we are pleased to have dr deanna crowds

deanna is a professor in the department

of bioengineering and therapeutic

science

in the school of pharmacy at the

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she received her bsn pharmacy from ohio

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deanna was a pratz fellow at the nih in

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the years including aaps new

investigator award in pharmacokinetics

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a fellow of aaps and aaas

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research interests are focused on

understanding the mechanisms underlying

interindividual variations drug

response and toxicity

deanna served

as the director of pharmaceutical

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graduate program at ucsf from 00 to

09

she is currently the deputy

editorinchief of clinical and

translational science please enjoy

todays lecture

im deanna kretz from the department of

bioengineering and therapeutic sciences

in the schools of pharmacy and medicine

at the university of california san

francisco

and im going to be talking to you today

about phase metabolism

so ill start with just an overview of

the characteristics of phase two

metabolism

followed by more details about three

specific conjugation reactions

glucuronidation sulfation

and glutathione conjugation and then

ill end with a summary of what ive

covered

so just to orient everyone

to what were going to be talking about

today

so drugs and and others you know biotics

can be metabolized to phase one

metabolites largely by the cytochrome

p0 enzymes and this will be covered in

a separate lecture

but these xenobiotics and drugs can also

be

conjugated to what is referred to as a

phase two metabolite

this can be either a direct reaction

with the parent drug

or it can be conjugation of a previously

formed phase metabolite

and these phase metabolites then are

eliminated from the body either

in the urine or in the bile so this is

an interesting analysis of about 00

substrates where they looked at

the

sequential metabolism of these

substrates and captured all their

metabolites looking at about 00

distinct metabolites and on average this

was about six metabolites per substrate

and what they found is that the initial

metabolites that were formed were

largely phase one metabolites about 0

percent

of these initial re reactions were

catalyzed by phase one enzymes mostly

p0s about 0

were hydrolytic reactions and a little

over 0

were direct phase ii conjugation

reactions

but as these initial metabolites became

further metabolized

conjugation reactions or phase

reactions become quantitatively more

important

as second generation metabolites they

represent about 0

of the metabolites that were formed

and as these metabolites are metabolized

even further

to third and further generation

metabolites they approach about 0

of the metabolites that are formed

so for a phase ii reaction

functional groups on the drug or

xenobiotic interact with endogenous

substrates

and they do this through the use of

activated cofactors

the conjugation reactions that comprise

phase ii reactions are glucuronidation

sulfation

acetylation

methylation

amino acid conjugation and glutathione

conjugation

well talk about a couple of these in

more detail

the main purpose of these phase ii

reactions is to

increase significantly the

hydrophilicity

of these metabolites

which will then facilitate their

elimination

and typically these conjugation

reactions lead to elimination of the

conjugates into both the urine and the

bile

quantitatively glucuronidation and

sulfation are the most important

conjugation reactions for drugs

and glutathione conjugation

is very important

for eliminating highly reactive

electrophilic metabolites

so these phase two reactions occur

at nucleophilic sites in drugs and

xenobiotics

and conjugation at these nucleophilic

sites by

um

glucuronidases

sulfatases and amino acid conjugating

enzymes

leads to an increase in water solubility

that will facilitate their elimination

from the body

and conjugation of these nucleophilic

sites leading to

an acetylation or methylation

increases the nucleophilic reactivity of

these conjugates

in addition

conjugation can occur at electrophilic

sites

on drugs and xenobiotics

and these conjugates that are formed

eliminate the reactivity of these

electrophiles and they increase water

solubility

and this is glutathione conjugation

this is just a summary of the

conjugation reactions that occur

whats interesting here is unlike the

phase one reactions which largely occur

in the endoplasmic reticulum in the

microsomal fraction of the cell

most of the conjugation reactions occur

in the cytosol

