Inborn errors of metabolism: Tyrosinemia

Inborn errors of metabolism (IEMs) are inherited metabolic disorders that result from defects in genes coding for specific enzymes. This defect generates abnormal chemical reactions that disrupt the normal metabolic pathways resulting in the toxic accumulation of the substrate behind the block or a deficiency in the product. IEMs can be classified into defects in amino acids; such as phenylketonuria, homocystinuria, maple syrup urine disease, and tyrosinemia, in carbohydrates; such as galactosemia, in fatty acid oxidation; such as short, medium, and long chain fatty acid disorders, and in urea cycle; such as citrullinemia and argininemia. The main focus of this article will be on tyrosinemias.

Introduction

Most of the enzymatic alterations in IEMs are inherited as autosomal recessive disorders, the remainder being either autosomal dominant or X-linked. The recessive mode of inheritance occurs in an individual that acquires an abnormal allele from each heterozygous carrier parent ending up being homozygous for the abnormal gene. The child of two carrier parents may have a possibility of being 25% affected, 25% normal, or 50% as a heterozygous carrier.

It is thought that every human carries at least one abnormal recessive gene that may occur randomly during DNA replication. Marrying a relative increases the chance of both partners carrying the same abnormal gene, inherited from a common ancestor. In addition, the recessive gene abnormality varies in frequency among different racial groups. Those predisposed groups may perform population-based carrier testing and antenatal diagnosis may be offered for high-risk pregnancies. Therefore, the risk of these disorders is increased by consanguinity and within specific racial groups.

Overview and classification of Tyrosinemia

Tyrosinemia is a general term used to describe an inherited disorder that disrupts tyrosine metabolism, resulting in the accumulation of high amounts of tyrosine in the blood. Tyrosinemias are classified into three types: type I, or hepato-renal, type II, or oculocutaneous, type III, which consists of three sub-types. These sub-types are all concerned with a dysfunction in the 4-hydroxyphenylpyruvic acid dioxygenase (pHPPD) enzyme and include primary pHPPD dysfunction, transient newborn tyrosinemia (TNT), and hawksinsinuria. The incidence of each type of tyrosinemia differs among certain ethnic groups. For example, tyrosinemia type I is most notably presented in Quebec, where the carrier rate and
prevalence at birth were estimated to be 1 in 20 and 1 in 1846 live births, respectively (Grompe, 2010). Elsewhere, the prevalence has approximated at 1 in 100 000. (Nyhan & Ozand 1998)

**General Considerations of Tyrosine**

Tyrosine is an aromatic amino acid of a low solubility which forms insoluble crystals at high concentrations. Amino acids exist as stereoisomers, which can be dextrorotatory (D) and levorotatory (L). L-tyrosine is the only metabolic active form. L-tyrosine is essential for the synthesis of proteins, thyroid hormones, melanin, catecholamines, and dopamine. However, the predominant fate of tyrosine is its incorporation into proteins or metabolism via a series of reactions. (Scriver et al 2000).

The normal plasma L-tyrosine level ranges from 30 to 120 µmol/L. Values greater than 200 µmol/L are considered high, but clinical manifestations due to high tyrosine plasma levels only become apparent when plasma levels exceed 500 µmol/L. (Grompe 2010).

**Metabolism of L-Tyrosine**

The metabolism of tyrosine principally occurs in the cytoplasm of hepatocytes and renal proximal tubules. In humans, tyrosine is obtained from two sources, hydrolysis of dietary or tissue protein and hydroxylation of phenylalanine obtained from tissues or diet. Phenylalanine will be converted to tyrosine via phenylalanine hydroxylase (PAH). The formed tyrosine will then undergo catabolism initially via tyrosine aminotransferase (TAT) enzyme and is converted to 4-OH-phenylpyruvic acid (pHPP). Then pHPPdioxygenase (pHPPD) will convert pHPP into homogentisic acid (HA) via oxygenation and decarboxylation. Once HA is produced, it is then oxidized by HA oxidase (HAO) into malyacetoacetic acid (MAA), which gets transformed into fumarylacetoacetic acid (FAA) via MAA isomerase. Finally, the FAA hydrolase enzyme will break down FAA to yield fumarate, the Krebs cycle intermediate, and acetoacetate, which is ketogenic. Both the MAA and FAA will generate succinicacetoacetic acid, which gets converted into succinylacetone (SA).

**Types of Tyrosinemia**

The types of tyrosinemias are indicated in Fig 1.

**Type 1- Hepato-renal Tyrosinemia or Hereditary Tyrosinosis**

*Fumarylacetoacetate Hydrolase Deficiency (HRT)*

HRT is the most devastating inborn error in childhood that principally affects the liver, kidney and peripheral nerves. It involves a deficiency in the FAH enzyme preventing the breakdown of FAA into fumaric acid and acetoacetic acid. Thus, FAA will be converted into the toxic metabolite SA, which is a specific biomarker identified in the blood and urine of HRT patient. SA can also inhibit ALA-D that is responsible for the heme biosynthesis preventing the conversion of ALA to porphobilinogen. Typically, HRT is characterized by progressive liver disease, renal tubular dysfunction and hypophosphatemic rickets. The most common presentation in early infancy is failure to thrive or hepatomegaly. The earliest and most severe presentation is the liver manifestations. The patient can present with acute hepatic crisis or chronic liver disease that resembles cirrhosis. If untreated, HRT patients may die of acute liver failure before two years or from chronic liver failure or Hepatocellular carcinoma (HCC) before the end of 20 years. The renal clinical manifestations are thought to arise from the effect of MAA. The kidney develops typical Fanconi syndrome associated with metabolic acidosis. The urine of such sick individuals contains phosphates, glucose and proteins. Their urine smells of sweet cabbage odor. The phosphate loss into urine can result in rickets that is resistant to vitamin D supplementation. The neurological symptoms associated with HRT resemble porphyria and parasthesia. HCC is considered to be the major complication of HRT.

