INTRODUCTION

With advancing technology and scientific discoveries, more drugs are added to the market that display high selectivity for specific molecular targets. The end result is controlled management of the disease without numerous side effects. In order to understand the drugs on the market and their mechanism(s) of action, a thorough understanding of cell biology, biochemistry, and physiology in normal vs pathological cells is required. Reinforcing these concepts through laboratories and lectures and applying them to a real disease in a pharmacy practice setting gives our students a broader and more thorough understanding of cell biology and its relation to disease and their careers. This lecture and accompanying pharmacy practice assignment are presented to our students to give them an understanding of the role of microtubules in basic cellular processes. The overall purpose of this paper is to enhance student learning of lecture material by use of pharmacy practice exercises. In the pharmacy practice assignment, the students are asked to apply their knowledge of microtubules in treating gout with colchicine, a drug that acts at the level of microtubules. The student objectives for this topic are as follows:

1. Describe the relationship of microtubules to other organelles within the cell.
2. Describe the function of microtubules in maintaining cell architecture.
3. Describe the mechanism of microtubule assembly within the cell.
4. Describe the function of microtubules in intracellular transport including endocytosis and exocytosis and in cellular motility.
5. Describe the role of microtubules in cellular movement including ameboid locomotion and ciliary movement.
7. Apply the concepts of microtubules to treating disease in a pharmacy practice setting.

INSTRUCTIONAL METHODS

PHBMS 355 Human Anatomy, Physiology and Pathology I is a required 4-credit course that is offered sequentially with PHBMS 356 Human Anatomy, Physiology and Pathology II. PHBMS 355 is taught in
the fall semester of the third year of a 6-year (0–6) entry level PharmD curriculum. *Human Anatomy, Physiology and Pathology I* has a laboratory component that reinforces gross anatomy and histology and also teaches the clinical appraisals of physiological functions. It is also during this laboratory time that students get a “hands on” learning experience using real tissue and equipment. The pathology component of the course was added over 4 years ago out of a need to decrease our stand-alone, 1-credit courses, and thus reduce the course load on our students. Therefore, PHBMS 357, *Introduction to Disease*, and all of its competencies and content was integrated into either PHBMS 355 or PHBMS 356. In addition, the credit lost from the 1-credit course was added to the spring semester PHBMS 356 course, turning it into a 5-credit course rather than a 4-credit course. Also, rather than teaching from 2 textbooks, the course is now taught from 3 textbooks. In addition to using multiple texts, this course is also taught by 3 different instructors who are experts in the content areas they teach. This mechanism of teaching brings variety to the course and ensures that the students are receiving the highest level of education and instruction.

Even though the integration of pathology into PHBMS 355 and 356 was done out of need, it has been found, over the years, to enhance the learning and teaching components of the course. The application of basic anatomical and physiological processes to disease states reinforces the fundamentals of anatomy and physiology and keeps the student in a highly engaged state, which results in a greater level of learning and understanding. Also, within the last year, another teaching modality was introduced into PHBMS 355 and 356 and was developed through our Center for Pharmacy Practice. The goal for the Center for Pharmacy Practice was to develop exercises that integrate, reinforce, and enhance the process of learning. All too often, students are left with the feeling, “why do I have to learn this material?” or “I’m never going to use this in real life.” The exercises help to bridge the gap between student perceptions of lecture material and the use of the material in a pharmacy practice environment. Even though the students are in the early stages of their pharmacy training (third year of a 6-year program), getting them into a pharmacy practice setting and having them apply the information learned in their courses to the practice of pharmacy continues to reinforce the value and importance of their courses to their pharmacy careers. This, in turn, enhances their learning of the lecture material through direct application of these principles.

