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Breast Cancer

Last lecture, we talked about breast cancer and its related chemotherapy. We said that we start by hitting hard, essentially by using those two drugs: Doxorubicin, which is the main drug to be used in breast cancer, but it has serious side effects limiting its use, mainly causing cardiotoxicity. We also use Cyclophosphamide (alkylating agent), which binds to DNA and produces strand breaks, but also it has a degree of toxicity (cystitis).

Cancer treatments' toxicity problem is derived from the fact that they are unselective; they target every dividing cell in your body, and they actually affect normal replicating cells more than they affect cancer cells (which have certain phenotypes allowing them to evade apoptosis and making them less susceptible – resistant actually- to many of these agents).

After finishing 4 cycles, we start giving a drug called Paclitaxel (mitotic spindle inhibitor), and our strategy this time is different, we try to produce a cell cycle specific activity –on the M phase-. We may give 4 to 8 cycles depending on the aggressiveness of the cancer.

In many cases (20% of breast cancer patients), there is gene amplification and over expression of *HER2* on the cell surface, and this constitutes a tempting target for treatment. We use a drug called Trastuzumab (Herceptin). It is very expensive, but it's a nice drug, because it's an example of targeted therapy (so different from other anticancer drugs). Hence, it is not associated with serious side effects, not even the four main side effects produced by anticancers (alopecia/bone marrow suppression leading to anaemia/ GI disturbances like nausea, vomiting and diarrhoea/ immunocompromisation). It's absolutely wrong to give a HER-2 negative patient this drug (useless). Note that this drug is not given with Doxorubicin, due to cardiotoxicity exacerbation (synergic effect).

There are some types of cancers (mainly prostate, endometrial and breast cancer) that depend on hormones. The problem with breast cancer is that it has a driver which is estrogen, which somehow increases the growth of cancer cells, and cancer cells produce too much ER (estrogen receptor) on their surface. Note that not all breast cancer patients are ER positive (80-85%). So our target is to inhibit this effect, by using one of these mechanisms:

1-using a hormone with an opposing action (physiological antagonism).

2-Hormonal antagonists.--> give Tamoxifen

3- Inhibition of synthesis of the hormone.

Note that when estrogen level in the female body decreases, this will produce osteoporosis (same as in menopause).

Tamoxifen is a Selective estrogen receptor modulator (**SERM**), so not truly classified as agonist or antagonist, it's a modulator. It has estrogenic and anti-estrogenic effects on various tissues. The good news is that its effect on bone tissue is estrogenic, while having anti-estrogenic effects on cancer cells. The problem of Tamoxifen is that it has a partial agonist activity on the endometrium, thus increasing risk of endometrial cancer.

- Tamoxifen side effects include hot flushes, depression, increased risk of uterine cancer and blood clots.
- Taken daily by mouth for 5 years (***to prevent recurrence***)
- Clinical trials have shown that taking this drug more than 5 years on a daily basis may increase the risk of endometrial cancer by 1-2% (if increase duration→ increase risk). So Tamoxifen is **only** to be used for 5 years.
- When used prophylatically, Tamoxifen has been shown to decrease the incidence of breast cancer in women who are at high risk for developing the disease (such as *BRCA-1* mutations, which increases the risk of breast cancer by 40%). The good news is that breast cancer is the only cancer that we can use prophylaxis with its regard. Since a lot of the cases are ER positive, Tamoxifen is a valuable prophylactic target. We prophylact for 5 years. The question is when? This is determined by the doctor (the oncologist) taking some factors into consideration like the geographic area. For example, places like China and south east Asia, the cancer onset is early (30-35 years). In our region, for example, cancer onset is between 40-48 years, and in the west it's sometimes more late than that (depending on the genetic makeup). So we (here in Jordan) may use Tamoxifen prophylaxis around the age of 35-40.

Pharmacogenetics

- There is a branch in medicine called Personalised Medicine (prescribing a drug for a patient based on the genetic makeup of the patient). This is because of the genetic variations between individuals. One way of this variation is what is called single nucleotide polymorphism. (Change in one nucleotide like T becoming C).

