Old Herborn University Seminar Monograph 36: The microbiome and cancer.
A summary of the lectures given during the 36th Old Herborn University Seminar
Writing committee: Peter J. Heidt, Tillmann Rusch and Volker Rusch.
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RELATIONSHIP BETWEEN GUT MICROBIOTA AND SYSTEMIC CHEMOTHERAPY

Introduction

Dr. Aadra Bhatt (Division of Gastroenterology & Hepatology, University of North Carolina at Chapel Hill, USA) presented data about the relationship between gut bacteria and systemic chemotherapy.

Medications can influence intestinal bacteria

Over the last years there is an increase in research that describes the reciprocal relationship between medications and the intestinal bacteria. It is known that medications can influence the microbiota by altering intestinal pH and the osmotic balance, and many medications can have an impact on barrier integrity. Medications can also affect nutrient availability for the microbiota and many drugs, including a class of antipsychotics, have been recently described to have bacteriostatic side-effects. So even drugs of which we do not think as being antibiotic can influence the vitality of our microbiome.

Intestinal bacteria can influence medications

However, another important observation is that microbiota can alter medications. Microbiota can alter nearly every aspect of drug metabolism that includes absorption, metabolism, distribution and excretion, which are basically the cornerstones of pharmacokinetics.

A number of host factors can regulate how we respond to medications. These include our age, our biological sex, physiological states such as pregnancy, our environment, our underlying genetics and ethnicity. These factors are unmodifiable. Ultimately these contributors to drug response are fixed and not changeable. However, intestinal bacteria are major contributors to drug response and this is important because microbiota are modifiable. They are one of the few modifiable contributors to drug response. This is a field that requires intense investigation because ultimately it can improve drug usage, drug tolerability, and drug access.

Antibiotics do attack our bacteria while those same bacteria are really important for homeostasis of the whole biont, for maintaining our health and for who we are as people. The goal of Dr. Bhatt's research is to identify the mechanisms by which microbiota alter drug metabolism and selectively target this with the purpose to preserve the integrity of the entire microbiome.

Pharmaco-microbiomics

Pharmaco-microbiomics is the study of microbiota and drug interactions. This is a very new and upcoming field. Dr. Bhatt's laboratory has studied drug microbiota interactions as to improve precision medicine by targeting bacteria and in particular specific bacterial functions. Dr. Bhatt gave a few specific examples of how bacteria influence drug metabolism and she started with two medications that are very widely used but they are not used in the context of cancer (Figure 1). One is digoxin, a cardio-protective drug that is converted by an enzyme expressed by a bacterium called *Eggerthella lenta*. There is a reduction of the one double bond in digoxin which converts digoxin into its inactive form called dihydrodigoxin. This is an example by which this bacterial function can be selectively targeted to preserve the efficacy of digoxin. A converse example is the activation of a compound called sulfasalazine which is used to treat ulcerative colitis. There is a class of bacterial enzymes called azoreductases that convert the azo-bond

