In February 2013, the U.S. Food and Drug Administration approved Kadcyla (ado-trastuzumab emtansine), the first antibody-drug conjugate approved for human epidermal growth factor receptor 2 (HER-2) positive metastatic breast cancer\(^1,2\). Manufactured by Genentech, Kadcyla contains the established agent Herceptin (trastuzumab, approved in 1998 and also manufactured by Genentech), a recombinant monoclonal antibody product developed to target the human epidermal growth factor receptor 2 (HER-2) overexpressed by certain cancer cells\(^3\), which is linked to a microtubule inhibitory drug DM1 via a thioether linker\(^2\).

The HER-2/neu (c-erbB-2) gene encodes a transmembrane tyrosine kinase receptor protein from the epidermal growth factor receptor (EFG) family\(^4\). Various investigations since the late 1980s have evaluated abnormalities of the HER-2/neu gene in patients with breast cancer, finding correlations between HER-2/neu gene amplifications and disease outcome\(^5,6,4\). HER-2 over-expressing tumors account for 25-30% of breast cancers\(^7,3\) and display aggressive cancer progression\(^7,6\) and shortened survival\(^8\). Treatment with targeted therapies against HER-2, alone and in combination with cytotoxic chemotherapy has been shown to prolong survival, increase overall response rates and increase time to recurrence in patients with HER-2/neu over-expressing primary tumors\(^7,6\). Previously approved drugs for HER-2 positive breast cancer includes the humanized HER-2 antibody trastuzumab, (Herceptin, 1998), lapatinib (Tykerb, 2007), a dual tyrosine kinase inhibitory binding HER-2 & the EFG, and most recently pertuzumab (Perjeta, 2012) a HER-2 dimerization inhibitor\(^4,5,10\).

The coupling of cytotoxic agents to monoclonal antibodies is a recent novel approach in pharmaceutical development and an alternative to therapy antitumor antibodies alone.\(^10\). These antibody drug conjugates (ADCs) combine the targeting ability of antitumor antibodies with the potent cell killing activity of small molecule via a biodegradable linker, and have become a major focus of targeted cancer medicine. ADCs are expected to offer an advantage over traditional chemotherapy because by delivering the cytotoxic agent directly to the cancer cells, normal tissue exposure is minimized, leading to potentially fewer side effects and a more favorable toxicity profile\(^11,12\). Currently, other conjugates approved...
by the U.S. FDA include Adcetris (Brentuximab vedotin, for relapsed Hodgkin and anaplastic large cell lymphoma) and Mylotarg (gemtuzumab ozogamicin), although the latter was withdrawn in June 2010, ten years after it was approved for acute myeloid leukemia\textsuperscript{12,13,14}.

Kadcyla \cite{Kadcyla_2013} is a targeted ADC for the HER-2/neu gene, that combines trastuzumab, an antitumor humanized monoclonal antibody with the cytotoxin DM1, a maytansine derivative and microtubule inhibitor. Emtansine refers to the complex formed by DM1 and and MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate)) the thioether linkage between the two complexes\textsuperscript{2}.

Derivatives of maytansine, an antimitotic drug, bind directly to microtubules, similar to the traditionally used vinca alkaloid antineoplastic agents (e.g. vincristine, vinblastine), disrupting the microtubule network and causing cell cycle arrest and cell death. The conjugated design of ado-trastuzumab emtansine allows for intracellular release of DM1-containing cytotoxins when the drug binds to the HER-2 receptor, thus delivering DM1 directly to HER-2-overexpressing tumor cells only and sparing healthy cells\textsuperscript{2,10}.

The FDA approval of ado-trastuzumab emtansine was based on a multicenter, phase III open labeled trial where 991 patients with unresectable locally advanced or metastatic breast cancer, shown were randomized to receive either ado-trastuzumab emtansine or lapatinib (Tykerb, GlaxoSmithKline) and capecitabine (Xeloda, Genentech). All subjects had confirmed HER-2 positive cancer (assessed by IHC analysis and/or FISH), had previously been treated with a taxane and trastuzumab- based regimen, and experienced progression of disease during or following treatment. Patients in the study group received ado-trastuzumab emtansine 3.6 mg/kg as an intravenous (IV) infusion every 21 days, and patients in the lapatinib/capecitabine group received 1250 mg lapatinib orally once daily with 1000 mg/m\textsuperscript{2} capecitabine twice daily on days 1-14 of the 21 day cycle. Patients who had received prior treatment with ado-trastuzumab emtansine, lapatinib or capecitabine were excluded from the study. Median survival rates were 30.9 months with ado-trastuzumab emtansine compared to 25.1 months with lapatinib + capecitabine\textsuperscript{2}. Median progression-free survival also improved by 50% in patients who received ado-trastuzumab emtansine\textsuperscript{8}.

