Paracetamol ADME

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

14/11/2022
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 82

This document contains 1 pathway and 13 reactions (see Table of Contents)

https://reactome.org
Paracetamol (APAP, aka acetaminophen or N-acetyl-p-aminophenol) is an analgesic drug used for to treat mild to moderate pain and as an antipyretic agent. It is one of the most widely used drugs in the world and is available alone or in combination with other drugs for pain relief, fever and allergy. It is thought to act through the inhibition of cyclooxygenases 1 and 2 (Graham et al. 2013, Esh et al. 2021). Paracetamol is generally safe at therapeutic doses but in overdose cases, it causes mitochondrial dysfunction and centrilobular necrosis in the liver which can lead to death.

APAP has a high oral bioavailability (~88%), is well absorbed and reaches peak blood concentrations after 90 minutes after ingestion. APAP binds plasma proteins to a small extent and has a plasma half-life of 1.5-3 hours. Most of the drug is eliminated by glucuronidate and sulfate conjugation (~55% and ~30% respectively) in the liver or as unchanged drug (~5%) (Forrest et al. 1982). A small amount (5-15%) is oxidised to the reactive metabolite N-acetyl-para-benzoquinone imine (NAPQI). NAPQI is usually detoxified by binding to liver glutathione but in overdose cases, glutathione is depleted and NAPQI instead, binds to sulphhydryl groups on proteins, leading to liver damage. ABCC2, ABCC3, ABCC4 and ABCG2 transporters mediate the efflux of APAP metabolites out of cells (McGill & Jaeschke 2013).

Literature references


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Paracetamol (APAP) is a weak acid (pKa ~9.5) so at physiological pH it is mostly neutral and can be rapidly absorbed from the duodenum into the bloodstream and from there into hepatocytes. The rate of gastric emptying can be used as a measure of plasma APAP levels (Nimmo et al. 1973, Heading et al. 1983). In humans, the half-life of APAP in blood after a therapeutic dose is 1.5hrs to 3hrs (Cummings et al. 1967).

**Followed by:** UGTs glucuronate APAP to APAP-GlcA, SULT dimers sulfate APAP to APAP-SO3, CYP2E1 monooxygenates APAP to NAPQI

**Literature references**


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UGTs glucuronate APAP to APAP-GlcA

Location: Paracetamol ADME

Stable identifier: R-HSA-158546

Type: transition

Compartments: endoplasmic reticulum membrane, cytosol

The liver, and to a lesser extent the kidney and intestine, are the major organs implicated in the elimination of acetaminophen (APAP, aka paracetamol) (McGill & Jaeschke 2013). Glucuronidation is the main route of APAP elimination, accounting for 45-55% of APAP metabolism, and is mediated by UGT1A1, UGT1A6, UGT1A9, UGT2B15 in the liver and UGT1A10 in the gut (Mutlib et al. 2006, Court et al. 2001, Navarro et al. 2011). Glucuronidation renders APAP more water-soluble, facilitating its elimination from the body.

Preceded by: APAP translocates from extracellular region to cytosol


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SULT dimers sulfate APAP to APAP-SO3

Location: Paracetamol ADME

Stable identifier: R-HSA-9753277

Type: transition

Compartments: cytosol

After a therapeutic dose, a large fraction (30-44%) of acetaminophen (APAP) can be converted to inactive sulfate conjugates. Cytosolic sulfotransferases (SULTs) carry out the sulfation conjugation of APAP. SULTs transfer a sulfo group from PAPS to APAP, making it more polar and therefore easier to eliminate. SULTs 1A1, 1A3, 1A4, 1C4, 1E1 and 2A1 are known to have sulfotransferase activity towards APAP (Reiter & Weinshilboum 1992, Adjei et al. 2008, Mazaleuskaya et al. 2015). SULTs are expressed in many tissues but Yamamoto et al. found higher sulfating activity in the liver and intestine than that found in the lung and kidney (Yamamoto et al. 2015).