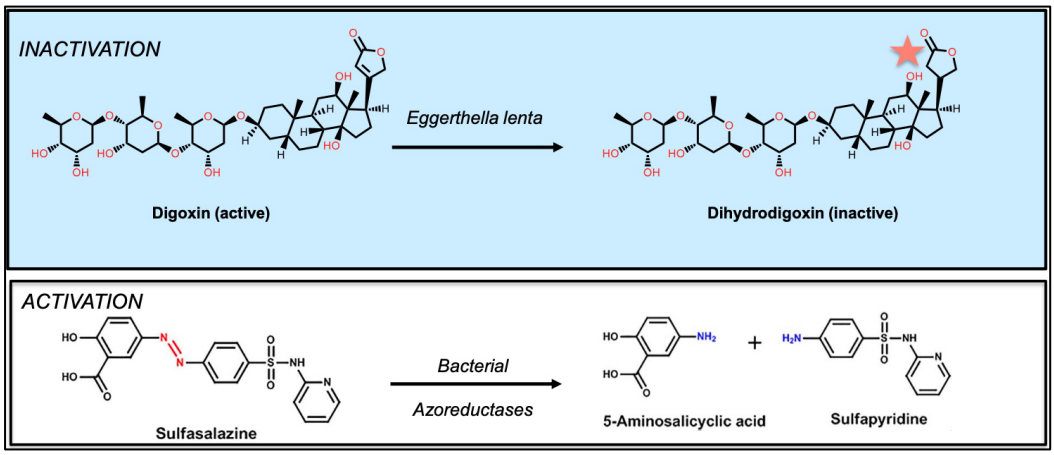

Figure 1: Gut bacterial enzymes directly alter drug efficacy. (Figure adapted from *Ervin* et al., 2020).

and release the protective compound called 5-aminosalicyclic acid which is an immunomodulatory compound that exerts the beneficial effects of sulfasalazine for people with ulcerative colitis.

Dr Bhatt recently published that patients suffering from ulcerative colitis that don't have this class of bacterial azoreductases in their gut are not actually going to derive any benefit from sulfasalazine treatment.

Another example of bacterial modification of a medication is levodopa converted by *Helicobacter pylori* into dopamine. Dopamine is unable to cross the blood-brain-barrier and patients who have Parkinson's disease early stage derive no benefit from levopoda treatment if they also have a concurrent *Helicobacter pylori* infection.

Bacterial enzymes can also increase the gastro-intestinal toxicity of 5-fluorouracil (5FU), which is a very widely used cancer drug which can also increase the nephrotoxicity of acetaminophen (paracetamol). Bacterial conversion of paracetamol into para-aminophenol results in a toxic metabolite that can cause kidney damage.

Bacterial enzymes can also convert molecules like para-cresol, which is generated during bacterial fermentation of protein in the human large intestine into the molecule called para-cresol sulphate. Para-cresol sulphate compete for the same detoxification enzymes that our body uses to detoxify paracetamol. This competition causes accumulation of toxic metabolites of paracetamol that again can exert nephrotoxicity.

It is also known from studies in germfree mice that germfree mice have a high expression of the constitutive androstane receptor (CAR) which is important for drug metabolism and this actually causes different responses to anaesthetics that are used for surgery.

One of the most exciting examples of how drug metabolism can affect host disease is that of choline. Choline is a dietary compound found very highly concentrated in red meat and eggs. Bacteria convert choline into trimethylamine (TMA) which is then subsequently oxidised by a bacterial enzyme responsible for drug metabolism called flavin mononucleotide (FMN) and generates the molecule called trimethylamine N-oxide (TMAO) which is cardiotoxic and linked to cardiovascular disease. TMAO has been very strongly associated with atherosclerosis.

