted rabbits should be made out in the recent future years for research use.

TRANSGENIC RABBITS PRODUCED BY LENTIVIRUS TECHNOLOGY

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Previous works opened the door for the use of replication defective lentiviral vectors for transgenic applications. Since then numerous transgenic laboratory and livestock animal was created by HIV based lentiviral vectors and various tissue specific cellular and viral promoters have been examined to direct transgene expression. To establish lentiviral transgenesis method at the laboratory of Agricultural Biotechnology Center a HIV-1 based lentiviral vector was used to create transgenic BALB/c mice by perivitelline injection. The GFP expression was driven by the human EF1a promoter. Up to the sixth generation only one newborn showed eGFP inactivation. The established GFP+ BALB/c mouse strain is expected to be extremely useful in various immunological experiments. Lentiviral vectors derived SIV have now been generated in several laboratories. Characterization of these vectors showed that they are similar to HIV derived vectors with respect to the insertion of transgenes in non-proliferating cells. We have used a SIV based lentiviral vector with GFP indicator gene placed under the CAG promoter to create transgenic rabbits. GFP expressing transgenic rabbits were created with 20% efficiency. Transgene expression showed mosaic pattern, but included derivatives of all three primary germ layers. Species specific differences in the efficiency of lentiviral transgenesis will be highlighted.

TRANSGENIC RABBITS FOR TRANSLATIONAL RESEARCH (TRTR)

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Translational research also called “the bench-to-beside” research refers to those studies that bring the achievements derived form the basic sciences directly to the clinical applications including new drugs, devices, and diagnostic tools. It goes without saying that any experiments aiming at this strategy is critical for medical sciences. Until now, the mouse has been becoming the major experimental tool for medical research because it is small and easily-use and many transgenic and knock-out mice are available. It is also quick for young scientists and students to get results and publish using mice. However, in many fields e.g. cardiovascular and lipid metabolism research, alternative animal models are required for translational research since suitable mice are not available or even impossible due to the differences between human and murine. Mice are different from human in terms of their lipid metabolism system and cardiovascular physiology therefore their use in translational medicine is quite

limited. We have been using transgenic rabbits for the study of hypercholesterolemia and atherosclerosis. Now, we are investigating many genes as therapeutic targets for the treatment of hypercholesterolemia and atherosclerosis. In this symposium, I will introduce the application of transgenic rabbits for the development of diagnostic and therapeutic strategies.

β-AMYLOID 1-42 PEPTIDE IMMUNIZATION IN A RABBIT MODEL OF ALZHEIMER'S DISEASE

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Immunotherapeutic strategies aimed at reducing or eliminating β-amyloid (Ab) pathology have been successfully used in transgenic mouse models of Alzheimer's disease (AD) and represent a dramatic departure from existing experimental approaches. Unfortunately, when applied to humans with the disease, a small subset of treated patients showed encephalopathy associated with damaging inflammatory responses to the Ab vaccine. A promising and understudied animal model of AD is the cholesterol-fed rabbit. The amino acid sequence of Ab that forms extracellular Ab plaques and intracellular soluble Ab is 97% identical in rabbits and humans. Cholesterol-fed rabbits develop a number of pathological indices of AD that are accelerated when a trace amount of copper is added to the drinking water. In our first experiment, a total of 18 young male New Zealand white rabbits were tested, with 14 rabbits fed 2% cholesterol added to their normal diet and 0.12 mg/liter copper added to their distilled drinking water for 10 wk ("AD model rabbits"). Four rabbits were fed a normal diet of rabbit chow and distilled water ("control rabbits"). Ten AD model rabbits and 4 control rabbits received the immunotherapy with was β-amyloid 1-42 peptide (Ab1-42) conjugated to keyhole limpet hemocyanin (KLH) in PBS. 6 wk (n=5/group of AD model rabbits; n=4/group control rabbits) or 8 wk (n=5/group of AD model rabbits) after initiation of the diet, 0.5 ml of Ab1-42-KLH preparation was mixed with 0.22 ml of Freund's complete adjuvant and injected subcutaneously. Rabbits were boosted every two weeks for a total of five immunizations with Ab1-42-KLH in Freund's incomplete adjuvant. Blood was drawn before the first inoculation and before the 3rd, 4th, and 5th immunizations. Serum antibody titers were measured following each blood sampling using an Ab1-42-specific ELISA. Results confirmed that cholesterol-fed rabbits treated with Ab immunotherapy generate high titer anti-Ab responses similar to those seen in control rabbits. Brain Ab was reduced by 50 to 80 percent in inoculated rabbits. In our second experiment, in addition to inoculation we tested the rabbits on a task severely impaired in AD. Although brain Ab was reduced significantly by inoculation, cognitive performance was not improved.

