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Review

Metabolism and interactions of antileprosy drugs

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ABSTRACT

Leprosy is a chronic infectious disease caused my *Mycobacterium leprae* that primarily affects peripheral nervous system and extremities and is prevalent in tropical countries. Treatment for leprosy with multidrug regimens is very effective compared to monotherapy especially in multibacillary cases. The three major antileprosy drugs currently in use are 4, 4′-diaminodiphenyl sulfone (DDS, dapsone), rifampicin, and clofazimine. During multidrug therapy, the potent antibiotic rifampicin induces the metabolism of dapsone, which results in decreased plasma half-life of dapsone and its metabolites. Furthermore, rifampicin induces its own metabolism and decreases its half-life during monotherapy. Rifampicin upregulates several hepatic microsomal drug-metabolizing enzymes, especially cytochrome P450 (CYP) family that in turn induce the metabolism of dapsone. Clofazimine lacks significant induction of any drug-metabolizing enzyme including CYP family and does not interact with dapsone metabolism. Rifampicin does not induce clofazimine metabolism during combination treatment. Administration of dapsone in the acetylated form (acedapsone) can release the drug slowly into circulation up to 75 days and could be useful for the effective treatment of paucibacillary cases along with rifampicin. This review summarizes the major aspects of antileprosy drug metabolism and drug interactions and the role of cytochrome P450 family of drug metabolizing enzymes, especially CYP3A4 during multidrug regimens for the treatment of leprosy.

1. Introduction

Leprosy is a chronic disease and is prevalent in tropical countries, especially in India, Philippines, and Brazil, and poses a public health problem [1]. It was first identified and reported by a Norwegian physician G.A. Hansen in 1873 and since then it is also known as “Hansen's disease” [2]. Leprosy is a contagious disease, but its morbidity is low because a large portion of the population is naturally resistant to the disease [3]. It is transmitted by close and prolonged contact through inhalation of the bacilli present in nasal secretion or through skin erosions [3–4]. Diagnosis of leprosy is based on clinical examination, bacilloscopy, and histopathology [5]. Early diagnosis and treatment are very important to prevent transmission and for a complete curative therapy without deformities.

Leprosy is caused by a rod-shaped slow-growing acid-fast aerobic bacteria called *Mycobacterium leprae* (*M. leprae*). Recently, another species named *Mycobacterium lepromatosis* has been reported [6–8]. It causes diffuse lepromatous leprosy (DL), a unique form of leprosy endemic in Mexico [9]. Lately, a case of diffuse lepromatous leprosy with Lucio phenomenon was reported from India [10]. They have identified the causative agent as *M. leprae* and not *M. lepromatosis* using polymerase chain reaction. *M. leprae* primarily infects histiocytes (non-motile tissue macrophages) in the dermis and Schwann cells in the peripheral nerves. It may take up to 5–20 years to develop the clinical symptoms after the initial infection, which depends on the type of leprosy [11]. During this period, the bacillus silently multiply in the infected areas and destroy most portions of peripheral nervous system [12]. This could result in decrease or loss of sensitively and may destroy parts of extremities due to repeated injuries and unnoticed wounds.

The biochemical and molecular properties of *M. leprae* are not yet clear owing to the uncultivable nature of the bacterium. Due to extensive loss of genes necessary for independent growth, *M. leprae* and *M. lepromatosis* are obligate intracellular pathogens and uncultivable in the laboratory [13]. The first full genome sequence of *M. leprae* completed in 2001, which revealed that only half of the small genome contains protein-coding genes, while the remaining portion consists of pseudogenes and non-coding regions [14]. Many of the *M. leprae* pseudogenes are the result of stop codon insertions that could be happened by the dysfunction of sigma factors or the insertion of repetitive sequences derived from transposons [15–16]. It is estimated that *M. leprae* has lost approximately 2000 genes from its genome [14]. *M. leprae* can only grow as a parasite in animals with lower body

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temperature, such as nine-banded armadillos, footpads of immunocompromised mice, or the extremities of a human body [17–18]. The loss of genes involved in vital metabolic pathways such as energy metabolism, limiting the carbon sources the bacteria can use, and interruption in respiration pathways could explain the uncultivable nature of *M. leprae* [19]. The complex and unique cell wall of *M. leprae* made up of peptidoglycan, arabinogalactan, and mycolic acid that are covalently linked and difficult to destroy could be one of the reasons for extremely slow replication rate [20–21].

