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# Hyperprolactinemia

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One of the most common metabolic disorders among both men and women is hyperprolactinemia. Hyperprolactinemia, by definition, is the persistent elevation of serum prolactin levels (Kaye 265). Prolactin is a hormone produced and secreted by lactotroph cells in the anterior pituitary gland (Kaye 266). The normal upper limit serum prolactin level is 20ng/mL in women and slightly lower in men (Kaye 265). Prolactin hormone is similar to growth hormone but has several different functions in the body. It is known that prolactin induces lobuloalveolar growth of the mammary gland and stimulates lactogenesis, commonly referred to as milk production (Prolactin). It is also known that prolactin has molecular heterogeneity. This means that there are different molecular forms of prolactin with varying degrees of bioactivity (Crosignani 1117). Hyperprolactinemia can be caused by numerous factors from certain drugs to adenomas. Two specific causes of hyperprolactinemia, ethanol consumption and administration of anti-psychotics, have been examined to determine exactly what causes increased prolactin production. It is important to study the molecular aspects of increased prolactin production in order to effectively treat those affected. The disorder has also been shown to be asymptotic or symptomatic. Symptoms range widely and are more common in women than in men. Though symptoms can suggest hyperprolactinemia, laboratory tests are necessary to confirm a diagnosis. Once diagnosis is confirmed there are several different treatment options for patients and physicians to consider. Treatment ranges from observation to surgery depending on the severity and cause of the disorder. There are no defined preventative measures for hyperprolactinemia. There are, however,

suggestions such as ceasing ingestion of certain medications known to cause elevated prolactin levels if abnormal serum prolactin levels are reached. Another aspect of hyperprolactinemia is its involvement in autoimmune diseases. There are indications that high levels of serum prolactin in the body effect aspects of immune function. Hyperprolactinemia is thought to play a role in Sjogren's syndrome and systemic lupus erythematosus, an auto-immune thyroiditis, as well as other auto-immune diseases (Velkeniers 1102). The immune system is also thought to produce auto-antibodies against high levels of serum prolactin.

The onset of hyperprolactinemia can be caused by numerous things. Hyperprolactinemic patients can be broken down into three groups: those without a pituitary lesion, those with a microadenoma, and those with a macroadenoma (Rebar 1100-01). When no pituitary lesion is present, high levels of prolactin can be caused by numerous things. Physiologically, pregnancy, early nursing, stress, sleep, nipple stimulation, and the ingestion of certain foods can cause serum levels to increase above normal (Biller & Luciano 1078). There are also several medications that can cause hyperprolactinemia. Dopamine receptor antagonists and dopamine-depleting agents can cause prolactin levels to increase. (Luciano 1087) Prolactin levels are regulated through a mechanism where hypothalamic dopamine inhibits the synthesis and secretion of prolactin by binding receptors on lactotrophs (Prolactin). If there is a lack of dopamine in the body, excess prolactin can be produced and secreted. Hormones such as estrogen, antiandrogens, and opiates can also increase serum prolactin levels. Several preexisting diseases are also known to increase serum prolactin levels. Hypothalamic and pituitary stalk disease, primary hypothyroidism, chronic renal failure, cirrhosis, chest wall trauma,

and seizures can all cause hyperprolactinemia (Luciano 1087). Cirrhosis and chronic renal failure cause hyperprolactinemia by inducing an increased level of prolactin secretion and a decreased metabolic clearance rate. Often times when there is no adenoma present in hyperprolactinemic patients an exact cause for elevated prolactin levels cannot be pinpointed. (Kaye 265)

The major causes of hyperprolactinemia are adenomas. There are two different types of adenomas associated with hyperprolactinemia: microadenomas and macroadenomas. Microadenomas are defined as lesions less than one centimeter in diameter and macroadenomas are defined as lesions greater than or equal to one centimeter (Rebar 1102). Collectively, the different types of pituitary lesions causing hyperprolactinemia are referred to as prolactinomas. Hyperprolactinemia caused by a microadenoma is considered less threatening to a patient than macroadenoma induced hyperprolactinemia because macroadenomas are more prone to increasing in size.

Adenomas, certain drugs, and even certain foods have all been shown to cause hyperprolactinemia. While these causes of hyperprolactinemia are all apparent, there are underlying causes of increased prolactin production. The molecular causes of increased prolactin production are oftentimes studied in laboratory animals. If scientists can specifically determine what causes increased prolactin production and cell proliferation they may be able to better treat hyperprolactinemic patients. It has been suggested that alcohol consumption can increase the amount of prolactin produced in the pituitary causing hyperprolactinemia. A study using female rats was conducted at Rutgers, The State University of New Jersey, to determine how alcohol (ethanol) affects lactotrope growth and prolactin production. Anti-psychotics are another cause of increased

prolactin production. A paper entitled Prolactin Levels and Adverse Events in Patients Treated with Risperidone (Kleinberg) examines the effect of Risperidone on prolactin levels and the risk associated with anti-psychotic drug induced hyperprolactinemia. Studies done on two drugs, alcohol and anti-psychotics, examine ways in which hyperprolactinemia is brought about at the molecular level and the consequences of the disorder.

Although it is known that alcoholism can cause hyperprolactinemia the mechanism by which this occurs is unknown. A study performed at the Center of Alcohol Studies and Department of Animal Sciences at Rutgers University and reported in a paper entitled Ethanol Induces Hyperprolactinemia by Increasing Prolactin Release and Lactotrope Growth in Female Rats (De) examined whether increased pituitary production of prolactin was caused by an increase in cell number and/or cell production of prolactin in the pituitary. Several clinical studies have shown chronic alcoholism elevates serum prolactin levels. Alcohol has also been demonstrated to raise serum prolactin in nonhuman primates and various laboratory animals in studies conducted in the past. The positive correlation between alcohol and increased serum prolactin levels led researchers to investigate exactly how ethanol increased prolactin production. This information could lead to a better understanding of how to treat elevated prolactin levels.

In this study, scientists addressed:

> Whether alcohol-induced hyperprolactinemia is the result of increased pituitary production of prolactin as a result of increased cell number and/or increased cell production of prolactin in the pituitary ... and whether ethanol acts directly on the pituitary lactotropes and whether

ethanol's actions on lactotropes is dependent on the presence of estradiol (De 1421-2).

Estradiol is a potent estrogen hormone synthesized in the ovaries. It is present in the placenta, testis, and possibly the adrenal cortex. (On-line Medical Dictionary)

The affect of alcohol (ethanol) on rats in various conditions was tested in this study. Control strains were used for comparison to ethanol fed rats. Fischer-344 rats were kept in a controlled environment and used for this experiment. A portion of the rats were ovariectomized while the remaining rats kept their ovaries. A portion of the ovariectomized rats had an estradiol capsule implanted inside of them during surgery. The remaining ovariectomized rats had empty capsule inserts and served as controls to compare the effects of estradiol on the action of ethanol. At the end of the experiment, the test rats were euthenized and their trunk blood and pituitary glands were extracted for analysis. The anterior pituitaries of twenty-four ovariectomized rats with estradiol capsules were treated for lactotrophic cell extraction. These cells were treated with various doses of ethanol and their level of prolactin production was measured by radioimmunoassay (RIA). RIA is an analysis system for testing antigen-antibody reactions using radioactivity to label the antibody or antigen to determine the relative abundance of the desired antibody or antigen (On-line Medical Dictionary). Other lactotropes were treated with ethanol and/or estradiol and assayed for cell proliferation.

The mitogenic affect of ethanol was determined in this experiment by measuring the wet weight and protein content of the pituitary. In cyclic female rats it was demonstrated that an ethanol diet significantly increased the weight of the anterior pituitary when compared to those rats fed diets without ethanol after fifteen days.