**Type II- Oculocutaneous Tyrosinemia or Richner-Hanhart Syndrome**

*Tyrosine Aminotransferase Deficiency (OCT)*

OCT is a disorder of tyrosine metabolism leading to skin, eye and in some cases neurologic symptoms. This type of tyrosinemia is caused by a deficiency of the hepatic TAT. This enzyme converts tyrosine to pHPP, which is the rate limiting step in the tyrosine metabolic pathway. The main biochemical finding is the elevated tyrosine plasma concentration that is typically more than 1000 µmol/L, extremely higher than in other forms. The clinical manifestations involve lacrimation, photophobia, pain and redness in the eyes, ulcerations in the cornea, erythematous papular lesions on the skin of the palms and soles and severe mental retardation. Both the ocular and cutaneous symptoms occur as a re-
result of the excessive tyrosine accumulation and are usually apparent in the first year of life, although may develop in adulthood. Those symptoms will not resolve unless the tyrosine plasma concentrations are below 550-700 µmol/L. The neurologic features of OCT include mainly mental retardation, which accounts for approximately 50% of patients, and rarely convulsions or behavioral problems (Scriver et al 2000).

**Type III- 4-hydroxyphenylpyruvic acid Dioxygenase deficiency; pHPPDD**

**A, Hawkinsinuria**
Hawkinsinuria is an interesting rare metabolic disorder with a benign and treatable nature. This disorder involves the presence of an amino acid, known as hawkinsin named after the first family reported with this disorder. Hawkinsin is produced from an epoxy intermediate, which is formed between the reaction of pHPP and homogentisic acid. Patients suffering from the deficiency of this intermediate step in the pHPPD reaction are mainly asymptomatic and can only be detected by having the biochemical phenotype of hawkinsinuria.

**B, Transient Newborn Tyrosinemia (TNT)**
TNT is the most common amino acid disorder affecting newborns and it is not genetically determined. It involves a problem of delayed developmental synthesis of pHPPD. This will lead to an imbalance in the rate of tyrosine catabolism and incomplete activity of the immature pHPPD. The newborn will present with isolated transient hyper-tyrosinemia, which is associated with lethargy and reduced motor activity in the neonatal period.

**C, Primary Deficiency of pHPPD**
This is a rare type of tyrosinemia involving a primary inherited dysfunction of the pHPPD enzyme. Affected individuals exhibit isolated hypertyrosinemia and increased urinary excretion pHPL, pHPP, and pHPA. Infants deficient in the pHPPD enzyme are usually asymptomatic; however, may show evidence of altered central nervous system manifestations, such as ataxia, convulsions, cerebral atrophy and neuronal demyelination.

**Diagnostic Methods of Tyrosinemia**

Diagnosis of tyrosinemia in sick infants is based on measuring several diagnostic markers in blood, urine and amniotic fluid. Tyrosine, methionine, SA, AFP and ALA may be considered for the diagnosis of tyrosinemia. Also, measurement of enzyme activities, such as ALA-D and FAH may be used to confirm tyrosinemia. Elevated concentrations of tyrosine in dried blood spots (DBS) is not considered a definite confirmation for the diagnosis of HRT, however detection of the toxic SA is important to confirm HRT. On the other hand, detection of high levels of tyrosine in DBS may be considered appropriate for the diagnosis of OCT and TNT.

Two types of diagnostic methods exist: chemical and instrumental. The chemical diagnostic methods involve a chemical reaction detected by a change in color of the sample. The instrumental methods are the more commonly used analytical techniques for detecting the presence of tyrosinemia including tandem mass spectrometry (MS/MS) (Figure 2), capillary electrophoresis (Cansever et al 2005). Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS can be used for the detection of SA in the amniotic fluid in case of pregnant women and in plasma of sick infants treated with 2-(2-nitro-4 trifluoromethylbenzoyl -1,3-cyclohexanedio (NTBC) a mainstay therapeutic agent for HRT. (Cyr et al 2006).

An additional method of diagnosis is Enzyme-Linked Immunosorbtent Assay (ELISA), which is based on measurement of the FAH activity in blood cells for diagnosis of HRT. However, measurement of FAH activity alone is not appropriate to establish a diagnosis of HRT. Therefore, measuring SA levels in both blood and urine is nec-
Management of Tyrosinemia

Dietary Modification
It is necessary to restrict the dietary intake of phenylalanine and tyrosine in all tyrosinemias. The requirement of these amino acids varies among individual patients depending on the growth rate, adequacy of energy and protein intakes. Therefore, it is best to ensure that the selected dietary management appropriately maintains the blood concentrations of tyrosine between 200-500 µmol/L and phenylalanine between 20-80 µmol/L. If the blood concentration of phenylalanine is too low (<20 µmol/L), additional calculated amounts of phenylalanine should be added to the milk or food of the sick infant to prevent malnutrition.

It is important to recognize that this dietary approach does not prevent SA production, liver or renal disease progression, or reduce the risk of developing HCC or neurological disorders. The dietary modification is helpful in preventing the dermatological and ophthalmological manifestations that arise due to high plasma tyrosine levels. The administration of ascorbic acid (50-200 mg/day orally for 1-2 weeks) may benefit those with pHPPD deficiency or immaturity because it is an important co-factor for pHPPD activity. Also, infants with pHPPD deficiency can apparently escape symptoms, which have been associated with cow’s milk or standard milk formulas, by depending on breast milk as the primary nutritional source.

Use of pHPPD inhibitor 2-[Nitro-4-Trifluoromethylbenzoyl]-1,3-Cyclohexanedione (NTBC) or Nitisinone (Orfadin®)
NTBC was approved by the Food and Drug Administration (FDA) in April 2002 for the management of HRT. NTBC is a potent reversible and competitive inhibitor of pHPPD, thus prevents the accumulation of FAA and MAA and their conversion to SA, which is toxic to hepatocytes and renal tissue.

Although phase II or III trials are not conducted to determine the clinical effects of NTBC, it has been approved for HRT patients because of its significant improvement of liver function. Thus, NTBC is only available in an international study protocol. This is based on an international survey that included 300 patients that had better overall survival (88%) because it prevented the early onset of HCC especially in infants younger than 2 months who presented with HRT (Holme & Lindstedt 1998). Therefore, it is best to prescribe NTBC as soon as diagnosis of HRT is confirmed to decrease the risk of HCC development.