with the exception of glucuronidation

which also occurs in the endoplasmic

reticulum

there are specific enzymes that are

needed to catalyze each of these

reactions as well as specific cofactors

well talk in more detail about a few of

these reactions

quantitatively the most important

conjugation reaction is glucuronidation

followed by

sulfation

and then glutathione conjugation

and so those are the three that were

going to talk about in more detail

and acetylation and methylation

amino acid conjugation they can occur on

drugs but quantitatively theyre much

less important

so we will start with glucuronidation

and glucuronidation can occur on a

nucleophilic oxygen which is represented

here

but it can also occur on a nucleophilic

nitrogen

sulfur or carbon group

the cofactor for this reaction

is uridine fibrin diphospho alpha d

glucuronic acid or udpga

the structure is shown here

and this glucuronic acid moiety at this

end of the molecule

will

react with the drug to form this

glucuronic acid conjugate

this alpha deep with uronic acid

forms a beta glucuronide

through a backside attack

and these glucuronide conjugates are

mostly charged at physiological ph with

a pka of about three to four

this is catalyzed by a glucuronazole

transferase enzyme

these enzymes the udp glucuronocele

transferases are referred to as udpgt or

new gts

these are microsomal proteins the only

conjugation reaction that occurs in the

endoplasmic reticulum

as i just mentioned the cofactor is

udpga

and importantly these are low affinity

enzymes but they are high capacity

and that is why they are quantitatively

the most important

for phase metabolism

and glucuronidation is typically the

most common phase reaction that youll

find

there are human isoforms of the ugts

and the most important for human drug

metabolism are ggta

a

a a a9 and ugtb

this is some data from bhagwa prasads

laboratory at washington state

university where they have used

proteomics to quantify

the protein level of the ugts both in

the liver as well as in the intestine

and you can see a different distribution

of these ugts in these two tissues

so for example in the liver

ugtb is the most abundant isoform

followed by ugtb

where in the intestine ugtb

is the most abundant isoform

it represented a very small fraction of

what was present in the liver

and this is important for understanding

which of these isoforms are going to be

more important for

glucuronidation

in the liver and intestine

heres an example of the most common

glucuronidation which is glucoronidation

of oxygen groups

and this is an example with codeine and

morphine

so codeine shown down here can actually

be directly glucuronodated by

ugtb to the codeine glucuronide

metabolite

it is also in a minor pathway

phase one reaction catalyzed by sipd

converted to morphine

which is highly responsible for the

analgesic effect of codeine

and morphine in turn

can be glucuronidated

at both the three position

as well as the sixth position to form

morphine three glucuronide and morphine

six

glucuronide and you can see these are

both catalyzed by the same ugt enzyme

ugtb

and the major pathway is the formation

of morphine glyceronide

with only a minor amount going to the

sixth glucuronide

another example of o glucuronidation is

with bilirubin

both of the carboxylic acid groups on

bilirubin

can be glugronigated

in this case by ugta

and we find both the bilirubin

monoglucuronides

as well as the bilirubin died from

humonides being formed

this is an interesting pathway its a

mechanism for elimination of bilirubin

and

this enzyme ugta

is actually

not active at birth and so in neonates

it takes several days for a ugta

activity to be turned on and that is why

in neonates there can be accumulation of

bilirubin thats responsible for

jaundice

after a couple days ugta activity is

usually

sufficient to eliminate

bilirubin from these neonates

i mentioned that the glucuronide

conjugates that are formed are going to

be highly charged at physiological ph

and because of this negative charge

they require transporters to cross cell

membranes

this is important for

secretion of glucuronides into the bile

especially for larger molecular weight

glucuronide conjugates

they can be highly secreted into the

bile after theyre formed in the

hepatocyte

and the two transporters that are most

important for glucuronide secretion in

the bile are the breast cancer

resistance protein our bcrp

and the multi drug resistance associated

protein

are mrp

for glucuronides that are formed in the

hepatocyte to circulate in the plasma

and be able to for example get to the

kidney for renal elimination

they also require a transporter on the

vasolateral membrane of the hepatocytes

and mrp

has been shown to be critical for the