Those are examples by which bacterial metabolism directly generates

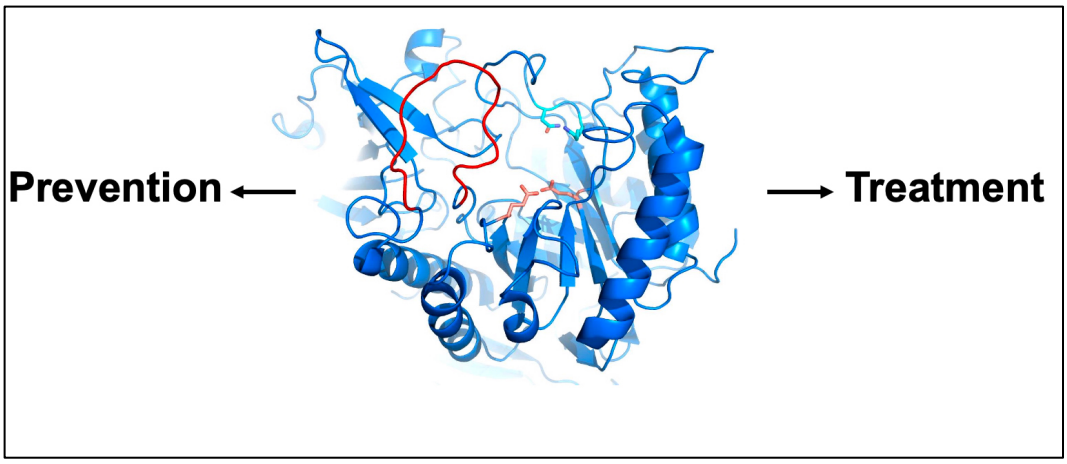

Figure 2: Monomer of *E. coli* ß-glucuronidase. (Figure from *Walace* et al., Science 2010)

molecules that can exert various kinds of toxicity or altered drug responses. There is an additional mechanism how bacterial enzymes interact with phase II conjugates that our body generates in response to detoxifying compounds. Whenever we take a medication that is hydrophobic, these hydrophobic molecules are conjugated in the liver by a class of enzymes called uridine-diphosphate-glucuronosyltransferases (UGT's) with a small 6-carbon sugar: glucuronic acid. This conjugation generates a hydrophilic molecule called hydrophilic glucuronide conjugated compound, making it easier for the body to excrete through urine or bile. There are additional Phase II conjugation reactions including sulfation.It are not just xenobiotics that are being recycled in this way. Antibiotics and substances that we make, such as hormones and transmitters like serotonin are also recycled by the same mechanisms. So these conjugated molecules are inactive and they are unable to exert their chemical effects and are considered to be inactive. The generation of these hydrophilic compounds allows them to be easily eliminated through urine or faeces. When they are eliminated through the faeces they encounter a class of bacterial enzymes called ß-glucuronidases which, as the name suggests, hydrolyses the glucuronide conjugate from these inactive molecules and convert them into active molecules in the gut. This is because glucuronic acid is a source of carbon in the highly competitive environment in the gut. Bacterial ß-glucuronidase or GUS is a non-essential carbon scavenging enzyme that is essential in humans because its deficiency causes a type of lysosomal storage disease called Sly syndrome. However, in bacteria is GUS a non-essential enzyme that is involved in carbon scavenging. It is not essential because when it is knocked-out of a lab strain of *E. coli*, the "knock-out GUS" shows the same sort of fitness and growth as the wild-type strain.

The loop

Figure 2 shows a monomer of *E. coli* ß-glucuronidase and deep within the enzyme is this catalytic site which is able to actually bind to a glucuronic acid molecule and adjacent to the catalytic site is this red floppy motif which is called "the loop". The loop is like a molecular clamp that holds the glucuronide conjugate really close to the active site so that the hydrolysis reaction can occur very efficiently. The catalytic site of ß-glucuronidase is highly