Preceded by: APAP translocates from extracellular region to cytosol


Literature references


Editions

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2021-10-13 Reviewed Huddart, R.
**ABCC2,ABCG2 transport APAP-GlcA, APAP-SO3**

**Location:** Paracetamol ADME

**Stable identifier:** R-HSA-9753278

**Type:** transition

**Compartments:** plasma membrane, extracellular region, cytosol

Excretion of glucuronidated acetaminophen (APAP-GlcA) and sulfated acetaminophen (APAP-SO3) into bile involves the ATP-binding cassette sub-family C member 2 transporter (ABCC2) and the broad substrate specificity ATP-binding cassette transporter (ABCG2), both found in the canalicular membrane of hepatocytes where they secrete drug conjugates into bile (Giacomini et al. 2010, Kidron et al. 2012, review McGill & Jaeschke 2013).

**Preceded by:** SULT dimers sulfate APAP to APAP-SO3, UGTs glucuronate APAP to APAP-GlcA

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ABCC3, ABCC4 transport APAP-GlcA, APAP-SO3

**Location:** Paracetamol ADME

**Stable identifier:** R-HSA-9753283

**Type:** transition

**Compartments:** plasma membrane, extracellular region, cytosol

Glucuronidated acetaminophen (APAP-GlcA) and sulfated acetaminophen (APAP-SO3) can also be secreted into blood via the ATP-binding cassette sub-family C member 3 and 4 transporters (ABCC3 and ABCC4), both found at the basolateral surface of hepatocytes (Giacomini et al. 2010, review McGill & Jaeschke 2013, Koenderink et al. 2020).

**Preceded by:** SULT dimers sulfate APAP to APAP-SO3, UGTs glucuronate APAP to APAP-GlcA

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A small percentage (5-15%) of acetaminophen (APAP) is converted to the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) primarily by cytochrome P450 CYP2E1 (Manyike et al. 2000). Other P450s such as CYP1A2 and CYP3A4 may also be capable of mediating this transformation (Thummel et al. 1993, Zaher et al. 1998). In human volunteers, increasing therapeutic doses of APAP were found to increase the turnover of glutathione (GSH) (Lauterburg & Mitchell 1987) and APAP-protein adducts could be measured from APAP overdose patients (Davern et al. 2006). Cysteine residues in proteins are the major targets for covalent modification by NAPQI, the primary cause of APAP-induced hepatotoxicity (Streeter et al. 1984). APAP-induced hepatotoxicity occurs when APAP is taken as an acute oral dose exceeding 150 mg/kg in children and 7.5 grams in adults within 24 hours. Acutely increased levels of APAP saturates Phase II glucuronidation and sulfation detoxification pathways in the liver, shifting APAP to Phase I oxidation and producing high levels of NAPQI. Excess NAPQI overwhelms the glutathione conjugation pathway leading to glutathione store depletion in the liver. Excess NAPQI leads to increased oxidative stress with the formation of toxic free radicals which bind to cellular proteins forming APAP protein adducts leading to DNA fragmentation, especially in the mitochondria (Yoon et al. 2016).

The antituberculosis drug isoniazid can induce CYP2E1, which increases APAP oxidation, promotes GSH depletion and NAPQI formation and ultimately leads to increased hepatotoxicity (Epstein et al. 1991, Zand et al. 1993).

**Preceded by:** APAP translocates from extracellular region to cytosol

**Followed by:** GSTs transfer GSH to NAPQI to form APAP-SG, NAPQI binds proteins in hepatocytes

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NAPQI binds proteins in hepatocytes

**Location:** Paracetamol ADME

**Stable identifier:** R-HSA-9753916

**Type:** uncertain

**Compartments:** cytosol

After a toxic dose of paracetamol (APAP), the normal sulfation and glucuronidation pathways get saturated and higher proportions of the drug get oxidized to N-acetyl-p-benzoquinine imine (NAPQI). Higher levels of NAPQI eventually deplete the protective glutathione stores and start to form protein adducts by binding to cysteine groups on cellular proteins (James et al. 2009). NAPQI primarily targets mitochondrial proteins and ion channels leading to the loss of energy production, ion misbalance and cell death, eventually leading to hepatotoxicity (reviews - James et al. 2003, Hodgman & Garrard 2012)

**Preceded by:** CYP2E1 monooxygenates APAP to NAPQI

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The effects of paracetamol (APAP) poisoning can be reversed by administration of N-acetyl-L-cysteine (NAC) within 15hrs of APAP intake. This treatment is effective in preventing liver damage, hepatic failure, renal damage, and death (Prescott et al. 1977, Prescott 1981). NAC provides L-cysteine (L-Cys) for the formation of the tripeptide glutathione, which can conjugate the reactive APAP metabolite N-acetyl-p-benzoquinine imine (NAPQI), thereby reducing damage. NAC is thought to be deacetylated to L-Cys by aminocylase 1 (ACY1), a dimeric enzyme highly expressed in the kidneys (Stocker et al. 2012, review Pedre et al. 2021).