WHHHL RABBITS, HISTORY AND APPLICATIONS

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The Watanabe heritable hyperlipidemic (WHHL) rabbit is an animal model for hypercholesterolemia due to a deficiency of low-density lipoprotein (LDL) receptors. In 1973, a male mutant Japanese white rabbit showing hyperlipidemia was found by Watanabe (1927-2008). After 6 years of breeding, Watanabe developed a mutant strain and designated as the Watanabe heritable hyperlipidemic (WHHL) rabbit. Since the lipoprotein metabolism of rabbits resembles humans, WHHL rabbits were used in studies of lipoprotein metabolism and one of the users of WHHL rabbits won the Nobel Prize in 1985. WHHL rabbits also contributed to elucidate the mechanism of initiation and progression of atherosclerosis. Furthermore, WHHL rabbits were used to develop compounds for hypercholesterolemia and atherosclerosis. One of the compounds is statin, which is taken by more than 20 million patients in the world. WHHL rabbits are useful for translational researches about hypercholesterolemia and atherosclerosis. In the original WHHL rabbits, atherosclerosis is developed in the aorta. However, the incidence of coronary atherosclerosis was low. To develop coronary atherosclerosis, selective breeding was carried out (1980-1993). By the selective breeding, the coronary plaques enlarged and the incidence was increased. However, incidence of myocardial infarction was still low. To develop myocardial infarction, the second selective breeding was carried out (1993-2000) and the myocardial infarction-prone WHHL (WHHLMI) rabbit was developed in 2000. In this strain, the cumulative incidence of myocardial infarction was more than 90% at the age of 30 mo and the coronary plaques showed the features of vulnerable plaques in humans. WHHLMI rabbits have been used in studies to develop devices and/or compounds for imaging vulnerable plaques *in vivo* and compounds for vulnerable plaques. However, development of acute coronary syndromes is unusual in the WHHLMI rabbits. In the near future, we would like to develop a rabbit model for acute coronary syndromes by development of transgenic WHHLMI rabbits and/or administration of compounds. WHHLMI rabbits will be contributing to studies of atherosclerosis and the related diseases.

TECHNOLOGY SUITABLE FOR RABBIT SEMEN CONSERVATION

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For short-term preservation, rabbit semen can be stored at a low temperature for several days. It is reported that rabbit semen was stored for 2-3 d at 4-5˚C in a refrigerator. Furthermore, it has been previously reported that rabbit sperm can be effectively stored in a gelatin-supplemented extender at 15˚C for up to 5 d and retain sufficient fertility. For long-term preservation, rabbit semen needs to be frozen and stored in liquid nitrogen, similar to the technique for other laboratory and domestic animals. Indeed, cryopreservation of semen has recently become an important technique for the conservation of laboratory animals as bioresources, especially for mutant and genetically modified animals. Until now, various methods for rabbit semen freezing have been reported. Glycerol, ethylene glycol (EG), dimethylsulfoxide (DMSO) and some amides have been reportedly used as cryoprotectants, with successful recovery of fertile motile sperm. However, no standard method or protocol for cryopreservation of rabbit semen has been determined. To create a rabbit resource banking system, it will be necessary to establish a standard method for cryopreservation of rabbit semen. In our facility, rabbit sperm freezing has been carried out using the egg yolk-acetamide extender. Following our protocol, motility of post-thawed sperm is 34.7±9.5%. When using 20×10^6 motile sperm post-thawing for artificial insemination (AI), we have achieved a pregnancy rate of 60-80% with a mean litter size of 4-5 kits. Cryopreservation of semen can be useful for preservation of a rabbit strain because of its low cost and long-term stability. In this meeting, we would like to introduce our protocol for freezing rabbit semen by focusing on some important aspects of the method that increase efficiency after freezing and thawing.