- This is present in all human beings, so there are no 2 humans on earth with the exact same DNA-except identical twins-. A source of genetic material change is single nucleotide polymorphism**s,** because every 700-1000 nucleotides in the human genome, we'll find a different nucleotide between different individuals. By knowing that we as humans have around 3 billion nucleotides in our genome, then there are around half a million nucleotides different between different humans. This is what makes us different from each other and gives each human his/her distinctive characteristics.

- So as we all (as humans) have originally-from the first father of humanity- the same DNA, different humans and their progenies developed different single nucleotide polymorphisms in different locations in the genome, making this a source of human variation.

- An important scenario is that a single nucleotide polymorphism occurred in an exon (the coding part that will exit the nucleus as mRNA and get translated into a protein), then a codon will change, so the corresponding amino acid will change, and eventually this may change the protein.

- If the affected protein is a drug target (like a certain receptor), when this change occurs and the protein shape (3D structure) changes, then we'll expect that the binding of the drug to it will be altered (not normal, so the affinity may change, the complementarity in shape and interactions may change). Now imagine if the change was in a metabolizing enzyme, like *CYP450,* the activity will be changed (may increase or decrease). Now imagine that the change occurred in a transporter (P-glycoprotein), the function will change.

- All of this will lead us to an important subject which is Personalised Medicine. I have to know if my target is functioning or not, will there be a binding or not? As an example, 16% of Jordanians do not respond to

Salbutamol (β2 agonist) when given to them. The reason is that one amino acid-numbered 16- in the β2 receptor primary structure changed from Arg to Gly. So the receptor shape is altered, and giving Salbutamol doesn't lead to good binding and no effect is seen. So by nature, some people don't show bronchodilation when taking Salbutamol, because the target is not there (very loose binding) and we give them other drugs for the desired effect.

- Now what if the mutation is in a metabolizing enzyme? Let's look at Tamoxifen. Tamoxifen is a prodrug; it needs activation by *CYP2D6* to be converted to the active form which is **Endoxifen.**

  Note: there are 2 pathways for the activation of Tamoxifen, the major one depends on *CYP2D6* mainly, while the other requires several *CYP450* enzymes. Each pathway results in a certain metabolite before production of Endoxifen. Endoxifen is 100 times more active than the metabolites or Tamoxifen itself.

- If there's a Single nucleotide polymorphism in the *CYP2D6* gene, this will result in weak activation of the drug. This individual is called poor *CYP2D6* metabolizer. These are individuals who lost the **2 alleles** of the enzyme gene. Now, if an individual loses only **1 allele**, then he's called intermediate *CYP2D6* metabolizer (some residual enzyme activity is present, but not as efficient as if the 2 alleles are present). While an individual with 2 normal, functional alleles is called an extensive *CYP2D6* metabolizer.

- Some humans are called ultra-rapid metabolizers, they don't have just 2 copies of the enzyme gene, they have more (4 or8 or 12…). These have **higher metabolic capacity** than the extensive metabolizers.

- For a drug like Tamoxifen, it's very important to know where does the patient stand. Is she a poor/intermediate/extensive/ultra rapid metabolizer? This will actually determine the needed dose and will affect her life (esp. if used as prophylaxis, cuz it's not easy to give a patient a large dose only to prophylact her). So I must take a blood sample from her to identify her genetic characteristics.
  If she's a poor metabolizer→ the drug is not being activated, so I must increase the dose.
  If intermediate→ increase the dose but not like the poor type.
  If extensive→ keep the dose as it is. The dose is made for these.

If ultra rapid→ decrease the dose.