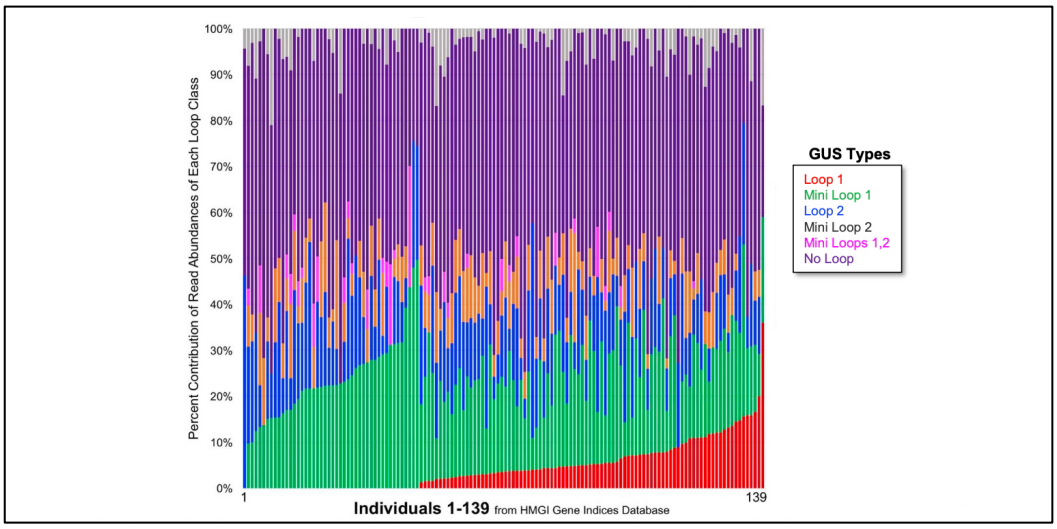

Figure 3: GUS types in 139 healthy individuals in the Human Microbiome Project catalogue. (Figure from *Pollet* et al., Structure 2017).

conserved among all bacteria and this catalytic site is used almost as a "bait" to delf through the Human Microbiome Project. When looking through the faecal database of the Human Microbiome Project, using this catalytic site as "bait", a number of structural features of ß-glucuronidase can be identified that cluster into six specific types, which are termed "Loop 1" such as those expressed by *E. coli*, "Loop 2" such as those expressed by sero-Bacteroides, "No Loop", "Mini-Loop 1" and "Mini-Loop 2" and bacteria that have both "Mini-Loop 1" and "Mini-Loop 2". Each one of these loop structures is essential for determining the substrate specificity. The specific loop motive directs the type of substrate that each of these bacterial enzymes has a specificity towards.

Over the last few years we have been really delving deeply into what are the specific substrates for each of these loop types. Every bacterium in every phylum have their unique GUS, so we know this is a very widely expressed bacterial enzyme. What all the structural and molecular work has helped to understand is that these features are absent in the million ortholog ß-glucuronidases and this is important because it allows to selectively target the bacterial isoforms while leaving the million enzymes unperturbed. This is very important because GUSes are essential for how we process various polysaccharides.

Each one of the bars in Figure 3 is an individual from the Human Microbiome Project. There are 139 bars and the different colours represent a specific loop type. Only about 2/3 of all individuals express "Loop 1" GUSes. This is really important because "Loop 1" GUSes, are the specific bacterial enzymes that are involved in deconjugating drug glucuronide-conjugates.

Cancer drugs and gastrointestinal toxicity

Table 1 shows a list of FDA approved cancer medications that are all detoxified in the liver by conjugation with glucuronic acid. Important is that all of these drugs are causing gastrointestinal toxicities, specifically diarrhoea. Irinotecan, as an example, is used for treating colorectal cancer and also sometimes pancreatic cancer; either alone but most usually in combination with other

Table 1: Cancer drugs detoxified by glucuronic acid conjugation via Phase II metabolism cause gastrointestinal toxicity

Dasatanib	Bicalutamide	Mycophenolate	Vandetanib	Daunorubicin
Irinotecan	Sorafenib	Epirubicin	Olaparenib	Cyclophosphamide
5-Fluorouracil	Bevacizumab	Vorinostat	Etoposide	Bortazomib
Anastrozole	Panobinostat	Afatanib	Axitinib	Fulvestrant
Bexarotene	Regorafenib	Capecitabine		

compounds. Irinotecan is administered intravenously. It is first converted into a molecule called SN38 by plasma carboxylesterases. SN38 has almost a 10,000 fold higher affinity than irinotecan to bind its cellular target which is topo-isomerase which is an enzyme that is important for unwinding DNA during DNA replication. Irinotecan or SN38 can selectively target highly proliferative cells such as cancer cells. The gut is a highly proliferative organ that turns over once every five days which is a high rate of proliferation. Irinotecan is known to cause severe diarrhoea of which the only way to resolve it is to suspend therapy. Stopping treatment with their anti-cancer drug is of course for someone undergoing treatment for cancer not a really good idea.