CURRENT STATUS OF RABBIT GENOME AND GENOMICS

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Research on the rabbit genome is still limited but the growing number of molecular tools and sequencing data are likely to provide highly valuable information in a very near future. Our aim will be to summarize available tools and data, to give an overview of the current genome wide programs and to present results on the analysis of a large genomic segment spanning the major histocompatibility complex (MHC) locus. A comparative map between rabbit and man has been drawn and refined by a cytogenetic map comprising more than 350 genes. A microsatellite-based linkage map has been anchored onto the cytogenetic map. Collections of genomic DNA fragments cloned into bacterial artificial chromosomes (BACs) have been constructed and partial genome wide expression arrays are commercially available. In the frame of the Mammalian Genome Project launched by the Broad Institute, a two-fold coverage of the rabbit genome sequence is publicly available. The scientific community combined efforts to ask for a deeper coverage of the rabbit genome and a seven-fold coverage of the genomic sequence will be released soon. End sequencing of 384 cytogenetically mapped BAC clones have been included in this project in order to help contig anchoring onto the maps. A new project aiming at identifying single nucleotide polymorphisms (SNPs) by sequencing several rabbit breeds has started. Sequencing of the MHC locus is in progress and analysis on the segments containing class I and II genes provide highly interesting data on the rabbit-specific organization of this well conserved locus in Mammals. The development of genomic tools dedicated to the rabbit will renew research on this species and should on the one hand enhance the use of the rabbit as a relevant alternative to mouse and on the other hand include the rabbit in functional comparative mapping studies in Mammals.

TRANSGENIC RABBITS EXPRESSING HUMAN C-REACTIVE PROTEIN

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Increased levels of plasma C-reactive protein (CRP) predict cardiovascular events but whether CRP itself is a causal factor in the pathogenesis of atherosclerosis has been a subject of debate. To study the physiological functions of CRP and its relationship with atherosclerosis, we created two transgenic (Tg) rabbits expressing human CRP (hCRP) gene under the control of liver-specific elements from the human

apolipoprotein E gene with 4 copies of the chicken beta-globin insulator. Human transgenic CRP was specifically expressed in the liver of Tg rabbits as confirmed by Northern blots and immunohistochemical staining. Plasma levels of hCRP were 0.8 mg/L and 50 mg/L in two Tg founder rabbits. Western blot analysis revealed that plasma hCRP of Tg rabbits existed as a pentamer (pentraxin) and became a monomer on SDS-PAGE under reducing conditions. Furthermore, we isolated hCRP from Tg rabbit plasma and showed that hCRP can activate the rabbit complement in the presence of enzymatically modified low density lipoproteins. Taken together, these results showed that Tg rabbits expressed functional hCRP. We are now examining whether these Tg rabbits are susceptible to atherosclerosis.

ON THE ROLE OF LIF AND BFGF IN RABBIT ESC LINE DERIVATION, CHARACTERIZATION OF PLURIPOTENCY MAKER EXPRESSION IN RABBIT ESC LINES

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Embryonic stem cells serve as source material for the production of genetically modified animals and they can be potentially applied in cell therapy and tissue engineering. Rabbit ES cells would be invaluable tool both for creating second generation transgenic models of human diseases using gene-targeted technology and testing stem cell therapies for human applications. Rabbit ES like cells exhibit flattened monolayer colonies, as reported for human ESC. The use of human ES cell lines is restricted, since destruction of developing human embryos is required for their establishment. As a consequence of this limitation and the advantages of using rabbit ESC, getting deeper insight into generation of stable rabbit ES cell lines and characterization of pluripotency markers is highly important. It has been shown that LIF is dispensable for undifferentiated status maintenance of rabbit ES cells whereas an earlier report has demonstrated improved rabbit ES-like establishment in the presence of LIF.