The risk of developing HCC is still evident in NTBC-treated patients and no evidence is available to support that the prevention of HCC can be achieved when using NTBC early in life. Some HRT patients when started on NTBC showed better liver histology and decreased levels of AFP. Other patients showed a reduction in AFP levels followed by a sudden rise of AFP levels with liver histology suggestive of HCC. Therefore, it is possible to state that patients whose AFP levels slowly decrease or is never maintained on a normal value are at higher risk for developing HCC.

NTBC is usually orally given as 1 mg/kg/day and is adjusted to maintain blood drug concentration within the therapeutic range, which is between 40-60 µmol/L. The calculated dosage is usually given in two divided doses, but stable individuals may take it as a single dose owing to its long half-life (50-60 h). If the biochemical parameters did not reach normal after one month of treatment initiation, the dose of NTBC may be increased to 1.5 mg/kg/day or to a maximum of 2 mg/kg/day if needed.

The side effect to benefit ratio is low for NTBC. The rare side effects include transient low platelet and neutrophil count resolved without any intervention and photophobia which will subside following the decrease of tyrosine blood concentrations. Since, NTBC works by inhibiting the catabolism of tyrosine, it is important to maintain a tyrosine-free diet to prevent the precipitation of tyrosine crystals in the cornea forming ocular lesions.

Monitoring of biochemical parameters must be performed monthly for the first year, then every 3 months thereafter. These parameters include plasma amino acids, blood and urinary SA, liver function tests, complete blood count (CBC) and differential,
and serum AFP. The serum AFP may take several months to normalize after NTBC initiation, thus it is best to monitor the trend of decline, which should be continuously downwards. SA levels require about 3 months to be completely normalized.

**Surgery: Liver transplantation**

Liver transplantation was the only definitive therapy for HRT before the availability of NTBC as a therapeutic agent. Liver transplantation carries high morbidity and mortality, thus reserved for those infants who present with severe liver failure and fail to respond to NTBC or have documented evidence of malignant changes in hepatic tissue. Some patients developed HCC despite NTBC treatment for many years. This necessitates intensive follow-up for possible HCC development. Follow-up is usually done by monitoring biochemical markers, including AFP plasma levels.

Although, the long-term effect of NTBC on liver function is still unknown, those patients that underwent a liver transplantation may also benefit from low-dose NTBC to prevent the progression of renal tissue destruction by SA. The augmentation of NTBC may be helpful because liver transplantation alone will not enhance renal tubular function due to the presence of residual extrahepatic FAH deficiency probably in the kidney. (Mieles et al 1990)

Liver transplantation may be accompanied by complications. One of them is the long-term immunosuppression required in transplant recipients, which is associated with a mortality rate reaching 10% or higher (King et al 2008). Another complications of liver transplantation is graft rejection, which may also result in recipient’s death.

**Newborn screening (NS)**

Screening should be done for all infants between 24 and 72 h of birth by pricking the heel of the newborn to obtain a blood sample on filter paper. Each country offers variable genetic NS programs depending on the regional prevalence of a specific IEM.

Internationally, NS is an essential and preventative public health program that permits early detection and intervention of otherwise potentially life-threatening or debilitating inherited disorders. Once a positive result is obtained further diagnostic testing is essential to provide a definite diagnosis since early therapeutic intervention lowers morbidity and mortality.

The use of MS/MS for NS is advantageous, generating fast, low false positive and negative results, high throughput, and with a high sensitivity and specificity allowing simultaneous screening of a range of IEMs in a single assay.

In Kuwait, NS program was established in Kuwait Medical Genetic Center to provide metabolic screening service for four governmental hospitals: Maternity, Farwaniya, Adan and Jahra hospitals. NS is mainly conducted to rule out PKU and congenital hypothyroidism (CH) (Bastaki et al 2006). Screening for other IEMs including amino acids, organic acids, fatty acid oxidation defects was introduced using MS/MS and performed at the Faculty of Pharmacy (FOP), Kuwait University (KU). Therefore, it is of concern to widen the scope of NS by implementing MS/MS as a routine screening tool on a national basis. This is necessary because of the high rate of consanguineous marriage in Arab regions. Since the introduction of metabolic screening service at FOP, fifteen cases of tyrosinemia have been diagnosed, two of which were classified as typical HRT and thirteen were considered as transient neonatal cases.

One of the limitations regarding NS in Kuwait is that not all newborns are tested and the priority is for newborns in the screening care unit. Another limitation is the sample collection and handling, such as insufficient blood sampling or incorrect time of specimen collection. Also, there is a lack of awareness of the carrier parents about the consequences of their inborn infant. The solution is that health care professionals, especially the community pharmacists, should provide genetic counseling and educate high-risk family members about the potential risks to offspring.

Prenatal testing is recommended for high risk pregnancies by analyzing DNA extracted from fetal cells using amniocentesis, which can be performed at 15 to 18 weeks of gestation, or by chorionic villus sam-
pling at 10 to 12 weeks of gestation. Also, prenatal diagnosis of HRT can be made by identifying the presence of SA in amniotic fluid or by measuring FAH enzyme activity in cultured amniocytes. (King et al 2008)

References

1. Which of the following conditions is an in-born error of metabolism?
   A. maple syrup urine disease
   B. tyrosinemia
   C. phenylketonuria
   D. homocystinuria
   E. all of the above

2. In hepato-renal tyrosinemia (HRT), which of the following enzymes is deficient?
   A. glucose-6-phosphate dehydrogenase
   B. amylase
   C. pepsin
   D. fumarylacetoacetate hydrolase
   E. none of the above

3. The usual oral dose of NTBC for the management of hepato-renal tyrosinemia is
   A. 3mg/kg/day
   B. 5mg/kg/day
   C. 1mg/kg/day
   D. 4mg/kg/day
   E. 0.5mg/kg/day

Is there a problem?
A 34 year old man, came to the pharmacy with the prescription shown below, for his dyslipidemia. Is there any major error in the prescription?