The structure of the PHMBS 355 course, *Human Anatomy, Physiology and Pathology I*, was based upon a very simple guiding principle: start simple and continue to build on, reinforce, and apply the basic principles to teach the more complex processes. In keeping with this, PHBMS 355 begins with lectures focusing on basic cell biology including genetics (13 lectures), followed by 6 lectures devoted to teaching pathophysiology at the level of the cell and genetic diseases. Next, 5 lectures were devoted to teaching the fundamentals of cell membranes, transport, and primary tissue, followed immediately by 6 lectures on inflammation, cellular repair, disorders in growth, and benign and malignant neoplasms. Next, 8 lectures were devoted to the integumentary system and bone and muscle contraction, which then lead nicely into the study of one of the organ systems, the nervous system, to which 13 lectures were devoted. Building on the concepts learned from the cell, progressing to tissues, then to organs, and finally to whole organ systems, enhances learning at each level of instruction by showing these interrelationships. The second semester course, PHBMS 356 *Human Anatomy, Physiology and Pathology II*, continues where the first course ended, focusing on the other organ systems: immune, blood, cardiovascular, renal, pulmonary, gastrointestinal, endocrine, and reproductive.

The laboratories are designed to enhance some of the concepts learned from the lectures; thus, they are placed strategically after selected lectures. For example, following lectures on cell membranes, the students perform experiments on diffusion. During the other laboratory sessions, they also gain practical experience by learning how to use microscopes, dissect tissue, and perform diagnostic tests. Along with the laboratories, the Center for Pharmacy Practice exercise is also strategically placed within this curriculum. Following the completion of the lectures on microtubules, for example, the students are then required to complete an assignment through the Center for Pharmacy Practice. The students, unscheduled, visit the Center to complete the exercises that were developed for that semester; each semester will have a different assignment highlighting a different concept learned from their lectures. The student answers a series of questions relating to the information presented in lecture and then proceeds to fill a prescription for that drug using computer software and an automated dispensing technology that is available in licensed pharmacies. Upon completion of the exercise, students have a clearer understanding of not only their lecture material, but also its application to pharmacy practice, thus enhancing their learning experience. In this example, and following the
lectures on microtubules, the students are asked to answer questions about gout and fill a prescription for colchicine. The students have 1 month to complete the exercise, which is to be done independently within the confines of the Center for Pharmacy Practice using the resources provided by the Center. The resources provided to the students are the same ones provided to pharmacists in a real pharmacy. These include patient information leaflets, Drug Facts and Comparisons, Pharmacotherapy, Drug Information Handbook, Micromedex, USPDI Information for the Patient, and the Patient Counseling Manual, as well as their class notes.

Besides learning from a traditional lecture format, using laboratory sessions, and applying their knowledge to a pharmacy practice setting, other methods are employed in this course to enhance student learning. Each instructor and student has access to a CD-ROM (provided along with the Saladin text1) that contains appealing visual aids, animations, quiz questions, and helpful study guides. The instructors can use the CD-ROMs themselves to highlight key concepts in lectures that are better demonstrated using animations and pictures keeping the quote, “a picture speaks a thousand words,” in mind. The students, on the other hand, can use the CD-ROMs to practice and study the material in the privacy of their homes. In addition, the students are given another interactive CD-ROM entitled the Essential Study Partner (also provided along with the Saladin text1) that reinforces key concepts learned during lecture in a question/answer and sample-testing format. In addition, the instructors use colorful handouts (provided along with the Saladin text1) and also give quizzes in the form of extra credit. Giving the quizzes in this form is extremely effective for the following reasons: (1) it allows the instructor to get instantaneous feedback on student learning, (2) it encourages consistent and steady student study habits, (3) it maintains high student attendance in class, and (4) it prepares the student for the test in a less stressful manner.

BACKGROUND
The Relationship of Microtubules to other Organelles Within the Cell

Normal cellular function relies on many factors including those proteins involved in maintaining homeostatic conditions. Even though numerous proteins and processes are involved, including those contributed by the other organelles, like the endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, peroxisomes, ribosomes, and nucleus, the ability of a cell to maintain its proper nutrient source and also maintain cell viability is controlled, in part, by microtubules. Microtubules also function in maintaining cell morphology and in cell division, cellular movement, and cellular trafficking called endocytosis and exocytosis.1

