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## Abbreviations

## Abbreviations:

ABC: ATP-binding casette

ATP: adenosine triphosphate

AUC: area under the curve

Bcl: B-cell lymphoma

BCRP: breast cancer resistance protein

BDMC: bis-demethoxycurcumin

C.: Curcuma

Cl: clearance

COX: cyclooxygenase

CurA: NADPH-dependent curcumin/dihydrocurcumin reductase

CYP: cytochrome P450

DHC: dihydrocurcumin

DMC: demethoxycurcumin

DMSO: dimethylsulfoxide

E.: Escherichia

EGFR: endothelial growth factor receptor

ER: endoplasmic reticulum

FA: ferulic acid

GSH: glutathione

HPLC: high performance liquid chromatography

IC: inhibitory concentration

IL: interleukin

i.p. intraperitoneal

i.v. intravenous

Jak: Janus kinase

LD: lethal dose

LOX: lipoxygenase

MCP: Monocyte chemoattractant protein

MDR: multi drug resistance

MIC: minimal inhibitory concentration

MRP: multi drug resistance protein

MXR: mitoxantrone resistance protein

NADPH: nicotinamide adenine dinucleotide phosphate

NF-kβ: nuclear factor-kappa light chain enhancer-of activated B-cells

NSAID: non-steroidal anti-inflammatory drug

OTC: over-the-counter

PBS: phosphate buffered saline

PCF: Phosphate chemical fertilizer

pRb: retinoblastoma protein

RNA: ribonucleic acid

ROS: reactive oxygen species

SERM: selective estrogen receptor modulator

SOD: superoxide dismutase

Stat: signal transducer and activator of transcription

SULT: sulfotransferase

## Abbreviations

t½: Half life

t½ Ka: absorption half life

t½ ß: elimination half life

THC: tetrahydrocurcumin

TNF: tumor necrosis factor

TOF-MS: Time of flight mass spectrometry

TPA: 12-O-tetradecanoylphorbol-13-acetate

UDP: uridine diphosphate

WHO: World Health Organization

Y.: Yersinia

# 1. Abstract

## 1.1 Abstract

The Curcuma longa plant has been in use in traditional Asian medicine for centuries. In recent years the major compound and characteristic yellow pigment curcumin has experienced wide attention in medical research. Several of the beneficial effects for that it has been used traditionally have been verified by different studies, including its anti-oxidant, anti-inflammatory, anti-proliferative and anti-microbial activity. Further a wide range of molecular targets has been identified. Also enzymes and transporters that have an impact on drug metabolism interact with curcumin which can possibly lead to drug-drug interactions. Some of them are members of the family of ABC-transporters and have the ability of actively promote immediate substance efflux and therefore inhibit drug entry into cells, leading to therapy failure. Especially the inhibition of MRP1, BCRP and P-gp is in the main focus of cancer therapy in order to find a way of lowering resistance against cytostatics and minimize failing in medical treatment. Although high potential has been attested to curcumin there are several limiting factors, particularly the strong first pass effect and the resulting low bioavailability, that have prevented wide application hitherto and make further investigations necessary.

On the other side the proofed inhibition of the CYP-450 system, as well as of efflux proteins can also lead to undesirable side effects. Through increase of bioavailability the plasma concentrations of other drugs are altered when co-administered to curcumin. This is clinical relevant because interactions with a wide range of drugs, including warfarin, clopidogrel, losartan, loratadine, celiprolol, docetaxel, etoposide, midazolam, norfloxacin, sulphasalazine, tacrolimus and tamoxifen have been proven in experiments. Especially when great amounts of curcuma are administered orally in traditional Asian medicine there is the risk of interactions with modern medical drugs. Therefore strict drug monitoring is particularly necessary for drugs with a narrow therapeutic window and dose adjustment should be considered when they are taken together with curcumin or Curcuma longa products.

# 1. Abstract


## 1.2. Zusammenfassung

Seit Jahrhunderten wird das Rhizom von Curcuma longa in asiatischen Ländern für medizinische und kulinarische Zwecke genutzt. In den letzten Jahren hat vor allem ein Inhaltsstoff, das charakteristisch gelbe Curcumin besondere Aufmerksamkeit in der medizinischen Forschung erfahren. Viele der ihm in der traditionellen Medizin nachgesagten positiven Effekte, darunter die anti-oxidative, entzündungshemmende, antiproliferative und antimikrobielle Wirkung konnten bewiesen und viele verschiedene molekulare Angriffspunkte identifiziert werden. Zu diesen gehören auch verschiedene Enzyme und Transporter, die den Metabolismus anderer Medikamente beeinflussen können, wie beispielsweise die Familie der ABC-Transporter, deren Mitglieder die Fähigkeit haben, Moleküle durch sofortiges Ausschleusen am Eindringen in die Zelle zu hindern, wodurch verschiedene Medikamente in ihrer Wirkung herabgesetzt werden. Besonders der durch Curcumin hervorgerufenen Hemmung des MRP1, BCRP und P-gp wird in der Krebsforschung große Aufmerksamkeit geschenkt, mit der Hoffnung dadurch die Resistenz gegenüber Cytostatika senken und Therapieversagen minimieren zu können. Obwohl dem Stoff ein großes therapeutisches Potential attestiert wird, gibt es jedoch einige limitierende Faktoren, wie insbesondere den starken First Pass Effekt und die damit einhergehende geringe Bioverfügbarkeit, die eine breite Anwendung bis jetzt verhindert haben und weitere Forschung notwendig machen.

Auf der anderen Seite, kann die Inhibierung von Effluxproteinen, sowie die bewiesene Hemmung des CYP450-Systems auch zu unerwünschten Nebenwirkungen führen, da es durch die erhöhte Bioverfügbarkeit zu einem Konzentrationsanstieg gleichzeitig verabreichter Arzneistoffe kommt. Dies ist klinisch relevant, da verschiedene Studien Wechselwirkungen mit vielen unterschiedlichen Arzneistoffen, wie unter anderem Warfarin, Clopidogrel, Losartan, Loratadin, Celiprolol, Docetaxel, Etoposid, Midazolam, Norfloxacin, Sulfasalazin, Tacrolimus und Tamoxifen gezeigt haben. Dadurch kann es vor allem bei der oralen Verabreichung großer Mengen Curcuma in der traditionellen asiatischen Medizin zu Interaktionen mit schulmedizinischen Präparaten kommen, was deshalb genauer Untersuchungen bedarf. Daher ist vor allem bei Arzneistoffen mit geringer therapeutischer Breite striktes Drug Monitoring notwendig und eine Dosisanpassung

sollte in Erwägung gezogen werden, wenn sie gemeinsam mit Curcumin oder Curcuma longa Produkten verabreicht werden.

## 2. Aim of the work

## 2. Aim of the work

Curcumin, the major compound of Curcuma longa has been proven to have many beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-microbial and anti-proliferative activity.[1] Nevertheless there are several limiting factors for clinical use, particularly the strong first pass effect and the resulting low bioavailability.[36] Under the wide range of molecular targets also drug metabolizing CYP450 enzymes and efflux proteins, especially MRP1, BCRP and P-gp have been identified.[49] Inhibition of these targets might constitute a risk for drug-drug interactions. Different in vivo and in vitro studies reported influence of curcumin on a wide range of medical drugs. Because of the manifold structures curcumin interacts with in the human body the knowledge of its main metabolic pathways is essential for clinical research and for the estimation of possible drug interactions and their clinical relevance.[63], [64], [66]

In the present work I tried to collect important data concerning the metabolism of curcumin on the one hand and reported drug interactions in different experiments on the other. I searched for literature in different databases and discussed the clinical relevance and potential impacts of the findings on medical therapy.

# 3. Introduction:

## 3.1. Curcuma longa:

The perennial monocotyledonous Curcuma longa plant is one of an estimated 100 curcuma species that belong to the group of Zingiberaceae and are mainly found throughout Asian and African countries.[(1),(2)] The species differ not only in their morphology concerning the appearance of their organs below and above ground level, but also in their content of biologically active molecules.[(1)]

Curcuma longa L. syn. Curcuma domestica, that can further be divided in several varieties, is the most cultivated species, with more than 150.000 hectares of cultivation area in India alone. India is the leading international producer and exporter of turmeric spice. In order to increase profits, about 20 different genetically improved variations of C. longa have been developed. Other economically valuable species are C. aromatica, which also has medicinal purposes, in addition to C. zedoaria, C. pierreana, C. caesia, C. kwangsiensis, C. ochrorhiza, C. montana, C. angustifolia, C. decipiens, C. rubescens, C. alismatifolia, C. roscoeana, C. amada, C. malabarica, C. caulina and C. haritha that are used in folk medicine, traditional medicine, toiletries, arrowroot manufacturing, for decoration, and as kitchen spices. In the perfume industry and aroma therapy, turmeric oil is used for its characteristic flavor and color.[(1)]

The genus Curcuma has its origin in the Indo-Malayan region and is found in many areas of Africa, Australia, and most notably Asia, from sea level up to an altitude of 2000m. India, where at least 10 curcuma species are endemic, is the country with the highest occurrence of different species with a total of 41 species recorded there. Worldwide, the root powder of Curcuma longa is utilized as a spice in Asian, and especially Indian cuisine, but also as a coloring agent in the food industry because of its intense yellow pigment. In Buddhist and Hindu countries, turmeric has also been used for religious rites and medical treatment since ancient times, with the first records dating back to 1000-1500 BC. Even in the modern era, turmeric is still used in Hindu worship ceremonies, as well as in traditional Asian medicine, along with other plant products such as lime, sandal powder, mustard oil, garlic and ginger. The red colored powder that can sometimes be seen, results when turmeric is mixed with boric acid and the contained curcumin is transformed into rubrocurcumin or

rosocyanin. It is believed that the religious use of the plant enhanced its distribution over Asia and Africa.[1]

Because of its content of many biologically active molecules, the curcuma longa plant is widely used in traditional Asian medicine via oral administration against bilary and hepatic disorders, as well as anorexia, rheumatism and diarrhea. A turmeric paste is often applied locally on diabetic wounds, injuries, skin disorders, joint pain and insect- or snake bites, whereas fumes of dried turmeric are inhaled for respiratory infections and sinusitis. For veterinary purposes, it is used against parasitic infections, hair loss, injuries, mastitis and skin diseases. Many positive effects have already been verified by several studies, e.g. anti-inflammatory, anti-cancerogenic, anti-rheumatic, choleretic, anti-diabetic and antimicrobial properties, to mention a few.[1]

Responsible for the biological activity of Curcuma are curcuminoids, especially curcumin (about 77% in commercial curcumin), demethoxycurcumin (17%) and bisdemethoxy-curcumin (6%).[1],[3]

Curcumin is the main compound that is found in all Curcuma species, except C. zedoaria.[4] Besides curcumin, the Curcuma species also contain essential oil and oleoresin in different concentrations, with Curcuma longa being rich in all three compounds, containing 2-5% of essential oil, 2-7% of curcumin and 7-15% of oleoresin. Generally speaking, other species contain much lower concentrations of all of these substances, but environmental factors also seem to play an important role concerning the composition of the essential oil and the concentration of the chemical compounds.[1]

### 3.2. Structure of Curcumin and its derivatives:

Curcumin or Diferuloylmethane (= [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepta-diene-3,5-dione] is a diarylheptanoide with a low molecular weight of 368.38 Daltons and a melting point of 183°C. This hydrophobic polyphenole with a bis-α,β-unsatuared β-diketone structure is the major component of the rhizome of Curcuma longa and is responsible for its yellow color. The pigment is caused by a system of conjugated double bonds in the molecule.[5],[6] Like the gingerols, the curcuminoids are biosynthesized from phenylalanine via the polyketide pathway which is catalyzed by different enzymes.[7]

After first isolation of the molecule in 1815 by Vogel and Pelletier, nearly a decade passed before the structure was determined in 1910.[8]

Curcumin is soluble in nonpolar and polar organic solvents like DMSO, ethanol or acetone, but not in aqueous acidic or neutral solutions.[5]

Solutions of curcumin change color depending on the pH level. In the protonated form (pH<1) they show a red, in the neutral form (pH 1-7) a yellow and above pH 7.5 an orange color. Furthermore the molecule is reported to have at least two, probably three, different pKa values and an octanol-water partition coefficient of 3.29. [5]

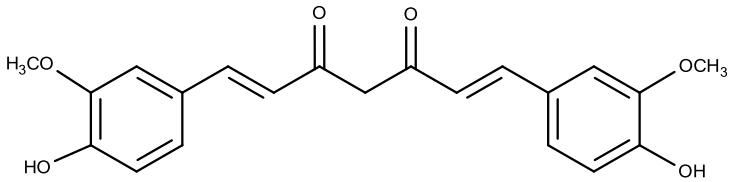



Fig. 1. Curcumin [5]

The major chemical characteristics of the curcumin molecule are the two phenolic groups, the ß-dicarbonyl moieties, the two methoxy groups and the conjugated double bonds. This structure is responsible for the compound's ability to exhibit Keto-enol tautomerism and accepting Michael addition at the α,β-unsaturated carbonyl group.[9] It has been proven that curcumin has a higher activity than its metabolites where at least one of the mentioned functional groups is lacking, which indicates that these structures are essential for biological activities.[8] With its electrophilic dienone structure, the curcumin molecule has the ability of reacting with free SH-groups of cysteins in proteins and break in the building of disulfide bridges.[10]