KLM HOSPITAL
Patient Name: Mr. Ahmad Ali          Age: 34 years
Address: Street No. 7
Rx
Simvastatin 40mg tablet
Orally at night
Send 1 packet
Gemfibrozil 600mg tablet
Orally twice daily
Send 1 packet
Dr. MXD
Signature                Date: 25/05/12

Answer:
The concomitant use of gemfibrozil and simvastatin may increase the risk of developing myopathy/rhabdomyolysis and is contraindicated.
(Source: MICROMEDEX Database and British National Formulary 63)
Acetaminophen toxicity

Owing to its status as the most widely used pharmaceutical analgesic and antipyretic agent in the United States and the world (contained in more than 100 products), acetaminophen (\(N\)-acetyl-p-aminophenol; APAP) is one of the most common pharmaceuticals associated with both intentional and unintentional poisoning and toxicity, as reported to the American Association of Poison Control Centers. Acetaminophen toxicity is reported as the most common cause of hepatic failure requiring liver transplantation in the UK. In the United States, acetaminophen toxicity has not only replaced viral hepatitis as the most common cause of acute hepatic failure, but it is also the second most common cause of liver failure requiring transplantation.

Acetaminophen is better known as paracetamol. This agent is available in the United States as 325-mg and 500-mg immediate-release (IR) tablets, and as a 650-mg extended-release (ER) preparation marketed for the treatment of arthritis. Various children's dissolvable, chewable, suspension and elixir formulations are available. Furthermore, acetaminophen is a component of many over-the-counter (OTC) cold and analgesic medications and prescription combinations, including codeine-acetaminophen (Tylenol #3) and oxycodone-acetaminophen (eg, Percocet). Hepatotoxicity associated with acetaminophen misuse and overdose is well recognised.

Acetaminophen is rapidly absorbed from the stomach and small intestine and primarily metabolised by conjugation in the liver to non-toxic, water-soluble compounds that are eliminated in the urine. The maximum recommended daily dose is 4 g in adults and 90 mg/kg in children. Toxicity is associated with a single acute ingestion of 150 mg/kg or approximately 7-10 g in adults. However, when dosing recommendations are followed properly, the risk of hepatotoxicity is extremely small.

In acute overdose or when the maximum daily dose is exceeded over a prolonged period, metabolism by conjugation becomes saturated, and excess acetaminophen is oxidatively metabolised by the CYP enzymes (CYP2E1, 1A2, 2A6, 3A4) to the reactive metabolite NAPQI. This has an extremely short half-life and is rapidly conjugated with glutathione, a sulphhydryl donor, and is excreted through the kidneys. Under conditions of excessive formation, or reduction in glutathione stores by approximately 70%, NAPQI covalently binds to the cysteiny1 sulphhydryl groups of cellular proteins, forming adducts. An ensuing cascade of oxidative damage, mitochondrial dysfunction, and the subsequent inflammatory response propagate hepatocellular injury, death, and liver necrosis. Similar enzymatic reactions occur in extra-hepatic organs, such as the kidney, and can contribute to some degree of dysfunction in that organ.

The antidote for acetaminophen poisoning is \(N\)-acetylcysteine (NAC), which is a precursor of glutathione and as such, increases glutathione conjugation of NAPQI. NAC also enhances sulfate conjugation of unmetabolised acetaminophen, functions as an anti-inflammatory and antioxidant and has positive inotropic effects. In addition, NAC increases local nitric oxide concentrations and promotes microcirculatory blood flow, enhancing local oxygen delivery to peripheral tissues. The microvascular effects of NAC therapy are associated with a decrease in morbidity and mortality even when NAC is administered in the setting of established hepatotoxicity. NAC is maximally hepatoprotective when administered within 8 h of ingestion. When indicated, however, NAC should be administered regardless of the time since the overdose. Therapy with NAC has been shown to decrease mortality rates in late-presenting patients with fulminant hepatic failure, even in the absence of measurable serum acetaminophen levels.
In 2009, the US FDA announced requirements for non-prescription and prescription containing medication to provide new information regarding acetaminophen-induced hepatotoxicity. Additionally, the FDA is examining possible removal of acetaminophen from some popular analgesic combination products (eg, Vicodin) and possibly lowering the maximum daily dose. In January 2011, the FDA further announced it was asking manufacturers of prescription acetaminophen combination products to limit the maximum amount of the drug in these products to 325 mg per tablet, capsule, or other dosage unit in the belief that such a limitation will reduce the risk of hepatotoxicity from acetaminophen overdosing.

Source: http://emedicine.medscape.com/article/820200-overview

Antibiotics in animals we eat

For as many decades as antibiotic resistance has thwarted the cure of bacterial infections, scientists have pondered the origins of resistance genes and how they became such a problem. Fingers were pointed at the overprescription of antibiotics in human medicine. Not long after their discovery, however, these miracle drugs were applied not only to sick humans and animals, but to healthy ones as well. Nowhere is this practice more prevalent and controversial than in animal husbandry, where animal feeds laced with small amounts of antibiotic are provided over extended periods of rearing. Labeled as “growth promotion” and employed primarily in large, concentrated feedlots for poultry, swine, and cattle, this nontherapeutic application appeared to fatten the animals faster, prevent rampant herd disease, and help bring healthy animals to market more quickly. While US farmers and other stakeholders have argued tenaciously for the continuation of subtherapeutic dosing, Europeans adopted the “precautionary principle,” instituting sequential bans on the practice beginning in the mid-1990s. Evidence of the negative consequences of low-dose antibiotic feeding has been mounting. Since 1976, several persuasive scientific studies have illustrated how animals fed low-dose antibiotics not only propagate resistant bacteria, but spread these resistant strains to farmers, their families, community residents, and ultimately, hospitalized patients. Particularly worrisome is the continued use in animals of antibiotics that are close structural relatives of those that are used in human medicine. It is feared that, in time, these drugs will lose potency as bacteria express “cross-resistance” to the related drugs. Some researchers have countered that the resistant bacterial strains found in serious hospital infections bear little or no resemblance to the strains found in farm animals. They argue that eliminating antibiotics on the farm would harm animal health, result in economic loss, and have little or no impact on reducing human morbidity and mortality. However, they overlook the transferable genetic elements (e.g., bacterial plasmids, transposons, phages) that can readily share DNA segments bearing resistance genes. These elements pass among strains, species, and even diverse bacterial genera, rearranging and accumulating even more resistance genes.