The Function of Microtubules in Maintaining Cell Architecture

The cytoskeleton consists of protein filaments (long, globular chains of tubulin heterodimers) and cylinders or “tubes” called microtubules that determine the shape of the cell, aid in structural support, move substances into and out of the cell, and contribute to movements of the cell as a whole. It can form a very dense supportive scaffold in the cytoplasm and is arranged in such a way that there is continuity from the extracellular fluid into the cytoplasm.1 The cytoskeleton is composed of microfilaments, intermediate filaments, and microtubules. Microfilaments are ~6 nm thick and are made of the protein actin. They form a network on the cytoplasmic side of the plasma membrane called the membrane skeleton or exoskeleton. Actin plays a role in supporting microvilli, and in conjunction with myosin, another protein, actin is also responsible for muscle contraction. Intermediate filaments, which are thicker and stiffer than microfilaments, are involved in cell-to-cell attachment via the formation of junctions. Microtubules, the third type of cytoskeleton, are a cylinder made of 13 parallel strands called protofilaments. Each protofilament is a long chain of globular proteins called tubulin. Microtubules radiate from the centrosome, hold organelles in place, maintain cell shape and rigidity, and act as guide tracks to transport substances throughout the cell with the help of the motor proteins, dynein and kinesin. Microtubules are involved in ciliary and flagellar structure and movement and are also involved in mitosis by forming the mitotic spindle that guides chromosome movement during cell division.1

The Mechanism of Microtubule Assembly Within the Cell

The half-life of microtubules is quite short, ~10 minutes. In fact, as microtubules are forming, part of the microtubule is already breaking down. This is called dynamic instability.4 The mechanism of microtubule polymerization is shown in Figure 1. The first step in microtubule polymerization is the formation of tubulin heterodimers that result when an alpha (α) and beta (β) tubulin monomer combine. Next, these chains, consisting of α/β tubulin heterodimers, form sheets that wrap
around, forming the protofilament. Finally, when 13 parallel strands of these protofilaments come together they form the more rigid tube-like structure called the microtubule.1,4

The Function of Microtubules in Intracellular Transport

Using vesicles, cells have adopted specialized processes to move large particles, droplets of fluid, or numerous molecules at once through the membrane. The process by which vesicles bring matter into cells is called endocytosis, and the process by which material is released from the cell is called exocytosis. There are 2 basic forms of endocytosis: pinocytosis and phagocytosis. Pinocytosis, or cell drinking, is a process of ingesting droplets of extracellular fluid containing small proteins. Pinocytosis occurs in all human cells and begins when invaginations along the plasma membrane occur, forming pits. These pits soon separate from the surface membrane completely and form small vesicles within the cytoplasm, which still contain the droplets of the fluid and small proteins.1,5

Phagocytosis is the process by which cells engulf particles such as bacteria and other large macromolecules. Only specialized cells have the capabilities to phagocytize macromolecules. Cells involved in the immune system like neutrophils, for example, protect the body from infection by phagocytizing and killing bacteria. Using a specialized form of movement called ameboid locomotion (discussed below), a neutrophil crawls about in the connective tissues and, when it encounters a bacterium, surrounds it with its pseudopods and traps it in a phagosome, a cytoplasmic vessel.1 Following phagocytosis, a lysosome merges with the phagosome within the cytoplasm, forming a structure called a phagolysosome. Through the action of various hydrolytic enzymes contained within the lysosome, digestion of the engulfed molecule ensues. In general, phagocytosis is a way of keeping the tissues free of debris and infectious microorganisms. The movement of the vesicle into the cell and towards the nucleus is accomplished through a motor protein called dynein. Following the digestion of the macromolecule within the vesicle, the unused or residual material is expelled from the cell by the process of exocytosis and moved along the microtubule out of the cell by the motor protein, kinesin (Figure 2).1,5

Receptor-mediated endocytosis is a more select form of either phagocytosis or pinocytosis. This form of endocytosis relies on receptors found on the plasma membrane that bind to specific proteins found in the extracellular fluid to promote their ingestion into the cell. The receptors then cluster together on the plasma membrane and the membrane invaginates once again, forming a pit that is coated with a peripheral membrane protein called
clathrin. The pit soon pinches off to form a clathrin-coated vesicle in the cytoplasm, a process called clathrin-mediated endocytosis (Figure 3).1,5

Exocytosis is a process of discharging material from the cell. A secretory vessel in the cell migrates to the surface, carried along the microtubule track by the motor protein, kinesin. The vesicle binds to peripheral proteins of the plasma membrane and then fuses with the vessel and allows it to release its contents. Exocytosis serves some very important functions within the cell: (1) it aids in the secretion of important substances (eg, hormones) needed elsewhere in the body, (2) it rids the cell of residual undigested material, and (3) it replenishes the cell membrane that either is lost through endocytosis or that has become damaged and needs to be replaced.1

The Role of Microtubules in Cellular Movement

Microtubules are also very important in cellular movement. There are 2 forms of cellular movement in the body: ciliary movement and ameboid locomotion. Their use is dependent upon the type and function of the cell.