2. Classification of leprosy

The World Health Organization (WHO) distinguishes leprosy into two classes only for the purpose of treatment regimens as "paucibacillary" (no bacteria on skin smears else five or fewer lesions) and "multibacillary" (presence of bacteria on any smear else more than five lesions) based upon proliferation of bacteria and skin lesion count [22–23].

Another more widely accepted classification of leprosy for clinical studies and research purposes is Ridley-Jopling scale [24]. They postulated leprosy into five classes viz TT, BT, BB, BL, and LL based on an expression of resistance to the infection or the patient's immunological status on lepromin test. They also stated about a group named indeterminate type, which is purely macular condition where plaques and nodules never occur. The diagnosis of this group is originally discussed by Currie [25]. Another class of leprosy named diffuse lepromatous leprosy or diffuse leprosy (DL) caused by *M. lepromatosis* is also added in the group. Diffuse leprosy could also be caused by *M. leprae*. Therefore, at present leprosy could be classified into 7 groups on a scale increasing severity of the disease.

The classification of leprosy is also depends on bacterial index (BI) on a skin smear [26]. The BI is an expression of the extent of bacillary load in the patient. It is calculated by counting six to eight stained smears under 100x oil immersion lens prepared with Ziehl-Neelsen method. The results are expressed on a logarithmic scale as follows: 1+ at least 1 bacillus in every 100 fields; 2+ at least 1 bacillus in every 10 fields; 3+ at least 1 bacillus in every field; 4+ at least 10 bacilli in every field; 5+ at least 100 bacilli in every field; 6+ at least 1000 bacilli in every field.

2.1. Indeterminate leprosy (IL)

Indeterminate type is a pure macular condition where plaques and nodules never occur. The macules are usually hypopigmented and few in number and slight impairment of sensation may be present [24]. Indeterminate leprosy is more predominant in Brazil [27–28]. The lesions may sometime heal by themselves or could progress to a more severe type.

2.2. Tuberculoid leprosy (TT)

Tuberculoid leprosy is characterized by solitary skin lesions that are asymmetrically distributed with clear demarcated edges [29].This is the mild type of leprosy and it occurs in hosts with strong cellular immunity. The classic lesion is a large erythematous plaque with a sharply raised outer edge, which slopes gradually toward a flattened center with reduced sensation and alopecia. A thickened peripheral nerve could be palpable around a lesion, and the thickening may be gross and irregular. Tuberculoid leprosy could heal spontaneously or may progress to a more severe form.

2.3. Borderline tuberculoid leprosy (BT)

Borderline tuberculoid leprosy is similar to tuberculoid type except that the skin lesions are smaller and more numerous. Satellite lesions are frequent near the larger lesions or presence of finger like projections from the edges of the plaques or macules into the normal skin [30]. Lesions may vary in size, shape, and color in the same patient from hypochromic to reddish. The BI varies from negative to 2+ positive [26]. Borderline tuberculoid is "low resistant tuberculoid leprosy" and could revert to tuberculoid or advance to severe form.

2.4. Borderline borderline leprosy (BB)

Borderline leprosy is characterized with numerous skin lesions that are irregularly shaped reddish plaques [31]. The lesions are intermediate in number and size between tuberculoid and lepromatous leprosy and depict a moderate degree of numbness. Borderline leprosy is rare and could move rapidly to tuberculoid or lepromatous type. The BI is usually strongly positive (2+ to 4+).

2.5. Borderline lepromatous leprosy (BL)

Borderline lepromatous leprosy is with numerous, disseminated symmetrically distributed skin lesions, raised bumps, plaques, and nodules [32]. The lesion edges are irregular and invade into normal skin. Type 1 and type 2 leprosy reactions are frequently present [33]. Obvious lepromatous features such as madarosis, keratitis, nasal ulceration, saddle-nose deformity, and leonine facies are absent. Bacterial index is strongly positive in all cases.