Ethanol treatments were also shown to increase the amount of protein produced by the anterior pituitary. After thirty days of an ethanol diet, rats showed an even more significant increase in wet weight and protein content. The total protein level of rats treated with ethanol was significantly higher in estradiol treated rats than in those administered ethanol alone indicating that estradiol potentates the effects of ethanol.

Analysis of the pituitary of ethanol fed rats revealed an increased number of proliferating lactotrophic cells after fifteen days. The serum prolactin levels of rats fed an ethanol diet and a regular diet were also compared. Serum levels were higher in all rats fed an ethanol diet. Overall, rats administered estradiol has serum prolactin levels higher than cyclic and ovariectomized rats that were not given estradiol capsules.

The effect of ethanol on isolated lactotropes and mixed anterior pituitary cell cultures was also analyzed *in vitro*. It was previously shown that estradiol increases lactotropic cell proliferation by increasing cell-to-cell communication among pituitary cells. Cultures of cells were tested to determine if ethanol similarly affected cell proliferation. Mixed anterior pituitary cells showed increased proliferation in the presence of estradiol and ethanol together. A culture of enriched lactotropes alone did not show an increase in cell proliferation with the addition of ethanol and estradiol. The data gathered in this experiment demonstrated that "ethanol increased lactotrope proliferation only when these cells maintain communication with other pituitary cells" (De 1426). The amount of prolactin secreted by cells in culture was also analyzed under different conditions. Cells treated with ethanol and estradiol together produced much higher levels of prolactin than those cells treated with ethanol alone.

All of the results compiled in the duration of this experiment indicated that ethanol affected the production of prolactin in a fashion similar to that of estradiol. Ethanol was shown to increase both serum prolactin levels and the number of mitogenic lactotropes in the anterior pituitary in a time dependent fashion. Administration of ethanol also appeared to potentate the affects of estradiol in the rats. Both ethanol and estradiol were shown to increase prolactin levels by directly affecting lactotrophic cells and increase the number of proliferating lactotropes indirectly by altering cell-to-cell communication between different cell types in the anterior pituitary. The increase in the mitogenic action of lactotropes was proven in this experiment by several examples. First, the wet weight and protein content of the anterior pituitaries both increased indicating a higher number of cells present. Secondly, the amount of cells shown to be undergoing DNA synthesis after ethanol administration indicated that the number of lactotropes dividing in the pituitary approximately doubled. Ethanol was also shown to increase the amount of prolactin excreted from lactotropes. Because ethanol increased prolactin production *in vitro* in enriched lactotropes alone it is believed that ethanol directly acts on the cells. It is still not clear exactly how ethanol causes increased prolactin release. It was shown that estradiol promoted the amount of prolactin released by cells administered ethanol. This only occurred in mixed cell cultures indicating that cell-to-cell communication may be necessary for ethanol and estradiol to fully interact to increase prolactin levels and lactotrope proliferation.

The exact mechanism in which ethanol affects lactotropes has not yet been determined. It is believed that it may involve cytokines, or intercellular mediators, produced locally in the pituitary. One cytokine, Transforming Growth Factor –[beta]-1

(TGF-[beta]-1), has been shown to inhibit prolactin production and cell proliferation. The production of this cytokine is inhibited by estradiol administration and further reduced by ethanol administration. This has lead researchers to hypothesize that ethanol's affect on the pituitary may be connected to modification of TGF-[beta]-1 production and action. The data gathered in this experiment suggests that "ethanol consumption may promote or aggravate the pathology of existed pituitary tumors" (De 1429).

Anti-psychotics are used to treat a number of illnesses and are often accompanied by several adverse side affects. One side effect noted with the use of all neuroleptics, or anti-psychotics, is increased serum prolactin levels. These drugs act as dopamine receptor antagonists. By definition, antagonists are drugs that bind a cell receptor without eliciting a biological response (On-line Medical Dictionary). They inhibit the action of dopamine on $D_2$ receptors on the surface of pituitary lactotropes causing an increase in prolactin production (Kleinberg 57). Early dopamine blocking drugs used to treat psychosis were all shown to relieve psychosis, cause extrapyramidal side effects, and elevate prolactin levels by antagonizing $D_2$ receptors. It was first shown in 1971 that chlorpromazine, one of the earliest dopamine-blocking drugs caused prolactin levels to increase above normal in humans (Kleinberg 57). More recently drugs such as Risperidone, Olanzapine, and Clozapine have been used to treat psychosis. These drugs are combined serotonin (5-hydroxytryptamine-2-[5-$HT_2$]) and dopamine (5-$HT_{2/D2}$) receptor antagonists. These drugs, which have a lower incidence of extrapyramidal side effects, still increase serum prolactin levels leading researchers to explore the causes of increased prolactin production for the sake of patients' health. (Kleinberg 57-8)

The main concern with anti-psychotic drug induced hyperprolactinemia is the possible side effects brought about by increased prolactin levels. There are several side effects of concern. These include amenorrhea, galactorrhea, and decreased libido in women, and erectile dysfunction, ejaculatory dysfunction, gynecomastia, and decreased libido in men (Kleinberg 57-8). It was previously shown that drugs with a mode of action similar to Risperidone increased serum prolactin levels to a greater extent than previously used neuroleptics. Studies were conducted on Risperidone and haloperidol to determine if the side effects from hyperprolactinemia occurring in patients treated with $HT_2/D_2$ receptor antagonists (Risperidone) was more severe than those occurring in patients treated with $D_2$ receptor antagonists (haloperidol). There was great concern that "Risperidone could cause an unacceptably high incidence of amenorrhea and galactorrhea as a result of elevated serum prolactin levels" (Kleinberg 58).

Because of the long term affects of disorders such as amenorrhea it was important to determine whether or not it was safe to administer Risperidone to psychotic patients. Amenorrhea and galactorrhea both cause a reduction in sex steroids. Prolonged reduction of estrogen, for example, can lead to premature osteoporosis. The compiled data suggests that Risperidone treatment, while it does raise prolactin levels higher than other anti-psychotics, is safe for the long term health of patients. This is also beneficial because there are less extrapyramidal side effects incurred during treatment with Risperidone as compared to anti-psychotics that are only $D_2$ antagonists. It was also important to rule out a greater incidence of hyperprolactinemic side effects for two other reasons. First, detrimental sexual side effects oftentimes cause patients to stop taking their medication. Side effects such as sexual dysfunction and ejaculatory or erectile dysfunction inhibit

sexual fulfillment and may lead to infertility. These side effects are detrimental to the already fragile mental state of people taking these medications. Secondly, hyperprolactinemia induced by anti-psychotics could mask a prolactinoma or other nonneuroleptic cause of hyperprolactinemia. It was concluded that all patients with elevated serum prolactin levels should be monitored for existing causes of hyperprolactinemia, such as a prolactinoma, regardless of medication they are taking. If such a condition is found to exist, it should be treated along with the patient's psychosis. (Kleinberg 62)

Though hyperprolactinemia has been diagnosed in less than one percent of the general population, it is still thought of as one of the most common pituitary disorders (Kaye 265). In some patients the disorder is asymptomatic and in others a variety of symptoms are apparent. In the early 1970's it was discovered that high levels of prolactin are associated with abnormal menstrual cycles in females and gonadal disorders in males (Luciano 1085). Symptoms of hyperprolactinemia are generally more apparent in women than in men. Hypogonadism is commonly associated with hyperprolactinemia and can cause many of the symptoms associated with raised prolactin levels. These symptoms can include oligomenorrhea or amenorrhea, and/or galactorrhea. (Kaye 265) Amenorrhea is a lack of menstrual cycles and galactorrhea is excessive or spontaneous secretion of milk (Prolactin). Oligomenorrhea is infrequent menstruation with markedly diminished menstrual flow (On-line Medical Dictionary). Developing amenorrhea as a result of hyperprolactinemia must be considered because it puts women at greater risk of developing osteoporosis (Kaye 265). Other symptoms of hyperprolactinemia in women can include decreased libido, hirsutism, acne, infertility, habitual abortion, and osteopenia

(Biller & Luciano 1080). Hirsutism is a condition of abnormal hairiness, most commonly involving male hair pattern distribution in women (On-line Medical Dictionary). Infertility due to hyperprolactinemia is most often caused by chronic anovulation, defective folliculogenesis, and luteal phase inadequacy (Jones 835). According to the On-line Medical Dictionary, the luteal phase is the post ovulatory phase of a woman's menstrual cycle. During this phase progesterone is produced which causes the uterine lining to secrete substances to support the implantation and growth of an early embryo.