Based on our previous results, we investigate the role of LIF and bFGF in rabbit ESC maintenance which might help to establish stable rabbit germ line competent ESC lines. In addition we characterize the expression patterns of the pluripotency markers (Nanog, Oct4, cd×2 and LIFR). We described recently, that the miR-290-295 cluster can retain mouse ES cells in their pluripotent state, primarily by regulating cell cycle at multiple points and at multiple targets. The aim of our forthcoming experiments is to determine the homologues rabbit miRNAs associated with pluripotency of ES cells. We are also interested to explore the stem cell specific miRNA expression patterns in different rabbit stem cells and embryonic tissues.

LEVELS AND PATTERNS OF GENETIC DIVERSITY AMONG AND WITHIN RABBIT DOMESTIC BREEDS: INSIGHTS INTO THE DEMESTICATION PROCESS AND BREED FORMATION

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Although widely used as a model species very little is known about the impact of domestication in the rabbit genome. In order to help building up this model system and shed light on the rabbit domestication process and breed development, we gathered sequence information on 9 autosomal and 7 X-linked loci, and we genotyped 25 allozymes and 9 microsatellites in a panel of 13 breeds. The results obtained were compared with data from wild rabbit populations from France and Iberian Peninsula. We report five main findings. First, in agreement with historical records our results indicate that domesticated rabbits most likely originated from a single domestication event in France. Second, consistent with a domestication bottleneck, domestic rabbits show a significant decrease of nucleotide diversity (between 20 and 50%) when compared with French wild rabbit populations. Nevertheless, levels of genetic diversity (approximately 0.2%) are at least two times higher than in humans. Third, unlike most mammalian species, the comparison of the amount of nucleotide diversity captured during domestication for autosomal and X-linked loci suggest similar contributions of males and females to the domestic rabbit gene pool. Fourth, rabbit breeds are well differentiated and the power to correctly assign individuals to each breed using clustering algorithms was high, with an average of 96%. Finally, breeds with more than one breeder origin were divided into subgroups, suggesting strong intrabreed stratification. This situation may result from extreme bottlenecks associated with the establishment of breeding stocks among breeders and in some cases results in higher genetic differentiation within breed than observed between distinct breeds. In light of these results we discuss the potential of the rabbit as a model to understand the process of domestication.

ISOLATION AND IDENTIFICATION OF BORDETELLA BRONCHISEPTICA FROM NEW ZEALAND WHITE RABBITS

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To investigate the incidence of experimental rabbits infected with Bordetella bronchiseptica, and then research some biological characteristics of these bacteria. Bordetella bronchiseptica were isolated and identified from nasal cavities of 63 rabbits with rhinitis in four experimental rabbits fields around Nanjing, and some characteristics of the isolates including toxicity to ICR mice, pathogenicity to infant rabbits, the growth and culture characterization, antibiotics sensitivity .etc were researched. 19 Bordetella bronchiseptica strains were isolated, and the rate was 30.16%. Among these bacteria, 15, 1, 3 were characterized as high, low and no pathogenicity to ICR mice, respectively. Antibiotics sensitivity tests of 5 high pathogenic bacteria strains showed these bacteria had highly susceptible to Neomycin, Chloramphenicol, Erythrocin and Tobramycin, but they were resistant to Furazolidone, Lincomycin and Streptomycin, and the sensitivity to other antibiotics was different. Bordetella bronchiseptica was one of the important bacteria causing rabbit rhinitis. The positive rate was high, and related with seasons. These isolates were high virulence to ICR mice and pathogenicity to infant rabbits. Their characterization of culture, morphology and antibiotics sensitivity were specific.