In neutral and acidic medium the keto-form is preferred, while it forms a stable enol in alkaline environment.[3] It is also reported that because the central C-atom is highly activated in the keto form through the neighboring O-atoms and the delocalized electrons in this system, curcumin works as an H-atom donor at pH 3-7. In contrast, it donates electrons above pH 8.[6] A different study reported that the enol form is generally dominant in solutions and in solid form because of its higher stability compared to the keto form with an energy difference of 7,75kcal/mol.[10],[11] The preference of the enol form could be explained by the extension of the backbone of the structure with its conjugated diene groups, as well as intramolecular H-bonds.[11]

Furthermore it is reported that the enol form shows a red shift of its absorption maximum compared with the diketone. It has been calculated that the enol has a planar structure which leads to a resonance within the two benzene structures while the keto form has a twisted conformation. The importance of the conjugated diene system was also proven by exchanging both phenolic OH groups of curcumin against $OCH_3$ groups creating methylcurcumin. This change had no impact on the conjugated system of the molecule and therefore showed little difference concerning the absorption maximum. In contrast, the curcumin metabolite tetrahydrocurcumin, which does not have any conjugated diene groups in its carbon backbone, shows a blue shift of its absorption maximum to 315nm. The same study also reported that there is a difference in the absorption spectrum concerning the deprotonation of the enolic or the two phenolic protons of curcumin. While the dissociation of the enolic proton only has a marginal impact on the absorption maximum, the deprotonation of a phenolic proton leads to a strong bathochromic shift. It is suggested that the enolic proton is the most active of all three dissociable protons and has the highest acidity, which plays an important role in the radical scavenging activity of curcumin via proton transfer.[11]

There are several structures through which curcumin is capable of forming H-bonds and interacting with its surrounding. First, the ß-dicarbonyl moiety that also has the ability of forming complexes with multivalent cations, as well as the phenolic OH groups that can work as H-bond acceptors and donors. Furthermore the ether groups can accept H-bonds and different C-C bonds allow rotamerization.[9]

Because of its high logP, that is a marker for its hydrophobicity, curcumin can interact with several lipophilic structures like biomembranes, but also hydrophobic amino acids in proteins.[9]

For maximization of its hydrophobic part, curcumin has the ability of adopting several conformations and can interact with side chains of aromatic amino acids over its phenyl ring through van der Waals interactions. This lipophilic structure with a phenolic and a carbonyl moiety also gives the molecule the ability of interacting with macromolecules like DNA through H-bondings.[12]

The two most important derivatives of curcumin (Curcumin I) are Demethoxycurcumin (Curcumin II) and Bisdemethoxycurcumin (Curcumin III).[5],[13] Further the existence of cyclocurcumin (Curcumin IV) is reported, but this molecule, which makes up

approximately 10% of the curcumin composition, has little to no biological effect.[2] In addition, many minor metabolites exist, including dihydrocurcumin, hexahydrocurcumin, octahydrocurcumin and their glucuronides, and curcumin sulfate. Many studies suggest that these metabolites also have an impact on the biological activities of turmeric, especially on the anti-oxidative anti-inflammatory activity.[10]

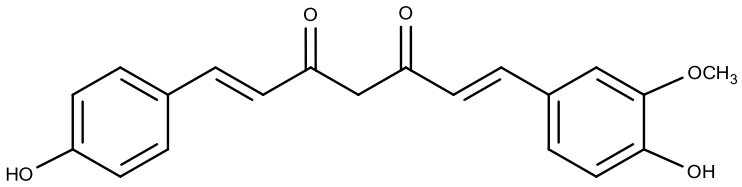



Fig. 2: Demethoxycurcumin [5]

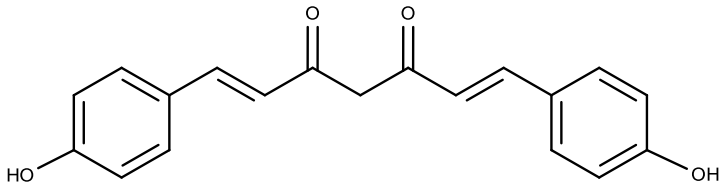



Fig. 3: Bisdemethoxycurcumin [5]

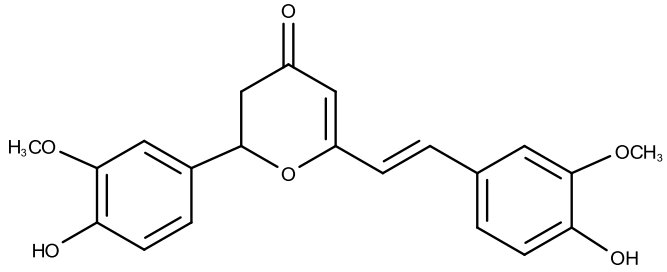



Fig. 4: Cyclocurcumin [12]

# 4. Stability of curcumin and its metabolites:

### 4.1. Degradation of Curcumin:

Curcumin is a very strong antioxidant and radical scavenging agent that has the ability to react with several physiologically produced oxygen-, nitrogen-, and sulfur-centered radicals, as well as non-physiological radicals like azide-, dibromide-, Triton-X-, halocarbonperoxyl- and galvinoxyl radicals. [9]

It is proposed that curcumin is degraded to DHC via the CurA enzyme that is found in intestinal bacteria and has the ability of exclusively reducing C=C bonds, not C=O bonds.[10]

DHC is then converted into THC over dihydro-DMC via the same pathway.[14],[15]

Tetrahydrocurcumin, which does not have any conjugated double bonds in its backbone structure, is white or colorless in contrast to the mother molecule with its strong yellow color caused by the conjugated diene groups.[10]

Curcumin, DHC and dihydro-DMC can then be further degraded via intestinal microorganisms into ferulic acid. DMC and BDMC are proposed to be degraded to dihydro-DMC and dihydro-BDMC and finally tetrahydro-DMC and tetrahydro-BDMC.[15]

Ferulic acid is, just like vanillic acid, reported to be an important metabolite that occurs in different excretion products after oral consumption of bread enriched with encapsulated curcumin.[5]

These findings are consistent with Wang et al. who found three minor degradation products (vanillin, feruloyl methane, and ferulic acid) when investigating the stability of curcumin in 0,1 phosphate buffer after 1 hour incubation time at 37°C. In the HPLC-analysis next to the peak of vanillin the peak of an unknown substance, which turned out to be the major compound, was detected. With extended incubation times, the vanillin level increased and subsequently became the major decomposition product while the level of the unknown compound decreased. This compound that had been shown to be unstable in phosphate buffer at high temperatures (60°C) was later identified via electrospray and electron impact mass spectrometry as trans-6-(4´-hydroxy-3`methoxyphenyl)-4-dioxo-5-hexenal.[16] In comparison with vanillin and ferulic acid docking studies on xanthine oxidase forecast a stronger activity for this molecule.[17]

## 4.Stability of curcuminoids and its metabolites

A different group that investigated degradation products formed in fermentation cultures with different E. coli strains found DHC and THC as minor metabolites, while ferulic acid and a curcumin-l-cysteine adduct were the major metabolites.[15]

Because Curcumin is a chromophore, photochemical degradation also plays an important role in the modification of the molecule. It absorbs light at visible wavelengths, not only in daylight, but also in artificial light, even if UV- light and oxygen are not present.[9]

It is very unstable in daylight, and when using clear instead of amber glassware, a 5% decrease in absorbance occurs at typical sample preparation time. After sunlight exposure of the methanolic and ethanolic extract, as well as of the solid form, vanillin, vanillic acid, ferulic acid and ferulic aldehyde were found. [16]

Several studies investigated Curcumin degradation in various buffer systems at different pH (1-11) and temperatures (31,5 and 37 °C). Besides vanillin, which was the major product, two other molecules were found: ferulic acid and feruloyl methane. It was suggested that non- absorbed curcuminoids get metabolized to different phenolic acids by gut bacteria.[18]

Curcumin is known to be more stable under acidic conditions than in neutral or alkaline media. It is proposed that the reason for that might lie in the structure of conjugated double bonds that lead to an enhanced stability in acidic media. Under basic conditions in contrast, the phenolic group gets deprotonated and consequently a destruction of the molecule follows.[16]

Wang et al. were able to show that the stability and degradation of curcumin depends on the pH of the surrounding media. Curcumin degradation was investigated in different pH values between 3 and 10 and turned out to follow first order kinetics in all of these pH values at 37°C and constant ionic strength. At neutral-alkaline buffers, 90% of curcumin showed a rapid decomposition because the phenolic OH-group experiences deprotonation which leads to destruction. At pH 7,4 curcumin is not stable in phosphate buffer solution, but the stability could be increased when glutathione, ascorbic acid, N-acetylcysteine and rat liver microsomes were added or when just the pH value was lowered. Curcumin is not very stable under neutral and basic conditions, but behaves more stably in acidic conditions similar to those present in the stomach. Furthermore it is reported that although curcumin is degraded very fast in 0.1 M phosphate buffer and serum free medium, it is relatively

stable in medium containing 10% fetal calf serum, as well as in human blood. There after an incubation time of 8 hours, not even 20% decomposed within 1 hour. Though it was reported that no curcumin could be found in the blood 2-12 hours after oral application in rats and that in phosphate buffer curcumin loses its absorbance at 420nm. This suggests that it is unstable under physiologic conditions.[16]

Several studies that investigated the features of the glycosylated form of curcumin as it is found after metabolic biotransformation, showed that it is soluble in water. During the glycosylation process, the conjugated double bonds are reduced, which leads to an increase in stability but also a loss of the characteristic color. This makes a higher bioavailability, pharmacological activity as well as stability in food and medical formulations very likely for sugar derivatives of curcumin.[19]

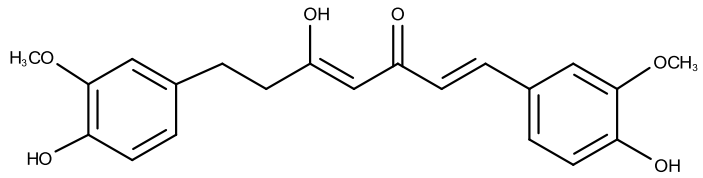
Tetrahydrocurcumin in contrast has a high stability in 0,1 M phosphate buffer solutions at different pH levels and was also more stable at pH 7,2 compared to curcumin. [10],[20] This aligns with the findings of Hoehle et al. who report that the important reductive metabolites of curcumin have a much higher stability than curcumin itself with THC being the most stable one before BDMC and DMC[21]

Other studies show that the maximum plasma concentration in humans does not exceed the low nanomolar range (>160mmol/l) even at high doses (12g).[22],[23]

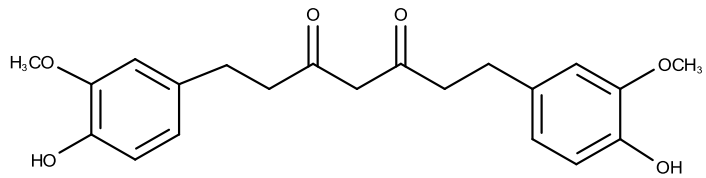
At body temperature, the degradation process follows first order kinetics, while it follows second order kinetics at a temperature of 31,5 °C.[18] During a 10 minute exposure to heat (70°C), curcumin proved stable. At higher temperatures the decomposition of the molecule starts and increases if the temperature rises up to 100°C. After 10 or 20 minutes in boiling water there is a 27% or 32% loss of curcumin concentration.[5]

Suryani et al. reported that when curcumin is incubated with bacteria for 36 hours without PCF, a decrease of the curcumin level occurs in the first 8-10 hours before the values stabilize for the remainder of the incubation time. In the presence of PCF, curcumin values decrease in the first 12 hours and then also remain constant.[15]

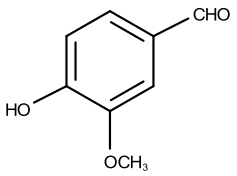
Degradation products of curcumin:



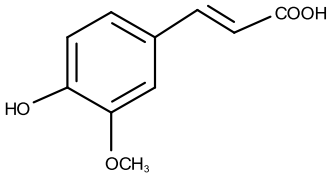

dihydrocurcumin



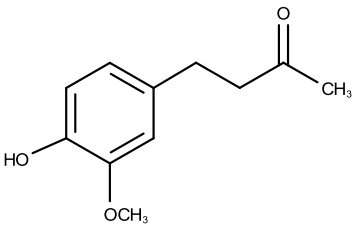

tetrahydrocurcumin




vanillin




ferulic acid




feruloyl methane

Fig. 5.: Degradation products of curcumin [24]

### 4.2. Analgesic drugs, Interaction through changes in stability and bioactivity

Choi et al. investigated the interaction of different analgesic over-the-counter drugs with curcurmin and analyzed changes in stability and bioactivity in the intestine. It could be shown that the presence of acetylsalicylic acid, as well as of ibuprofene, and acetaminophen (paracetamol) enhanced the stability of curcurmin which led to a

longer half-life (from 145 min to 494 min). Also the yellow color, that is caused by curcurmin and is proportional to its concentration, showed a higher stability when measuring the absorbance at 405 nm.[25]

Interestingly also just the mimicry of gastric conditions (0,01N HCl) led to an increase of the half-life of curcurmin (495 min). The addition of acetylsalicylic acid had less impact on the half-life but also increased it significantly. An explanation therefore might be that curcurmin is unstable under basic conditions but shows an enhanced stability with a decrease of the pH. When adding a sour compound like acetylsalicylic acid ($pK_a$ ~3,5) to a neutral or alkaline medium the lowering effect on the pH is much stronger than it would be in an environment that has been acidic from the beginning.[25]

Another explanation for curcurmins increased stability in the presence of acetylsalicylic acid might be that this drug is reported to have an antioxidative activity and to be capable of radical scavenging which could reduce the degradation of curcurmin in oxidative processes.[25]