When a macroadenoma is present in a patient they may also experience mass effects such as headaches, visual loss, cranial neuropathies, hypopituitarism, seizures, and cerebrospinal fluid rhinorrhea (Biller & Luciano 1080). For clarification: Cranial neuropathies involve functional disturbances and/or pathological changes in the peripheral nervous system and is also the term used to designate noninflammitory lesion in the peripheral nervous system; hypopituitarism is a medical condition where the pituitary gland produces lower than normal levels of hormones; and cerebrospinal fluid rhinorrhea is defined as the discharge of cerebrospinal fluid through the nose (On-line Medical Dictionary).

Men normally show fewer symptoms of hyperprolactinemia than women and the disorder itself is less common in men. Symptoms in men can include decreased sperm production, decreased libido and energy, gynecomastia, loss of pubic hair, osteoporosis, loss of muscle mass, and impotence (Biller & Luciano 1080). Gynecomastia is the excessive development of the male breasts (On-Line Medical Dictionary). Most often, hyperprolactinemia is associated with hypogonadism in men, which can cause impotence in the advanced stage (Jones 836).

In order for a physician to diagnose hyperprolactinemia, a medical examination and laboratory testing are necessary. Most patients with diagnosed hyperprolactinemia go to a physician only after showing symptoms such as infertility, irregular periods, or impotence. In many cases, hyperprolactinemia may go undiagnosed due to a lack of symptoms. In order to determine whether or not a patient has hyperprolactinemia, and the underlying cause of the disorder, the patient should have a complete medical examination by a physician and laboratory testing of hormone levels.

The physical examination conducted when a patient is suspected of having hyperprolactinemia should be very extensive. If a patient is showing symptoms of hyperprolactinemia, such as galactorrhea or amenorrhea, a blood test should be administered to measure serum prolactin levels and test for pregnancy (Biller & Luciano 1080). If prolactin levels are high and the pregnancy test is negative, a second test should be administered to determine whether or not prolactin levels are consistently high. This needs to be done because stress and exercise can temporarily elevate prolactin levels (Kaye 266). Exercise, stress, and pregnancy induced hyperprolactinemia are examples of physiologic hyperprolactinemia and oftentimes prolactin levels will return to normal naturally (Biller 1096). A detailed history of the patient should also be obtained during examination to determine any past factors that could contribute to the disorder.

For women, neurologic status, a thyroid evaluation, and a thorough pelvic and breast examination should be given. During the examination, the presence of any optic problems should be distinguished as well as scarring from past trauma to eliminate the possibility of neurological problems. The patient should also be checked for the presence of herpes zoster or any chest or breast lesions that could stimulate the production of

prolactin. The consistency and size of the thyroid should also be evaluated. (Jones 836) During the examination it is critical to check for the presence of hypothyroidism, renal failure, and hepatic dysfunction as a cause of elevated prolactin levels (Kaye 266). Hepatic dysfunction is the impaired ability of the liver to properly conduct its function in metabolism (On-line Medical Dictionary). It is also important to note all medications the patient is currently taking due to the fact that some medications can stimulate prolactin production producing what is referred to as pharmacologic hyperprolactinemia (Biller 1096). If none of the previously mentioned causes of hyperprolactinemia are apparent in the patient, thyroid and kidney function and liver enzymes should be tested (Kaye 266). For males, it is more common to present symptoms later in the course of the disorder with larger tumors already developed (Rebar 1101).

If all of the previously mentioned tests reveal negative results, magnetic resonance imaging (MRI) or computed tomography (CT) should be conducted on the patient's sella turcica to determine the presence of an adenoma (Biller 1097). The sella turcica is the area contained at the base of the skull that holds the pituitary gland (On-line Medical Dictionary). A MRI generally produces better pictures of the sella and should be used if available. Prolactinomas less than three millimeters in diameter may not be detected by the MRI scan (Kaye 266), but for practical purposes the MRI will detect whether or not an adenoma is present. If the prolactin level of a patient is greater than 200 ng/mL a MRI should always be performed to exclude a mass lesion (Biller & Luciano 1080). A MRI can reveal several different causes of hyperprolactinemia. A prolactinoma could be observed as well as stalk compression or, occasionally, an infiltrative process involving the pituitary gland (Kaye 266). The distinction between a

microadenoma (<1cm) and a macroadenoma (≥1cm) is considered arbitrary but has been found very useful in diagnosis. Compared to macroadenomas, microadenomas have a much lower risk of increasing in size. If a microadenoma is detected it should still be closely monitored because it could be the beginning stages of a macroadenoma. Macroadenomas are also more dangerous than microadenomas due to the mass effects they can cause such as vision problems and headaches. Sellar lesions other than prolactinomas can cause hyperprolactinemia but are rather uncommon. The presence of other lesions should also be checked during imaging because unlike prolactinomas, which can usually be treated with medication, most other sellar lesions require removal using surgical procedures. Lesions known to increase serum prolactin levels, other than prolactinomas, can include germinomas, craniopharyngiomas, meningiomas, cordomas, and lymphocytic hypophysitis. (Biller 1098) In order to clarify these lesions, their definitions from the On-line Medical Dictionary are as follows: germinomas are tumors stemming from germ cells in the brain; craniopharyngiomas are a type of primary brain tumor that stems from the pituitary gland and secrete large amounts of pituitary hormones; and meningiomas are common benign tumors stemming from the pia-arachnoid cells of the meninges, and can occur along the sphenoid ridge that contains the pituitary gland. Commonly, if a true prolactinoma is present the serum prolactin level corresponds with the size of the adenoma (Biller 1098). If the level of prolactin secretion is only mildly above average and there is a large lesion present in the patient there is a strong likelihood that the adenoma is not a prolactinoma and further testing should be considered to evaluate the lesion (Biller 1098). If all of the recommended diagnostic radiological and serologic examinations do not reveal the cause of elevated prolactin

levels the disorder is referred to as idiopathic hyperprolactinemia. Under these circumstances, treatment may or may not be administered depending on symptoms and severity of the condition. (Kaye 266)

As a result of all of the possible causes of hyperprolactinemia, it can be rather difficult, if not impossible, to pinpoint the direct cause of the problem. Guidelines for the diagnosis of a patient demonstrating symptoms of hyperprolactinemia including amenorrhea or irregular menses are outlined in the article *Hyperprolactinemia* by James Jones and are as follows:

First a pregnancy test should be done. If it is positive, elevated prolactin levels can be attributed to the pregnancy. If the pregnancy test is negative, follicle-stimulating hormone, luteinizing hormone, and prolactin levels should be measured. Follicle-stimulating hormone is produced by the anterior pituitary and stimulates the development of eggs and the release of estrogens in females and the development of sperm in males; luteinizing hormone controls sex hormones in both females and males (On-line Medical Dictionary). If prolactin levels appear normal and follicle-stimulating hormone and luteinizing hormone levels are low or normal the patient most likely has hypothalamic hypogonadotropic amenorrhea. In this case the amenorrhea is not a result or symptom of hyperprolactinemia. If the follicle stimulating hormone levels are greater than 40mIU/mL and prolactin levels are normal the patient may have ovarian failure. If serum prolactin levels are high upon initial testing a repeat of test should be done to confirm that the levels are consistently high. If prolactin levels remain high and follicle stimulating hormone levels are low or normal and luteinizing hormone levels are high the patient could be suffering from polycystic ovary syndrome. This syndrome is a clinical

syndrome characterized by amenorrhea or oligomenorrhea, anovulation and associated with bilateral polycystic ovaries (On-Line Medical Dictionary). If follicle stimulating hormone and luteinizing hormone levels are both normal or low and prolactin levels are high, the next step in diagnosis is testing the patient's thyroid-stimulating hormone (THS), triiodothyronine (T3), and thyroxine (T4) levels. THS is secreted by the anterior pituitary and activates cyclic AMP production in thyroid cells leading to production and release of thyroid hormones T3 and T4. T3 and T4 are the two main hormones produced by the thyroid gland (On-line Medical Dictionary). If thyroid-stimulating hormone levels are high and triiodothyronine and thyroxine levels are low or normal, the patient is most likely experiencing thyroid failure and has elevated prolactin levels as a result. If THS, T3, and T4 levels are all normal or low, a CT or MRI of the sella should be conducted. If the sella is normal the patient is experiencing idiopathic hyperprolactinemia. If the patient has a pituitary lesion less than 1cm in diameter the diagnosis is a microadenoma. If parapituitary changes are present in the MRI or CT the patient most likely has a Craniopharyngioma (a primary brain tumor that develops in the pituitary gland) such as a sarcoidosis aneurysm. Sarcoidosis is a disease of unknown etiology in which chronic inflammatory granulomatous lesions are present on the pituitary gland (On-line Medical Dictionary). If the patient has a pituitary lesion greater than 1 cm and/or an enlargement or erosion of the sella the diagnosis is determined to be a macroadenoma. Another possible cause of elevated prolactin levels is "empty sella" syndrome. This is a condition of the normal or increased sella with intrasellar herniation of subarachnoid space that causes symptoms such as headache, visual disorders and cerebrospinal fluid rhinorrhea (On-line Medical Dictionary).

Once it has been determined that a patient has hyperprolactinemia and the underlying cause(s) has been determined or narrowed down, the correct treatment option for the individual must be considered. There are many guidelines for treatment due to the several causes and symptoms of the disorder. The main reasons treatment is indicated are to diminish bothersome or dangerous symptoms the patient may be experiencing and lower serum prolactin levels. Dopamine agonists are generally the type of treatment used for hyperprolactinemic patients. These agonists can reduce serum prolactin levels, reduce the size of prolactinomas and restore gonadal function (Webster 1105). Dopamine agonists function by binding to dopamine receptors and activating them (On-line Medical Dictionary). Bromocriptine, Cabergoline, Quinagolide and Pergolide mesylate (Permax) are all dopamine agonists that have demonstrated effectiveness in the treatment of hyperprolactinemia (Kaye 265).

Bromocriptine mesylate (Parlodel) was the first dopamine agonist to be used in practice in the 1970s (Webster 1105). It has been shown to reduce prolactin levels, inhibit prolactin secretion, and reduce the size of tumors in 70% to 100% of patients (Kaye 267). Bromocriptine may also produce side effects including nausea, headache and dizziness. Due to these adverse effects, nearly 12% of hyperprolactinemic patients cannot tolerate taking the drug and must be treated by other means (Webster 1105). Treatment with bromocriptine should begin with 2.5 mg of the drug taken orally before bed. Once therapy has started the dose may be increased by 2.5 mg every week until normal serum prolactin levels are reached or symptoms are diminished. In general, patients take 2.5mg two to three times per day to achieve normal prolactin levels. (Kaye 267) Bromocriptine has been the mainstay of dopamine agonists effective in

hyperprolactinemia treatment. Recently two other dopamine agonists, cabergoline and quinagolide, have been developed and may take its place due to the adverse side effects and frequent administration required of bromocriptine (Webster 1105-6).

Cabergoline, an ergoline derivative, has a high affinity and selectivity for dopamine $D_2$ receptors. This differentiates cabergoline from bromocriptine, which has an affinity for $D_1$ receptors. Cabergoline also has a longer half-life than bromocriptine allowing for once or twice weekly doses. Studies have shown that 0.6 mg of cabergoline is equivalent to 2.5 mg of bromocriptine and reduces serum prolactin levels by about 70% of the pretreatment level. (Webster 1106) The European Cabergoline Study Group did several studies with hyperprolactinemic females using cabergoline treatment. In one study, less than one percent of the patients administered cabergoline could not tolerate the drug, a much lower percentage than those administered bromocriptine. In another study 3% of the patients discontinued use of cabergoline due to the severity of adverse side effects. In the same study, more than 90% of the women who came into the study amenorrheic or oligomenorrheic developed regular menses and/or became pregnant during the study. Adverse effects of cabergoline commonly include dizziness, headache, nausea, weakness, and fatigue. (Webster 1106) After several more studies were done it was determined that the new dopamine agonist, cabergoline, was significantly more effective in restoring prolactin levels and gonadal function and was tolerated better than bromocriptine (Webster 1107). The use of bromocriptine is still the most widespread treatment of hyperprolactinemia, but cabergoline, which was developed less than a decade ago shows sign of being a more effective treatment for hyperprolactinemia.

Quinagolide is another dopamine agonist that was developed around the same time as cabergoline. Quinagolide is a nonergot $D_2$ agonist and has a half-life in between that of bromocriptine and cabergoline. Due to this, the drug can be taken once daily to effectively treat hyperprolactinemia (Webster 1107). In three studies conducted comparing bromocriptine and quinagolide, only 25 to 200 μg of quinagolide was needed per day as compared to 1.25 to 20 mg of bromocriptine required per day for effective treatment. This indicates the greater efficiency of quinagolide in returning serum prolactin levels to the normal range. During these studies, less people had to terminate quinagolide treatment than bromocriptine treatment due to adverse side effects. As of December 1999, only one study had been conducted comparing quinagolide and cabergoline. In this study it appeared that quinagolide was more effective and better tolerated than cabergoline but more evidence is needed to effectively state quinagolide's superiority to cabergoline in treating hyperprolactinemia. (Webster 1108)

Pergolide mesylate is another dopamine agonist that has shown effectiveness in treating hyperprolactinemia in studies. It has been shown that for treatment of hyperprolactinemia, pergolide is as safe and effective as bromocriptine. Pergolide is also more efficient that bromocriptine, only needing to be taken once daily. It is also less expensive. (Kaye 267) As of December 1999 pergolide was licensed only for treatment of Parkinson's disease by the United States' Food and Drug Administration and the United Kingdom (Webster 1106).

Though there are several different dopamine agonist treatments for hyperprolactinemia, bromocriptine is still the primary medication available in the United States. As of December 1999, Cabergoline was licensed only in the United Kingdom and

other European countries for the treatment of hyperprolactinemia (Webster 1107). Dopamine agonists are generally the most popular form of treatment for hyperprolactinemic patients, but estrogen therapy or the use of oral contraceptives have also been shown to normalize serum prolactin levels.

When determining treatment for female patients with hyperprolactinemia it is important to considered whether or not the patient currently desires to become pregnant. As mentioned, elevated prolactin levels can cause infertility for several reasons. If pregnancy is not desired, sometimes only observation, not treatment, is required. For men, if an adenoma is detected, it is important to determine its size because they are usually larger than those found in females. The majority of indications for therapy are similar in both men and women, but special consideration needs to be taken if a women wishes to become pregnant.