Ciliary movement. As the name implies, cilia use this form of movement. Cilia are hairlike processes ~7 to 10 µm long. Nearly every human cell has a single, non-motile primary cilium a few micrometers long.1 Even though the function of many of the cilium on cells is still unknown, their roles in balance in the inner ear, absorbing light in the retina of the eye, ovulation, and keeping the airways cleared of debris are better understood. Cilia beat in waves that sweep across the surface of the epithelium, always in the same direction. Each cilium bends stiffly forward and produces a power stroke that pushes along the mucus or other matter. As one cilium initiates its power stroke, a domino effect occurs whereby the cilium in front of it begins its power stroke, and the one in
front of it begins its stroke, and so on. This produces a wave-like pattern. After the completion of the power stroke, a recovery stroke occurs whereby the cilium is pulled back to the upright position, ready to flex again.1,5

Cilia have a basic structure, which consists of an array of thin protein cylinders called microtubules. They are arranged as a 9 + 2 structure where 2 central microtubules are surrounded by a ring of 9 microtubule pairs. The central microtubules do not penetrate into the cell, whereas the peripheral microtubules do, thus anchoring the cilium in place. Each pair of peripheral microtubules contains 2 dynein arms. Dynein, a motor protein, not only functions in endocytosis but also functions in ciliary movement. By using energy extracted from ATP, dynein causes the cilium to bend toward the front through a crawling motion. Besides the cilia, microtubules also are responsible for the whip-like motion of the flagella of sperm, thus enabling their movement.1

Ameboid locomotion. Besides ciliary movement, another form of movement used by cells is called ameboid locomotion. This form of movement can be initiated by chemotaxis, or movement towards a substance, and also requires endocytosis and exocytosis to occur. Once a substance is released into a surrounding area, the cell will initiate its movement towards that substance by protruding the pseudopodium. As shown in Figure 4, when the pseudopodium is protruded, it must then attach to a surface or matrix like fibronectin. The pseudopodium attaches to the matrix using receptors that are exposed through the process of exocytosis. Movement in the forward position can only be accomplished if the anchoring receptors on the posterior end of the cell have been endocytosed, thus preventing their contact with the matrix. Next, contraction of actin fibrillae within the pseudopodium occurs similar to the contraction of a Slinky once stretched and the cell lurches forward. This process of exocytosis, anchoring, endocytosis, and contraction of actin and myosin is repeated until the cell reaches its target area.5

Drug Therapies Targeted at Microtubules for the Treatment of Disease

Besides functioning in normal cellular processes, microtubules have either been implicated in the etiology of disease or have been the target of drug therapy for the management of some diseases. In this section and also because pathophysiology is one of the components taught in this course, some diseases are highlighted to reinforce the principles learned about microtubules. Even though microtubule derangements have been implicated in numerous diseases, only a select few of the diseases are chosen to highlight the principles learned during their lectures. These diseases include: cancer, gout, Familial Mediterranean fever (familial paroxysmal polyserositis) and Inflammatory Amyloidosis. These diseases are chosen because microtubules are the primary targets of drug therapy used to manage these conditions.