During the trial, the most frequently observed adverse effects with ado-trastuzumab emtansine include fatigue, nausea, musculoskeletal pain, thrombocytopenia, headache, increased transaminases and constipation\textsuperscript{8}. Other reported serious toxicities include hepatotoxicity and reduced left ventricular ejection fraction (LVEF). All patients taking ado-trastuzumab emtansine should have serum transaminases, bilirubin, and LVEF regularly monitored and treatment should be withheld or discontinued for clinically significant reductions in cardiac or liver function. Pregnancy should be verified prior to initiating treatment, and appropriate contraception use is advised in women of childbearing age as ado-trastuzumab emtansine has also been reported to cause embryo-fetal death or birth defects.
New Developments in Antibody-Drug Conjugates for Cancer Treatment: Kadcyla® for Late Stage Breast Cancer
Aleena M. Cherian

Kadcyla (ado-trastuzumab emtansine) cannot be used interchangeably with Herceptin (trastuzumab) 1, 2.

The NCCN Guidelines for breast cancer were updated in March 2013 to recommend ado-trastuzumab emtansine as a preferred therapeutic option for HER-2+ recurrent or metastatic disease in patients previously treated with trastuzumab 13. There are also ongoing phase II studies for the use of ado-trastuzumab emtansine as adjuvant or neoadjuvant therapy in earlier stages of HER-2+ cancers 16, 17. More information is available at www.kadcyla.com.

References

“There are also ongoing phase II studies for the use of ado-trastuzumab emtansine as adjuvant or neoadjuvant therapy in earlier stages of HER-2+ cancers.”
Note from the Chair–Elect

Hope your all enjoying spring after our long hard winter. Of course now that the sun has come out to stay its time to start thinking about the AACC annual meeting. I want to let you know about two activities we have planned for Meeting Week 2014. The first is the time and date for our annual Division meeting and luncheon. This will take place on Monday 7/28 from 12:00 until 2:00 pm and will be held in the Wrigley room of the Hyatt Regency Hotel. This is always a good place to catch up with distant colleagues and here the latest of what is up with the Division.

I would also like to invite you to join Dr. Patrick Kyle and I on our second try at a poster walk on Tuesday 7/29 from 1:00 till 2:00pm. As you may recall we attempted this last year with modest success and this year we’re looking to increase the number of participants as well as showing our support for Division members actively engaged in research. This is a great way to see what is hot in our area and to interact with students and colleagues regarding “new” science.

The TDM/Tox division will be recognized by Dr. Steve Wong, AACC President.

Please submit your Division Election ballots in time! The ballots will be electronically sent out by the end of May.

I look forward to seeing you all in Chicago!

Jim Ritchie

UPCOMING MEETINGS OF INTEREST

AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY (AACC)
Annual Meeting
July 27—31, 2014, Chicago, IL.
www.aacc.org

THE AMERICAN ACADEMY OF CLINICAL TOXICOLOGY
North American Congress of Clinical Toxicology (NACCT)
October 17—21, 2014, Sheraton New Orleans Hotel, New Orleans, LA.
www.clintox.org

SOCIETY OF FORENSIC TOXICOLOGISTS (SOFT)
Annual Meeting
October 2014, Grand Rapids, MI.
www.soft-tox.org
Yohimbine (YH) is a widely available alkaloid that has been used as an aphrodisiac, mild hallucinogen, dietary supplement and erectile dysfunction (ED) drug. It has also been reported to increase salivation so could potentially be a treatment for dry mouth (i.e. xerostomia) (1). While it appears that the drug is relatively well-tolerated in low doses, a dose dependent toxicity exists and multiple case reports highlight its adverse effects, some of which can be life threatening (2). Although much has been written about YH, data regarding its toxicity and serious adverse reactions continue to emerge. Due to its side effect profile, recent popularity as a bodybuilding supplement and consistent marketing as an aphrodisiac, a brief reexamination of the drug will be undertaken.

It is important to note that there are two commercially available forms of this drug. The most common form is an over-the-counter (OTC) herbal extract; also known as Yohimbe for the tree that is its primary source. The other form is a purified salt of YH derived from Yohimbe tree bark. This drug is FDA approved and has been prescribed since the 1930’s for the treatment of ED (3). Unfortunately these two forms are often discussed interchangeably which may give the erroneous impression that they are synonymous. Most of what is known about YH is based on clinical trials on the purified salt and characteristics of this manufactured form may not necessarily be applicable to the natural extract; however, there is a great degree of overlap between them. This short review will focus on the known pharmacology and side effects of YH, based on clinical trials and published case reports, with a brief discussion of its efficacy.