Furthermore the study investigated the difference in the change of the degradation pattern of curcurmin, demethoxycurcurmin and bisdemethoxycurcurmin under treatment with acetylsalicylic acid, ibuprofen and acetaminophen. Degradation of Curcurmin which was shown to be the most unstable of all three compounds in PBS, was prevented through the addition of each drug, while the levels of the two other compounds (DMC and BMC) did not show a significant change. Also the stability of curcurmin was enhanced rather than DMC or BMC. [25]

On the other hand the study also analyzed the effect of curcurmin on over-the-counter drugs (acetylsalicylic acid and acetaminophen). Neither the amount of decrease in concentration of the generally unstable acetylsalicylic acid nor the increase of its hydrolization product salicylic acid was significantly changed in the presence of curcurmin. The stability of acetaminophen was not affected by curcurmin either.[25]

Since all three drugs, as well as curcurmin showed cytotoxic activity on INT 407 normal intestinal cells and HCT 116 colon cancer cells the influence on their cytotoxic properties when co-treated with each other was investigated. Curcurmin, that was also reported to gain stability in the presence of each of the tested drugs, showed a significant increase in cytotoxic activity, especially with acetylsalicylic acid. With

ibuprofene and acetaminophen the reported cytotoxic properties were also enhanced, except at a concentration of 2nM. Choi et al. confirmed a synergistic effect between curcurmin doses higher than 10µM and acetylsalicylic acid, ibuprofen and acetaminophen.[25] This aligns with a different study where curcumin and diclofenac were co-administered and showed an enhanced anti-neoplastic and apoptotic effect on colon cancer cells than both substances alone.[26]

Furthermore a positive influence of the OTC drugs on the decreasing effect of curcurmin on intracellular thiol compounds, including GSH was shown. In the HCT 116 cells the effect was again stronger than in the INT 407 cells. In the same study it is reported that neither in combination with acetylsalicylic acid or ibuprofen nor curcurmin alone had any significant effect on the reducing potential of the culture media.[25]

# 5. Biological activities of curcuminoids

## 5.1. Toxicity

Curcumin is considered safe according to the WHO. The acute oral toxicity was evaluated in animals, resulting in a $LD_{50}$ of 3.0g/kg in rats, mice, guinea pigs and monkeys.[12]

In a different study, rats were fed approximately 1,2g/kg curcurmin over two weeks with no toxic side effects.[27]

The acceptable daily intake of curcumin as a food additive was determined with 0-3 mg/kg by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[2]

A dose escalation study was done in order to determine the maximum tolerated dose in twenty-four healthy human volunteers. Doses starting from 500mg over 1000, 2000, 4000, 6000, 8000, 10000 up to 12000mg were administered to male and female adults. Only marginal non-dose-related effects occurred in seven subjects. In serum analyses of patients who took doses up to 8000mg, no curcumin could be detected. Only in one subject who was administered 10g and in one who was administered 12g low serum concentrations of 50,5ng/ml and 51,2ng/ml occurred after four hours. This suggests that even a single dose of 12g curcumin is safe.[28] In humans only minimal toxicity of Curcumin was reported causing diarrhea, headaches, yellow colored feces and skin rash in approximately 30% of patients.[12]

## 5.2. Biological activities

### 5.2.a. Anti-oxidative activity:

One of the major structure related activities of curcumin is its anti-oxidative effect through radical scavenging. The curcumin molecule is one of the most potent natural radical scavengers and has a stronger antioxidant activity than even vitamin E.[29],[30]

Yang et al. studied the impact of curcumin, but also DMC and bis-DMC on erythrocytes under hyperglycemic conditions. Diabetes goes along with severe oxidative stress that is caused through high blood glucose levels, an increase in sorbitol concentration and glycation of different proteins. This leads to lesion or malformation of erythrocytes. The results showed that all three curcuminoids are able to alleviate these effects and reduce oxidative stress significantly. They lead to reduction of radical generation and lipid-peroxidation, prevent protein glycosylation,

and raise the activity of the superoxide dismutase (SOD) and the glutathione (GSH) level. Some research suggests, that apart from the two phenolic groups, the β-diketone structure is involved in this mechanism and might work as a radical scavenger under division of the C-C chain.[29] Furthermore the methoxy and the phenolic hydrogen group are reported to form intramolecular H-bonds with each other and therefore facilitate H-atom seclusion.[30] Curcumin and DMC lead to a higher rise of the GSH level than BDMC while THC and turmerones did not have an impact on GSH levels at all, which indicates that conjugated double bonds in the backbone are essential for this effect.[30] Other studies suggest that THC has a stronger impact on the induction of several enzymes, including the GSH-peroxidase and glutathione-S-transferase. Also a higher radical scavenging and anti-oxidant activity is reported for THC, while curcumin has both anti-oxidant and pro-oxidant features.[10] The presence of curcuminoids, but not THC, increases the levels of reactive oxygen species(ROS) which leads to caspase-dependent and -independent apoptosis.[10],[30],[31] In this connection curcumin and DMC are less potent than BDMC, which showed the highest ROS values. According to these results, it is believed that the phenyl methoxy groups might have a negative influence on ROS production.[30],[31] Also investigated were the antioxidant activity of processed turmeric concerning its radical scavenging ability, the oxygen radical absorbance capacity (ORAC), the inhibition of lipid peroxidation and the ferric reducing antioxidant power (FRAP). The study showed that ethanol extracts generally have a higher antioxidant potential and radical scavenging activity for both raw and processed turmeric than aqueous extracts which might be due to the superior solubility of curcuminoids in ethanol. Also the aqueous boiled extracts had a higher antioxidant potential than the stirred aqueous extracts. The aqueous curcumin extract did not inhibit lipid peroxidation significantly while the boiled extract showed a protection of 56,16% and the ethanol extract one of 93,23%. Interestingly it is also reported that only the deeply yellow colored Curcuma longa rhizomes which have higher curcumin concentrations than the rhizomes of other curcuma species show significant radical scavenging activity. This indicates the importance of curcumin for these effects.[32]

## 5.Biological activities of curcuminoids

### 5.2.b. Anti-inflammatory activity:

Curcumin not only has very strong antioxidant properties but also an anti-inflammatory activity. Through modulation of various signaling pathways like the JAK/STAT and NF-kβ pathway, the expression of genes that code for different pro-inflammatory cytokines (e.g. IL-1,-2,-6,-8,-12, TNFα, MCP-1) is down-regulated, as well as the activation of different inflammation-related enzymes like cyclooxygenases (COX), lipoxygenases (LOX), the inducible nitric oxide synthase (iNOS) and the xanthine oxidase[33]

Through the TNFα-inhibition it also develops anti-diabetic activity. TNFα is a pro-inflammatory cytokine that is associated with hypertension, obesity, high glucose levels, and decreasing insulin sensitivity.[33]

It is believed that the hydroxyl and especially the methoxyl groups of the molecule are the responsible moieties for these effects, but the conjugated double bonds in the carbon chain are also important. This corresponds with the finding that curcumin has a much higher potency than its derivatives DMC and BDMC for inhibiting transcription factors that are related to inflammatory effects, such as TNFα and NFkβ. THC that contains both methyl groups, but does not have conjugated double bonds in its backbone structure, is much less effective than curcumin and does not have strong anti-inflammatory activity. Interestingly the suppressing activity concerning tumor cell proliferation is comparable for curcumin, DMC and BDMC, but THC has a much weaker potency. This may indicate that the methoxy groups are less important in this connection.[34]

Other authors suggest that the difference between curcumin and THC in cellular lipid accumulation might result in different cellular uptake of the two substances. Curcumin experiences fast uptake and metabolism while THC uptake is much slower. This correlates with the changes in lipid levels. Curcumin, but not THC has been proven to raise expression of a fatty acid binding protein and a fatty acid transporter which leads to higher lipid levels in the macrophages. In contrast to THC curcumin was also shown to upregulate a transcription factor that is related with lipid transport and stress resistance. Therefore it might have a positive influence on the protection of inflammatory vascular cells from damage.[10]

Several studies have proven the action of curcumin against different chronic inflammatory diseases such as cardiovascular diseases, diabetes,neurodegenerative

diseases, rheumatoid arthritis, asthma, inflammatory bowel disease, allergies and cancer.[34] Furthermore Curcumin was shown to reduce sepsis in rats and significantly enhance the survival time.[34]

### 5.2.c. Anti-proliferative properties:

Another important feature of curcumin is its anti-proliferative activity against tumor cells, which is reportedly related with the anti-oxidant capability. The molecule has been shown to induce apoptosis over several pathways through rapid generation of ROS. After release from the mitochondria which is caused by the ROS generation, the apoptosis inducing factor (AIF) migrates into the nucleus and induces a caspase-dependent apoptosis. Additionally pro-apoptotic proteins such as p53 and p21 can be activated to inhibit cyclin D, retinoblastoma protein (pRb) and other proteins that are involved in the cell cycle.[30] Also p53 and caspase independent induction of apoptosis has been found which indicates that there are manifold ways in which this molecule can interact with cell proliferation.[31],[35] Because of its lipophilic feature, curcumin has the ability to pass through the cellular membrane and accumulate in the endoplasmic reticulum (ER) and the envelope of the nucleus. During this process the membrane integrity can be damaged because the phosphatidyl-serine molecules are turned from the inner to the outer cell surface. This is reported to be in connection with free radical release.[35],[36] Furthermore the molecule was shown to upregulate a range of other pro-apoptotic proteins from the Bcl-2 family, such as Bax, Bak, Bim, Noxa and Puma, while it leads to a down-regulation of Bcl-2, Bcl-xL and inhibitor of apoptosis protein (IAP) which are anti-apoptotic proteins. Also cytochrome c release, caspase-3 activation, increase of $Ca^{2+}$ concentration, Akt inhibition and the reduction of metal cations like $Fe^{3+}$ and $Cu^{2+}$ reportedly play an important role in the curcumin-induced apoptosis. It is believed that curcumin induces apoptosis through swelling of the mitochondria which leads to an increase in permeability.[35],[36]

Curcumin, DMC, and BMC were found to have an analogical effect on cell proliferation of different cell lines and Curcumin, Curcumin mix and DMC on inhibition of TPA-induced tumor promotion in the skin of mice. BDMC in contrast was less effective in experiments. THC, that was also reported not to inhibit NF-kβ activation, only showed marginal activity on cell proliferation. Nevertheless no significant correlation between NF-kβ inhibition, ROS generation and cell proliferation was

found.[30] THC is suggested by several authors to have lower anti-cancer activity than curcurmin.[10]

A prominent field in curcumin research is also the molecules ability of sensitizing cancer cells to chemotherapy which allows the administration of cytostatics in lower doses and therefore reduces the dose-dependent side effects.[37]

**5.2.d. Anti-microbial activity:**

Many studies that investigated the antimicrobial characteristics of curcumin have verified its antiseptic features in that turmeric has been used for centuries in Asian cultures. For example, Curcumin has been shown to have the capability of reducing the activity of the cariogenic bacteria streptococcus mutans and the highly infective staphylococcus aureus in vitro by inhibiting sortase A. This enzyme catalyzes the linkage of different surface proteins, like the Pac protein, to the cell wall, which is an important step for the interaction of gram-positive bacteria with tissues and other cells. Curcumin showed a dose-dependent antibacterial effect with a minimal inhibitory concentration (MIC) of 125µmol/l. Since experimental results showed that Pac occurs in the supernatant when 15µmol/l curcumin were added to the media before incubation, it is believed that curcumin reduces the activity of sortase A at this concentration already. Because of the missing linkage to the cell surface proteins are then released into the culture media. Furthermore a significant decrease of the bacterial biofilm formation was seen at curcumin concentrations of 15µmol/l without any impact on the growth of the bacteria. Therefore it is suggested that curcumin might have inhibitory effects under its determined MIC already.[38] The anti-microbial effects of curcumin have also been proven in a different study where Curcumin and its β-diglucoside were tested concerning their activity against two gram-negative bacteria strains of Escherichia Coli and Yersinia enterocolitica. An antibacterial activity was seen for both molecules, but did differ for the two strains. For curcumin a higher dosis was needed to reach 100% inhibition of E.coli in comparison with the glucoside (0,611µM versus 0,469µM). For Y. enterocolitica in contrast curcumin showed 100% inhibition at 0,678µM while 0,867µM of the glucoside were required for a similar effect. For two gram-positive bacteria strains also contrary results occurred. Curcumin was less effective than its glucoside concerning the inhibition of E.Coli. For B. cereus it was more effective at high concentrations (0,135µM) and less effective at

low concentrations (0,07µM) compared to the glucosylated derivative.[19]

Furthermore curcumin has been shown to have antiviral activity and inhibit the entry of hepatitis C virus (HCV) in human hepatocytes, without having any impact on virus replication. This suppressing effect might be associated with the EGFR-downregulation by curcumin. THC in contrast did not show antiviral activity in experiments.[10]

Curcumin is also reported to inhibit Influenza Type A virus through interaction with the viral hemagglutinin. Again THC is less effective in this connection resulting in the suggestion that the presence of double bonds in the backbone structure of curcumin promotes the interfering with the hemagglutination activity.[10]

