《心肌缺血-再灌注损伤实验整合课程》教学大纲（实验）

（基础医学专业用）

Preface

The experimental integration curriculum for myocardial ischemia reperfusion injury is a new experimental course for students majoring in basic medicine. The basic theory of ischemia-reperfusion injury was learned in the experimental class, and the basic knowledge and theories were mastered. Further exploration and learning were carried out in the experimental class to consolidate the basic theoretical knowledge and understand the frontiers of scientific research on ischemia-reperfusion injury. It is through the experimental course that students can use the instrument correctly, preliminarily master the operation methods of common instruments, understand the common experimental methods of clinical and scientific research, and preliminarily master the design methods of animal experiment. Students are trained to be serious about scientific work, strict working methods and practical working style, and gradually be able to objectively observe, compare, analyze and comprehensively solve practical problems. The purpose of the course is to improve students' creativity and provide basic skills for future clinical practice and scientific research.

The experimental integration curriculum for myocardial ischemia reperfusion injury is studying living animals or in vitro tissues and organs. Under the guidance of teachers, create disease models and observe experimental therapeutics, collect samples of blood and heart, do histology and molecular biology analysis by using the collected samples.

The content of the experimental integration curriculum for myocardial ischemia reperfusion injury is based on BL-410 biological signal systems. Students learn the use of common clinical and laboratory instruments such as enzyme marker, PCR instrument and slicer. Through the experiment, the students should master the new technologies and new methods of computer-aided fundamental medical research.

This syllabus applies to students majored in basic medicine.

Total teaching hours of this course: 36 teaching hours, experiment: 33 teaching hours, self-directed learning: 3 teaching hours.

Assessment methods: experiment assessment (attendance, experiment reports and operations) accounts for 85% and self-directed learning accounts for 15%.

Practice 1 Create the model of myocardial ischemia-reperfusion injury in rabbit

1. Purpose and requirement

(1) To learn the method of creating the myocardial ischemia-reperfusion injury model.

(2) To learn the change of ECG in myocardial ischemia-reperfusion injury.

(3) To observe the effect of ischemia preconditioning on myocardial ischemia-reperfusion injury in rabbits.

(4) To learn how to use the biological function experiment system and ventilator.

(5) To learn how to do cardiac perfusion.

2. Experimental content

(1) Experimental animal: rabbit

(2) Experimental method

① Anesthetize and fix the rabbit.

② Operation: Tracheal intubation and common carotid artery intubation.

③ Connect ECG electrodes and ventilators.

④ Record the normal ECG, take the normal blood.

⑤ Create the myocardial ischemia-reperfusion injury model.

⑥ Observe the effects of myocardial ischemia, ischemia preconditioning and myocardial ischemia reperfusion injury on ECG in rabbit.

⑦ TTC staining.

⑧ Collect blood and heart samples.

3. Teaching hours

9

4. Teaching methods

Experimental teaching

Practice 2 Prepare paraffin specimens of myocardial tissue

1. Purpose and requirement

To learn how to prepare paraffin specimens.

2. Experimental content

(1) The myocardial tissue is dehydrated and transparent.

(2) Myocardial tissue is waxed.

(3) Myocardial tissue is embedded in paraffin.

3. Teaching hours

4

4. Teaching methods

Experimental teaching

Practice 3 Prepare paraffin section of myocardial tissue

1. Purpose and requirement

(1) To learn how to prepare paraffin section of myocardial tissue.

(2) To learn how to use a slicer.

2. Experimental content

(1) Fix specimens.

(2) Fix the blade on the holder.

(3) Adjust the section thickness of the slicer.

(4) Adjust the distance between the cutting surface of the wax block and the blade.

(5) Slice.

(6) Flatten slicing.

(7) Frame slicing.

(8) Baking slicing.

3. Teaching hours

3

4. Teaching methods

Experimental teaching

Practice 4 Hematoxylin-Eosin staining

1. Purpose and requirement

(1) To learn the principle and steps of H＆E staining.

(2) To observe the structural characteristics of myocardial tissue and differences between normal myocardial tissue and ischemia-reperfusion injured myocardial tissue.

2. Experimental contents

(1) Dewax paraffin section.

(2) Stain section with hematoxylin.

(3) Hydrochloric acid alcohol separation.

(4) Wash in tap water.

(5) Stain section with eosin.

(6) Dehydrate, transparentize, mount the section.

(7) Observe under a microscope.

3. Teaching hours

2

4. Teaching methods

Experimental teaching

Practice 5 Chromotropic acid 2R-bright green staining

1. Purpose and requirement

(1) To learn the principle and steps of chromotropic acid 2R-bright green staining.

(2) To observe the structural characteristics of myocardial tissue and differences between normal myocardial tissue and ischemia-reperfusion injured myocardial tissue.

2. Experimental contents

(1) Dewax paraffin section.

(2) Stain section with chromotropic acid 2R.

(3) Wash in glacial acetic acid.

(4) Stain section with bright green.

(5) Wash in tap water.

(6) Dehydrate, transparentize, mount the section.

(7) Observe under a microscope.

3. Teaching hours

3

4. Teaching methods

Experimental teaching

Practice 6 Detect p-p38 MAPK expression by Western blot

1. Purpose and requirement

(1) To learn how to extract protein from myocardial tissue.

(2) To learn how to determine protein concentration with the BCA assay and other analyses.

(3) To learn the principle and steps of Western blot analysis.

2. Experimental contents

(1) Extract protein from myocardial tissue.

(2) Determine protein concentration with BCA Protein Assay Kit.

(3) Load protein samples to the gel.

(4) Separate by using electrophoresis.

(5) Transfer.

(6) Block.

(7) Incubate in antibody solution.

(8) Blotting.

(9) Analyze the results.

3. Teaching hours

8

4. Teaching methods

Experimental teaching

Practice 7 Detect troponin and actate dehydrogenase in serum by ELISA

1. Purpose and requirement

(1) To learn the principle and steps of ELISA.

(2) To learn how to use enzyme marker.

2. Experimental contents

(1) Load the sample.

(2) Incubate.

(3) Cargille.

(4) Wash.

(5) Add enzyme.

(6) Incubate.

(7) Wash.

### (8) Coloration.

(9) Detect the result with enzyme marker.

3. Teaching hours

4

4. Teaching methods

Experimental teaching

Self-directed learning

1. Purpose and requirement

(1) To improve the ability of comprehensive analysis of experimental results.

(2) To improve the ability to find and solve problems in the process of scientific research through the discussion of experiment results.

(3) To learn research ideas.

(4) To understand the research design for ischemia-reperfusion injury.

2. Experimental contents

(1) Introduce the content, requirements, forms and assessment methods of the self-directed learning.

(2) Report all the experimental results in this course, analyze the possible reasons for different experimental results, and further give the suggestions or experimental design to improve this experiment group by group.

3. Teaching hours

3

4. Teaching methods

Self-directed learning

5. Self-directed learning

(1)Self-directed learning arrangement (0 teaching hours): Inform students of the teaching requirements, forms and assessment methods of self-directed learning.

(2) Self-directed learning and question-answering arrangements (0 teaching hours) : Students use the internet and the library to actively search and collect relevant reference books and literature to learn and understand relevant knowledge; Teachers provide guidance through email, QQ, wechat, and telephone..

(3) Report and discussion in class (3 teaching hours).