While still declining to issue an all-out ban on subtherapeutic feeding, the US Food and Drug Administration has taken measured steps in the right direction. First, in 2005, the agency prohibited the use of fluoroquinolones in poultry, and just in January 2012, it prohibited certain off-label uses of cephalosporins in livestock generally. It is a matter of concern, however, that the FDA does not address the ongoing use of penicillins and tetracyclines as growth promoters. Thus, we are still a long way from the steps needed to safeguard precious classes of drugs for effective treatment of human disease. Alternatives to antibiotics in animal husbandry including improving hygiene practices and reducing overcrowding, need to be more fully explored for implementation.

Adapted from: http://the-scientist.com/2012/04/01/antibiotics-in-the-animals-we-eat/
Occupational risk of anti-neoplastic drugs

Because most antineoplastic medications have non-selective mechanisms of action, these agents affect non-cancerous as well as cancerous cells, resulting in numerous adverse effects. When secondary cancers began to develop in patients treated with these drugs, concern was raised that healthcare workers also could be at risk for harmful effects from antineoplastic agents as a result of occupational exposure such as in storage, transportation, and disposal of the drugs.

With the use of antineoplastics, the number of healthcare workers who are potentially exposed to hazardous drugs has increased. However, workers in these areas may not be aware of the risks or may not be properly trained in safe handling of these agents.

Workers potentially exposed to hazardous drugs include the following:

- Shipping and receiving personnel- when handling contaminated medication vials and/or spills;
- Pharmacists and pharmacy technicians- when compounding and checking drug preparations or counting out tablets;
- Nursing personnel- when administering agents and handling contaminated wastes;
- Physicians- when treating patients with or administering hazardous drugs;
- Operating room personnel- when administering medications in the operating room;
- Home healthcare personnel- when transporting drugs, treating patients, and handling wastes;
- Workers in nursing home and long-term care facilities;
- Environmental services personnel- when removing contaminated waste and cleaning contaminated areas or dealing with medication spills;
- Research laboratory personnel- during drug research and development
- Personnel in veterinary practices- when hazardous agents are administered to animals.

Workers may be exposed to a drug throughout its life cycle, from manufacturing, to transport and distribution, to use in healthcare or home care settings, and to waste disposal. Several studies have documented that the outer surface of medication vials can be coated with the contents of the vials when they come from the manufacturer. Vial breakage during shipping is an additional source of exposure for shipping and receiving personnel.

The preparation (compounding) and administration of hazardous drugs have resulted in workplace contamination and exposure of healthcare workers to these agents, especially when complex manipulations are required, such as reconstitution of an intravenous (IV) drug, spiking an IV bag, priming an IV line, and other procedures. Uncoated tablets pose a risk for dermal and inhalation exposure if not properly handled. Cutting, crushing, or other manipulation of coated or uncoated tablets and capsules for pediatric, geriatric, or other uses may also expose workers to unsafe substances.

In pharmacies, most surfaces where antineoplastic medications are prepared will typically be contaminated with these substances. Surfaces such as biological safety cabinets, compounding isolators, countertops, floors, trays, carts, IV bags, computer keyboards, and others have all been found to be contaminated. Nursing stations and patient treatment areas show similar contamination on countertops, tables, chairs, carts, floors, infusion pumps, and patient restrooms.

Following this type of exposure, healthcare workers have been shown to absorb these drugs systemically as evidenced by excretion of the substances in their urine. In some cases, workers who did not physically handle a specific medication have experienced incidental exposure either by inhalation or inadvertent contact.

It has been postulated that dermal contact of contaminated surfaces accounts for most worker exposure, although the production of aerosols and dried drug residue may lead to exposure by inhalation. Another possible route of exposure is hand-to-
mouth after touching contaminated surfaces. Workers who don’t wear personal protective equipment, such as gloves, gowns, and respiratory protection, in the vicinity of drug preparation or administration activities are likely to come into contact with drug-contaminated surfaces many times during the workday. In addition, drug residues have been shown to migrate outside of areas where they are handled, and found in places such as hallways or on computers, posing an exposure risk to unsuspecting workers.

To keep exposure to hazardous agents to a minimum, the best recommendations are those that have been followed in industrial hygiene for many years:

- Use of proper engineering controls
  * Class II, type B2 biological safety cabinets that are 100% vented to the outside or compounding aseptic containment isolators that protect both the drug product and the worker
  * Clean room technology according to US Pharmacopeia Chapter 797 recommendations for hazardous drugs
  * Closed-system transfer devices for drug compounding and administration
  * Implement proper administrative controls, such as training and educating workers, worker evaluation, and other factors that can affect worker safety

Use of the best quality personal protective equipment, such as double gloves, especially gloves recommended for use with toxic agents, non-permeable gowns that are disposed of following each use, and respiratory protection when potential exists for inhalation of hazardous drugs.

However, either from lack of awareness or disregard of safe-handling recommendations, many workplaces are still being contaminated with a number of these drugs, sometimes at high concentrations, and workers continue to be exposed. Several factors may account for sustained worker exposure:

- Contaminated medication vials from the manufacturer continue to enter the pharmacy or other receiving area;
- Increased number of patients requiring treatment with hazardous agents as a result of an enlarging and aging population;
- The ability to use higher doses of medications as a result of amelioration of adverse effects caused by various agents;
- The use of several drugs in combination;
- The use of antineoplastic agents in specialties other than hematology-oncology;
- The use of antineoplastic drugs for certain procedures, such as intraperitoneal administration, isolated limb perfusion, bladder instillation, and others; and the increasing number of hazardous drugs as new, more potent medications are approved.

Unintended gaps, lapses, or breaches in safe working practices can have serious adverse effects. Vials can be dropped, IV bags can leak, IV lines can become loose or separated, and bedpans can spill, all of which can result in substantial exposure of healthcare workers to unsafe substances. Even in the best of facilities, workers and employers need to be aware that work environments, such as shipping and receiving, pharmacies, nursing stations, and patient treatment areas, are among the most likely to be contaminated with these toxic agents.