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- To sum up, all human beings are one of the following :

1. Poor metabolizer (PM)

- has low metabolic capacity
- Has two mutant alleles

2. Intermediate metabolizer (IM)

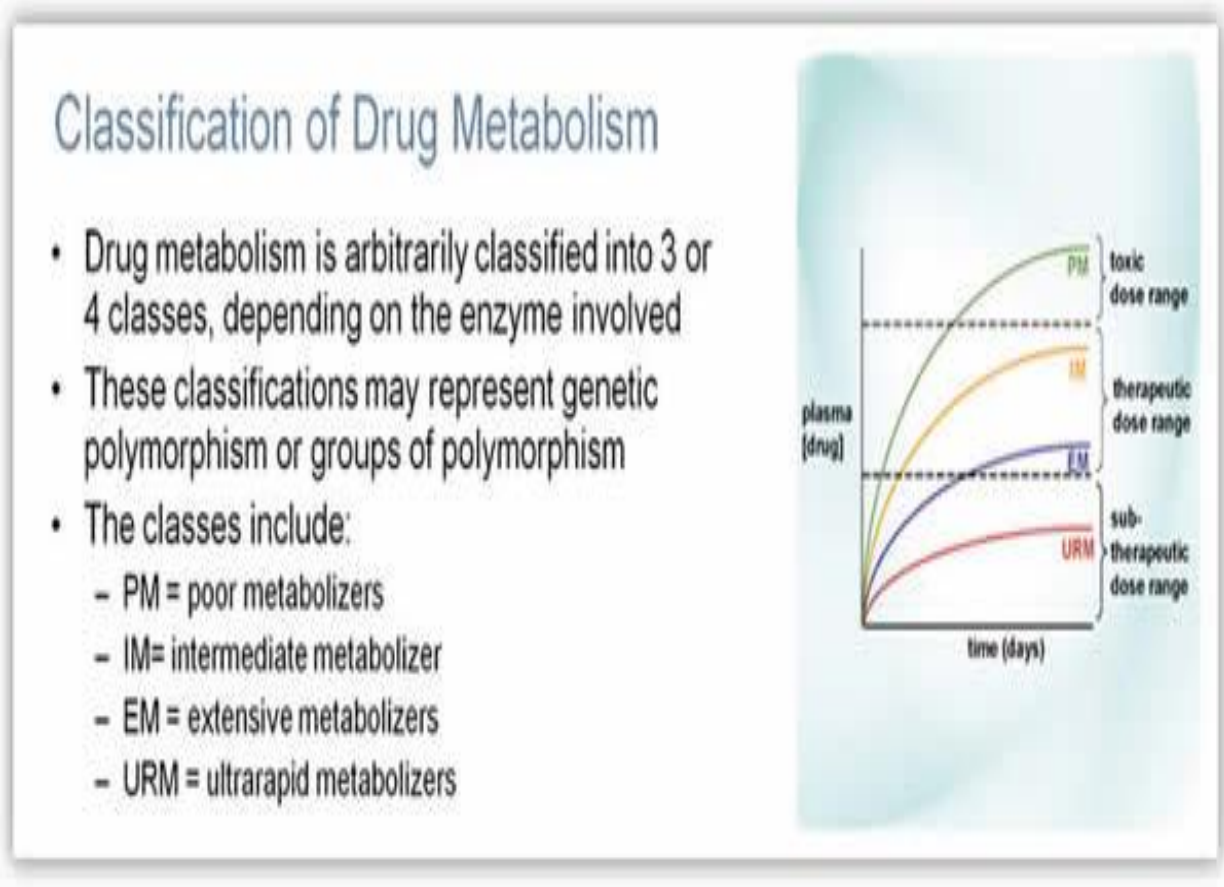
- has metabolic capacity between PM and EM
- has one reduced activity allele and one normal

- **3. Extensive metabolizer (EM)**

- has regular metabolic capacity
- has at least one and no more than two normal functioning alleles

- **4. Ultra rapid metabolizer (UM)**

- has higher metabolic capacity than EM
- Has multiple copies of functional alleles



*For a normal drug which is **inactivated** by the enzyme, it's the opposite

of Tamoxifen. If poor→ accumulation so I must decrease the dose.
If intermediate→also decrease the dose (but not like poor).
If extensive→ leave everything as it is.
If ultra rapid→ very fast clearance so I must increase the dose.