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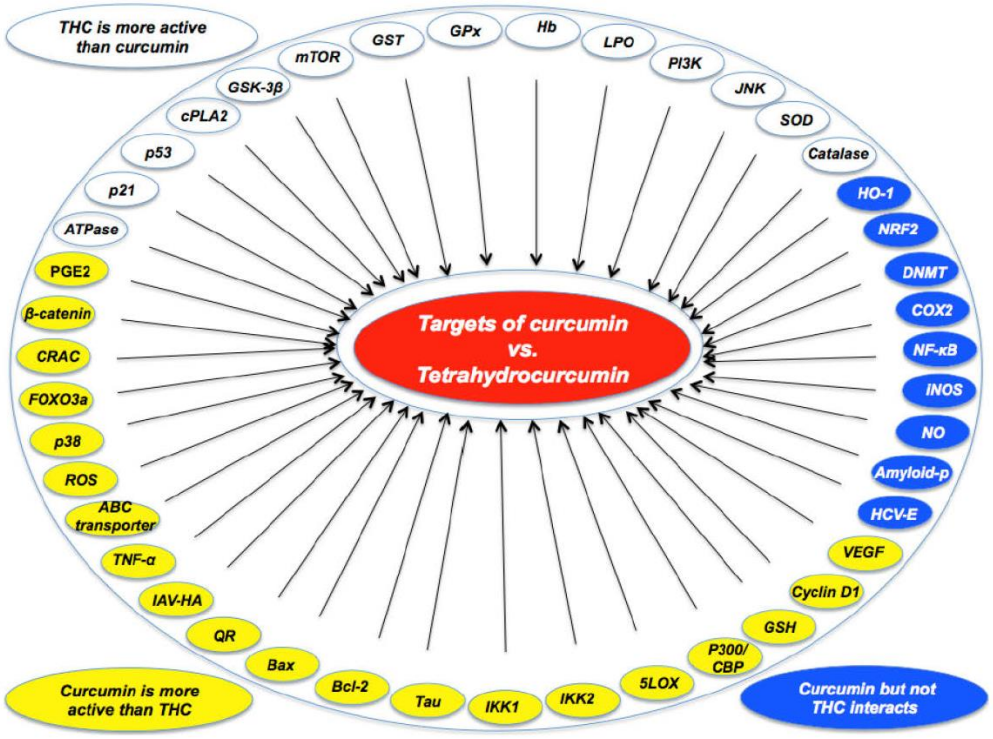


Fig. 6: Different molecular targets of curcumin and tetrahydrocurcumin. [10]

Yellow: Curcumin is more active than THC. Blue: Curcumin, but not THC interacts. White: THC is more active than curcumin.

## 6. Bioavailability

Low bioavailability is one of the most limiting factors in medical drug treatment and can be caused by many sources, including rapid metabolism, clearance or elimination, low absorption rates and tissue distribution, inactivity of metabolites or low intrinsic activity.[3]

Orally applied curcuminoids are absorbed very poorly and, because of their hydrophobicity, metabolized very fast in vivo. This leads to a scant bioavailability which is reportedly only 1% in rats.[8],[39] Because of this strong first pass metabolism the plasma concentration is extremely low and the molecule gets excreted very fast.[39]

It is also believed that daily curcumin consumption decreases the absorption rate because of a curcumin accumulation in the intestinal mucosa.[23], [40]

In human blood maximum concentrations of orally applied curcumin do not exceed low nanomolar values (<160nmol/L), even after administration of 10g or 12g.[20],[28]

Low plasma concentrations do not only occur in humans, but also mouse models, although the curcumin serum levels are reported to not be directly comparable between different species.[3]

After i.v. administration of 10mg/kg of curcumin in rats higher maximum serum levels (0,36±0,005µg/ml) resulted than after oral administration of 500µg/kg (0,06±0,01µg/ml).[39] This indicates the importance of the application route for the bioavailability.[3]

In a mice study by Pan et al. curcumin was given orally (1g/kg) and reached a plasma concentration of 0,13µg/ml after 15 minutes. The maximum concentration (0,22µg/ml) was measured after one hour and was below the detection limit five hours later. When applied intraperitoneally (i.p.) the curcumin concentration was significantly higher (2,25 µg/ml) after 15 minutes than it was one hour after oral application, but showed a fast decrease within one hour.[20] Also the tissue concentrations were investigated in this study. In brain tissue hardly any Curcumin was found after one hour whereas it had a concentration of 26,9 µg/g and 26,06 µg/g in the liver and spleen. The highest concentration was found in the intestines (117 µg/g) after one hour.[20]

In a different study, 1g/kg was administered orally to rats in order to investigate concentrations in excretion products. Hardly any curcumin was found in the urine while 75% were excreted via the feces.[41]

Further Marczylo et al., who compared the bioavailability of curcumin with a new formulation of curcumin with phosphatidylcholine, report a maximum concentration of 6,5±4,5 nM in rat serum half an hour after oral application of 340mg/kg curcumin. Rats that received the formulation with phosphatidylcholine, showed an enhanced systemic bioavailability with fivefold higher area under the curve (AUC) levels, in comparison to those that received unformulated curcumin.[42] After administration of a new formulation of curcumin as micronized powder in liquid micelles containing curcurmin powder and Tween-80, the bioavailability in humans was also increased, but differences between sexes occurred. Women showed a higher absorption of Curcumin, but smaller distribution volumes than men.[22]

Administration of curcumin in enriched bread resulted in a lower serum concentration (130nmol/L) than administration of free curcumin (10nmol/L). Enrichment of bread with encapsulated curcumin led to an increase of the bioavailability.[18]

In several studies a reduction of the first pass metabolism was accomplished through addition of different substances like piperine, that is known to lower glucuronation.[3],[18] During phase II metabolism, orally applied curcuminoids are glucuronated and/or sulfonated in order to enhance their hydrophilicity and fasten their elimination from the body. In contrast to that curcumin that is given i.p. is reduced to tetrahydro-, hexahydro-, and octahydrocurcumin.[24]

It is suggested that after curcumin is glucuronated during absorption, it can be distributed into different organs very easily because of the lipophilic structure of the glucuronide. This might explain the low plasma concentrations that occur after oral administration.[43]

# 7. Metabolism

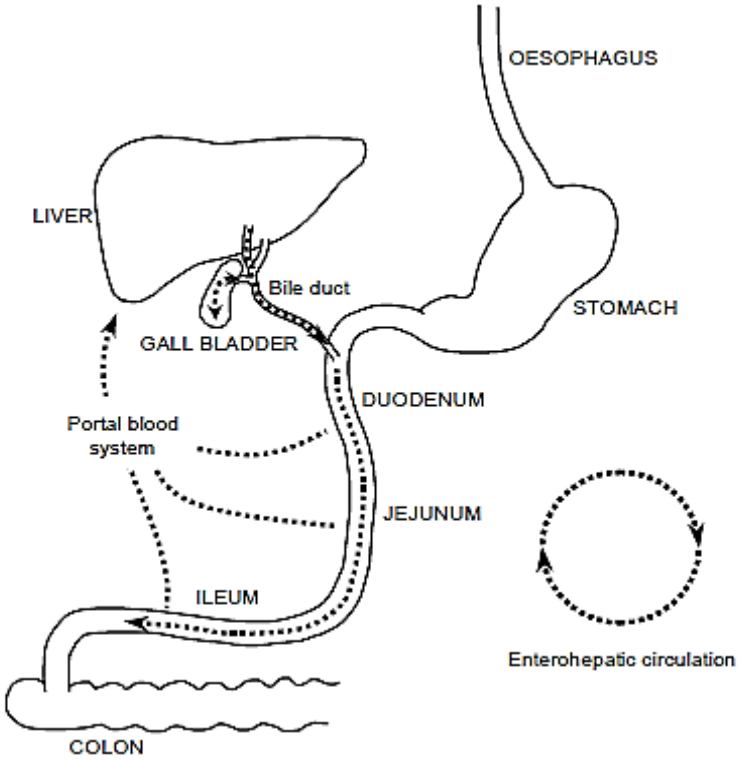


Fig. 7.: Enterohepatic circulation.[44]

After ingestion food ingredients are absorbed in the intestine and transported to the liver over the portal blood flow. Compounds that experience extraction in the liver are then transported over the canalicular membrane into the bile duct from where they reenter the small intestine.

## 7.1. Phase I metabolism

An important element of the curcumin metabolism is Escherichia Coli bacteria which are found in human feces and produce the NADPH-dependent curcumin/ dihydrocurcumin reductase (CurA). This enzyme, that contains two identical subunits and has a molecular mass of 82kDa, depends on NADPH and works as a catalyst for the exclusive reduction of C=C double bonds. This leads to the reduction of curcumin to dihydrocurcumin and finally to tetrahydrocurcumin. The structure of CurA is related to the Zn-independent medium-chain dehydrogenase/reductase (MDR) superfamily, where also the alcohol dehydrogenases belong to. In contrast to many other MDR family members, it is suggested that CurA is metal free, but has, as many other

related structures, the ability of binding NADPH in its center. This is mediated through a glycine-rich motif that is important for the binding of the pyrophosphate group and that is found in many different oxidoreductases. Another remarkable fact about CurA is its structural stability at a wide pH range (pH 4,5-12,0) [14]

After incubating different CUR A positive E.Coli with curcumin at pH 6.00-6.27, a fast degradation to ferulic acid, feruloyl methane and vanillin occurred within 30 minutes. This result suggests that bacteria might be responsible for the production of these metabolites.[15]

In the same study also tetrahydrocurcumin, ferulic acid, dihydrocurcumin and the curcumin adduct curcumin-L-cysteine were found in the organic fraction, whereas no metabolites occurred in the aqueous fraction. L-Cysteine had been added to the growth medium (mMCB) in order to reduce the potential for oxidative and reductive reactions. It is proposed that the curcumin-L-cysteine adduct can exclusively be produced in the presence of bacteria through a NADPH catalyzed Michael-addition.[15]

Further Lou et al. found different metabolites in human intestinal bacteria in vitro using ultra-performance liquid chromatography coupled with quadrupole TOF-MS. The three major metabolites were a hydroxylated, a hydroxylated and acetylated and a demethylated, reduced and hydroxylated derivative.[45]

After incubation over 36 hours in mMCB in presence or absence of PCF (phosphate chemical fertilizer) strong differences concerning the ability of curcumin metabolism in different bacteria strains are reported. Escherichia fergusonii (E469) was shown to have a much higher metabolizing potential for curcurmin, with a metabolism of 37,3% in absence and 23,2% in presence of PCF, in comparison with E. coli E739, that only metabolized 19,9% without PCF addition and 15,8% with PCF. Similar results were observed for E. coli E10B that metabolized 20,8% in absence and 16,1% in presence of PCF. While curcumin concentrations decreased after incubation, DCM values were significantly altered in similar amounts.[15]

# 7. Metabolism

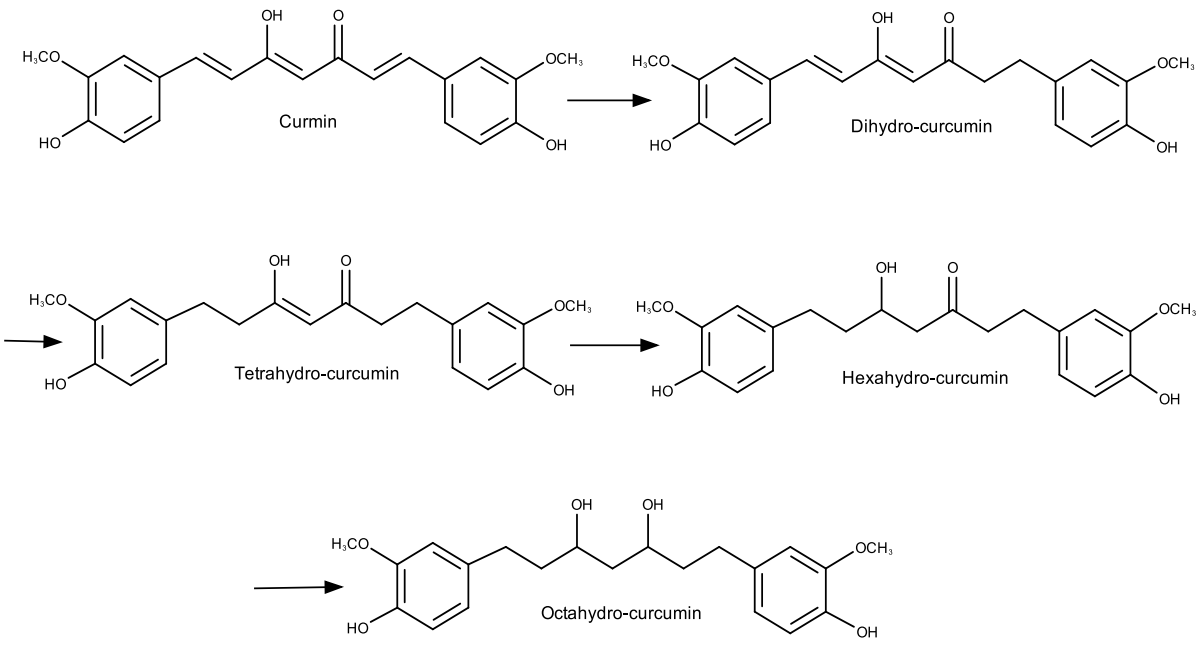


Fig. 8.: Metabolism of curcuminoids in rat liver [21]

Although most experiments have been performed in rat liver similar results can be expected for humans because rat liver is a well-established animal model for studies of metabolic reactions.