Yohimbe refers to the evergreen tree *Pausinystalia yohimbe* (formerly known as *Corynanthe yohimbe*) indigenous to Western Africa from which YH is derived (1, 4). Some common names for prescribed YH include Aphrodyne, Yocon, and Plain Prowess while the herbal extract is sold under a broad variety of names. Some of these OTC herbal products are illustrated in Figure 1. Due to the increasing international popularity of YH supplements the Yohimbe tree has been overharvested and is threatened with extinction. An additional botanical source of YH is the flowering plant *Rauvolfia serpentina* (Indian snakeroot) that is native to India and East Asia (1).

Historically, YH has been used for several centuries in Africa as an aphrodisiac and to enhance sexual performance. Other uses were to treat fevers, leprosy, and heart problems, and warriors used it as a stimulant before battle. The powder was smoked to induce hallucinations, and YH poultices were placed on the skin as an antiseptic and analgesic. In the 1890s YH was first used as a medicine in Europe and it is currently prescribed in the U.S. to treat impotence.

**Pharmacology**

YH (17α-hydroxyyohimb-an-16α-carboxylic acid methyl ester) is the principal indole alkaloid of over 50 isolated thus far from Yohimbe tree bark. It has selective α₂-adrenergic receptor antagonist properties and weaker α₁-receptor antagonist properties (4). It has a molecular weight of 354.44 g/mol, chemical formula of C₂₁H₂₆N₂O₃ and its chemical structure is depicted in Figure 2. The standard adult therapeutic dose prescribed for ED is 5.4 mg tablets taken three times a day. After oral ingestion YH is rapidly absorbed by the gastrointestinal tract within 45-60 minutes and is primarily cleared by the liver, with less than 0.1% of a dose recovered unchanged in the urine within 24 hours (4, 5). A 10 mg oral dose has been shown to produce a peak blood level of 289 ng/mL at one hour and 115 ng/mL at four hours (6). There is some variability reported in the plasma T½ but the overall mean is around 0.6 hours. YH undergoes extensive first pass metabolism and ~82% is bound to plasma proteins. Two phenolic metabolites appear in urine within 24 hours of ingestion; 11-hydroxy YH and 10-hydroxy YH (7).
Laboratorians tasked with the detection and quantification of amphetamine and cathinone derivatives will continually be in a state of catch-up as dozens of biologically active variants are known or can be readily designed and synthesized, which elude current detection methods.

Medical Uses & Efficacy

YH has been approved for use in the U.S. to treat ED, although its effectiveness has been highly controversial. This drug has also been abused on the streets as a purported aphrodisiac and mild hallucinogen, while more recently it has gained significant popularity in the body building community for its lipolytic and stimulant effects that allegedly increase athletic performance (2). Based upon limited experimental data YH may have a potential future clinical use as a treatment for dry mouth. The public commonly views OTC YH as a readily available ED treatment that is far less expensive than newer ED medications like the cGMP-specific phosphodiesterase type 5 enzyme inhibitors (ex: Viagra/ Sildenafil). YH is thought to promote penile tumescence by increasing the release of nitric oxide, and by antagonizing the stimulation of α2-adrenergic receptors by norepinephrine at the presynaptic site, causing decreased intracellular Ca2+ and increased relaxation of penile smooth muscle (4). As a natural aphrodisiac, YH’s performance appears to be quite mixed at best. Multiple YH studies from the 1980’s and 1990’s were conducted on patients with varying forms of ED (organic, psychological or drug-induced). Some studies showed statistically significant improvement in erectile function compared to placebo; however, in those same studies less remarkable results were seen when patients were crossed over from placebo to the treatment group. The most noteworthy studies included one with 215 males (ages 26-78) with ED and various underlying medical illnesses, who were treated with YH daily for 6 weeks (4). Approximately 62% subjectively reported no improvement, 33% reported partial improvement, and 5% had full improvement. Of the group with no or partial subjective improvement, about half were administered a higher dose for up to 11 months and 52% then reported further improvement. A more objective measure of erectile performance with YH treatment was obtained in a 1997 study on 85 males with ED but no clear underlying etiology (4). Penile rigidity was measured by polysomnography and demonstrated a 71% improvement in penile response in the YH treated group versus 45% in the placebo group. Further supporting its potential to promote penile tumescence is a case report of a refractory sustained, painful erection (i.e. priapism) for over 20 hours in a 42-year-old male with no underlying cause other than ingesting the YH supplement the previous day. Ultimately pharmacological intervention was unsuccessful to correct the priapism and surgery was required (8).