2.6. Lepromatous leprosy (LL)

Lepromatous leprosy is due to the failure of the activation of T1 helper cells, which is necessary to destroy *M. leprae* on infection [34–35]. Since cell-mediated immunity is absent in lepromatous leprosy, *M. leprae* proliferates freely and results in widespread systemic disease. The initial skin lesions are erythematous or hypopigmented disseminated small pale macules with poorly defined borders and large number of bacilli [36]. The progressive infiltration of the face makes skin folds more evident giving a typical clinical aspect known as facies leonina. Loss of eyebrows, beginning from the external part results in madarosis. Leproma, a mass of histiocytes containing large quantities of *M. leprae*, forms in the peripheral nerves, eyes and lymph nodes. As the disease progresses, peripheral nerves may be enlarged and impairment of sensation may occur on hands, feet and other involved areas with deformities.

2.7. Diffuse lepromatous leprosy (DL)

Diffuse lepromatous leprosy is a clinical variation of lepromatous leprosy and is first described by Lucio and Alvarado in 1852 and re-identified by Latapí in 1936 [37]. It is common in Mexico (23% leprosy cases) and in Costa Rica and almost absent in other countries. It is characterized by a diffuse infiltration of the disease throughout the skin, which never transforms into nodules, a complete alopecia of eyebrows and eyelashes as well as body hair, an anhydrotic and dysesthesic zones of the skin, and a peculiar type of lepra reaction named Lucio's phenomenon or necrotic erythema [38]. Recently, cases of diffuse lepromatous leprosy with Lucio's phenomenon caused by *M. leprae* were also reported from India [10,39].

2.8. Treatment for leprosy

The treatment for leprosy depends on whether the patient belongs to paucibacillary or multibacillary group [22,40–41]. In places where facilities for bacteriological examination of skin smears are not available, patients with five or less (≤ 5) poorly pigmented lesions are classified as paucibacillary and patients with more than five (>5) lesions as multibacillary leprosy. Places where skin smear facilities are available, patients showing negative smears at all sites are grouped as paucibacillary and those showing positive smears at any site are

grouped as multibacillary. Paucibacillary group is considered as non-infectious and positive for lepromin test, while multibacillary group is infectious and negative for lepromin test.

Leprosy is treated with a combination of drugs including strong antibiotics [42]. This strategy helps to prevent the development of drug resistance by *M. leprae,* which is a common problem during long-term treatment. Since *M. leprae* preferably invades the Schwann cells that surround peripheral axons, if left untreated, the nerve damage can result in paralysis and crippling of hands and feet. Therefore, early diagnosis and timely treatment is very important to prevent permanent nerve damage and paralysis. Even though, multidrug regimens kill the bacteria and cure the disease, the treatment does not reverse nerve damage or physical deformities that occurred before the diagnosis.

3. Antileprosy drugs currently in use

The three major antileprosy drugs currently in use are diaminodiphenyl sulfone (dapsone), rifampicin, and clofazimine. These three are the first-line antileprosy drugs effectively used in the treatment of leprosy for several decades. Rifampicin is also used as the first-line drug for the treatment of tuberculosis.

3.1. Diaminodiphenyl sulfone (dapsone, DDS)

Diaminodiphenyl sulfone (dapsone) was first synthesized by Fromm and Wittmann in 1908 [43]. Its chemical structure is illustrated in Fig. 1A. It was first introduced for the treatment of leprosy in 1940′s by Cochrane and Muir in India and Dove and Davey in Nigeria [44]. Ingestion of one tablet of 100 mg gives a peak blood level 500–600 fold higher than the minimal inhibitory concentration (MIC) against *M. leprae* [45]. Dapsone inhibits the synthesis of dihydrofolic acid in bacteria in competition with *para*-aminobenzoic acid for the active site of the enzyme dihydropteroate synthase [46]. Several proven cases of *M. leprae* resistance to dapsone were reported in late 1950′s and early 60′s [47–48]. Missense mutations at codon 53 (ACC) or 55 (CCC) coding threonine or proline in *folP1* is responsible for dapsone resistance [49–50]. The development of mouse footpad assay by Shepard in 1960 provided great opportunity for the study of antileprosy drugs and drug resistance [51]. The introduction of potent antibiotics rifampicin and clofazimine and multidrug regimens for the treatment of leprosy solved the problem of bacterial resistance to dapsone.