Adapted from: www.medscape.com/viewarticle/738076

Cancer gene testing gains momentum

Norway is set to become the first country to incorporate genome sequencing into its national healthcare system. This nation will use ‘next-generation’ DNA sequencers to trawl for mutations in tumours that might reveal which cancer treatments would be most effective.

In its three-year pilot phase, the Norwegian Cancer Genomics Consortium will sequence the tumour
genomes of 1,000 patients in the hope of influenc-
ing their treatments. It will also look at another
3,000 previously obtained tumour biopsies to get a
better idea of the mutations in different cancers,
and how they influence a patient's response to a
drug. Tumour biopsies must be of a high quality to
be used in genetic testing or sequencing.

In a second phase, the project will build the la-
boratory, clinical and computing infrastructure
needed to bring such care to the 25,000 Norwe-
gians who are diagnosed with cancer each year.
Norway eventually aims to sequence each cancer
patient's tumour to provide personalized treat-
ments.

Similar projects are under way in the UK and at
research hospitals in the USA, France and else-
where. But Norway's will be among the first to
look for tumour mutations using next-generation
DNA sequencing rather than conventional genetic
testing.

The team first plans to sequence 1,000 genes that
are commonly mutated in cancers, including a
handful for which drugs targeting the mutated gene
products are available or in clinical testing. But the
researchers will eventually sequence all of the hu-
man genome's 20,000 or so protein-coding genes,
collectively known as the exome.

**Saving on sequencing**

More than a dozen drugs that specifically target the
products of cancer-causing mutations are on the
market, but many more are in development. Exome
sequencing could identify, in a single test, the
drugs from which a patient is most likely to bene-
fit. Under the Norwegian programme, physicians
will receive this information, and it will be up to
them and their patients to use it.

Mutations in other genes could predict whether a
patient with cancer is likely to develop resistance
to a targeted drug, or help to explain why a certain
drug isn't working. Norway's challenge will be to
sequence cancer genomes quickly and accurately
enough to guide physicians. The genetic tests that
are currently used to determine whether someone
should be prescribed a particular drug have typical-
ly received a stamp of approval from regulators,
and are performed in certified labs. Clinical-quality
genome sequencing, by contrast, is still in its infan-
cy and few hospitals are yet equipped to offer it.
Every patient with cancer in Norway is tracked un-
der a single system. This will allow oncologists to
easily draw on the experiences of other patients

who have received experimental therapies targeted to
their cancer mutation.

Researchers at Massachu-
setts General Hospital in
Boston designed a single test
that analyses 15 genes target-
ed by existing cancer drugs.
The hospital now tests 60
patients a week, and is col-
laborating with a company to market the test com-
mercially. Instead of assessing tumours for a single
mutation that will indicate whether a drug is likely
to work or not, the hospital tests patients for some
150 mutations in more than a dozen cancer-causing
genes, with the results being used to guide novel
treatments, clinical trials and basic research. This
form of personalized medicine tailors treatments on
the basis of the molecular and genetic characteristics
of a patient's cancer cells, potentially improving the
treatment's outcome.

A UK pilot project for mass genetic screening of
cancers will begin soon. The project will combine
personalized medicine and centralized research,
with the aim of benefiting patients and scien-
tists. The programme will enrol 9,000 people with
breast, colorectal, lung, prostate, ovarian and skin
cancer in the first, two-year phase of the project.
Surplus material from biopsies on the tumours will
be sent to three centralized laboratories and tested
for specific genes and mutations. Eventually it is
hoped that this information can be used to tailor
treatments to a patient's cancer. At the same time,
the data will be held centrally and offer researchers
a resource for improving medicine from the top
down.

France's national cancer institute INCA is leading
a programme to detect biomarkers in lung and colorectal cancers and melanoma. But most existing programmes are focused either on the patient care aspect or on the research side of things.

**Huge benefits**

Creating something similar with thousands of cancer samples and data could bring huge benefits to researchers. If you have a sample set of 3,000 patients, say, where you have all of the DNA already extracted, you have some idea of what treatment they've gone for and their outcomes.

**Personal approach**

The Stratified Medicine Programme is part of a wider thrust towards personalized medicine, which has seen some doctors moving beyond the gene-specific tests proposed under this initiative to whole genome sequencing for patients (see: US clinics quietly embrace whole-genome sequencing). However, for a nationalized health system such as that in the UK, a gene-specific approach is likely to be far more cost effective to apply widely.

This programme is likely to be increasingly important as, for example, drug companies look to identify which patients will benefit from expensive targeted therapies before they are administered. This form of ‘stratified medicine’ uses genetic information to group patients according to their likely response to a particular treatment. The tests, which will look for several dozen mutations in about a dozen genes linked to cancer, will be carried out on people with lung, breast, colorectal, prostate or ovarian cancers, or metastatic melanoma, who are being treated at six NHS hospitals. Therapies that target specific tumour-causing mutations have already been approved, or are on the verge of approval, for most of these conditions.

Testing a clinical sample for so many mutations at once is a challenge in itself. By genotyping patients for a broad array of cancer-causing mutations, the new tests will make it easier to assign subjects to clinical trials.

At Massachusetts General, a broad genetic test detects a mutation in a gene called BRAF that is already known to be commonly mutated in metastatic melanoma. Finding such mutations in people with lung and colon cancer made it possible to put them in a trial of an experimental treatment targeting that gene.

Data could reveal how drugs targeting one molecular pathway are affected by mutations in another gene.

This will pave the way for expanding genetic testing to more patients and other conditions, such as diabetes, AIDS and even psychiatric disorders. Cancer offers a good testing ground for personalized medicine, because numerous targeted therapies already exist.

Adapted from: http://www.nature.com/news/norway-bom-bom-cancer-gene-tests-to-the-clinic-1.9949

---

**IN THE NEWS**

**Experimental drug shows promise against type 2 diabetes**

A recent report in the Lancet (26 Feb 2012) highlighted Phase 2 clinical data of an investigational type 2 diabetes therapy using the GPR40 agonist TAK-875 developed by Takeda Pharmaceutical Company Limited.