VAGINAL IRRITATION TEST FOR RUBBER CONDOM IN THE JAPANESE WHITE RABBIT MODEL

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The rabbit is specified as an appropriate animal for evaluating potential irritation by the current ISO standards, the average scores difference of test article and control can be determined. In this study, to evaluate the biocompatibility of medical devices, we investigated the potential possibility of vaginal irritation for rubber condom. The rabbit was carefully restrained by an assistant and the back legs were secured to expose the perineum. The vagina of rabbit was exposed. The sample was gently inserted into the vagina. The sample was retained in vagina for half hour. The Negative Control material was operated as the same way. The above procedure was repeated at 24 h intervals every day for five consecutive d. The signs of rabbit vaginal mucous membrane were noted and recorded. The rabbits were killed by over dose pentobarbitalum natrium. The entire vagina was dissected, opened longitudinally and examined for signs of irritation. The cervical, central and caudal portions of each vagina were fixed in 10% formalin, paraffin embedded, sliced and HE stained. The histological changes were observed and scored. The scores for microscopic evaluation for all the rabbits in the test group are added and divided by the number of observations to obtain a test group average score. The control group average score was subtracted the test group average score to obtain the irritation index. Macroscopic evaluation: no erythoma and oedema were observed on rabbit's vagina and perineum. No liquid overflowed from test and control group rabbit's vagina, and no erythema and oedema was observed on the vagina mucous membrane after the vagina was dissected. The mean score of the test article was calculated to be 2.2 while the control article was 1.1. The difference of the mean score of the test and control rabbits was 1.1. The irritation index was calculated to be 1.1. The index was more than 1.0 and less than 4.0. The degree of response was slight. The vagina irritation test result of test sample was met the requirements of ISO standard.

PYROGEN TEST FOR HYDROXYAPATITE IN THE JAPANESE WHITE RABBIT MODEL

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The rabbit is specified as an appropriate animal for evaluating potential febrile response by the Pharmacopoeia of china and USA. To evaluate the febrile response of the materials, we investigated the potential possibility of febrile response of the test article. Material preparation: hydroxyapatite, its form is about 10×20 mm rectangle, and its thickness is about 3 mm. The sample is non-aseptic package and was stored in room temperature, sealed. The test article was prepared by the test article extract liquid. The test article extract liquid was prepared under the conditions with 0.2 g/mL, 121ºC, 1h. The extract medium is normal saline. The sample quantity is 27.63 g. The equipments which were touched to the extracted liquid were eliminated pyrogen under the condition of 250ºC, half hour. Assay method: The 3 rabbits were

taken to measure normal temperature. Within 15 min after the normal temperature was measured, the sample extract liquid was warm-up to 38ºC and injected into ear edge vein of the rabbits, the dosage was 10mL/kg body weight. The temperatures rise of each test rabbits was less than 0.6ºC, and the sum of temperature rise in three test rabbits was less than 1.4ºC. Under the condition of this study, the febrile response of the test rabbits were not observed after injected the sample extracted liquid. The test results are met ISO10993.11-2006, the Pharmacopoeia of USA (28th edition), and Pharmacopoeia of China (2005).