In the article Following Patients Under Treatment for Hyperprolactinemia (Rebar), treatment of patients without pituitary lesions, with microadenomas, and with macroadenomas is considered along with the desire for pregnancy. The following paragraphs outline the treatment options given to physicians as described in the article by Robert Rebar.

Patients without adenomas have a low risk for developing one. If pregnancy is not desired by the individual it is usually only necessary to observe the patient on a regular basis, an ongoing treatment program known as expectant management. Prolactin levels should be tested yearly in the patient. If levels continue to rise, the MRI or CT procedure should be repeated. For women also experiencing amenorrhea it is important to measure bone density during check-ups due to the risk of osteoporosis associated with

this condition. Dopamine agonists such as bromocriptine should be used if the patient is experiencing reduced bone density, symptoms of hyperprolactinemia such as irregular menses, or decides they want to become pregnant. Estrogen therapy may also be used to treat hyperprolactinemia in women who do not wish to currently become pregnant. If estrogen is used, prolactin levels should be measured after three months, six months, and one year. After the initial year, prolactin levels should be measured yearly. If prolactin levels continue to rise, estrogen therapy should be halted and another treatment should be considered.

If a hyperprolactinemic patient with no pituitary lesion wishes to conceive, a dopamine agonist should be used starting at the lowest possible dose. Prolactin levels should be measured weekly and treatment should be used until prolactin levels return to a normal range. If the patient still has symptoms after prolactin levels have resumed to normal a different dopamine agonist could be considered or intravaginal bromocriptine can be used. A patient trying to conceive should use contraception until she has her first menstrual period. This is done in order to better determine the time of conception. As soon as the patient tests positive for pregnancy, use of the dopamine agonist therapy should be discontinued.

A second class of hyperprolactinemic patients are those with microadenomas (<1cm in diameter). If a patient with a microadenoma does not wish to become pregnant and is asymptomatic and/or ovulating, oftentimes only observation is necessary. This can be used because microadenomas rarely grow in size. The patient must be carefully monitored however because at the time of the MRI the adenoma could appear less than one centimeter but be a developing macroadenoma. Patients with microadenomas also

need their bone density measured and need to be observed for any appearance of physiologic symptoms brought on by chronic hyperprolactinemia. Patients with microadenomas under expectant management should have their prolactin levels measured at three months, six months, twelve months, and yearly thereafter. If the physician decides to treat a patient with a dopamine agonist, they should be started at the 2.5 mg dose and gradually increase doses as explained previously. If the patient's prolactin level remains steady and in the normal range after one to two years of treatment, the treatment may be ended. After abandoning treatment the patient should be monitored yearly for the continuation of normal prolactin levels and normal menstruation.

When a patient with a microadenoma desires to become pregnant, dopamine agonists are the best medication. As with patients without adenomas, use of medication should be discontinued after a positive pregnancy test. Studies have shown that there is little risk of pregnancy complications in women who have taken medication, but its use during pregnancy is still very controversial. After a woman has given birth, she may again need therapy to regulate prolactin levels.

Patients diagnosed with macroadenomas ($\geq$ 1 cm in diameter) are at the highest risk for developing mass effects due to the size of the macroadenoma and severe symptoms due to extremely high prolactin levels. If the patient does not wish to currently become pregnant, they can generally be treated with a dopamine agonist. Once the patient responds to therapy, prolactin levels should be tested regularly and sellar imaging (MRI or CT) should be conducted to monitor any sign of further adenoma growth. If the patient's symptoms do not dissolve, if prolactin levels do not return to normal, or if the adenoma increases in size it may be necessary to refer the patient to a neurosurgeon. If

the patient experiences mass effects such as visual impairment or cranial nerve palsy they should be referred to a neurosurgeon immediately.

If a patient with a macroadenoma desires to become pregnant dopamine agonists can be used, but their use is controversial. The macroadenoma may become symptomatic during pregnancy causing problems for the mother and possibly the fetus. If a patient has a macroadenoma, some physicians continue use of dopamine agonists during pregnancy while others do not. Though studies have shown that the drugs put the fetus at little risk, leaving a woman on a treatment program during pregnancy is very controversial. With macroadenomas, surgery before pregnancy is an option. The size of macroadenomas can increase during pregnancy putting the woman at an increased risk of mass effects and hyperprolactinemic symptoms. Partial or complete transsphenoidal resection of the mass may be done. It is suggested that the smallest amount possible be taken out to avoid a complete hypophysectomy (the surgical removal or destruction of the pituitary gland as defined by the On-line Medical Dictionary). It is also very important to evaluate the woman's pituitary function following surgery before pregnancy is attempted.

It is important to remember that an elevated prolactin level is only a laboratory number. If a patient has no adverse effects from a high prolactin level, treatment is sometimes unnecessary. Indication for treatment, besides the desire to become pregnant, usually relies on symptoms such as infertility, abnormal menses, anovulation, galactorrhea, dysparenunia (defined as difficult or painful coitus by the On-line Medical Dictionary), hypogonadism, acne, hirsutism, headaches, and diminished libido (Olive 1092). If visual field impairment or cranial nerve palsies occur in a patient, treatment must be administered due to the high risk of these symptoms. Treatment ranges from

dopamine agonist therapy to surgery depending on the individual case. For hyperprolactinemic patients, the goal of treatment is primarily to diminish adverse side effects harmful to the patient and secondly to restore normal prolactin levels. (Olive 1093) If there are no adverse side effects due to elevated serum prolactin levels in the patient it may only be necessary to observe them carefully. Studies have shown that in some women prolactin levels can revert back to normal spontaneously. This can often be predicted by symptoms the patient is presenting. (Sanfilippo 1112) If chronic hyperprolactinemia is left untreated however, it may cause long term effects in the patient. Some possible long term effects of chronic hyperprolactinemia include osteoporosis due to loss of bone density, hypoestrogenism (decreased estrogen levels), the disruption of hormone functions, and mass effects if a macroadenoma is present. It is normally recommended that treatment with a dopamine agonist be used to treat hyperprolactinemic patients to avoid the possible effects of chronic hyperprolactinemia (Sanfilippo 1113-14). Generally, surgery is only necessary if a patient desires to become pregnant, has complications such as mass effects, or does not respond to medication or cannot tolerate the medication (Biller & Luciano 1081). Sudden hemorrhage into an adenoma can also occur. While this is very rare it is classified as a neurosurgical emergency and requires immediate surgery (Biller & Luciano 1082).

Prolactin has been shown in several instances to play a role in autoimmune diseases and cause a higher incidence of autoantibodies (Steinfeld 1717). Prolactin has several functions in the body and is produced by several different types of cells under different conditions. Two autoimmune diseases in which prolactin has been implicated in playing a role are Sjogren's Syndrome (SS) and Systemic Lupus Erythematosus (SLE).

Sjogren's Syndrome is a chronic autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands clinically leading to keratocunjunctivitis sicca and xerostomia (dry eyes and dry mouth) (Steinfeld 239). SLE affects mostly young women of childbearing age and has been observed to be sex-linked in both its cause and pathogenesis (Lahita 352). Prolactin is present at the sites of both of these diseases and plays a significant role in their pathogenesis. Understanding how prolactin can act in the production of auto-antibodies and continuation of autoimmune disease is important in understanding how to treat these diseases and exactly how prolactin functions in the body.