These data demonstrated that at doses ranging from 6.25 to 200 mg/day, TAK-875 significantly lowered blood HbA1c levels over a 12-week period versus placebo. This was achieved without significant increase in the incidence of hypoglycemia. TAK-875 is the first GPR40 agonist to reach late stage (Phase 3) clinical development, and completed studies have claimed glucose-lowering effects in patients with type 2 diabetes by stimulating glucose-dependent insulin secretion. Type 2 diabetes is the more prevalent form of the disease, accounting for about 90% of cases. Often tied to obesity, it involves a gradual decline in how insulin responds to changes in blood glucose. TAK-875 designed to enhance the secretion of insulin in response to such changes, which means that it has no effect on insulin secretion when blood sugar levels are normal - potentially reducing the risk for hypoglycemia.

This drug acts in a different way from other diabetes drug classes such as sulfonylureas.
Oxford Nanopore introduces a method to read DNA strands through nanopores and debuts a disposable sequencer that fits in the palm of a hand. Oxford Nanopore Technologies unveiled two new DNA sequencing systems, the high-throughput GridION unit and the portable MinION unit the size of a USB stick.

The systems rely on nanopore sequencing, a method for reading long, unbroken strands of DNA and RNA. A proprietary enzyme pulls the strands through specially engineered nanopores in a polymer membrane. Meanwhile, an electronic chip senses disruptions in electrical current as uniquely identifiable combinations of nucleotide bases pass through each nanopore. At the meeting, company representatives presented data that demonstrated that the 48-kilobase genome of the lambda bacteriophage could be sequenced as a complete fragment.

The nanopore chips process DNA at a rate of 20 -400 bases per second per pore, which is significantly quicker than current sequencing systems. However, the systems have a high error rate of 4%. Before the products are officially launched, the company aims to trim the error down to 0.1-2% by developing better nanopores.

The GridION nodes contain a consumable cartridge with 2000 nanopores, capable of processing tens of gigabytes of sequence data in a 24-hour period. Multiple nodes can be linked together for greater computing power. The portable and disposable MinION will feature 500 nanopores and cost less than $900. While the company has not priced the GridION yet, it expects to charge a cost per gigabase competitive with other systems.

Such an affordable technology could transform diagnostic medicine. “We could scan patients and see what viruses or bacteria they have,” said David Deamer, who developed the original idea for nanopore sequencing more than 20 years ago. Soon after, Deamer went on to collaborate with physical scientist John Kasianowicz of the National Institute of Standards and Technology and chemical biologist Hagan Bayley who were studying a pore called alpha-hemolysin. In a paper published in the PNAS in 1996, the group described the nanopore sequencing technique. In 2005, Bayley joined technologist Gordon Sanghera to found the company that would become Oxford Nanopore Technologies.

Nanopores are very well suited to simple sample preparation and a new type of informatics workflow where the system is programmed to run the experiment until sufficient data has been gathered to conclude the experiment.

By 2013, Oxford Nanopore hopes to sequence an entire human genome in just 15 minutes. This will require 20 GridION nodes equipped with 8,000 nanopores each.

Adapted from:

Once-daily triple HIV drug

August 10, 2011 - FDA granted approval of Complera, a once-daily, 3-drug combination for treatment-naive HIV-infected patients.

Complera is a single tablet that contains rilpivirine (Edurant, Janssen Pharmaceuticals), a nonnucleoside reverse transcriptase inhibitor (NNRTI), and tenofovir (Viread, Gilead Sciences) and emtricitabine (Emtriva, Gilead Sciences), both nucleoside reverse transcriptase inhibitors (NRTIs).

A bioequivalence study demonstrated that the tri-
A single-drug tablet achieved the same blood levels as each of the drugs administered individually. The recommended dose of Complera is 1 tablet, containing emtricitabine 200 mg, rilpivirine 25 mg, and tenofovir 300 mg, once daily, to be taken with food.

**Cautions, adverse reactions**

FDA issued the following cautions in announcing the drug's approval:

- More patients with HIV-1 RNA levels greater than 100,000 copies/ml at the start of therapy experienced virologic failure vs patients with lower HIV-1 RNA levels at the start of therapy with rilpivirine.
- The virologic failure rate in rilpivirine-treated participants conferred a higher rate of overall treatment resistance and cross-resistance to the NNRTI class compared with efavirenz.
- More patients treated with rilpivirine went on to have lamivudine/emtricitabine-associated resistance compared with efavirenz.

The most common grade 2 to 4 adverse drug reactions to rilpivirine, occurring in 2% or more of patients, are insomnia and headache. The most common adverse drug reactions to emtricitabine and tenofovir disoproxil fumarate, occurring in 10% or more of patients, are diarrhea, nausea, fatigue, headache, dizziness, depression, insomnia, abnormal dreams, and rash.

Emtricitabine/rilpivirine/tenofovir DF is contraindicated in patients taking the following drugs:

- the anticonvulsants carbamazepine, oxcarbazepine, phenobarbital, and phenytoin
- the antimycobacterials rifabutin, rifampin, and rifapentine
- proton pump inhibitors, such as esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole
- more than a single dose of systemic dexamethasone
- St. John's wort (*Hypericum perforatum*)

These drugs may cause significant decreases in rilpivirine plasma concentrations and may result in a loss of virologic response and possible resistance to rilpivirine or to all NNRTIs.

Additional FDA warnings related to the drug indicate that caution is needed regarding prescribing Complera with drugs with a known risk for torsade de pointes, and that monitoring bone mineral density should be considered in patients with a history of pathologic fracture or other risk factors for osteoporosis or bone loss. Severe depressive disorders have been reported with Complera; if these occur, immediate medical evaluation is recommended for severe depressive disorders.