The evidence supporting the OTC YH efficacy has led some researchers to support its use as an ED therapeutic option (9). However, others have concluded that the current data doesn’t support its efficacy (10). The 2005 American Urological Association guidelines on ED management concluded that YH isn’t a recommended treatment based on their comprehensive review of the data (1).
Dry mouth is another clinical condition for which YH is reported to be therapeutically beneficial. Many prescribed drugs such as antidepressants (e.g., tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors), and certain diseases like Sjögren’s Syndrome often produce dry mouth. YH has been shown to possess the ability to activate cholinergic pathways that leads to increased salivary secretion (4). Experimental administration of YH to patients suffering from dry mouth secondary to TCA administration led to significant increases in saliva production for up to 4 hours at relatively low doses (6). This YH application may have therapeutic implications and warrants further studies.

In recent years YH has also become a popular weight lifting supplement allegedly capable of decreasing body fat and being an appetite suppressor (2, 4). A 6 month dual gender study to determine its impact on body weight and fat distribution showed no effect (4), and other studies have found no demonstrable metabolic enhancements (2). Further, a 3 week study involving athletes showed YH had no effect on body muscle mass and no consistent impact on sports performance (2).

Toxicity and Laboratory Analysis
Overall, YH appears to be well-tolerated by most in low to moderate doses; however, its side effects and potential toxicity at higher doses cannot be ignored. Common symptoms reported with YH overdose include anxiety, drowsiness, disorientation, tremors and seizures.

Figure 1. Some commercially available YH extract products.
There exists a limited amount of published literature about toxic levels of YH in blood and the first fatal levels were just recently published (11). No antidote currently exists for YH overdoses and treatment is largely symptomatic. The California Poison Control System identified 238 cases of YH-associated adverse reactions from 2000-2006 with 98.7% involving the herbal supplement (12). The average age of complainants was 39 years and 77% were males. Commonly cited reasons for YH ingestion were sexual enhancement (27.7%), weight loss (9.2%), and stimulant effects (7.6%). Typical reported adverse drug events were 45.7% with gastrointestinal distress (including nausea, emesis, dizziness and abdominal pain), 43.1% tachycardia, 32.9% anxiety/agitation, 24.7% hypertension, 20.0% flushing/erythema, 14.0% diaphoresis, 13.2% tremor, 12.1% chest pain, 9.9% chills, 7.3% tachypnea/shortness of breath, 6.0% mydriasis, and 5.4% had altered mental status. Overdoses leading to neurotoxic effects have been seen from YH doses of 200-5,000 mg. It is worth noting that the first generation hypertension drug reserpine has a chemical structure with similarities to YH (Figure 2). Like YH, reserpine is an indole alkaloid that is naturally derived from the Rauvolfia serpentina plant. It is rarely prescribed today due to the development of more efficacious drugs and safety concerns.

Several case reports of life-threatening events attributed to YH ingestion have been published, and we will describe four. A case of acute neurotoxicity occurred in a 37-year-old male after ingesting ~5 g of YH during a body-building competition. He arrived at the hospital in an unconscious state; other symptoms included marked hypertension, tachycardia, and seizures (13). Supportive treatment involved intubation and the administration of diazepam and clonidine. He fully recovered after 12 hours. At 3 hours post-ingestion his YH blood level was 5,240 ng/mL. A 16-year-old girl ingested 250 mg of YH and developed weakness, generalized parasthesia, decreased coordination and a dissociative state within 20 minutes (14). These complications had resolved by 36 hours. A 38-year-old man ingested 350 mg of YH for ED with no other co-ingestions, he developed atrial fibrillation and tachycardia that resolved within 24 hours (15). The patient appeared confused, lethargic and had retrograde amnesia that lasted 4 days. In the last case study, a 42-year-old man after ingesting 16.2 mg of YH developed a desquamative skin eruption with lupus-like syndrome and progressive renal failure, but recovered after receiving supportive treatment (16).

Deaths associated with YH use are extremely rare, with only two reported in 2003 by the American Association of Poison Control Centers (12). No additional information was provided about these fatalities such as the presence of other co-ingested drugs like ephedra. In October 2013 two fatal case studies were published that were attributed solely to YH ingestion (11).
In the laboratory, YH has been detected in whole blood, plasma, serum and many other biological fluids via liquid or gas chromatography and an assortment of detectors. At present, mass spectrometry is the typical detection system used and only a few reference labs have a validated quantitative method.

YH continues to be a widely used dietary supplement marketed towards males with claims of enhanced sexual potency and athletic performance. The evidence supporting these claims is both mixed and inconclusive. There are significant concerns regarding safety and toxicity that clinicians and laboratorians should be aware of. Although the YH supplement is more easily accessible and significantly cheaper than prescription ED drugs, it is not without side effects or potential for adverse outcomes. Consumers are encouraged to become informed about these side effects and dangers prior to use, and to avoid exceeding dosage directions.

Figure 2. Structure of YH (top) and Reserpine (bottom).
References

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