- For a population like Jordan, a study was carried out by *Dr.Malik Zohlof* and his colleagues about the frequencies of *CYP2D6* genotypes on a representative group of Jordan University students, and the results were

Predicted Phenotype	**Count (192)**	**Frequency (%)**
Poor metabolizer	5	2.6
Intermediate metabolizer	41	21.1
Extensive metabolizer	120	62.5
Ultra rapid metabolizer	26	13.5

- So a drug like Tamoxifen really does worth it to know the genotype of the patient, especially that our population has 13.5% ultra-rapid (very high percentage).
- This difference in genotype between individuals can be a major source of side effects, because drugs (and their doses) found in the market are allocated to Extensive metabolizers, and the half life and the steady state of the drug is based on that. So a poor or an ultra-rapid metabolizer is prone to a jump in the concentration of the drug in the body (think about toxicity) or very fast clearance from the body. If there are sufficient cases with serious side effects, then the drug may be withdrawn from the market.

  <u>*Side note:*</u> a drug called Codeine (prodrug that is converted by *CYP2D6* to morphine) was usually prescribed for post-partum pain (if the mother feels pain). A woman who took it was an ultra-rapid *CYP2D6* metabolizer (but no one knew) so her body readily and efficiently produced high amounts of morphine, which is very lipophilic, and actually was taken up by the baby during breastfeeding→the baby had a respiratory arrest and died. After that it was prohibited to give Codeine post-partum. So it is very important to know where do you stand and what is the patient's genotype.

- A drug like Cyclosporine can have different concentrations when given to a group of people, because its level in blood is affected by many factors, such as age, sex, body-mass index, kidney function, liver function and the level of the metabolizing enzymes. All these factors together clearly state that the level of the drug **cannot** be the same for 2 individuals (too many factors→more variation) because no 2 persons can have all equal (same everything) regarding those factors.

- Where is this important? In ***Narrow-therapeutic index drugs***, the patient will either experience toxicity or loss of activity, and you are actually limited to a narrow window (must be careful with the concentration if we are talking about narrow therapeutic index), so you have to dose your patient according to his genotype.

- When talking about cancer, we have to be very careful. Cancer drugs are toxic, and any change in their concentration will make a difference. An important example is Tamoxifen:
  If ultra-rapid→ high endoxifen→good activity but **high side effects**.
  If poor→ you are taking that dose for nothing, **must increase dose**.

- Polymorphisms may also affect the transporter of the drug to its site of action. An example is a polymorphism in Apo-E (which is required for endocytosis in hepatocytes), which constitutes a means for Statin drug to enter liver cells and inhibit Cholesterol synthesis, thus lowering its plasma concentration. If Apo-E changed→no entering of drug→no inhibition→ no lowering of Cholesterol.

- Origin of these polymorphisms: (mainly for self-benefit (self reading))

*A lot of the polymorphisms found in China are not found in the Middle East, and Europe's polymorphisms are different as well, so different areas→different conditions→different selective pressure on DNA→ different polymorphisms to adapt different situations.

*There are some polymorphisms that have a high frequency in the Middle East, for example, that also occur (but to a lesser extent) in a place like Spain or south Europe-generally-,but you won't find it in North Europe, for example. This is due to the history of these nations (way back when Arab Muslims were in Al-Andalus (Spain), so those people had lived together and married from each other and passed their polymorphisms to their progeny. But Arab never went-generally speaking- to Northern Europe, so you'll find a totally different genotype).

*There's an origin for every polymorphism, if you follow the frequency of a particular polymorphism and the chronological sequence of events throughout history (how people moved from one place to another, and the fact that people marry and mix up their genes), you'll find a sequence on the map guiding you to the origin of it (usually it's the place

with the highest frequency of that polymorphism).

*So the distribution of Poor/intermediate/extensive/ultra-rapid differs from one place to another. You cannot say that Sweden population has 13.5% ultra-rapid *CYP2D6* metabolizers just because Jordan has that.

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To sum up:
Polymorphisms may affect the drug's target→Salbutamol
Polymorphisms may affect the drug's transport→Statin
Polymorphisms may affect the drug's metabolism→Codeine/Tamoxifen

All of this affects the activity of the drug, and this brings us back to *Pharmacodynamics/Pharmacokinetics*, and also shows the need for ***Personalised Medicine*** (adjustment of the dose for every single individual according to his genotype).

Remember

There is no pill that fits us all

THE END