elimination of most glucuronides from

the hepatocyte into the plasma

with some potential contribution from

mrp as well

glucuronides

that are secreted into the bile

they can also undergo this process of

enteropathic recirculation

this is illustrated here where you have

a parent rug such as a morphe

being converted to the glucuronide

this glucuronide if its

efficiently secreted into the bile by

mostly bcrp or mrp

these glucuronides then

will be dumped back into the intestine

where they can be converted by bacterial

betaglucuronodases

back into the parent drug

this parent drug then can be reabsorbed

back into the systemic circulation enter

through the portal vein into the liver

and go back out

into the systemic circulation

and so what you see with the center of

paddock recirculation

is you see a

second peak of suggesting that theres

additional absorption

of the drug

at some later time after the cmax from

the original absorption process

and so any kind of later

bump in the concentration time curve

um is an indication that you might have

intraopatic recirculation going on

heres an example of this beta

glucuronidase

reaction

this is with the morphine three beta

glucaronide which can be converted back

to

morphine

and

in this case morphine could end up back

into the systemic circulation again

glucuronides can also be eliminated from

the body in the urine

again because theyre going to be

charged at physiological ph

they will require transporters

to facilitate this secretion into the

urine

and this is a concerted effort of both

uptake transporters on the basolateral

membrane and eflex transporters on the

breast border membrane of the renal

epithelial cells

the major uptake transporter for

glucuronide conjugates is the organic

anion transporter or 0

with some contribution from the o

transporter as well

and on the brush border membrane

of the most important efflux

transporters mrp multidrug resistance

associated protein

with

both mrp and the mates also

transporting some of the furonides

mass balance studies provide information

about all of the metabolism thats

occurring within the human body

and these um quantify the metabolites in

plasma urine and feces to be able to

account for

all of the drug and its elimination

from these types of studies

we have evidence that there is

intestinal metabolism of glucuronides

heres an example of a c mass balance

study

of

a tgf beta r

kinase l inhibitor

this ly compound here

and you can see that there are multiple

routes of elimination of this compound

and for each of these metabolites its

indicated whether it was detected in

plasma

urine and or feces

heres an example

of a direct glucuronide being formed

from this m metabolite so you get this

m metabolite and then the um m the

glucuronide

of m is the m metabolite

the m metabolite is found in plasma

urine and feces

but the m metabolite is only formed in

found in the plasma and the urine

this suggests

that the m glucuronide

form in the intestine was hydrolyzed

back to m

and what you find coming out in the

feces is only

the

hydrolyzed glucuronide metabolite m

this is uh similarly shown here for this

mm combination

with the

m being found in the feces but the

glucuronide of m the m metabolite only

being found in the urine

so we know that this intestinal

metabolism by bacteria of glucuronides

is commonly

found for a lot of glucuronide

metabolites

there can be quite a bit of variability

in glucuronidation

inner individual variability

thats regulated

particularly by various disease states

in this particular example

these investigators were looking at the

pharmacokinetics of morphine as well as

the morphine six and three glucuronide

and they compared concentration time

profiles in healthy volunteers on the

left

to patients renal failure patients on

the right

and what you can see is that the

elimination

of morphine

as well as the morphine and

glucuroni metabolites

is

significantly impaired in the renal

failure patients

its been recognized for a number of

years that renal failure actually

affects hepatic metabolism particularly

phase one

metabolism and phase two metabolism

and so thats what is probably

represented by this delayed elimination

here of morphine

and then renal failure is also critical

for

elimination of metabolites so this long

sustained

accumulation of morphine and

glucuronide likely reflects the

inability of the kidneys to secrete

these morpheme glucuronides as well as

they do in healthy volunteers

so in this case renal failure causes

both a

decrease in the parent drug

metabolism as well as a decrease in the

elimination rate of the metabolites that

are formed

there are also differences in

glucuronide

levels of morphine that have been

demonstrated