ISOLATED AND CULTURED MSC OF NEW BORN RABBIT AS A MODEL TO STUDY THE INTERACTION BETWEEN CAPTOPRIL AND STEM CELLS

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Captopril is an effective antihypertensive drug. Recent studies revealed its promoting effects in stem cell transplantation treatment. However, some researches implied its opposite effects in certain cases. Because of the pluripotent differentiation ability and the convenience to amplify, mesenchymal stem cell (MSC) is commonly used in tissue engineering and pharmacological researching. In our study, we added captopril in different doses into isolated and cultured new born rabbit MSC. The cell's viability and the morphological characteristics were observed for exploring the dose-response relationship between captopril and MSC. The new born laboratory Japanese white rabbits were purchased from Medical Experimental Animal Center, Sichuan Province. Bone marrow MSC was isolated in sterile conditions. After passaged for 3 times, the MSC was trypsinized and re-suspended by α-MEM medium. 5×10^4/100 ul cells were injected into each hole of a 96-hole plate. 20 ug/mL to 2400 ug/mL captopril was added into the holes in triple. After 48 h cultured, the cell viability was detected by MTT method and the morphological characteristics were observed. MSC isolated from new born rabbit bone marrow grew well. At the end-point, more cells suspended morphologically in high dose captopril group, and each group presented obviously different in color 4 h after MTT addition. The supernatant was sucked up at uncentrifuged and 5 min 500 *g* centrifuged conditions and dimethyl sulfoxide was added in respectively. Then, the absorbance values were compared at 570 nm. The results showed that high dose captopril can promote the proliferation of MSC, instead of cell attachment. Dose 100-300 ug/mL may be able to guarantee the normal physiological function and proliferation of MSC. The effect of captopril on MSC is still in debates. In our research, the dose of captopril may be one of the answers to the divergences. Though dose 100-300 ug/mL seems to be the better choice in culture condition, it's too high to be got in practical situation. We have reasons to believe that as long as keeping in allowable scope, the higher the captopril dose is, the better the promoting effects of captopril on the MSC are. Due to the convenience, rabbit MSC shares the same value in exploring and solving similar problems.

RABBIT PLASMA AS A SUBSTITUTE OF HUMAN PLASMA FOR PARTIAL THROMBOPLASTIN TIME AND PROTHROMBIN TIME EVALUATION OF THE MEDICAL DEVICESCONTACTING WITH BLOOD

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For the security of medical devices contacting with blood, the International Organization for Standardization issued ISO 10993-4 standard. Though partial thromboplastin time (PTT) and prothrombin time (PT) are important evaluation indexes, the concrete methods and operation instruments are deficient in the standard. ASTM F 2382-04 draws a description of PTT test and suggests human plasma as the test reagent. Considering the shortcomings of the human plasma (inconvenience, limited supply and the possibility of the virus spread), animal plasma, especial rabbit plasma which is similar with human in many aspects, as the substitute is valuable. In our study, five kinds of materials in market were evaluated by PTT and PT with human and rabbit plasma respectively. The purpose of the study is to discuss the possibility of replacing human plasma by rabbit in coagulation test. The fresh Japanese White rabbit blood (anticoagulant by ACD) was collected and centrifuged. The rabbit plasma and human plasma from health volunteers were used for the 5 kinds of component materials contacting (37 ºC, 30 min) as suitable ratio. The clotting time of PT and PPT were recorded for each sample. The untouched plasma was set for the negative control and the percentage negative control of the sample means was calculated for the PTT criteria. The quartile interval of each group and the student t-test that between the materials with the negative control were employed for the extent of data variation and the statistically significant difference. PTT and PT varied according to the different species. The test sample acceptance

criteria are based on the percentage negative control in PTT. Although the thrombogenicity data was a little different, the interpretation was same both in rabbit and human plasma with all tested materials. The significant difference is the key criterion of PT test. One kind of materials showed statistical difference in rabbit plasma whereas two in human plasma. It suggested that the sensitivity of rabbit plasma might be less than human plasma in PT. Conclusion, the high consistent results promise rabbit plasma as a substitute of human plasma in coagulation test. But the interpretation and analysis of the small sample tests, PT test for example, should be done with caution.