3.1.1. Acedapsone

Acedapsone (4,4′-diacetyldiaminodiphenyl sulfone) is a long-acting intramuscular repository derivative of dapsone (Fig. 1B). It was first synthesized and purified by Ernest Fourneau and his colleagues of Pasteur Institute in 1937 [52]. Recently, its physical and chemical properties have been studied extensively [53]. Acedapsone possesses little activity against M. leprae, but it metabolizes into active dapsone. The half-life of acedapsone is 46 days, and that of the derived dapsone is 43 days [54]. The potent antileprosy activity of acedapsone was first demonstrated by Shepard in 1967 [55]. Later, Shepard et al [56] observed that a standard intramuscular dose of 225 mg of repository acedapsone in 1.5 ml suspension releases dapsone at a rate of approximately 2.4 mg/day. They noticed that the corresponding serum concentration of dapsone was 60 ng/ml, which is about 20-fold higher than the MIC of dapsone (3 ng/ml). We have observed that a single intramuscular injection of 225 mg acedapsone in 1.5 ml suspension of castor oil and benzylbenzoate released a mean concentration of 14.76 ng dapsone/ml plasma on day 75 in a cohort of 15 patients, which was 5-fold higher than the MIC of the drug against *M. leprae* [57]. The lowest dapsone level observed on day 75 in a single patient following acedapsone injection was 8.4 ng/ml plasma, which is around 3-fold higher than the MIC of dapsone against *M. leprae*. The mean peak dapsone level (85.36 ± 7.12 ng/ml plasma) was observed on day 7 following acedapsone injections [57].

3.1.2. Advantages of acedapsone over dapsone

Acedapsone (Hansolar, DADDS) is a diacetyl derivative of dapsone. An advantage of a single intramuscular injection of acedapsone over daily intake of dapsone tablet is that the patient could avoid the burden of daily drug intake for the chronic disease and can maintain the circulating drug level against *M. leprae* for long periods. This is especially useful in paucibacillary cases. A 300 mg intramuscular dose of acedapsone maintains blood dapsone levels well above the inhibitory concentration against M. leprae for approximately 100 days [58]. The

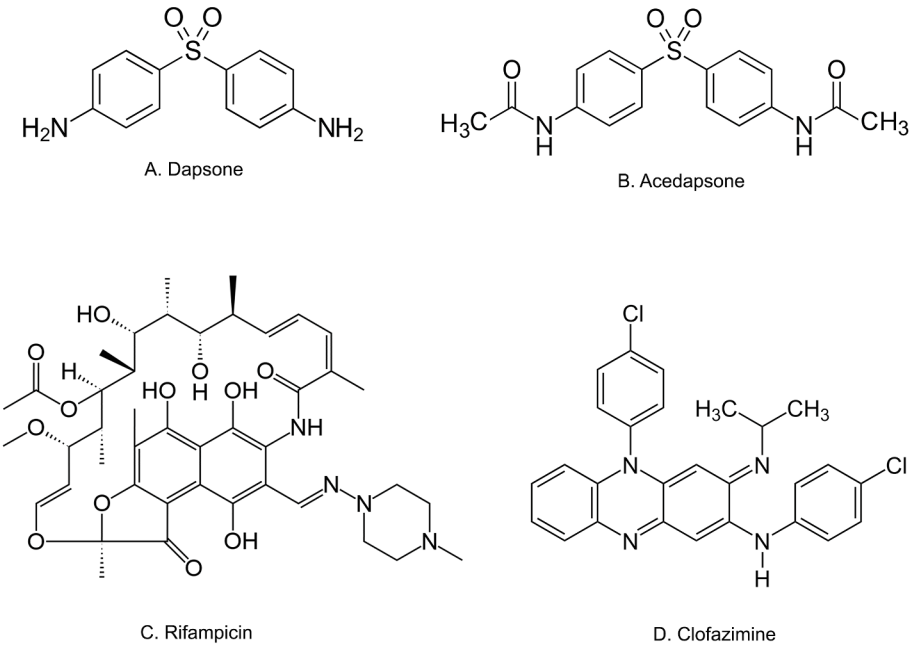

Fig. 1. Chemical structure of **(A)** 4,4′-diacetyldiaminodiphenyl sulfone (DDS, Dapsone), **(B)** diacetyl diacetyldiaminodiphenyl sulfone (DADDS, Acedapsone), **(C)** Rifampicin, and **(D)** Clofazimine (Lamprene).