## 7.2 Phase II metabolism -Conjugation

### 7.2.a. Glucuronation:

Glucuronation is the most important conjugation reaction in phase II of the curcumin metabolism and usually takes place on the phenolic hydroxyl groups.[46] If the molecule does not contain this structure only aliphatic glucuronides occur.[43] Like sulfonation, the glucuronation makes the molecule more hydrophilic and leads to excretion.[46]

Pan et al. suggested that about 99% of curcumin and at least 85% of THC found in the plasma are glucuronated.[20]

After oral application, curcumin is absorbed at the alimentary tract and then metabolized and conjugated before reaching the blood circulation in a glucuronated and or sulfonated form. THC - glucuronides discovered in the plasma after i. p. administration only seem to be minor metabolites.[20]

The maximum concentration of conjugated curcumins is reported to be reached after

one hour.[41]

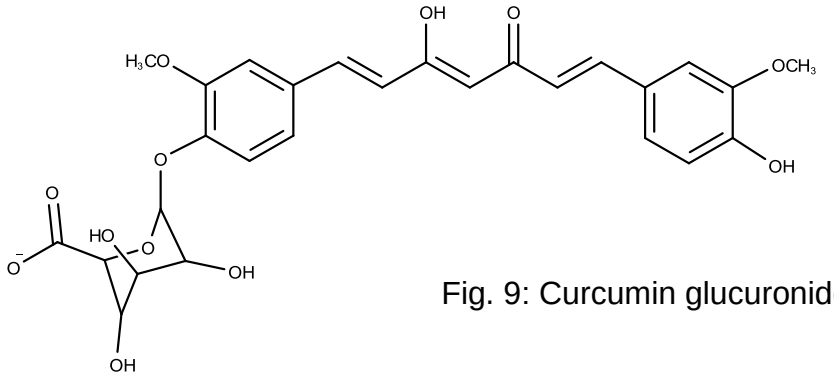


Fig. 9: Curcumin glucuronide [24]

Although most studies only report one curcumin glucuronide (Fig. 9), Pfeiffer et al. suggested that there are two different mono-glucuronides that emerge from curcumin.[43],[46]

Curcumin glucuronide A, the major one has a phenolic structure, whereas curcumin glucuronide B is reported to be alcoholic. In contrast to that Hexa-hydrocurcumin is only turned into a phenolic glucuronide.[43],[46]

The glucuronation reaction is catalyzed by the uridine-5´-diphospho-glucuronosyltransferase (UGT) superfamily. These enzymes, that are bound to the luminal membrane of the ER, transfer glycosyl groups of the uridine-5´-diphosphoglucuronic acid (UDPGA) onto various xenobiotics, including curcumin and its metabolites.[46]

Out of eight different UGTs, that all formed curcumin glucuronide A, and some also glucuronide B, the UGT1A1, 1A8 and 1A10 showed the highest activity in experiments. All three of them are found in the human intestinal microsomes, where also the activity for the curcumin glucuronation seems to be three times higher than in the liver.[43]

This is in agreement with Asai et al. who reported that although the UDP-glucuronosyl - transferase activity was higher in the liver than in the intestine, only traces of free curcumin could be found in the portal vein blood which suggests that free curcuminoids are not able to reach the liver when administered orally. Because of this fact it is very likely that the pharmacological activity of curcumin is mediated

through its metabolites.[41]

The stability of the curcumin glucuronides A and B is at least as low as the one of the aglycon. All three are eliminated within one hour. Bisdemethoxycurcumin degrades even faster than its glucuronic conjugate, while isocurcumin and isocurcumin glucuronide show a slow decomposition. Only hexahydrocurcumin and hexahydro-curcumin glucuronide are stable. The differences in stability might be explained by different substitution pattern of the aromatic rings. In order to be reactive the molecule structure must have two characteristics. First, the aliphatic chain has to have conjugated double bonds and second, the phenolic OH-group must be located in para position to this aliphatic chain. If these features are lacking, the molecule is more stable but also less reactive (e.g. iso-curcurmin where the phenolic groups are found in meta-position or hexahydrocurcumin which has a saturated aliphatic chain).[43]

### 7.2.b. Sulfonation:

With the sulfonation the phase II metabolism includes another important pathway that is essential for the biotransformation of curcurmin. Already glucuronated and unglucuronated molecules can further be sulfonated in order to fasten the elimination process.[41],[47]

These reactions are catalyzed trough the superfamily of sulfotransferase (SULT) enzymes, that are located in the cytosol of different cells. The most prevalent human tissue for the sulfonation of curcumin is the small intestine. [47] Interestingly liver, lung and kidney do not seem to play an important role on this connection, although an older study reports to have found the highest activity of sulfotransferases in the liver of rats.[41], [47]

The predominant enzyme on sulfonating Curcumin and Demethoxycurcumin is SULT1A3, which is found only in the small intestine, but not in the liver. A study investigated the activities of nine different SULT enzymes concerning curcumin sulfonation. SULT1C1, SULT1A3, SULT1E1 and SULT1B1 all exhibited measurable sulfonation activities in previously mentioned order for a curcumin concentration of 1,25µM. For higher concentrations (5µM and 20µM) SULT1A3 was the isoform with the highest activity. SULT1B1was found to exclusively catalyze the sulfonation of curcumin, but not of its metabolites.[47]

The dominant SULTs for Demethoxycurcumin are SULT1A3, 1C4 and 1E1, whereas for Bisdemethoxycurcumin only SULT1C4 and 1E1 are worth mentioning, with SULT1C4 being the more active isoform.(47)

Generally, it can be marked that SULT1E1 was found to be the enzyme with the highest catalytic efficiency for curcuminoids, especially to DMC.(47)

On the other hand Curcumin has been shown to inhibit several SULT isoforms like most notably the hepatic and extrahepatic phenol SULT1A1. Lu et al. explained this process with allosteric development of an unproductive dead end complex after curcurmin binding. This could be a risk factor for drug-drug interactions (47)

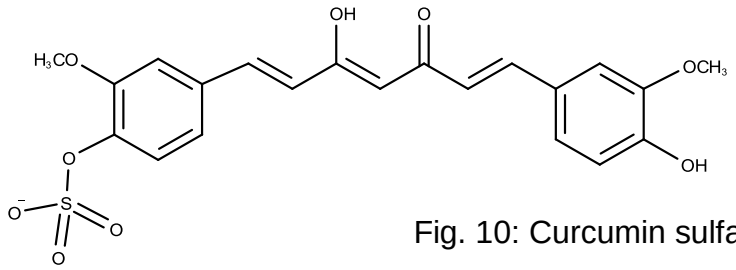


Fig. 10: Curcumin sulfate (24)

# 7. Metabolism

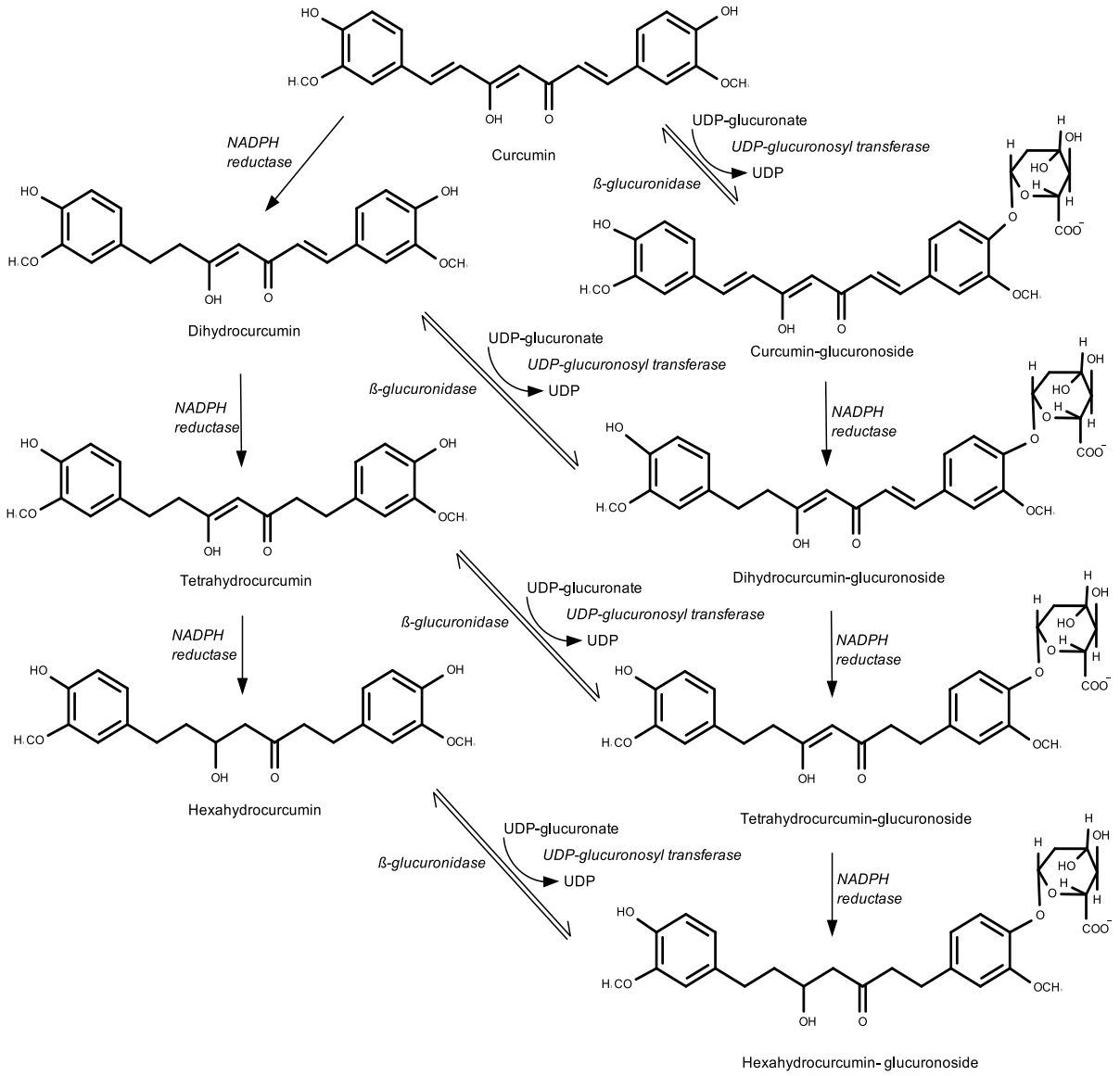


Fig. 11: Curcumin biotransformation in mouse. (48)

NADPH mediates curcumin reduction while ß-glucosidase hydrolyzes conjugated derivatives that occur as glucuronides in plasma.

# 8. Transport

## 8.1. Multidrug resistance (MDR)

One of the main challenges in modern medical treatment is the overcoming of drug resistance. Particularly in cancer treatment this mechanism plays an important role and impedes therapy significantly, being the most common reason for failure of therapy. The most important group of proteins that are involved in MDR are the ATP-binding cassette (ABC) transporters that have the ability of actively transporting drugs out of cancer cells.[49] The ABC-superfamily contains a very large group of proteins with different functions, including membrane transporters, ion channels and receptors.[50] In humans 49 members have been yet identified.[49] All members are characterized by a highly conserved ATP-binding cassette structure formed by 200-250 amino acids with two nucleotide-binding domains. According to their general sequence and structural homology they are divided into seven subfamilies (A to G).[50],[51] One of these subfamilies is the family of the xenobiotic efflux pumps, that are mostly located in physiological barriers like the kidneys, the blood-brain-barrier or the gut, but are also found in tumor tissue. Their function is to promote the efflux of possibly dangerous xenobiotics, including medical drugs, in order to prevent cells from damage.[49] These proteins, which are usually located on apical membranes, are one of the reasons that ingested compounds do not reach the blood and experience systemic distribution because they are immediately driven back to the intestinal lumen.[44] Because of their negative influence on therapy the search for inhibitors of these efflux pumps is an important task in medical research nowadays. The activities of the breast cancer resistance protein (BCRP), the multidrug resistance protein (MRP) and the P-glycoprotein (P-gp), that are all three members of this subfamily, have been proven to be negatively influenced by curcumin.[49]

## 8. Transport

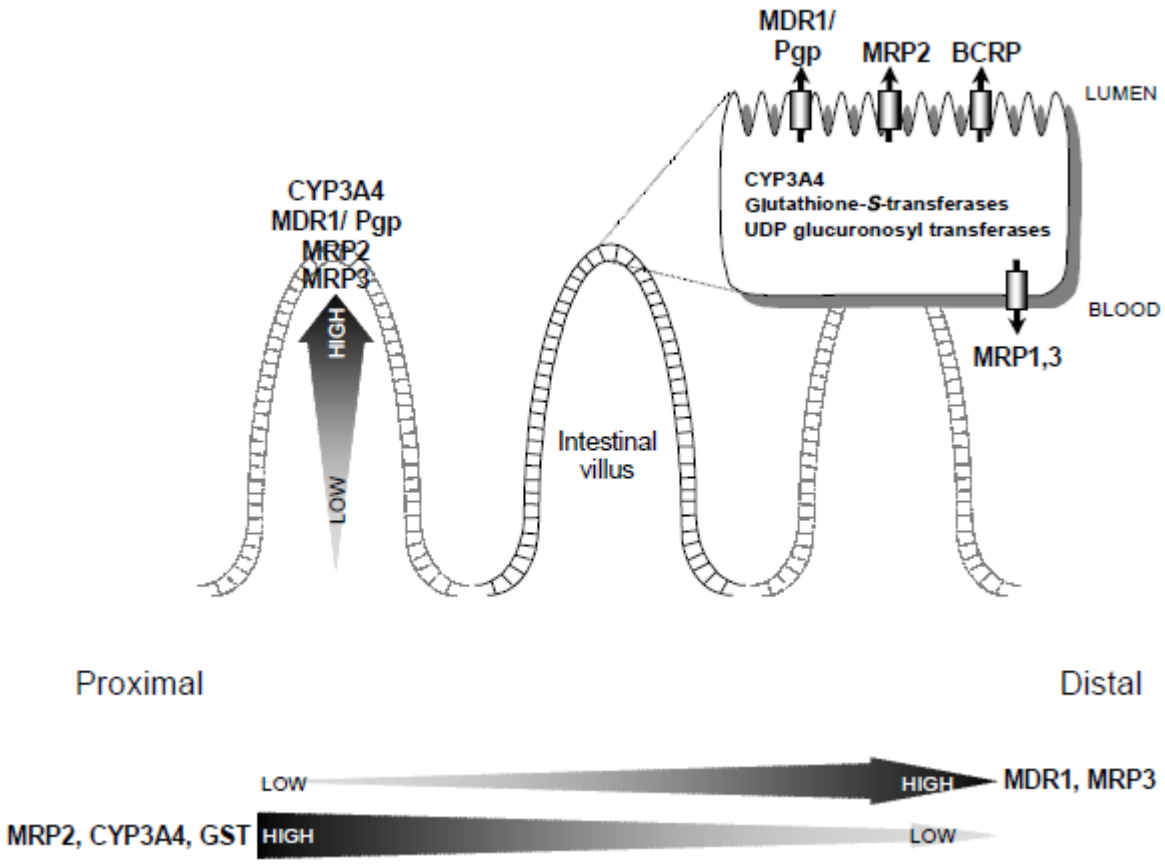


Fig. 12: Intestinal efflux mechanisms. [44]