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GSTs transfer GSH to NAPQI to form APAP-SG

Location: Paracetamol ADME

Stable identifier: R-HSA-9753280

Type: transition

Compartments: cytosol

N-acetyl-p-benzoquinone imine (NAPQI) can bind to the cysteine thiol of GSH, a critical mechanism of detoxification. The reaction of NAPQI with GSH can occur both spontaneously and enzymatically (Coles et al. 1998).

Preceded by: CYP2E1 monooxygenates APAP to NAPQI

Followed by: GGT dimers hydrolyse APAP-SG

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GGT (gamma-glutamyl transpeptidase) dimers associated with the plasma membrane (Hanigan & Frierson 1996) hydrolyze glutathione conjugates to form cysteinylglycyl conjugates (CysGly) and glutamate (L-Glu). GGT1 has been extensively characterized. The active dimeric form of the enzyme is generated by autohydrolysis (West et al. 2011) and in vitro can catalyze the reaction of GSH with a free amino acid or dipeptide to generate a gamma-glutamyl-amino acid and cysteinylglycine (Castonguay et al. 2007, Pawlak et al. 1989, Tate & Ross 1977, Thompson & Meister 1976). Based on amino acid sequence similarity, Heisterkamp et al. (2008) identified five additional dimeric proteins, GGT2, 3P, 5, 6, and 7, likely to catalyze the same reactions. West et al. (2013), however, found that GGT2 had no catalytic activity in vitro.

**Preceded by:** GSTs transfer GSH to NAPQI to form APAP-SG

**Followed by:** CNDP2:2Mn2+ dimer hydrolyses APAP-CysGly

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CNDP2:2Mn2+ dimer hydrolyses APAP-CysGly

**Location:** Paracetamol ADME

**Stable identifier:** R-HSA-9753632

**Type:** transition

**Compartments:** cytosol

Cytosolic, non-specific peptidase (CNDP2) can hydrolyse cysteinylglycine (CysGly) to release cysteine (L-Cys) and glycine (Gly) (Tuefel et al. 2003). CNDP2 is functional as a homodimer and is thought to require 2 Mn2+ ions per subunit.

**Preceded by:** GGT dimers hydrolyse APAP-SG

**Followed by:** NAT1,2 acetylate APAP-Cys to APAP-Mer, ABCC1,4,5 transport APAP-Cys,APAP-Mer from cytosol to extracellular region

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Human arylamine N-acetyltransferases (NATs) are expressed as two polymorphic isoforms, NAT1 and NAT2, which have toxicologically significant functions in the detoxification of xenobiotic arylamines by N-acetylation (Liu et al. 2009, Klaassen 2013). NAT1 is located in virtually every tissue whereas NAT2 is mainly expressed in the liver and gut. Slow acetylators of polymorphic NAT2 may suffer more often from side-effects of NAT substrates than fast acetylators due to its inhibition by many drugs (Chien et al. 1997).

**Preceded by:** CNDP2:2Mn2+ dimer hydrolyses APAP-CysGly

**Followed by:** ABCC1,4,5 transport APAP-Cys,APAP-Mer from cytosol to extracellular region

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**ABCC1,4,5 transport APAP-Cys,APAP-Mer from cytosol to extracellular region**

**Location:** Paracetamol ADME

**Stable identifier:** R-HSA-9753284

**Type:** transition

**Compartments:** plasma membrane, extracellular region, cytosol

The ATP-binding cassette sub-family C member transporters (ABCCs, aka multidrug resistance-associated proteins, MRPs) mediate the export of organic anions and drugs from cells. Induction of ABCC expression after APAP-induced liver failure may represent a compensatory mechanism of damaged hepatocytes to reduce accumulation of potentially toxic compounds. ABCC1, 4 and 5 are thought to efflux the cysteine- and mercapto-conjugates of paracetamol (APAP-Cys and APAP-Mer respectively) from hepatocytes (Barnes et al. 2007).

**Preceded by:** NAT1,2 acetylate APAP-Cys to APAP-Mer, CNDP2:2Mn2+ dimer hydrolyses APAP-CysGly

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