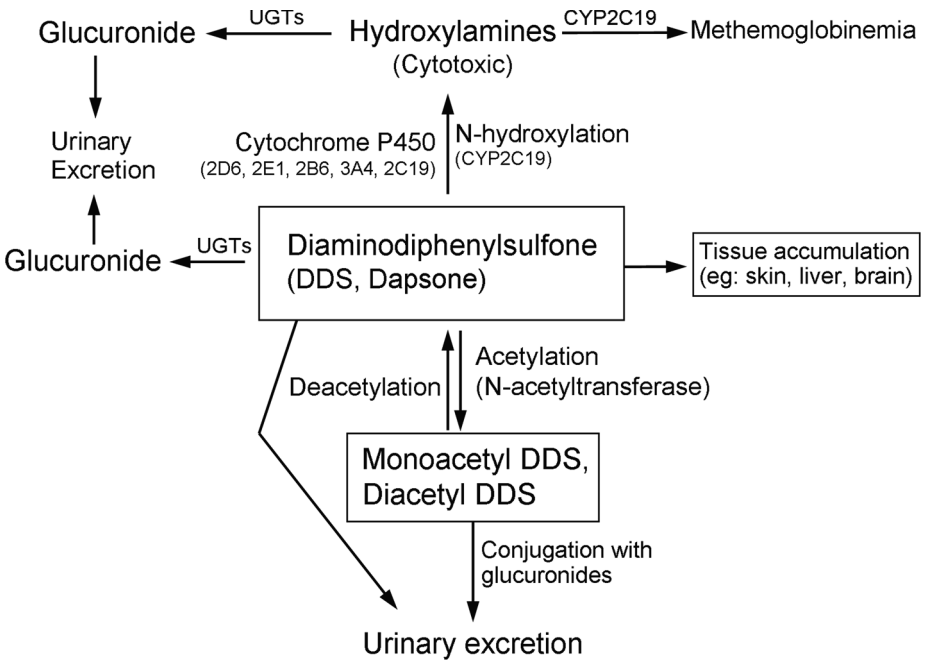

Fig. 2. The major metabolic pathways of orally ingested dapsone. After reaching in the liver, dapsone is metabolized either by N-hydroxylation to produce toxic hydroxylamines or by acetylation to produce nontoxic acetylated dapsone, which includes monoacetyl dapsone (MADDS) and diacetyl dapsone (DADDS). Cytochrome P450 2C19 (CYP2C19) is mainly responsible for the generation of dapsone hydroxylamine which could lead to methemoglobinemia. Both hydroxylated and non-hydroxylated dapsones conjugate with glucuronides and excreted through urine.

usual treatment regimen is five intramuscular injections per year at a dose of 225 mg of DADDS in 1.5 ml suspension of castor oil and benzylbenzoate [59]. The patient, doctor, and the associated technical staff need not worry about missing dose of the drug and related adverse events.

3.1.3. Metabolism of dapsone

About 80–85% of orally administered dapsone is absorbed slowly from the gastrointestinal tract and uniformly distributed to all tissues including muscles, liver, and kidneys and it crosses both blood–brain and placental barriers [60]. After gastrointestinal absorption, dapsone is transported to liver though portal circulation and is metabolized through acetylation or N-hydroxylation. The major metabolic pathways of dapsone are presented in Fig. 2. Dapsone is acetylated into monoacetyl and diacetyl dapsone (MADDS and DADDS) and the rate of acetylation depends on the acetylator phenotypes [61]. The acetylated dapsone could deacetylate into dapsone or excreted through urine, directly or after conjugated as N-glucuronides and N-sulphates [62]. The N-hydroxylation of dapsone by cytochrome *P*-450 enzymes, especially CYP2D6, 2B6, 3A4, and C19 yields hydroxylamines, a potentially toxic metabolite [63]. Dapsone metabolites produced by CYP2C19 isozyme could lead to methemoglobinemia, a condition caused by elevated levels of methemoglobin in the blood [64–65]. Most of the dapsone hydroxylamines formed are converted to glucuronides and excreted through urine [66]. One of the reasons of elevated β-glucuronidase levels in leprosy patients treated dapsone could be related to the increased glucuronide production during dapsone metabolism [67].