SN-38 is detoxified in the liver by an enzyme called UGT which binds glucuronic acid to SN-38 generating SN38-glucuronide which is inactive and unable to bind to the topoisomerase I enzyme. SN-38 is excreted via faeces where bacterial ß-glucuronidases encounter this glucuronic conjugate and hydrolyse it forming an SN38 molecule in a site where it probably should not be.

Several years ago, selective inhibitors of bacterial ß-glucuronidases were developed and when they were administered in concert with irinotecan to naïve mice, the weight loss and bleeding and diarrhoea that mice experience with irinotecan treatment could be stopped with co-administration of a GUS inhibitor.

When irinotecan is administered to mice and the activity of bacterial ß-glucuronidases is examined in the faeces (*in fimo*), an increase in total gut activity after administration is observed. As soon as a substrate is put into the mix, activation of the bacterial enzyme is a result. This was replicated using gnotobiotic facilities at UNC. Germfree wild-type C57 black six (C57BL6) mice were colonised with either a wild type *E. coli* strain or the isogenic mutant that lacks a functional ß-glucuronidase. Mice were colonised for a week and treated with a single dose of irinotecan, after which the proliferative pool of intestinal stem cells in the colon as well as ileum were examined (figure 4).

In these mice proliferation was qualified using *in vivo* BrdU (5-bromo-2'-deoxyuridine) labelling. In mice that were colonised with the wild type strain of *E. coli* and subsequently treated with this proliferation inhibitor, a reduced number of proliferative cells was observed (the dark spots in the lower micrographs of figure 4). Fewer or hardly any dark spots are seen in the colon of mice colonised with the wild type *E. coli* strain compared to the mice that were colonised with the isogenic ß-glucuronidase mutant (upper panel of the micrographs in figure 4). This also demonstrates the importance of bacterial ß-glucuronidase in exerting the toxicity in the gut of irinotecan.

Because there are very few ways to treat triple negative breast cancer (TNBC), two different mouse models of triple negative breast cancer were used: one was an xenograft model in which immunodeficient mice were injected

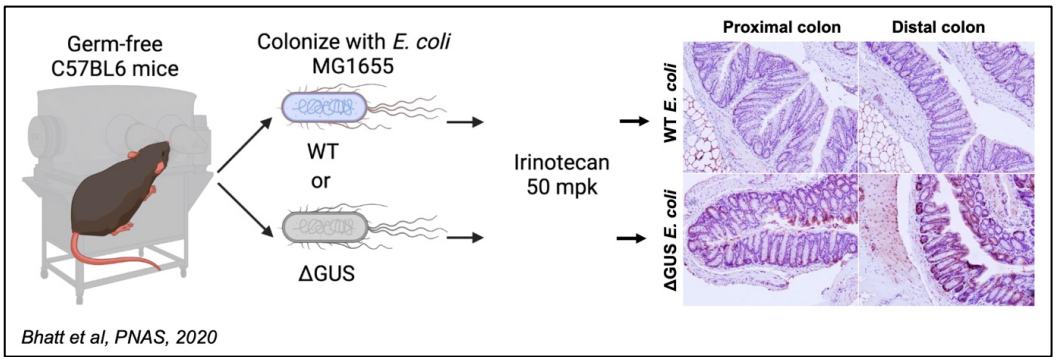

Figure 4: Germfree wild-type mice colonised with GUS-deficient *E. coli* are protected from irinotecan-mediated injury

with TNBC cell line, and the other model was a genetically engineered mouse model (GEMM) for TNBC in which the SV40 large T antigen drives mammary epithelial cell specific expression of the T antigen. In both models, tumours were allowed to develop to hundred cubic millimetres after which the study was initiated. The mice were randomised into 4 groups in which mice received irinotecan alone or with a next generation GUS-inhibitor in combination and of course the respective control groups (GUS-inhibitor alone or vehicle). In both models it was found that irinotecan alone was able to reduce tumour growth and the co-administration of the GUS-inhibitor did not change tumour volumes when measured serially (figure 5).