CLINICAL OBSERVATION ON RADIATION INJURY COMBINED WITH RADIAL FRACTURE RABBITS

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To investigate rabbits' mutual impact of radiation-induced injury and compound fractures in order to provide a basis for clinical treatment of combined injury. Whole body of rabbits was irradiated using 8Gy 60Coγ-ray. Within 2 h after exposure to irradiation, middle of the right-side radial fracture was caused artificially. In experiment, 3 groups of rabbits, each 8, weighing 1.3~2.4 kg, were set up, i.e. of radiation injury group, simple fracture group and combined injury group. 60 d after injury, day-to-day indexes such as activity status of animals, appetite, body temperature, skin-mucous membrane bleeding, stool and blood picture of animals were observed. Fracture X-ray film was checked once a week. Autopsy examination of dead rabbits was conducted on the day, and the survival rabbits were killed for autopsy examination 62 d after being injured. In the combined injury group, rabbits with temperature above 40ºC and the number of their activity variation difference were 50 and 87.5%, that of fracture group decreased to 0 and 25% (P<0.05). Mortality rate of combined injury group was 85.7%, and from autopsy of dead animals, petechial or patchy hemorrhage was found at their stomach, intestine, lung, heart and kidney; partial intestinal or tonsil infection rates reached 50%; and all animals of fracture group survived, without focus of infection and significant bleeding. 2 d after injury, total WBC of rabbits in combined injury group increased rapidly to 141.1% that of theirs before injury, and then decreased to 5.7~15.3% 4~15 d after injury. Later, on 60th d it rose gradually to value of 111.2% that before injury, and neutral myeloid cells renewed faster than the lymphocyte. In fracture group, total number of leukocytes and lymphocytes were basically shown as ascending response, with little change in red blood cells and hemoglobin. In fracture group, X-ray film showed periosteal reaction emerged at fracture location 20~27 d after injury, and same reaction occurred in combined injury group 34 d after injury; in terms of time of fracture callus connecting, that of combined injury group was 48 d after injury, being postponed for 1 to 2 wk compared with fracture group (P<0.05); as to time of bone absorption-reconstruction and disappearance of fracture lines, that of fracture group was 41~55 d, and that of combined injury group was postponed to 62 d after injury (P<0.01); in combined injury group, time of rehabilitation and remodeling of bone marrow cavity in good condition was also delayed for 1~2 wk compared with fracture group. Change of simple radiation injury group was shown with the case between combined injury group and the fracture group. Fracture union of such combined injury was significantly delayed compared with simple fracture group, showing severe clinical performance, with increased effect of a course.

IMPACT OF BMP AND TGF-B1 EXPRESSION ON HEALING OF RABBIT'S FRACTURE COMBINED WITH RADIATION INJURY

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To explore the impact of compression external fixation on the expression of bone morphogenetic protein (BMP) and transforming growth factor-β1 (TGF-β1) in rabbit subjected to compound fracture after radiation injury. 144 adult Japanese rabbits, weighing 1.8~2.7 kg each, were subjected to 15 Gy 60Coγ- ray local irradiation on theirs right hind legs, and subsequent transverse fracture of tibia made artificially. Half of these rabbits were treated with compression or non-compression fixation of fractures with semi-ring trough micro-external fixer respectively. After operation, immunohistochemical staining was adopted to observe the change of BMP and TGF-β1 expression. In the compression fixation group, 1st wk after treatment, BMP expression was located in the mesenchymal cells and fibroblasts, in 3rd wk the expression was increased remarkably, in 6th wk the expression was located in the mature cartilage cells, in 12th wk positive cells were mainly osteoblasts and a few osteocytes, and in 24th wk the staining was osteocytes. While, in the control group, in 2nd to 3rd wk, only mesenchymal cells and fibroblasts showed positive expression of BMP, in 6th wk the expression was only located in fibroblast-like mesenchymal cells and some cartilage cells, in cartilage cells (in 12th wk) and partial osteoblasts and osteocytes (in 24th wk). For TGF-β1, in the compression fixation group, 1st wk after treatment, staining was mainly located in mesenchymocytes, in

3rd~6th wk, some of the cartilage cells and osteoblasts showed positive expression, and in 12th wk osteoblasts, partial osteocytes and a few cartilage cells showed strongly positive expression, and to 24th wk osteocytes became mainly positive cells. However, in the control group, in 2nd to 3rd wk TGF-β1 was mainly expressed in mesenchymocytes, in 6th wk only a few cartilage cells and hypochromatic osteoblasts were postive, just in 12th wk more cartilage cells and osteoblasts showed stronger positive, and until 24th wk a small number of osteocytes and osteoblast cells were positive. Compression external fixation for rabbits's fracture combined with irradiation injury can significantly enhance the expression level of BMP and TGF-β1.