3.1.4. Methods for determination of dapsone and its metabolites in biological samples

The best method for accurate determination of dapsone (DDS) and its metabolites, monoacetyl dapsone (MADDS) and diacetyl dapsone (DADDS) is high performance liquid chromatography (HPLC) [68]. Dapsone along with its metabolites should be extracted from biological samples such as plasma and urine using HPLC grade acetonitrile. The second best and accurate method for determination of DDS, MADDS, and DADDS in human plasma and urine is spectrofluorimetry [69–70]. Another routine quantitative technique for estimation of dapsone (total acid labile metabolites) is colorimetric or spectrophotometric method [71]. The other sensitive methods for determination of dapsone, especially in urine samples are enzyme linked immunosorbent assay (ELISA) and tile test [72]. Additional methods for semiquantitative determination of dapsone in urines samples are haemagglutination inhibition (HI) test and paper spot test [73]. The simple paper spot test is especially useful to monitor patients compliance in field conditions.

3.2. Rifampicin

Rifampicin is a potent antibiotic employed for the treatment of several types of bacterial infections especially *Mycobacterium tuberculosis* and *M. leprae*. Rifampicin is produced by the gram-positive bacteria *Amycolatopsis rifamycinica* [74]. The chemical structure of rifampicin is depicted in Fig. 1C (Mol wt 822.94). Rifampicin inhibits bacterial DNA-dependent RNA polymerase that catalyzes the transcription of RNA from a DNA template [75]. It prevents formation of mRNA by physically blocking RNA elongation and thus synthesis of bacterial proteins. Crystal structure and biochemical data indicate that rifampicin binds to the RNA polymerase β-subunit within the DNA/RNA channel, but away from the active site [76]. A single 600 mg dose of rifampicin can reduce the number of viable bacilli to undetectable levels within a few days, with killing rates measured in excess of 99.9% after 1 month [77–78]. Mutations in the rpoB gene, which encodes the β-subunit of RNA polymerase, alter residues of the rifampicin-binding site on bacterial RNA polymerase resulting high-level resistance to rifampicin in M. leprae [79]. Rifampicin resistant strains of *M. leprae* with missense mutations (a change in single nucleotide in a codon) in the rpoB gene were observed among leprosy patients in Southern India [80]. Xpert MTB/RIF assay, a nucleic acid amplification test (NAAT), can detect rifampicin resistance in *Mycobacterium tuberculosis* and diagnose tuberculosis rapidly [81]. The mechanism of rifampicin resistance has been recently reviewed [82–83]. Despite development of bacterial resistance, rifampicin serves as the most potent and powerful drug against *M. leprae*.

3.2.1. Metabolism of rifampicin

After oral administration on an empty stomach, rifampicin is quickly absorbed from the gastrointestinal tract almost completely. A single dose of 600 mg rifampicin produces a peak serum concentration of about 10 μg/ml at around 2 h. The plasma half-life of rifampicin for 600 mg dose is around 2.5 h, which is highly influenced by hepatic microsomal enzymes [84]. After absorption, rifampicin is distributed throughout the body, and effective drug concentrations are attained in

most organs and body fluids, including the cerebrospinal fluid. About 60% to 90% of the drug is bound to plasma proteins [85]. Rifampicin is the most powerful inducer of the hepatic cytochrome P450 enzyme system, including isoenzymes CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, CYP3A5, and CYP3A7 [86]. Rifampicin accelerates its own metabolism due to the induction of own metabolizing enzymes [87,88]. Rifampicin induces the metabolism of many drugs including dapsone and consequently they became less effective by decreasing their plasma half-life. We have observed that the plasma levels of dapsone decreases during daily concurrent administration of 600 mg rifampicin during multidrug regimes for treatment of leprosy with a decrease of more than 70% on day 15 at 24 h after the dose [71]. An 80-fold induction of CYP3A4 was observed in cultured primary human hepatocytes after treatment with rifampicin, which was dose dependent [89]. Rifampicin causes proliferation of smooth endoplasmic reticulum of hepatocytes probably due to the extensive induction drug metabolizing enzymes, especially the cytochrome P450 family present on hepatic microsomes [90].