### 8.2. Multidrug resistance associated protein (MRP)

One of the main reasons for the development of drug resistance is the modulation of the human multidrug resistance related protein (MRP1/ABCC1). It is like P-gp and BCRP a member of the ABC superfamily and gives cells the ability to actively efflux drugs and to reduce their concentration beyond a toxic level. This pathway that on the one hand prevents the body of toxicities is on the other hand a major problem especially in cancer therapy. With increasing MRP concentrations the therapeutic doses cannot be reached and therefore therapy fails. MRP1 is a transmembrane receptor with intracellular epitopes and primarily transports GSH, oxidized GSH and organic anions conjugated with glucoronate and sulfate.[50],[52]

The protein is expressed in a wide range of cell types and organs, including tumor tissue.[52] Several studies investigated the effect of curcumin on MRP1 activity.[50], [52]

The sensitivity of etoposide in MRP1- transfected HEK 293 cells was shown to be

increased by the three curcumins I, II, and III with curcumin I being the most efficient. Curcuminoids also enhanced the accumulation of calcein-AM and fluo4-AM in these cells in a concentration dependent manner. Furthermore treatment with curcuminoids led to stimulation of the basal ATPase activity and inhibition of ATP-hydrolysis through MRP1, stimulated by quercetin, perhaps also through interaction with the substrate binding site, but without being substrates.[13], [50]

All three forms of curcuminoids showed similar effects on P-gp and MRP1 concerning the ATP hydrolysis. In lower concentrations they were able to stimulate the basal ATPase activity, but had an inhibiting effect at higher concentrations.[50]

ATP hydrolysis is on the one hand essential for drug transport by ABC transporters and on the other hand for resetting the transporter for a new catalytic cycle through change of conformation. Therefore inhibition of ATP hydrolysis by curcuminoids is an important mechanism for lowering drug resistance.[52] It is suggested that neither isolated curcuminoids nor the curcumin mixture are direct substrates of P-gp and MRP1 because no significant difference in the $IC_{50}$ occurred between the control and the MRP1 overexpressing HEK-cell line. Still curcuminoids showed a reversing effect on the resistance of the MRP1-HEK293 cells against vinblastine and etoposide, without affecting the cytotoxicity of these drugs. Also expression levels of MRP in the transfected cells did not change in the presence of curcumin. These results suggest that the reversal of drug resistance in the MRP1 overexpressing cells was caused through inhibition of the MRP1 function by curcuminoids.[50]

### 8.3. P-glycoprotein 1 (P-gp)

Another prominent protein is the P-gp (ABCB1) which is mainly found in the kidneys and adrenal gland and in lower concentrations in many other tissues including liver, small intestine, colon and lung.[44] This glycoprotein of 170kDa contains 1280 amino acids and is bound to plasma membranes on the apical side.[52],[53] It is encoded by the human MDR1 gene and consists of two identical halves with six transmembrane domains each, that build a drug–binding pore.[52] It has the function of an efflux pump and is known to transport many drugs that are in current clinical use (e.g. steroids, antiviral drugs and cardiac drugs). Also cytostatic drugs used in cancer therapy (e.g. vinblastine, doxorubicine, paclitaxel) work as substrates for this transporter.[49] After chemotherapeutic treatment, high concentrations of P-gp have been detected in

various tumor tissues, particularly in renal, hepatic, colorectal and adrenocortical cancer cells.[52] When Chearwae et al. investigated the cytotoxicity of Curcumin I-III extracts on KB-V1 cells (multidrug resistance cervical carcinoma cell line) and KB-3-1 cells (drug sensitive cervical carcinoma cell line) they found that the $IC_{50}$ levels did not differ significantly between the P-gp expressing KB-V1 cells and the not P-gp expressing KB-3-1 cells. They concluded that curcuminoids might not be transported via the multidrug transporter although they interact with the P-gp protein. When analyzing the impact of curcuminoids on the cytotoxicity of vinblastine in these two cell lines using verapamil as a modulator, it could be shown that all three curcuminoids only increased the cytotoxic effect in the KB-V1 cells with a radical decrease of the $IC_{50}$ from 1,7 to 3,0µM by a modulation of P-gp function. In the same study an efflux block of three fluorescent substrates (rhodamine123, calcein-AM, bodipy-FL-vinblastine) through curcumins in the P-gp expressing KB-V1 cells, but not in the KB-3-1 cells could be shown. [13] Chearwae et al. also reported that curcumin I modulates the P-gp function most efficiently of all curcuminoids and binds directly to the transporter. It is suggested to have the same binding site as verapamil or prazosin and not only inhibits the function of P-gp, but also the expression.[50] This aligns with the report of a concentration dependent inhibition of photoaffinity labeling of P-gp by IAAP ([$^{125}$I]-iodoarylazidoprazosin) through curcuminoids. Again curcumin I was the most effective of all three curcumins but less effective than the mixture.[13]

Hou et al. investigated the influence of different Curcuma extracts (C. longa, C.aromatica, C. zedoaria) and curcumin alone on the P-gp expression and function and found that all Curcuma extracts lead to a significant enhancement of P-gp activity through an up-regulation of P-gp expression and MDR1 mRNA levels. In contrast curcumin itself showed the complete opposite effect with a decrease in MDR1 mRNA and P-gp expression and inhibition of its activity. These results suggest that co-administration of curcuma drugs or curcumin might influence the P-gp activity and therefore the P-gp mediated drug transport in different ways.[53]

Furthermore the impact of the curcumin metabolite THC on the activity of different ABC transporters has been investigated and it was found that THC hast the ability of inhibiting P-gp function, as well as MRP1 and mitoxantrone resistance protein (MXR) function. In human breast cancer MCF-7 cells THC inhibited vinblastine efflux and increased drug accumulation significantly in a concentration dependent manner. It

also led to a stimulation of P-gp mediated ATPase activity and reduced the binding and incorporation of IAAP into P-gp which suggests that THC might interact directly with the drug binding site of the transporter protein. It is suggested that it binds at the same site as prazosin.[51]

Further it increased accumulation of calcein-AM and rhodamine in P-gp expressing drug resistant human cervical carcinoma cells KB-V1, as well as calcein – AM - accumulation in MRP1-HEK293 cells. Also mitoxantrone efflux in MXR expressing MCF7 cells was inhibited and the sensitivity against the drug enhanced. MXR mediated ATPase activity was stimulated in a concentration dependent manner. Because curcumin is metabolized to THC in vivo, the inhibiting activity of THC against ABC transporters may have an influence on co-administered drugs.[51]

### 8.4. Breast cancer resistance protein (BCRP)

The breast cancer resistance protein (BCRP/ABCG2) is a glycoprotein of 655 amino acids and also belongs to the group of ABC transporters. It is mainly found in the intestine, kidney, placenta and liver, where it is expressed in apical membranes. An overexpression of BCRP was also found in various tumor tissues, including colon, ovary, gastric, lung and breast cancer.[52],[54] High BCRP expression is an important cause for limitation of oral bioavailability of drugs and fast elimination into the urine and bile. Subjects with a gene mutation leading to lower BCRP expression show a significant higher bioavailability of different orally administered drugs. BCRP activity leads to a limitation of the penetration of drugs into the brain and testis. BCRP has a remarkable great substrate specificity which makes it to a major problem in therapy, because it can interact with a variety of drugs.[54]

A study investigated the effect of curcumin on sulfasalazine uptake and oral bioavailability in healthy human subjects, as well as in wild-type and BCRP (-/-) mice. It was found that curcumin is a potent inhibitor of the human BCRP when administered in doses of 300mg/kg and 400mg/kg and led to an eightfold increase of the AUC in wild-type, but not in BCRP (-/-) mice.[54] This aligns with the results of a different study that also proved that pretreatment with 400mg/kg curcumin enhances the bioavailability of orally administered sulfasalazine in mice. Furthermore the $T_{max}$, the $C_{max}$ and the apparent plasma half – life of sulfasalazine were significantly

increased and the apparent oral clearance strongly decreased. Furthermore the sulfasalazine plasma values were still high after 24h in curcumin-treated animals, while they were under detection limit in the control group.[55] Similar results were obtained in humans, where curcumin lead to a twofold increase when applied in micro-doses and a 3.2-fold increase at therapeutic doses. The increase after curcumin administration was significant for different doses of sulfasalazine (micro - dose and therapeutic dose). Compared to mice curcumin exhibited a tenfold higher potency in humans for in-vivo BCRP inhibition.[54]

Curcumin I has been shown to inhibit BCRP most effectively compared to the inhibition of two other major transporters MRP1 and P-gp. In an ex-vivo study using brain capillaries from rats, curcumin showed a concentration dependent inhibition of BCRP activity with an $IC_{50}$ of 19,4nM.[55] These results suggest that oral co-administration of curcumin leads to an enhancement of intestinal bioavailability of several drugs through inhibition of the BCRP and might therefore be useful in therapy.[54],[55]

# 9. Drug interactions

## 9.1. CYP 450 inhibition

Cytochrome P450 (CYP) enzymes, that are mainly found in liver and small intestine, are prominent factors regarding first pass metabolism, which is associated with low bioavailability of many substances. More than 50% of all drugs that are in current clinical use are metabolized via the CYP3A4 isoform. Consequently enzyme inhibition through xenobiotics is one of the main reasons for drug-drug interactions.[56]

Several studies investigated the CYP inhibition potential of Curcumin.[4],[57],[58] It was found that the molecule shows less potency for inhibiting CYP3A4 , CYP1A2 and CYP2D6 than demethoxycurcumin. Sesquiterpenes that were also found in C. aromatica showed an even higher inhibition of these three CYPs.[57]

In a different study CYP3A4 positive Caco-2cells were treated with curcumin which led to a decrease of the CYP3A4 catalytic activity of about 30-40% and a 38% decrease in CYP3A4 protein expression. Furthermore the cells were treated with two different extracts derived from Curcuma rhizomes (C. longa and C. zedoaria). This caused an 85-95% decrease in the CYP3A4 activity and a 60-70% decrease in CYP3A4 protein expression. Neither Curcuma extract nor curcumin treatment influenced the CYP3A4 mRNA expression.[4]

Also the impact of curcumin, as well as of a mix of its decomposition products and of four isolated decomposition products (vanillin, vanillic acid, ferulic aldehyde and ferulic acid) on five important human CYP450 enzymes (CYP1A2, CYP3A4, CYP2D6, CYP2C9, CYP2B6), that were expressed and isolated out of E.Coli cells, was investigated. [58]

Curcumin exhibited the highest potency against CYP2C9 with a concentration of 300µM leading to almost total inhibition. Similar results were found for CYP3A4 and CYP1A2, whereas the activity of CYP2D6 and CYP2B6 was only inhibited by 72% and 69,1%. It is suggested that these differences in inhibition are caused by different structures at the active sites of cytochrome proteins. The impact of curcumin on the metabolizing CYP enzymes was clearly stronger in this concentration than the one of its decomposition products (vanillin, vanillic acid, ferulic aldehyde and ferulic acid), that showed only weak inhibiting effects of 1-50% of CYP activities. The inhibition caused by a mixture of these four decomposition products was slightly higher with an inhibition of 57,4% for CYP3A4 and 74,8% for CYP2C9.[58]

Furthermore it is reported that curcumin caused an increase of $K_m$ values without changing the Vmax levels significantly in a MROD (methoxyresorufin-O-deethylase) assay with CYP1A2, as well as in a BROD (benzyloxyresorufin-O-debenzylase) assay with CYP2B6 and CYP3A4, while it led to a decrease of the $V_{max}$ levels with CYP2D6 and CYP2C9 without changing the $K_m$ values significantly. This indicates competitive inhibition for CYP1A2, CYP3A4 and CYP2B6 and non-competitive inhibition for CYP2D6 and CYP2C9. To proof that no mechanism-based inhibition occurs with any of the CYPs curcumin was pre-incubated with NADPH-fortified CYPs in a second experiment in this study. No increase of the inhibition potency could be seen, thus mechanism-based inhibition does not play a role with any of these CYPs.(58) Concerning drug-drug interactions Appiah-Opong et al. suggested that the strong inhibition of CYP3A4 through curcumin in vitro could lead to in vivo interactions at oral administration because of the high values of CYP3A4 in intestinal epithelial cells. When drugs are co-administered orally the intestinal exposure to the cytochrome proteins could lead to an enhanced bioavailability, followed by an increase of plasma concentrations. In contrast to that the low inhibition potency of the curcumin decomposition products towards the CYPs indicates that these metabolites are not likely to cause drug interactions.(58)

### 9.2. CYP interactions

Several studies investigated interactions with curcumin and substances that are also metabolized via the CYP450 system.