RESEARCH PROGRESS OF SPF RABBIT CULTIVATION AND BIOLOGICAL CHARACTERISTICS IN CHINA

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SPF laboratory rabbit is an international standard laboratory animal. SPF laboratory rabbit has been widely used in medical research, biology research and product testing field in developed countries. With the development of China's life science and medical research, laboratory rabbits are gradually reaching SPF level. Wang Yinhuai carried out cultivation and research of germfree rabbits in 1984, which laid a foundation for the development of SPF rabbits. Xue Jiabin, Zhang Yabin, Zhang Kuo established breeding stocks of SPF rabbits by cesarean section and artificial feeding in 2003, 2004 and 2006, respectively, which have promoted the standardization of production and breeding of SPF rabbits in China. He Zhongpin, Fang Yuanshu, Wenjing, Li Zengqiang and Liu Ke determined hematological and biochemical parameters of SPF New Zealand rabbits in 2000, 2003, 2005, 2008 and 2009, respectively. These researches verified that hematological and biochemical parameters of SPF New Zealand rabbits were affected by sex, age and microbiological background. Zhu Jiating, Yuanjin and Xiao Yuhua carried out studies on growth development and reproductive performance in 1996, 2003 and 2004, respectively. And they recorded and analyzed some indexes of growth development and reproductive performance. Zhang Jianing and Yuan Jin determined the main organ coefficients in SPF New Zealand rabbits in 2005 and 2006, respectively. And Yuan Jin compared and analyzed the difference of the main organ coefficients in SPF and conventional rabbits. These researches showed the main organ coefficients were affected by background and rearing methods. Yun shifeng and Xu wenyu carried out study on temperature of SPF New Zealand rabbits, and compared the difference between SPF and conventional New Zealand rabbits. At present, the cultivation and research work of China's SPF rabbits have achieved remarkable success. With the development of China's life sciences and medical research, and the demand promoting in qualified rabbits, the cultivation and other researches in SPF New Zealand rabbits will develop rapidly in China.

A RABBIT ILIAC ARTERY MODEL OF STENT IMPLANTATION IN BOTH LEFT AND RIGHT

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We reproduce a rabbit model of stent implantation through two different paths (1) to find whether the left or the right carotid artery path is more convenient (2) to summarize the key technique of stents implantation in a rabbit iliac artery model of both left and right. Eighteen Japanese white rabbits weighting 3.5~4.0 kg of both male and female were subdivided into two groups. Six rabbits in group one were placed stents in the left and the right iliac artery through the left carotid artery, while twelve rabbits in group two were placed stents through the right carotid artery in both iliac arteries. All the stents were 2.5 mm diameter and 18 mm length. Angiography was performed immediately before and after implantation and before the sacrifice of animals. The diameter of the iliac arteries of the animals was estimated by the investigator through the angiography prior to stent placement. The vessel-to-stent diameter ratio was within the desired range of 1:1.1 and 1:1.2. Stent placement was successful in 5 of 6 rabbits of group one (10 stents) and in 9 of 10 rabbits of group two (18 stents). One rabbit died before stent implantation and one failed to implant stent for the stent fell off from the balloon before placement in group two. There was no statistic difference of the model between the left and the right carotid artery path ($P>0.1$), however the right carotid artery path was more convenient than the left evaluated by investigator. The stent placement in a rabbit iliac artery model can be completed well through both the left and the right carotid artery. To gain a satisfied survival of animals, it is important to choose a befitting weight rabbit, a suitable diameter arterial sheath and an adapted length and diameter stent.