**Use in special populations**

The following should be considered regarding use of Complera in specific populations:

- The drug is pregnancy Category B, meaning that it should be used during pregnancy only if the potential benefit justifies the potential risk. Because of the potential for HIV transmission, women infected with HIV should be instructed not to breast-feed.
- Complera is not recommended for patients younger than 18 years, and clinical studies of the component drugs did not include enough participants 65 years and older to determine whether they respond differently than younger participants.
- Dose selection for elderly patients should be cautious, considering the higher prevalence of hepatic, renal, or cardiac impairment, and concomitant disease or other drug therapy.
- Because Complera is a fixed-dose combination, it should not be given to patients requiring dosage adjustment, such as those with moderate, severe, or end-stage renal impairment and creatinine clearance of less than 50 ml/min.
- No dosage adjustment of Complera is needed in patients with mild or moderate hepatic impairment, but it has not been studied in patients with severe hepatic impairment.
STATE OF KUWAIT
Pharmaceutical & Herbal Medicines Control and Registration Administration

New Pharmaceutical products approved from November 2011 to May 2012

- Acne Biotic Pain; Erythromycin-40mg, Zinc Acetate-12mg; The Arab Drug Co.
- Acuvail Ophthalmic Soln.(for single use); Ketorolac Tromethamine-4.5mg; Allergan.
- Adancor Tablets 10, 20mg; Nicorandil-10, 20mg; Merck KgaA.
- Amaryl M SR Tabs 2/500mg; Metformin HCL-500mg, Glimepiride-2mg; Handok Pharm. Co. Ltd.
- Appi-D Syrup; Pizotifen-0.5mg; Deef Pharm. Industries.
- Arzerra Conc. for Soln for Infn; 100mg, Ofatumumab-20mg; Glaxo Group Ltd.
- Benefix Powder & Solvent for Solution for Injection 250,500, 1000, 2000 IU; Nonacog Alfa- 250, 500, 1000, 2000 IU Sodium Chloride (0.234%)-5ml; Wyeth Europa Ltd.
- Brilinta Tabs 90mg; Ticagrelor-90mg; Astrazeneca AB.
- Bydureon pdr & solvent for PR Suspn; For Inj. 2mg, Exenatide-2mg, Carmellose Sodium-20mg, Sodium Chloride-5mg, Water for Inj. – 0.75ml; Eli-Lilly.
- Candistan Vaginal Cream 2%; Clotrimazole-20mg;The Arab Drug Co.
- Candistan Vaginal Tabs-200mg; Clotrimazole-200mg; The Arab Drug Co.
- Carboplan Solution for Infn 150mg/15ml and 450mg/45 ml; Carboplatin-150mg and 450mg; Vianex S.A
- Citrin Syrup; Cetirizine HCl-5mg; Deef Pharm. Ind. Co.
- Clinoleic Emulsion for Infn 20%; Olive Oil Refined-160mg, Soyabean Oil refined-40mg; Baxter SA.
- DBL Gemcitabine Soln for Injection 200mg/5.3ml and 1g/26.3ml and 2g/52.6ml; Gemcitabine-200mg, 1g, 2g; Hospira Pty. Ltd.
- Defonase Syrup; Loratadine-5mg, Pseudoephedrine-60mg; Deef Pharm. Ind. Co.
- Dexamethason - Rotexmedica Soln. for injection 5mg/ml; Dexamethasone Sodium Phosphate-5mg; Rotexmedica.
- Doloraz Capsules 400mg; Ibuprofen-400g; JPM.
- Dolorgan Infusion (1g/100ml); Paracetamol-1g; Hikma Pharma.
- Dompidone Tabs; Domperidone-10mg; The Arab Drug Co.
- Ecata Powder for conc. for Solution of Infusion 100mg; Anidulafungin-100mg; Pfizer Ltd.
- Elonva Solution for Inj. 100, 150ug, Cortifollitropin Alfa-100, 150ug; N.V. Organon.
- Enemax Enema; Monosodium Phospate-16gm, Disodium Phosphate-6gm; Amoun Pharma Co.
- Erbitin Solution for Infusion 500mg/100ml; Cetuximab -500mg; Merck KGaA.
- Erbitux Solution for Infusion 100mg/20ml and 250mg/50ml and 50mg/10ml; Cetuximab -100, 250, 50mg; Merck KGaA.
- Fexigra Tabs 120, 180mg; Fexofenadine-120, 180mg; Cipla Ltd.
- Flohale 50, 125 HFA Inhaler, Fluticasone Propionate -50, 125ug, Cipla Ltd.
- Foster Pressurised Inhalation Soln. 100/6mcg; Beclometasone Dipropionate-100mcg, Formoterol Fumarate Dihydrate-6ug; Chiesi Farmaceutici S.P.A.
- Funzol 150 Capsules; Fluconazole-150mg; JPM.
- Galacetam Tabs – 250, 500, 1000mg; Levetiracetam-250, 500, 1000mg; Galenicum Health SL.
- Galin Tabs 75mg; Clopidogrel-75mg; Galenicum Health SL.
- Galmedix Tablets 1mg; Anastrozole-1mg; Galenicum Health SL.
- Galpro FC Tabs 150, 300mg; Irbesartan-150, 300mg; Galenicum Health SL.
- Galtrol Tabs 2.5, 5, 7.5, 10mg; Olanzapine-2.5, 5, 7.5, 10mg; Galenicum Health SL.
- Gastro – Soothe tabs 10mg; Hyoscine Butylbromide-10mg; AFT Pharma Ltd.
- Haemodialysis Acid Conc. For Bicarbonate Dialysis (QPC-1, 2, 3), (QPF 1, 2, 3, 4, 5, 6, 7); Many ingre
The Kuwait Pharmacy Bulletin (ISSN 1028-0480) is published quarterly by the Faculty of Pharmacy, Kuwait University, and includes a list of recently approved drugs from the MOH. It aims to provide instructive reviews and topical news items on a range of drug related issues. It is widely distributed free within the university, to hospitals, polyclinics & private pharmacies as well as to other universities within the Gulf & Middle East region.

The information in this bulletin does not necessarily reflect the views of the editorship, nor should it be taken as an endorsement of any product that is mentioned herein. Articles are generally adapted from literature sources and rewritten or occasionally reproduced with permission from the appropriate sources.

Readers wishing their own copy may ask to be added to the mailing list by contacting the Executive Editor.

Executive Editor: Yunus Luqmani.  Assistant Editors: Leyla Hasan Sharaf, Samuel Koshy

Editorial Office: Faculty of Pharmacy, Health Sciences Centre, Kuwait University, PO Box 24923 Safat, 13110 Kuwait, Fax:25342087; email: yunus@hsc.edu.kw

Answers to: Test your knowledge

Correct answers:
1-E; 2-D; 3-C