Recent studies have implicated prolactin to be present in the salivary glands of Sjogren's syndrome patients. Forty-six percent of patients with primary Sjogren's syndrome were found to have hyperprolactinemia in a study conducted by Gutierrez et al, 1994 (Steinfeld 240). Evidence of hyperprolactinemia, especially that associated with the overproduction of big prolactin, can be a clinical sign of a more aggressive disease (Steinfeld 244). Big prolactin has not only been associated with SS but also with rheumatoid arthritis and SLE (Steinfeld 243). It was previously mentioned that prolactin has molecular heterogeneity. Recently, a big prolactin (60 kDa) was found to be neosynthesized and overexpressed in the glandular epithelial cells of minor salivary glands in SS patients (Steinfeld 1712). It has been suggested that prolactin induces autoantibody production in these patients due to the elevated levels of anti-Ro and anti-La antibodies associated with increased prolactin in acinar epithelial cells. Ro and La are nuclear proteins present in humans. Prolactin has been found to act as a cytokine and has been demonstrated to stimulate synthesis and production of proteases in certain cells. In

an article entitled Prolactin Up-Regulates Cathepsin B and D Expression in Minor Salivary Glands of Patients with Sjogren's Syndrome (Steinfeld), researchers investigated whether prolactin could modulate cathepsin B and D expression in the minor salivary glands of patients with SS. Cathepsins are intracellular proteolytic enzymes of animal tissues. Specifically, cathepsin B is a lysosomal cysteine proteinase which hydrolyzes proteins. Cathepsin D is an intracellular proteinase found in several tissues. It is involved in the catabolism of cartilage and connective tissue. (On-line Medical Dictionary)

It was found that there was a positive correlation between prolactin levels and the production of anti-Ro and anti-La antibodies; and the level of prolactin and the extent of symptoms in SS patients. According to the article, "Prolactin-like proteins are synthesized and overexpressed in glandular epithelial cells of labial salivary glands from SS patients and correlate with the aggressiveness of the disease" (239). Patients that expressed extraglandular manifestations showed higher levels of prolactin than those patients without symptoms. It was also observed that SS patients' acinar cells of the minor salivary glands expressed prolactin while healthy control subjects did not. (Steinfeld 1716). The researchers involved in this experiment hypothesized that "the continuous prolactin neosynthesis in minor salivary glands of SS patients, but not in those of healthy subjects, might modulate the level of expression of some proteases and so lead to a progressive destruction of the glandular epithelial cells because of this vicious circle" (Steinfeld 1716). The data collected in this experiment validated this hypothesis. Prolactin was shown to significantly up-regulate the expression of cathepsin B and D in a dose-dependent fashion. Since they also showed that prolactin is neosynthesized in the

glandular cells of the minor salivary glands of SS patents, but not in healthy patients, it was concluded that the increased level of prolactin allows for the continuous expression of cathepsins B and D and brings their proteolytic activities into play. The increased proteolytic activity of cathepsins B and D is not present in healthy patients because no prolactin is neosynthesized in their minor salivary glands. (Steinfeld 1716) This data is not only significant in Sjogren's Syndrome research. Cathepsin B and D are involved in the pathology of several diseases including chronic inflammatory diseases of the airways and joints and cancer which could also implicate the involvement of prolactin in these diseases. Mononuclear cells infiltrating the minor salivary glands of SS patients show increased cathepsin B-immunoreactivity in the presence of prolactin. This suggests that prolactin could also modulate the biological functions of these infiltrating cells and aid in the destruction of tissues (Steinfeld 1717). The authors' concluded that "the prolactin neosynthesis, which occurs for still unknown reasons in the minor salivary glands of patients with Sjogren's syndrome, could play a significant part in the destruction of acinar structures through the activation of proteinases, including at least cathepsins B and D" (Steinfeld 1717).

Hyperprolactinemia has also been associated with systemic lupus erythematosus, perhaps a more commonly known autoimmune disease that Sjogren's syndrome. Although not all SLE patients have hyperprolactinemia, elevated serum prolactin levels has been associated with the disease in some instances. Whether or not hyperprolactinemia and SLE are related is still controversial. Some researchers have shown positive correlation between elevated prolactin levels and SLE while others have shown that there is not a positive correlation between the two. While this is

controversial, it is known that the immune system and neuroendocrine system communicate through intracellular communication (Jara 748). Several studies suggest a link between prolactin and systemic lupus erythematosus. There is also indication that prolactin plays a role in the pathogenesis and clinical manifestations of SLE in humans. The following evidence gathered from numerous reports is presented in a review entitled Prolactin in Human Systemic Lupus Erythematosus (Jara) and suggests there is a connection between SLE and increased prolactin production:

1. Hyperprolactinemia (HPRL), which is often mild, has been found in 20 – 30% of SLE patients.

2. HPRL appears to be associated with clinical activity of SLE during pregnancy and in non-pregnant SLE patients...Recent clinical and experimental studies support the potential role of prolactin in clinical activity and severity of SLE.

3. HPRL secondary to microadenoma may trigger the onset of SLE in a subset of patients, especially when there is mild HPRL.

4. Defects in...dopamine metabolism, as well as the effect of cytokines on prolactin secretion, may explain in part the HPRL in SLE.

5. The findings of elevated urine PRL and urine IL-6 in active lupus nephritis and in cerebral spinal fluid in active central nervous system (CNS) involvement suggest a pathogenic role for prolactin and an abnormal communication between the immune and neuroendocrine systems in active SLE.

(753-54)

While there are still several questions to be answered about the relationship between prolactin and SLE, there are several indications that they are somehow linked. While not all SLE patients demonstrate elevated prolactin levels, a significant percentage do. The findings from these numerous experiments suggests that prolactin may "participate in different local and general immune/inflammatory processes and forms a bridge between the neuroendocrine and immune systems in SLE" (Jara 754).

Hyperprolactinemia is a common pituitary disorder among both men and women. Raised serum prolactin levels can be caused by numerous things ranging from exercise to an adenoma and there are a variety of different symptoms associated with the disorder. It can be seen in studies on ethanol and anti-psychotics that prolactin has several functions and hyperprolactinemia induced by the ingestion of substances is complex. Understanding the mechanisms which cause hyperprolactinemia is important in understanding how to properly treat the disorder and how to avoid taking substances that may aggravate the disorder. After a first blood test determines elevated prolactin levels, a second test should be done to confirm hyperprolactinemia. Once the diagnosis has been confirmed, treatment options vary depending on the individual with the disorder and their symptoms. Depending on the underlying cause of the disorder there are several different treatment options ranging from expectant management to surgery. For women, the desire to become pregnant is an important consideration in determining treatment. Though surgery is a treatment option, it is rarely used unless mass effects occur as a result of a macroadenoma. Generally, expectant management or treatment with a dopamine agonist such as bromocriptine is adequate for most patients. The purpose for treatment is to first diminish symptoms bothersome or harmful to the patient and secondly lower serum

prolactin levels. If hyperprolactinemia is left untreated, and elevated serum prolactin levels persist, adverse affects such as osteoporosis can occur. Hyperprolactinemia is a common disorder and can be the underlying cause of several ailments as well as a symptom of a greater underlying cause. Autoimmune diseases and hyperprolactinemia have been shown to be connected. Increased prolactin levels may worsen the deterioration caused by autoimmune diseases such as Sjogren's syndrome and systemic lupus erythematosus. It is important to understand how increased prolactin levels effect the progress of these diseases for proper treatment. If a patient shows signs of hyperprolactinemia it is important to test for, and treat, the disorder due to the adverse affects or discomfort the patient may experience from elevated serum prolactin levels.

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UHON 499 Thesis Project (2)
Fall '02: Dr. Douglas Fix's laboratory

Background

Mutations in the DNA of an organism can be caused by several different factors. Rarely, mistakes occur during DNA replication and are not repaired by any of the several DNA repair mechanisms. It is these un-repaired mistakes, or mutations, that over time can contribute to the evolution of organisms or to diseases such as cancer. Mutagens such as UV radiation increase the mutation frequency in organisms causing more mutations to occur than in cells not exposed to any mutagens.