The goal of drug therapy is to manage a particular disease state. Regarding microtubule-directed drug therapies, it is the dynamic state of the microtubule that is targeted (i.e., by preventing or promoting its depolymerization). In either scenario, the inability of a microtubule to polymerize or depolymerize will prevent its proper function within a cell. This will impair a cell’s ability to go through mitosis, transport molecules into or out of itself, or prevent its movement throughout the body. Most the microtubule-directed drug therapies prevent the locomotor and phagocytic properties of immune cells to produce an anti-inflammatory response. For example, colchicine is used to treat diseases like gout, Familial Mediterranean fever, and Inflammatory Amyloidosis because it reduces the inflammatory response within the body.6-9 The inability of the microtubule to properly assemble in the presence of colchicine prevents 2 essential components underlying ameboid locomotion, endocytosis and exocytosis, from occurring (Figure 4), thus reducing the inflammatory response. As in the case of gout, the inhibition of the migration of granulocytes into the inflamed area and a decrease in the phagocytic activity of these cells reduces the release of lactic acid and other proinflammatory enzymes that elicits an immune response.9 As for Familial Mediterranean fever and Inflammatory amyloidosis, diseases characterized by an enhanced immune response accompanied by a potentially life-threatening and inappropriate deposition of amyloid proteins into various organs including the kidney,6-9 colchicine is also an effective therapy due to its suppressing effects on the

Figure 4. The mechanism of ameboid locomotion. (Adapted from Textbook of Medical Physiology, 10th Edition. Guyton and Hall. The cell and its function. 2000: 21; Figure 2-16, with permission from Elsevier.)
immune system and amyloid body production.

In contrast to colchicine, drugs like paclitaxel (TAXOL), which prevent microtubule depolymerization or “promote” microtubule polymerization, are also effective in treating diseases like breast and ovarian cancers. Because these diseases are characterized by a high rate of cell proliferation, paclitaxel acts as an effective anticancer agent by targeting the mitotic capabilities of cell and thus interfering with cell division. As discussed previously, microtubules play an important role in cell division by forming the essential mitotic spindles to ensure proper chromosomal separation and cell division. Paclitaxel prevents normal cell division from occurring by forming mitotic bundles. This bundling impairs a cell’s ability to go through mitosis, resulting in a decrease in cell division.6-9

Application of Cell Biology Principles to the Practice of Pharmacy

During lectures on cell biology, a clear connection between cellular proteins and disease management by use of drugs is made. In the fall 2003 semester, the Center for Pharmacy Practice exercise focused on the application of a drug that targets microtubules. In particular, the students were first asked to answer questions about gout and how colchicine manages the symptoms of gout. Students were then required to fill a prescription for colchicine using computer software and an automated dispensing technology that is available in licensed pharmacies. The students were allowed to use a variety of reference materials to complete their Center for Pharmacy Practice exercises, references available at licensed pharmacies. An example of the completed prescription for colchicine and the questions accompanied by the answers given in the fall 2003 semester are shown below and in Figure 5:

Questions

1. What is this patient most likely using this medication to treat? [Answer: Colchicine is used to treat acute gouty arthritic attacks as well as preventing recurrences of such attacks.7,8]

2. What is hyperuricemia? Describe how this condition occurs in the body. [Answer: Hyperuricemia is an increase in serum uric acid. This can happen when uric acid is not excreted from the body as it should be. Normally, uric acid is balanced between its production and elimination. Most of the uric acid (two thirds of it) is excreted in the urine and the rest is eliminated in the GI tract.2,6]

3. What happens in the kidneys during this condition? What part do the kidneys play in this development? [Answer: The renal tubules have the capacity to both actively secrete urate ions and also actively resorb them. The active reabsorption occurs in the proximal tubules. In some patients, they have overly active reabsorption and consistently have an elevated urate ion concentration in the extracellular fluid.2,6]

4. In what role do polymorphonuclear leukocytes play? [Answer: Leukocytes are involved in the phagocytosis of urate crystals. When this happens, it results in a rapid lysis of cells and proteolytic enzymes are then discharged into the cytoplasm. An inflammatory reaction occurs that involves intense joint pain, erythema, warmth, and swelling. The patient may also experience fever.2,6]

5. Describe what signs and symptoms are seen with this condition? [Answer: In acute attacks, you
may see arthritis, nephrolithiasis, gouty nephropathy, and deposits of sodium urate in cartilage, tendon, and synovial membranes. There may be a rapid onset of pain, swelling, and inflammation.2,6

6. What is the most common area that gout affects first? What other areas may also be affected? [Answer: The most common area is the first metatarsophalangeal (MTP) joint, the “great toe.” The insteps, ankles, heels, knees, wrist, fingers, and elbows follow.2,6]