At the end of the study the tumours were dissected out of these mice. The total weight of the tumours also didn't differ in either group and in the GEMM it was found that the tumours were practically undetectable. This protection might be largely due to the prevention of diarrhoea. The diarrhoea that resulted even from irinotecan treatment in the mouse model was so severe that mice did lose up to 20% of their body weight, which is the humane cut off in the protocol. Co-administration of the GUS-inhibitor allowed a majority of the mice to remain diarrhoea-free for a longer time which resulted in preserved body

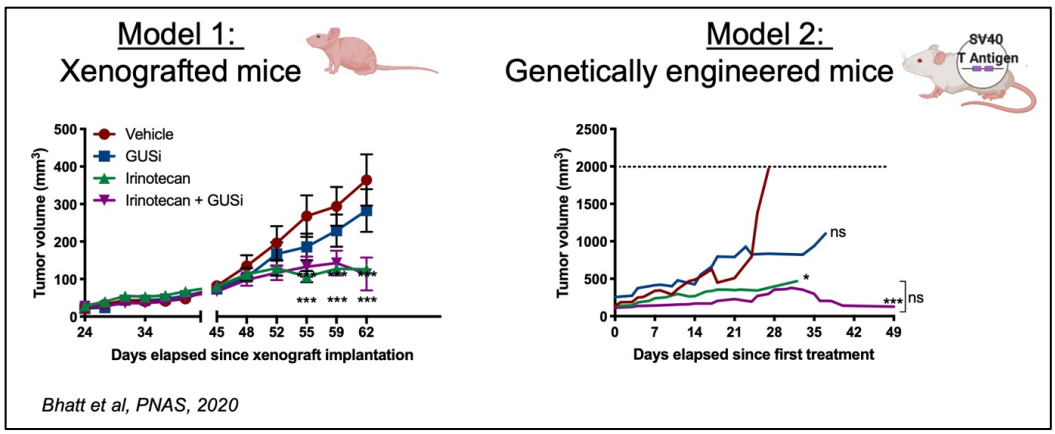

Figure 5: GUS-inhibitor co-treatment does not impede irinotecan's anti-tumour effects in murine models

weight, which again resulted in the mice to be able to withstand higher or larger number of doses of irinotecan. This is a big win because very often people fail treatment because of the side-effect and not because of the actual treatment on its own.

Personalised chemotherapy

The described experiments serve as a proof of concept that selectively modifying specific microbiota function, as opposed to wiping out entire classes of bacteria, might be a good way to improve drug response by reducing the toxic side effects that are exerted by the microbiota. This is an example of how we can use pharmaco-microbiomics to improve drug responses.

The long-term goal is to personalise chemotherapy to improve drug responses. *In fimo* drug reactivation rates may serve as a prognosticator of adverse drug responses. By quantifying the rate of turnover of glucuronides it will be possible to stratify individuals to be at high, medium or low risk of developing intestinal side-effects.

As mentioned earlier, multiple chemotherapy therapeutic drugs are detoxified by conjugation with glucuronic acid. But it is not just chemotherapy that is detoxified in this way. This might also be true for more drugs that, for instance, are being used for gout, and drugs like raloxifene that is being used for treating osteoporosis, or metformin that is being used for treatment of diabetes type 2. These are compounds that are detoxified by conjugation with glucuronic acid and many of them also have often diarrhoea side-effects.

Summary

- Bacterial drug metabolism can explain the inter-individual variability in drug responses.
- ß-glucuronidases can reactivate conjugated drug metabolites in the gut.
- Selective and non-lethal GUS-inhibitors can be a useful strategy to block drug-glucuronide activation in the gut.
- "Drugging the bug" can be an effective strategy to improve on drug response.
- Microbiome targeting can improve precision medicine.

This paper was reviewed by Dr. Aadra Bhatt before publishing.

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