UV radiation can cause pyrimidine dimers to form in between DNA nucleotides. A pyrimidine dimer is the chemical rearrangement of bonds between two adjacent pyrimidines. There are three ways in which the rings can fuse leading to different dimers. The most common dimer causes the formation of a cyclobutane ring. A cyclobutane pyrimidine dimer (CPD) is caused by bond formation between the 5-carbon atoms and 6-carbon atoms of two adjacent pyrimidines. Another form of the pyrimidine dimer can occur when a bond is formed between the 6-carbon on one pyrimidine and the 4-carbon on an adjacent pyrimidine. The 6-4 product has a rate of occurrence that is 10% as frequent as the cyclobutane dimer. The third photoproduct is known as TA*. This occurs at a frequency that is 1% of the occurrence of the CPD. When any of these dimers occur during replication a cell enters SOS and translesion synthesis occurs at the sight of the photoproduct. SOS is a response to DNA damage in which alternate genes are turned on for replication in order to repair the damaged DNA. During SOS, mutagenesis occurs at an increased rate due to the activation of alternate DNA polymerases that lack inherent repair mechanisms. The different dimers have been found to cause different frequencies

of mutation. Cyclobutane pyrimidine dimers are not highly mutagenic. 85% of the bypass events that occur as a result of a CPD do not cause a mutation. While the 6-4 and TA* photoproducts occur much less frequently than CPDs they are much more mutagenic. Only 9 – 18% of the bypass events that occur as a result of these products are *not* mutagenic. Each photoproduct has been shown to primarily cause a certain mutation. The cyclobutane dimer most often causes the sequence TT (T: Thymine) to mutate to TA (A: adenine). The 6-4 photoproduct generally causes TT to TC mutations (C: cytosine). The TA* product most commonly results in TA to TT mutations.

A special type of repair system called photoreactivation exists as a repair mechanism for pyrimidine dimers caused by UV irradiation. This system separates the bound nucleotides rather than replacing them. An enzyme known as photolyase binds to the fused bases and can separate them. The enzyme obtains the energy needed to separate the bases from a reduced flavin adenine dinucleotide ($FADH_2$) group in the enzyme. $FADH_2$ absorbs light of wavelengths between 350 and 500nm (visible light), hence the name photoreactivation. The photolyase binds the dimer and the absorption of light causes it to cleave the bond between the two pyrimidines, restoring the bases to their original form.

*Escherichia coli* can be manipulated in order to determine the specific mutations that occur as a result of pyrimidine dimers. *E. coli* strain FX-11 is a tyrosine negative strain (it cannot synthesize tyrosine). This particular strain of *E. coli* is Tyr- due to a nonsense mutation encoding a stop codon in the middle of the gene. When cells of this strain are exposed to UV light a portion of the cells mutate to become Tyr+ (can synthesize tyrosine). This is caused by back mutations that change the stop codon (UAA)

to another codon that allows complete translation of the gene (CAA, AAA, GAA, TTA, TCA, TAG, TAT, or TAC). FX-11 is also excision repair deficient causing the number of mutations that occur to be greater than a cell with this excision repair. The increased frequency of mutations caused by this deficiency makes it easier to study the different mutations that occur as a result of dimers. This strain of *E. coli* was further manipulated by altering the bases that surround the nonsense mutation producing a strain called FX-11-AT. In this strain there is an A on one end of the nonsense codon and a T at the opposite end:

---ATAAT---
---TATTA---

Previous experimentation with this strain has shown a high degree of TA to TT mutations consistent with the TA* photoproduct.

Experiments

Over the course of the semester I worked with *E. coli* FX-11-AT, FX-11-AT pKY 33, and FX-11-AT pXZ 1997. Strain FX-11-AT pKY 33 harbors a plasmid that encodes a CPD photolyase. The overexpression of this photolyase in the irradiated cells should cause fewer backmutants when the cells are exposed to visible light after they are exposed to UV light. Strain FX-11-AT pXZ 1997 also harbors a plasmid encoded photolyase. This inducible [6-4] photolyase may also cause fewer mutations to occur in cells exposed to visible light after UV exposure depending on the type of photoproducts produced. Four sets of data were collected using strains FX-11-AT and FX-11-AT pKY 33 and four sets of data were collected using strains FX-11-AT and FX-11-AT pXZ 1997 to determine mutation frequency after exposure to UV light and changes in frequency as a result of a photoreactivation.

FX-11-AT pKY 33 and FX-11-AT

Cultures of FX-11-AT and FX-11-AT pKY 33 were grown overnight in a 37°C shaker bath in 5 ml 1x A-0 plus 10 µl (microliters) tyrosine, 10 µl phenylalanine, and 10 µl 20% glucose. The pKY33 media also contained 5 µl Ampicillin (Amp). This strain must be grown in media containing Amp because of the plasmid it harbors. The plasmid encodes an ampicillin resistance gene on it and the addition of Amp to the media ensures the presence of the plasmid in the cells.

After the cells were grown overnight, the culture was diluted 1:20 in fresh 1x A-0/tyr/phe/glc and returned to the 37°C shaker bath. The optical density of the cells was monitored at 450 nm. The cells were grown to an optical density of 0.5 and harvested. This took approximately three to four hours. Eight milliliters of the culture was then centrifuged at 3500 rpm for 25 minutes. The supernatant was discarded and the cell pellets were resuspended in 4 ml fresh 1x A-0.

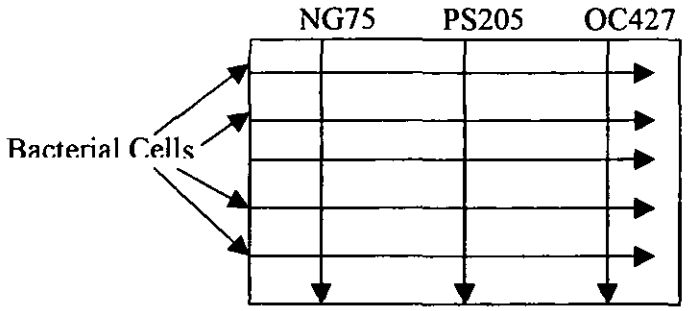
After resuspension in 4 ml A-0 the cells were ready for exposure to UV light. Cells were irradiated for zero or ten seconds for each strain. Ten seconds of exposure is equal to 1 $J/m^2$ of UV exposure. Fifty µl of each cell suspension for each exposure time was spread onto search plates (plates containing nutrient broth, leucine, phenylalanine, A-0, glucose, and agar). Two plates were prepared for each dosage in order to minimize pipetting errors. These plates are referred to as the Mutation plates. Spread plates were also prepared using dilutions of the original cultures at each time point. The suspension of FX-11-AT treated with zero seconds of UV exposure and 10 seconds of UV exposure was diluted to $10^6$ cells/ml when plated on search plates. FX-11-AT pKY 33 cells which received zero seconds of UV exposure were diluted $10^6$-fold and the cells that received 10 seconds of UV exposure were diluted $10^5$-fold when plated. Plates inoculated with

these dilutions are referred to as viability count plates and were used to count the number of viable cells in the cultures. Two viability plates for each strain at each dosage were made to take an average count of the viable cells.

The spread plates inoculated during the UV dosage procedure were incubated for 48 hours in a 37°C incubator. All plates were then counted. The data obtained from the plate counts was entered into an Excel spreadsheet that calculated the percent of viable cells and the mutation frequency for each UV dosage.

After counting the mutation plates, 30 colonies from each plate were randomly selected and inoculated onto isolation plates (plates containing leucine, phenylalanine, glucose, A-0 and agar). These colonies were grown overnight in a 37°C incubator. The colonies grown on the isolation plates were then used in a phage test to determine which colonies were back mutants.