Piperine that is found in black pepper, has the ability of increasing curcurmins low bioavailability. As mentioned in 5.2.b. curcurmin works as an inhibitor of human cytochromes, mainly CYP3A, CYP2C9, as well as UGT and SULT. Studies suggested that piperine also has an inhibiting activity on these enzymes and that the single but also combinatory use of these two drugs could influence metabolism of other therapeutic agents.(59)

The group of Volak L. et al. investigated this issue on three different drugs: midazolam (CYP3A metabolism), flurbiprofen (CYP2C9 metabolism) and paracetamol (dual UGT and SULT metabolism) in healthy human volunteers. Surprisingly the results showed that a short term therapy of a standard piperine-curcurminoid preparation for two days does not have a significant impact on the

metabolism of these drugs.[59]

Neither an important change in plasma $C_{max}$, nor in AUC, clearance, elimination half-life or metabolite concentrations of the three drugs occurred. It is suggested that the short duration of the treatment and/or the low levels of curcurmin and piperine conjugates that occurred, were responsible for the missing interactions.[59]

Other studies that investigated changes in CYP metabolism proved the impact of curcumin. When 1g of curcurmin was administered orally together with caffeine for two weeks the results showed a by 28% lowered CYP1A2-mediated caffeine metabolism and a by 49% altered CYP2A6 metabolism. [60]

Also the effect of curcumin on the plasma concentration of the antiplatelet drug clopidogrel, that needs CYP-mediated metabolism in order to be transformed into an active metabolite that has the ability of irreversibly inhibiting platelet aggregation, was investigated. Further the impact on a second antiplatelet drug, warfarin, that experiences CYP mediated in vivo oxidation, was studied. As expected the administration of high curcumin doses (100g/kg) lead to an increase in AUC and $C_{max}$ of warfarin and clopidogrel in rats, but still no significant change in the pharmacokinetic parameters occurred. It is suggested that the influence of curcumin on the pharmacokinetics of these drugs is not related with CYP metabolism, but with curcumins ability of inhibiting P-gp, a major drug efflux protein.[61]

In contrast rats that received midazolam after a four day treatment with curcumin (60mg/kg/d) showed an increased AUC and decreased $CL_{oral.}$ Midazolam is only a CYP substrate and not a P-gp substrate which leads to the conclusion that the changes in the pharmacokinetic profile were caused through inhibition of CYP activity.[62]

Also the bioavailability and plasma concentration of norfloxacin was increased after oral co-administration with curcumin in rabbits.[63]

Furthermore the metabolism of several other drugs, including loratadine, losartan, docetaxel, tacrolimus and tamoxifen has been found to be influenced by curcumin.[61], [62], [63],[64],[65], [66],[67],[68],[71]

In contrast to these findings also CYP3A4 induction has been reported by Hsieh et al. who administered curcumin to rats together with etoposide that is known to be a CYP3A4, as well as a P-gp substrate. According to this in vivo study curcumin decreased the bioavailability and plasma concentration of etoposide significantly. As

expected for low bioavailability and rapid metabolism a high concentration of glucuronides and sulfates were found in the serum. When analyzing these metabolites they surprisingly activated CYP3A4 in contrast to curcumin, DMC and BDMC that were proven to be inhibitors, which aligns with previous in vitro results. Thus it is suggested that because of the rapid metabolism and immediate conjugation the free curcuminoids do not have enough time to interact with the CYPs that are located in hepatocytes and enterocytes and that the inducing effect of the glucuronides exceeded the P-gp inhibition. This would be an explanation for the unexpected in vivo results in this study.[69] Nevertheless a different group reported that curcumin co-administration with etoposide in rats leads to an enhanced oral bioavailability of etoposide through CYP3A4 inhibition.[70]

Generally it has to be marked, that the described drug interactions are not caused by CYP inhibition alone, but also by inhibition of efflux transporters, especially P-gp in the small intestine.[63], [69], [70] Further investigation has to be done in order to determine the extent of involvement of the single mechanisms in these synergistic effects.

### 9.3. Interaction through inhibition of efflux transporters

As already described in 8.2. the inhibition of drug efflux transporters also plays an important role in drug interactions.

Besides CYP inhibition especially P-gp inhibition in hepatic and intestinal cells is made responsible for changes in pharmacokinetics after co-administration of drugs with curcumin. When 60mg/kg curcumin were given p.o. for four days a downregulation of P-gp expression in rat intestine was observed, while an up-regulation in the liver occurred. Also CYP3A4 levels were shown to not be regulated coordinately in both organs. This findings are in line with other reports about tissue-specific response to drug treatment.[62]

In rats that received celiprolol orally after four days of curcumin treatment a significant increase in $C_{max}$ and AUC and a decrease in systemic clearance was observed in comparison to the control group. A single dose of curcumin half an hour before drug administration did not have an impact on the pharmacokinetic profile of the drugs, suggesting that the changes in plasma concentration and bioavailability

were caused through an inhibition of P-gp and CYP enzymes.[62] Further P-gp efflux is mainly made responsible for the reported increase in bioavailability of different other drugs, such as etoposide, warfarin and clopidogrel in presence of curcumin. CYP inhibition only seems to play a minor role in this connection.[61],[70] Besides P-gp also the inhibition of other efflux transporters can lead to drug interactions. Oral curcumin co-administration has been shown to lead to an enhancement of sulphasalazine bioavailability in humans through inhibition of BCRP.[54]

As shown in table 2 CYP450 interactions with curcumin have been investigated with various drugs. Most in vivo experiments have been performed in rats, but a few also in rabbits and healthy human volunteers. Curcumin was always administered orally in different dosages and lead to a dose-dependent increase of bioavailability. In tab. 1and 2 a selection of different in vivo and in vitro results can be seen.

Tab. 1: Drug interactions in vivo

| Drug | System | Parameter | Curcumin dose | AUC control | AUC co-administered | Proposed mechanism | Ref. |
|---|---|---|---|---|---|---|---|
| Caffeine | Human, p.o. | $AUC_{(0-t)}$ ↑<br>$T_{max}$ ↔<br>t½ ↔<br>$C_{max}$ ↔ | 1g for 14days | 97,3±38,5 (µmol/L/ h) | 104,4±36,2 (µmol/L/ h) | CYP1A2 ↓ | (60) |
| Celiprolol | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑<br>$CL_{oral}$ ↓ | 60mg/kg for 5 days | 3570.91±106.26 (ng/ml/ h) | 4582.18±382.65 (ng/ml/ h) | P-gp ↓ | (62) |
| Clopidogrel | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑<br>CL ↓ | 100mg/kg for 7 days | 142.1±64.54 (µg/ml/ h) | 228.94±71.79 (µg/ml/ h) | P-gp ↓<br>CYP2C9 ↓<br>CYP3A4 ↓ | (61) |
| Docetaxel | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>$T_{max}$ ↔<br>t½ ↔<br>$C_{max}$ ↑ | 100mg/kg for 5 days | 282.6±18.4 (ng/ml/ h) | 2244.1±68.0 (ng/ml/ h) | P-gp ↓<br>CYP3A4 ↓ | (66) |

# 9. Drug interactions

Tab. 1 continuation

| Drug | System | Parameter | Curcumin dose | AUC control | AUC co-administered | Proposed mechanism | Ref. |
|---|---|---|---|---|---|---|---|
| Etoposide | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑<br>F ↑ | 8mg/kg single dose | 561±98 (ng/ml/ h) | 846±169 (ng/ml/ h) | P-gp ↓<br>CYP3A4 ↓ | (70) |
| Loratadine | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑ | 8mg/kg single dose | 926±167 (ng/ml/ h) | 1544±324 (ng/ml/ h) | P-gp ↓<br>CYP3A4 ↓ | (64) |
| Losartan | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>$T_{max}$ ↔<br>t½ ↔<br>$C_{max}$ ↑ | 100mg/kg for 7 days | 2080±837 (ng/ml x h) | 3460±1350 (ng/ml x h) | P-gp ↓<br>UGT1A1 ↓<br>UGT2B7 ↓ | (65) |
| Midazolam | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑<br>$CL_{oral}$ ↓ | 60mg/kg for 5 days | 476.88±109.50 (ng/ml/ h) | 1835.40±422.13 (ng/ml/ h) | CYP3A4 ↓ | (62) |
| Norfloxacin | Rabbit, p.o. | $AUC_{(0-t)}$ ↑<br>$T_{max}$ ↔<br>t½Ka↓<br>t½ß↑<br>$C_{max}$ ↑ | 60mg/kg for 3 days | 2.67±0.42 (µg/ml/ h) | 4.06±1.24 (µg/ml /h) | P-gp ↓<br>CYP3A4 ↓<br>UDP-glucuronosyl - transferase ↓ | (63) |
| Sulphasalazine | Human, p.o. | $AUC_{(0-t)}$ ↑<br>$T_{max}$ ↔<br>$C_{max}$ ↑ | 2g single dose | 5.89±0.71 (µg/ml/ h) | 19.0±2.7 (µg/ml/ h) | BCRP↓ | (54) |
| Tacrolimus | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑ | 170mg/kg single dose | - | - | P-gp ↓<br>CYP3A4 ↓ | (71) |
| Talinolol | Human, p.o. | $AUC_{(0-t)}$ ↓<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↓<br>$CL_{total}$ ↑ | 300mg/kg for 6 days | 1860.0±377.9 (ng/ml/ h) | 1246.0±328.2 (ng/ml/ h) | P-gp ↓<br>MRP2↑ | (67) |
| Tamoxifen | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑ | 10mg/kg single dose | 284±62 (ng/ml/ h) | 334±74 (ng/ml/ h) | P-gp ↓<br>CYP3A4 ↓ | (68) |
| Warfarin | Rat, p.o. | $AUC_{(0-t)}$ ↑<br>t½ ↔<br>$T_{max}$ ↔<br>$C_{max}$ ↑<br>CL ↓ | 100mg/kg for 7 days | 16.68±6.90 (µg/ml/ h) | 26.64±5.84 (µg/ml/ h) | P-gp↓ | (61) |

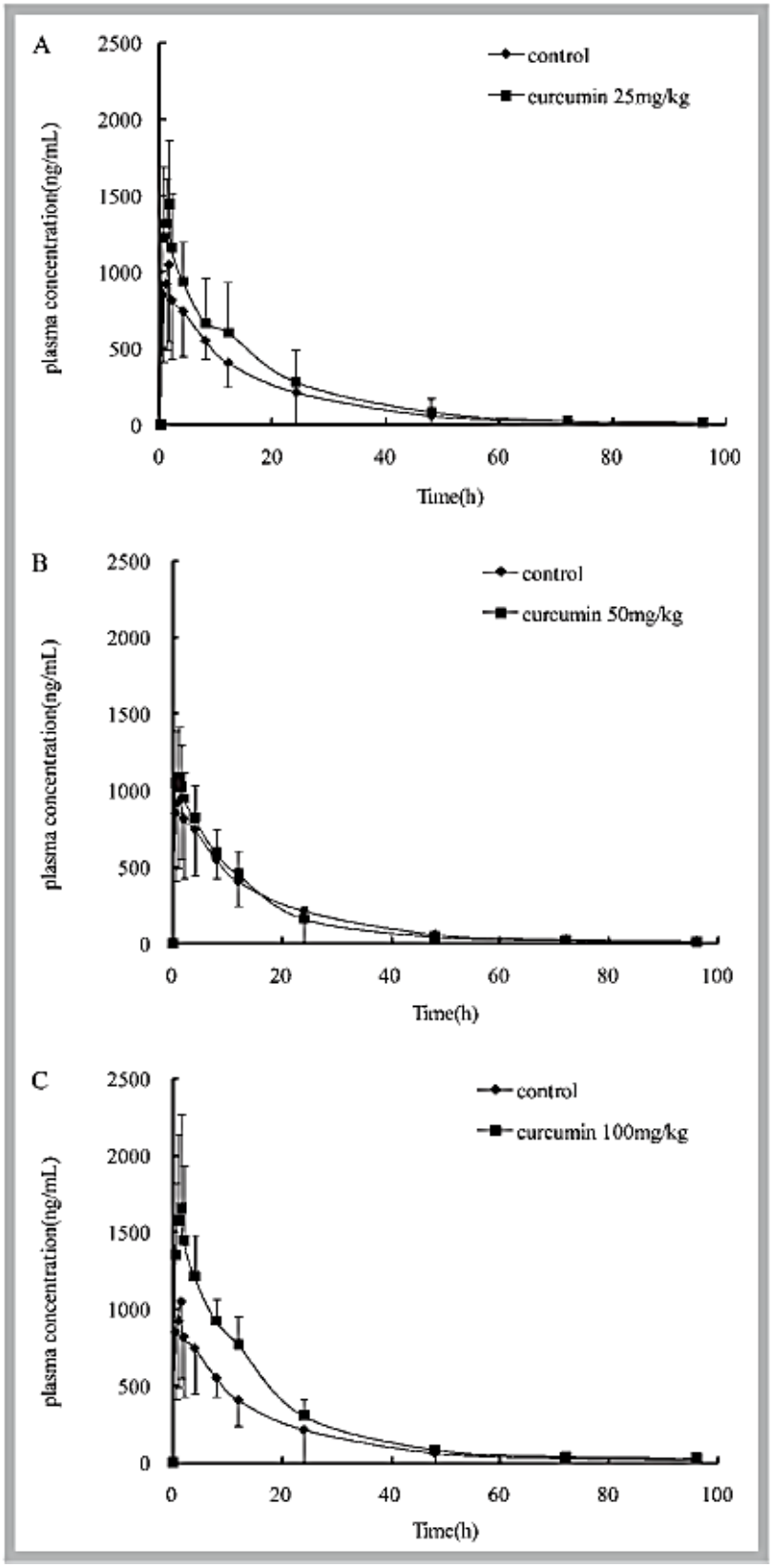


Fig. 13: Curcumin co-administration to warfarin in rats in vivo.[61]

Mean plasma concentration-time curves of warfarin without curcumin (control) or with curcumin in different doses (25, 50, 100mg/kg curcumin) Curcumin leads to a dose dependent increase of Warfarin bioavailability after oral administration in rats in vivo.