in patients with nonalcoholic steato

hepatitis or nash

in this case theres no effect in these

patients with liver disease

on the elimination of morphine

but what you do see is that both the

morphine three glucuronide and the

morphine six glucuronide levels

are higher

in patients with nash compared to the

healthy controls

and in fact theres a correlation

between

the

morphine glucuronide this is a

combination of both three and six

glucuroni

in either the cmax

or the auc

theres a correlation with those levels

and the severity of the liver disease

which is this nash

fibrosis score

this is actually

related to the fact that in patients

with nash they overexpress

the mrp transporter on the basolateral

membrane of the hepatocyte

leading to higher levels of the

glucuronides in the plasma

the mechanism of this increased

regulation of mrp

in nash patients is not completely

understood

but theres multiple lines of evidence

that suggests that this leads to

increased circulating levels

of glucuronite conjugates

in general we think of conjugation

reactions

occurring as a way to eliminate

the pharmacological activity

of a drug

but there are examples of active

glucuronide metabolites that have been

reported

and the best characterized of these is

this morphine glucuroni metabolite

its the minor glucuronide metabolite

formed

from morphine

but

it has its own pharmacological

activities and is an effective analgesic

in fact in this randomized controlled

trial with about 0 patients

in each arm of the study

they directly compared the analgesic

activity of morphine glucuronide

in the solid circles to

the standard of care morphe in these

open circles

and they compared

the ability of morphine glucuroni to

give effective

analgesia following a major abdominal

surgery

so you can see if you look at this

insert up here at early times the

morphine glucuronide does not give as

good of analgesia as morphine itself

so theres some delay in the analgesic

effect compared to the parent drug

morphine but after that theres very

little difference

and this clearly demonstrates that

morphine glucuronide is an effective

analgesia

you can see this as well

in data looking at patientcontrolled

analgesia

how many times do they

selfadminister morphine or morphine

glucuronide per hour is what is

plotted here on the yaxis

again at early times it looks like

morphine glucuronide might not be

quite as effective as morphine

but these differences disappear

or if anything the morphine sticks with

youronite actually looks even more

effective at later times

past surgery

so well now move on to briefly discuss

sulfation

another important conjugation or phase

two reaction

the substrates for sulfation

are very similar and

usually overlap with those for

glucuronidation

any drug that has a nucleophilic oxygen

or nitrogen

can form a sulfate conjugate

and this requires the cofactor three

prime

phosphoadenosine fibrin phosphosulfate

or paps which is shown here

and the sulfate group over here

will be transferred to the nucleophilic

site on the drug to form this sulfate

conjugate

this is catalyzed by sulfur transferases

and similar to

conjugates these sulfate conjugates are

going to be highly charged at

physiological ph

the sulfur transferases that catalyze

the sulfation conjugation reaction are

called salts

these are cytosolic proteins

as i just mentioned they require the

cofactor paths

and in contrast to the ugts these are

high affinity

but low capacity

and so what that means is at lower

concentrations the sulfur transferases

will be more important than the

glucuronazole transferases and youll

see more sulfation at lower

concentrations than glucuronidation

but as the capacity of the cell

phototransferases is approached

glucuronidation becomes quantitatively

more important

the major human salt isoforms are

noted here these are salt a

b a

e

and salt a

as i mentioned

sulfation and glucuronidation

often occur at the same positions

this is illustrated here

for

this nmda receptor antagonist

you can see that

if you look at metabolite and

metabolite 9

these are direct conjugation reactions

direct glucuronidation and direct

sulfation of the parent drug

at the same position

and if you look at metabolite and

metabolite

you can see

both glucuronidation and sulfation again

at the same position

occurring on

this phase one metabolite that was

formed by cytochrome p0 to d the m

and so this is very common

these nucleophilic oxygens and nitrogens

youll typically see a mix of both a

sulfate conjugate and a glutamine

conjugate occurring

the last conjugation reaction that i

want to highlight is glutathione

conjugation

here is the structure of glutathione

its a tripeptide

gamma glutamyl systole glycine

and glutathione is found at high