The phage test uses three T4 bacteriophages to infect cells that became tyrosine positive as a result of UV exposure. The T4 phage has a nonsense defect causing it to grow in cells containing a tRNA suppressor gene (it lyses cells with a suppressor gene). When this suppressor gene is present in cells, it allows them to grow on media lacking tyrosine as a result of suppressor mutations, not back mutations. We are looking for true back mutants in which the codon that was mutated to a stop codon is reverted back to a codon other than a stop codon by exposure to UV light. Three phages are used in this test, NG75, PS205, and OC427. Suspensions of the three phages were spread across the phage plate in one direction and cells from the isolation plates were streaked across the plate in the perpendicular direction.




Forty µl of the phage suspension was added to the plates and a mini glass spreader was used to spread the suspension evenly between the walls of the plate. Bacterial cells from the isolation streaks were streaked in a straight line across the plate using sterile toothpicks. These plates were then incubated overnight at 37°C. A positive result was indicated by cell lysis where the phage and cells overlap. Four different outcomes were mainly observed in this experiment:

| 75 | 205 | 427 | **Indication** |
|---|---|---|---|
| + | — | — | Back Mutant |
| + | + | + | Suppressor Mutant |
| + | — | + | Suppressor Mutant |
| — | + | + | Suppressor Mutant |

The isolation streaks that were observed to be back mutants during the phage test were re-streaked onto fresh isolation plates, incubated overnight at 37°C, and stored at 4°C for later use.

FX-11-AT pXZ 1997 and FX-11-AT

The same procedure was used to induce mutations and identify back mutants in strains FX-11-AT and FX-11-AT pXZ 1997 except that 30 µl of IPTG (isopropyl-thio-beta-D-galactoside) was added to the cultures when they reached an optical density of 0.2 and then they were grown to OD = 0.5 and harvested. The addition of IPTG to the strain harboring the pXZ 1997 plasmid is necessary because the photolyase encoded on the plasmid is inducible. It is controlled by a *tac* (*trp-lac*) promoter that is turned on by the

addition of IPTG, a gratuitous inducer of the *lac* promoter. The IPTG is added when the optical density is 0.2 to give the cells time to produce a sufficient amount of protein.

Several problems were encountered when I first started working with the FX-11-AT pXZ1997 strain. First, the cells were not growing at a rate similar to that of FX-11-AT without a plasmid. The previous plasmid harboring strain grew at approximately the same speed as the strain without the plasmid. A second observation we made was that the mutation frequency for the photo-reactivated plasmid strain did not differ from that of the plasmid strain without photo-reactivation. This was not what was expected because the photolyase encoded on the plasmid should repair mutations that occurred as a result of the UV exposure. To test the presence of the plasmid a plasmid extraction was preformed. After the plasmid DNA was extracted from the cells, a sample was run on an agarose gel along side a ladder and a sample known to contain the plasmid to determine if the plasmid was still in our culture. We did not see a band that corresponded to the expected size of our plasmid. This plasmid extraction indicated that the plasmid that should have been in our pXZ1997 strain may not have been present. We started again using a new isolate in which it was determined that the plasmid was present.

Using the new isolate we still did not see the expected difference in mutation frequency between the photo-reactivated and not photo-reactivated strains of FX-11-AT pXZ1997. Since we knew that our plasmid was in the cells we were using we decided to look at the presence of the protein in the induced cells. This was done using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Cultures of FX-11-AT and FX-11-AT pXZ 1997 were grown overnight and then inoculated into fresh media. Two cultures of each strain were inoculated. When the optical density of the cultures

reached 0.2, 30 µl of ITPG was added to one culture of FX-11-AT and one culture of FX-11-AT pXZ 1997. All four cultures were then grown to an optical density of 0.5 and harvested. The harvested cells were prepared for the SDS-PAGE by resuspension in a buffer solution and boiling for 10 minutes. The samples were loaded into the gel (6% running gel) and the proteins in the sample were to separated according to size. The gel was then stained with Coomassie Blue and then destained until the bands were visible. The gel showed a band at approximately 100 kDa only in the lane containing the induced FX-11-AT pXZ 1997 strain. This indicated that the photolyase protein was indeed being induced in the cells but that its presence was not affecting the frequency of mutation as significantly as we originally thought it would.

Results and Discussion

The data obtained from the viability plate counts for strains FX-11-AT pKY 33 and FX-11-AT showed a slight decrease in survival in the strain lacking the plasmid after exposure to UV irradiation. When these cells were exposed to photoreactive light the rate of survival did not change. The strain harboring the plasmid indicated a greater decease in survival when exposed to UV light. The higher mortality rate of the plasmid strain can be accounted for by the presence of the photolyase. The protein binds to the site of DNA damage after UV exposure. If the cells are not exposed to photoreactive light the protein remains attached to the DNA preventing the DNA polymerase from replicating past the damage. Cells harboring the pKY 33 plasmid exposed to photoreactive light (after UV exposure) showed a much higher rate of survival as was expected. (Figure 1, panel A).

Data collected from the phage tests using strains FX-11-AT pKY 33 and FX-11-AT is illustrated in Figure 1, panels B, C, and D. The rate of backmutations, Gln U

suppressor mutations, and Gln V suppressor mutations all increased in both strains when the cells were exposed to UV light. The increase in mutation frequency is a result of DNA damage from UV exposure. FX-11-AT pKY 33 exposed to photoreactive light (after UV exposure) showed fewer mutations than cells not exposed to photoreactive light. The photolyase in the pKY 33 strain repaired DNA damage that resulted from the UV radiation resulting in fewer mutations.

Data collected from viability plate counts of strains FX-11-AT pXZ 1997 and FX-11-AT indicated little difference in survival rates between the two strains. The presence of the photolyase did not seem to decrease the rate of survival in the absence of photoreactivation as it did in the pKY 33 strain. Photoreactivation of the plasmid strain did not increase the rate of survival as it did with FX-11-AT pKY 33. (Figure 2, panel A)

The mutation frequency of both FX-11-AT pXZ 1997 and FX-11-AT increased when cells were exposed to UV radiation. The frequency of backmutations and GlnV suppressor mutations in the strain harboring the plasmid decreased when the cells were exposed to photoreactive light after UV exposure. This can be accounted for by the presence of the photolyase in this strain which repairs DNA damage. The frequency of GlnU suppressor mutations in the plasmid strain increased after photoreactivation. The increase in frequency was not expected. The error in this data set was larger than with the data collected for FX-11-AT pKY 33. (Figure 2, panels B, C, D)

Conclusion

Analysis of cells exposed to UV radiation showed a decrease in survival rate after exposure to UV light. The presence of a photolyase was shown to increase the survival rate of cells exposed to photoreactive light after exposure to UV light in cells harboring the pKY 33 plasmid. Cells harboring the pXZ 1997 plasmid did not demonstrate this

same increase in survival after photoreactivation. The mutation frequency was shown to decrease in cells encoding a photolyase after photoreactivation. This was shown in FX-11-AT strains harboring the pKY 33 plasmid and the pXZ 1997 plasmid. I had originally planned on sequencing several of the back mutants isolated during the course of the semester to determine the exact mutations arising as a result of the UV mutagenesis. Due to complications that arose with the pXZ 1997 strain I did not get a chance to sequence any isolates. I did, however, determine that the inducible photolyase was present in cultures of FX-11-AT pXZ 1997 and does not seem to significantly affect the mutation frequency of photoreactivated cells when compared to cells that are not photoreactivated. Further analysis of the backmutants isolated in this experiment will be performed to determine the exact mutations arising from UV irradiation of *E. coli* FX-11-AT pXZ 1997 cells.