7. How does colchicine work against gout? [Answer: Colchicine decreases leukocyte motility and phagocytosis by acting at the level of the microtubules. Colchicine binds between the alpha and beta heterodimer to prevent microtubule polymerization. This impaired ability to polymerize microtubules prevents the leukocyte from migrating to the urate crystals in the joints and also in phagocytizing the urate decreasing the inflammatory response.4,6-8]

8. What is the maximum dose that a patient can take at one time for treatment of an acute attack? [Answer: The maximum dose that a patient can take is a total dose of 8 mg.7,8]

9. How long does a patient have to wait before initiating another course of therapy to treat another acute attack? [Answer: The patient must wait at least 3 days before starting another course of therapy.7,8]

10. Can this medication be administered any other way besides the oral route for an acute attack? If yes, give the dosage. [Answer: Yes, it can be administered IV. The dose is 1 to 3 mg, then 0.5 mg every 6 hours until a response is achieved, not to exceed 4 mg per week.7,8]

11. What is the prophylactic dose for preventing attacks of gout? [Answer: The prophylactic dose is 0.5 to 0.6 mg daily or every other day.7,8]

12. Would a patient that is pregnant be able to take this medication? [Answer: The oral route is considered a category C and the parenteral route is considered a category D. This medication should not be taken by a pregnant woman unless absolutely necessary.7,8]

**ASSESSMENT**

Assessment was performed through multiple-choice questions, a survey administered to the students following completion of their Center for Pharmacy Practice exercise, and other forms of evaluation including Teacher Evaluation Questionnaires (TEQs). The instructor placed 2 to 3 questions pertaining to microtubules on the students’ final examinations and compiled the data for 5 years. During the fall semesters of 1999, 2000, 2001, and 2002, the students were not exposed to the Center for Pharmacy Practice exercise, whereas in the fall semester of 2003, the students were exposed to these exercises. On the final examinations given during the fall semester of 1999, the average percentage of questions correctly answered by students was 79% (overall test average = 83%); in 2000, 86.5% (overall test average = 81%); in 2001, 90% (overall test average 83%); in 2002, 84.5% (overall test average= 77%), and in 2003, 88.6% (overall test average= 76%). When compared with the overall average score for the 5 years, the students in 1999 scored 4% lower; students in 2000 scored 5.5% higher; in 2001, 7% higher; in 2002, 7.5% higher; and finally, in 2003, 12.6% higher. Thus, the greatest improvement in scores occurred during the fall semester of 2003, the semester in which these Center for Pharmacy Practice exercises were incorporated.

The PHBMS 355 course has been well received by students. The overall average University TEQ score for this course is 4.53 on a scale ranging from “1” to “5,” with “5” being the best. In addition, School curricular survey data indicate that 97% and 82% of the students (104/105 completing the survey) thought that the course objectives were adequately met for PHBMS 355 and 355L (laboratory), respectively.

These next sets of data provide the results of a survey that was administered after the completion of the Center for Pharmacy Practice exercise. A total of 106 out of 150 students responded to the survey. Even though 41% of the students thought the exercise was marginally difficult and 42% felt that it was too long, 64% of the students thought that it helped them to understand concepts taught in the course, 70% thought that the exercises helped them to apply the material in class, and 84% thought that these exercises showed them how a pharmacist might use these concepts in day-to-day practice. Overall, the students understood the concepts presented in lecture and were able to relate the material learned in the classroom to a typical pharmacy practice scenario. The application of cell biology concepts to a pharmacy practice scenario was successful. This is reflected in a higher number of correct responses to test questions in the fall 2003 semester compared with previous semesters and also by the positive survey results.
Overall, the integration of the different teaching modalities into PHBMS 355, Human Anatomy, Physiology and Pathology I, greatly enhanced the learning process for our students. Not only did the students score higher on their examinations, they also learned the practical application of their education to their pharmacy careers. The students were more attentive, engaged, and enthusiastic. As a result, they approached their learning of the course material with motivation. This integrated form of teaching enhances our students’ understanding of basic physiology because of the application of these basic principles to specific disease states and then to the management of a specific disease (gout) by drug therapy (colchicine).

REFERENCES