## 9. Drug interactions

Further different in vitro studies that have been performed proofed curcumins ability of inhibiting CYP450 enzymes and efflux transporters.

Tab. 2: Drug interactions in vitro

| Drug | System | Parameter | Curcumin concentration | $IC_{50}$ | Proposed mechanism | Ref. |
|---|---|---|---|---|---|---|
| [$^3$H]-Digoxin | Caco-2-cells | Transepithelial transport A-B permeability ↑<br>Transepithelial transport B-A permeability↓<br>Transepithelial transport A-B permeability ↓<br>Transepithelial transport B-A permeability↑ | Curcumin 30µM<br>Curcuma drugs 0,1mg/ml | | P-gp ↓ MDR1 ↓<br>P-gp ↑ MDR1 ↑ | (53) |
| Nifedipine | Caco-2-cells | Nifedipine oxidation ↓ | Curcuma longa extract 50mg/ml | 0.019mg/ml | CYP3A4 ↓ | (53) |
| Rhodamine | Caco-2-cells | Rhodamine accumulation ↑<br>Rhodamine accumulation ↓ | Curcumin 30µM<br>Curcuma drugs 0,1mg/ml | | P-gp↓<br>P-gp↑ | (53) |
| Testosteron | Caco-2-cells | 6-ß-OH-TST formation↓ | Curcuma longa extract 50mg/ml | 0.019mg/ml | CYP3A4 ↓ | (53) |
| Vinblastine | KB-V1 cells | Vinblastine cytotoxicity ↑ | Curcumin 15µM<br>Curcumin mixture 15µM | 23.5±5.6<br>26.3±4.7 | P-gp↓<br>P-gp↓ | (13) |

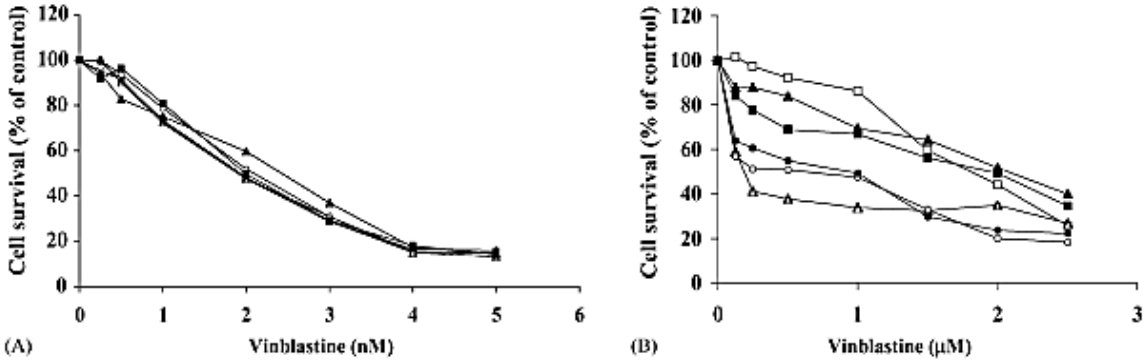


Fig. 14: Curcumin increases vinblastine cytotoxicity in vitro.[13]

Effect of curcumin I, II and III on the cytotoxicity of vinblastine. KB-3-1 cells (Panel A) and KB-V-1 cells (Panel B) with different concentrations of vinblastine alone (□) or with 15μM curcumin I (●), II (■) and III (▲), curcumin mixture (○) and 20μM verapamil (Δ).

### 9.4 Clinical relevance of drug interactions

In conclusion drug interactions caused by curcumin seem to be a synergistic result of two major mechanisms, the CYP inhibition on the one side and the inhibition of efflux transporters on the other side. Since these are main pathways in metabolism of a wide range of drugs, patients who take drugs with narrow therapeutic windows, such as anti-platelet drugs, should be warned about the risk of interactions and undergo strict therapeutic monitoring when additionally administering products that contain curcurminoids.[62]

The increase of biovailability and plasma concentrations of different drugs does not only have the potential for beneficial effects, but also for negative side effects that can occur when patients are co-treated with high doses of curcumin as they are found in ayurvedic or TCM herbal formulations. Since plant products are generally considered as safe many patients are unaware of the fact that co-administration of herbal medicinal products, but also food ingredients like fruit juices possibly has an impact on their medication and should be enlighten of the risks by their physicians or pharmacists. Curcumin and Curcuma longa products have been shown to cause drug interactions in different in vivo and in vitro systems when administered in high doses (Tab. 2 and 3). Therefore self-medication with high amounts of curcumin or Curcuma longa products should be avoided when modern medical drugs are taken

additionally. The occasional intake of Curcuma as a spice and food ingredient in low concentrations in contrast is not considered as dangerous when co-administered with drugs because such low amounts are unlikely to lead to drug interactions. Nevertheless further investigations should be done whether the daily consumption of higher amounts of Curcuma in Asian countries, especially in the Indian cuisine has an impact on the metabolism of medical drugs.

Another problem in treatment with herbals is the heterogeneous composition of biologically active ingredients. Location, weather and time of harvest are just a few of the factors that have an influence on the concentration of different molecules in the plant. In a qualitative and quantitative analysis of different curcuma samples from different organs, species and areas Li et al. found large differences concerning the concentration of curcuminoids. The rhizomes of Curcuma longa were shown to contain a larger quantity of curcuminoids than the tuberous roots of curuma longa and plants collected in the Sichuan province had a higher curcuminoid concentration than samples from other areas. [72] These results point up that the prediction of the exact effects of Curcuma consumption is very difficult and further investigations are necessary.

Because of its strong impact on different CYP450 enzymes and efflux transporters curcumin has a strong influence on bioavailability and leads to a more than 10% change of the AUC in high doses (see tab. 1+2). Therefore dose adjustment should be considered when co-administering curcumin in high amounts.

Tab. 3: Proposed dose adjustment for selected drugs in combination with curcumin

| Drug | Change in AUC (%) | Dose adjustment |
|---|---|---|
| Celiprolol | 28,34% | ↓ |
| Clopidogrel | 61.11% | ↓ |
| Docetaxel | 694,09% | ↓ |

| | | |
|---|---|---|
| Etoposide | 50,80% | ↓ |
| Loratadine | 66,73% | ↓ |
| Losartan | 66,34% | ↓ |
| Midazolam | 284,88% | ↓ |
| Norfloxacin | 52,06% | ↓ |
| Sulphasalazine | 222,58% | ↓ |
| Talinolol | - 33,01% | ↑ |
| Tamoxifen | 17,61% | ↓ |
| Warfarin | 59,71% | ↓ |

Furthermore there are suggestions that curcumin may lead to drug interactions with NSAIDs.

Acetylsalicylic acid (Aspirin*)*, but also NSAIDs that do not contain salicylate are mostly used against mild forms of pain. [73]

They inhibit the COX1 and 2 and therefore the production of prostaglandins and thromboxanes, but in contrast to Aspirin the effect of non-salicylate NSAIDs is reversible. One cause of the anti-inflammatory and antiplatelet activity of curcurmin is also the inhibition of prostaglandine and thromboxane synthesis, as well as stimulation of hydrocortisone release. There are suggestions that possible interactions between NSAIDs and Turmeric could lead to clotting disorders and a higher risk for the occurrence of bleedings. [73]

## 10. Discussion and conclusion

The rhizome of Curcuma longa has been used in traditional Asian medicine over centuries for its beneficial effects that have been verified by several studies in recent times.[1]

The characteristic yellow pigment of its main compound curcumin is caused by a system of conjugated double bonds in the structure of this hydrophobic polyphenole.[5] Furthermore the β-dicarbonyl moiety, as well as the two phenolic groups and the two methoxy groups make exhibition of keto-enol-tautomerism and interaction with the surrounding over H-bond formation and Michael addition possible.[9] Its chemical characteristics cause curcumin to not be very stable and experience fast photochemical degradation under daylight. Furthermore it is rapidly decomposed under neutral-basic condition and higher stability only occurs in acidic media.[16]

Although the molecule was found to not be toxic even at high doses and to have a high potential for treatment of various diseases, its wide application is still limited by an extremely low bioavailability and fast elimination from the body.[28], [39] After ingestion curcuminoids experience phase I metabolism over the CYP450 system immediately, followed by phase II metabolism where gluconation and sulfonation take place.[39], [41], [47], [57] This poor absorption from the gut after oral administration and the extremely low plasma concentrations put the search of new formulations in order to reduce first pass metabolism and increase the therapeutic efficiency in the main focus of many investigations.[18]

Curcumin has been proven to have a wide range of biological activities including anti-oxidant, anti-inflammatory, anti-proliferative and anti-microbial effects that are caused trough interactions with several structures and metabolic pathways in the human body.[1] Under these molecular targets also drug metabolizing CYP enzymes and efflux transporters, such as MRP, P-gp and BCRP have been identified.[49], [57] The mentioned transporters belong to the superfamily of ABC-transporters and have the ability of actively transporting xenobiotics out of cells in order to prevent damage. Because this mechanism also eliminates medical drugs from the cells, it constitutes a major problem in medical treatment, especially in cancer therapy.[49] Curcumin has been proven to inhibit those transporters, which gives it the ability to overcome multi

drug resistance and makes it to a potential adjuvant in treatment.[55] Several studies already verified this beneficial effect and showed that curcumin administration leads to an accumulation of different cytostatics in the cell. Consequently the drug efficiency increases and lower doses are needed. [54], [55] This mechanism of efflux inhibition, that one the one hand has the potential of becoming a useful tool in modern medical treatment, on the other hand also constitutes a high risk for drug-drug interactions.[62] Besides the inhibition of drug efflux transporters also CYP450 enzymes in the intestine are inhibited.[62] The CYP450 system is the main metabolic pathway for the largest part of medical drugs, with CYP3A4 alone metabolizing about 50% of all drugs in current clinical use.[56]

There are many drugs that are substrates for efflux transporters, especially for P-gp, as well as for CYP enzymes. If curcumin is co-administered with these substances it can cause undesirable side effects.[62] Several studies have proven these interactions with medical drugs, including midazolam, norfloxacin, etoposide, celiprolol, clopidogrel, losartan, loratadine, docetaxel, tamoxifen, sulphasalazine, tacrolimus and warfarin. Curcumin co-administration led to an increase in $C_{max}$ and AUC which are parameters for an enhanced bioavailability. This increase in plasma concentration leads to a higher therapeutic efficiency and therefore lower doses are needed. [54], [61], [62], [63], [64], [65], [66], [68], [70], [71] In contrast there is also a report that curcumin and curcuma drugs might have opposite effects on the activity of efflux proteins which makes precise studies essential in order to avoid side-effects.[53],[67] The range of drugs that are suggested to give interactions with curcumin is extremely wide and also includes substances with narrow therapeutic windows. [61], [62], [63], [70]

These findings have a high clinical relevance because in recent years a strong trend to self-medication with plant products is noticeable and especially traditional Asian medicine enjoys great popularity. Alternative medicine with the use of herbal products is generally considered as safe and therefore is often taken unaware of potential risks.[74],[75]

In traditional Asian medicine the daily consumption of great amounts of plant products is very frequent and information about the actual concentration of biologically active compounds in these formulations is often lacking. Influences like origin, weather and time of harvest cause differences in the composition of active ingredients and make the exact effects of the consumption of herbal products

unpredictable.[72] In case of the Curcuma longa rhizome this can lead to severe interactions with pharmaceutical drugs because of its great impact on metabolism.[73], [74], [75] Not only the inhibition of efflux proteins, but also the decrease of CYP enzyme activity might alter the concentration of co-administered drugs. That causes an over-dose through increase of therapeutic efficiency and can be dangerous. Especially for drugs with a very narrow therapeutic window, such as anti-platelet drugs this can lead to life threatening situations in extreme cases, which makes strict drug monitoring necessary. [61], [62], [63], [70] Several studies showed that curcumin interacts with a wide range of drugs that are metabolized over the CYP450 system or transported via efflux transporters, especially over P-gp, BCRP and MRP. When co-administering high doses of curcumin to different drugs in in-vivo studies, AUC increases of far more than 10% have been observed in many cases. Therefore dose adjustment should be considered when high amounts of curcumin are taken together with other drugs. Also patients should be explained the risks of self-medication with TCM or ayurvedic products containing Curcuma longa.

For the occasional use of Curcuma as a food ingredient or coloring additive in low concentrations no side effects are to be expected because an impact on therapy is unlikely in such low concentrations. Concerning the consumption of Curcuma as a spice in high amounts in the Asian cuisine further investigations should be done in order to determine the impact on drug treatment. Because of the proven influence of curcumin on the bioavailability of a big amount of drugs, interactions are very probable when regularly consuming curcumin-rich food and the doses of co-administered drugs should be adjusted.

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