concentrations in essentially every cell

in our body

often at millimolar concentrations

and that reflects its important role

in detoxifying any reactive

electrophiles

that might form in our cells

theres actually two pools of

glutathione within our cells theres a

smaller mitochondrial pool that has a

long halflife

but most of the glutathione is found in

the cytosol

this is the glutathione thats important

for drug conjugation

and it has a relatively

short halflife of two hours

glutathione conjugation

is catalyzed by the glutathione s

transferases or gsts

and these gsts

they

function as either homodimers or

heterodimers within their class

um the major isoforms are shown here so

for example in the alpha class

you could get homodimers or heterodimers

of any of these

catalyzing these reactions

kind of the classic example of

glutathione conjugation being critical

for the detoxification of a drug

is with the analgesic acetaminophen

the structure of acetaminophen or apap

is shown here

and under normal conditions at normal

doses of acetaminophen

you get both a glucuronide conjugate as

well as a sulfate conjugate that are

formed

a very minor pathway of acetaminophen

metabolism

is by cytochrome p0 e

with some contribution from sip a

to form this eventually this reactive

metabolite nap qi

because this is a reactive electrophilic

metabolite

it will be conjugated by glutathione

and this glutathione conjugation will

increase its hydrophilicity

leading to renal excretion

and also eliminate its reactivity

however if

part of the snap qi escapes this

glutathione conjugation

nab qi

can actually react with the

with the hepatocyte itself leading to

hepatotoxicity

so its understood that theres a lot of

inner individual variation in

acetaminophen metabolism that can

influence the risk of developing

hepatotoxicity

at normal doses the majority of

acetaminophen will be eliminated through

these glucuronidation and sulfation

pathways

however if this is compromised in any

way for example from genetic defects and

glucuronidation

with joe bears syndrome

then this pathway could be compromised

causing more drug to go through this

potentially toxic pathway

of course when you have an overdose

situation

you also will begin to saturate this

pathway

again causing more of the drug to go

through this reactive pathway

you can also have

particular conditions such as prolonged

fasting

chronic alcohol ingestion ingestion as

well as some drugs that are known to

induce cytochrome pe

that can lead to a larger percentage of

acetaminophen metabolism occurring to

this reactive napqi pathway

and when you have these situations and

you have a large amount of fqi being

formed you can also deplete your

glutathione stores

even though we have these millimolar

concentrations in the cell

they can be

depleted

under these overdose conditions

you can also have depletion of gsh due

to chronic liver disease as well as

chronic alcohol ingestion and under

conditions of malnourishment

and so in all of these cases that would

decrease the ability

of our bodies to glutathione conjugate

napqi

as a way to detoxify this compound and

then more napqi has the potential

to cause hepatotoxicity

so let me summarize the major points

that were made in this lecture

phase two metabolism

is an important mechanism for increasing

water solubility

and facilitating elimination of drugs

from the body both in the urine as well

as in the bile

most conjugation reaction occurs on

metabolites that have been formed

through a phase one reaction typically a

p0 needed phase one metabolite

although

there are examples of direct conjugation

of parent drugs for example morphine as

well

the most important conjugation reaction

quantitatively is glucuronidation

this is a high capacity

reaction

and this is followed by sulfation and

glutathione conjugation

because the purpose of these conjugation

reactions is to increase hydrophilicity

these conjugates typically are charged

at physiological ph

and require transporters for urinary and

biliary secretion

in general the conjugates that are

formed through these phase two reactions

are going to be pharmacologically

inactive

but there are examples the one that i

illustrated with six

the six um morphine six glucuronide that

shows pharmacological activity

glutathione conjugation is our most

important mechanism for detoxifying

electrophilic metabolites and preventing

toxicity of drugs and other xenobiotics

and we are beginning to recognize that

theres quite a bit of inner individual

variability

in

the glucuronidation and sulfation

reactions

both in their formation as well as their

elimination

and this can be influenced by disease

state

it can be influenced by genetics of the

enzymes involved

as well as drug drug interactions