3.2.2. Methods for determination of rifampicin in biological fluids

The simple and easy method for quantitative measurement of rifampicin in plasma and urine samples is spectrophotometric method [91]. Another best method for semi-quantitative determination of rifampicin in serum or urine samples is microbiological assay using Staphylococcus aureus (NCTC 10702) [92]. We have compared and evaluated microbiological assay and simple spectrophotometric method for determination of rifampicin in biological samples [93]. Rifampicin and three of its metabolites 25-desacetylrifampicin, 3- formylrifamycin SV, and 3-formyl-25-desacetylrifamycin SV in human plasma, urine and saliva could be accurately determined by high-performance liquid chromatography [94]. A rapid and sensitive liquid chromatography-mass spectrometry method was developed for the quantitative estimation of rifampicin in plasma after one-step extraction using ethyl acetate [95]. This technique would be very useful for the study of rifampicin metabolism and drug interactions in plasma samples.

3.3. Clofazimine

Clofazimine ([3-(p-chloroanilino-10-(p-chlorophenyl))-2, 10-dihydro-2-isopropylimino-phenazine, Mol wt 473.41) is a synthetic phenazine dye. The chemical structure of clofazimine is depicted in Fig. 1D. Clofazimine is used for the treatment of leprosy in combination with rifampicin and dapsone especially for multibacillary cases and erythema nodosum leprosum [96,97]. It is marketed in the brand name Lamprene by Novartis Pharmaceuticals. Clofazimine was initially known as B663 and was first synthesized in 1954 by a team of scientists at Trinity College, Dublin, UK. Clofazimine binds to the guanine bases of bacterial DNA templates, blocks DNA replication, and thus inhibits bacterial cell proliferation [98,99]. It was demonstrated that clofazimine attenuates antigen-induced Ca(2+) oscillations, suppress cytokine release, and prevent skin graft rejection by inhibiting Kv 1.3 channels with high potency and selectivity [100]. The common side effects of clofazimine are abdominal pain, diarrhea, itchiness, dry skin, and skin coloration. Clofazimine produces pink to brownish skin pigmentation in almost all patients within a few weeks and similar discoloration of most bodily fluids and secretions [101].

3.3.1. Metabolism of clofazimine

After intake, about 45–70% of clofazimine is absorbed from the gastrointestinal tract when clofazimine is administered as capsules containing a microcrystalline (micronized) suspension [102]. The absorption is depending on whether the drug is taken with or without food and better absorption was observed along with a protein rich diet [103]. The drug is highly lipophilic and is mainly distributed among fatty tissue and cells of the reticuloendothelial system through macrophages. A peak plasma concentration of 407.6 ng/g clofazimine was occurred between 4 and 8 h after a single oral dose of 200 mg of the drug taken 10 min after breakfast [104]. Clofazimine accumulates in high concentrations in the mesenteric lymph nodes, adipose tissue, adrenals, liver, lungs, gallbladder, bile, and spleen [104]. The biological half-life of clofazimine is about 70 days in human, a calculated value based on the urinary excretion of the drug [105,106]. No other data are available regarding half-life of clofazimine in human. In experimental mouse model of tuberculosis, the half-life of clofazimine in the serum has increased from 1.45 to 8.19 weeks when the duration of administration increased from 4 to 20 weeks [107].

Excretion of clofazimine is a very slow process and the drug accumulates in several human tissues and produce skin discoloration and pigmentation [108]. Clofazimine is excreted through urine, fecal matter, and breast milk. A substantial portion of the unchanged drug is excreted in feces [105]. A mean concentration of 1.33 μg/ml clofazimine was reported in breast milk after taking the drug either 50 mg daily or 100 mg on alternate days for a period of 1–18 months [109]. The metabolic pathways of clofazimine and urinary excretion of three major metabolites are presented in Fig. 3. The three major metabolites of clofazimine detected in patients urine employing mass spectrometry are unconjugated 3-(*p*-hydroxyanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-isopropyl-iminophenazine (metabolite I), β-D-glucopyranosiduronic acid conjugated 3-(hydroxy)-10-(*p*-chlorophenyl)-2,10-dihydro-2-isopropyl-iminophenazine (metabolite II), and 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-4,10-dihydro-4-hydorxy-2-isopropyl-iminophenazine (metabolite III) [110,111]. These metabolites appeared in urine within 24 h after administration of a single dose of 300 mg clofazimine [106]. A significant inhibitory effect of clofazimine was observed on the major drug-metabolizing enzyme CYP3A4 [112]. This would be beneficial during concurrent administration of clofazimine and rifampicin in order to reduce the metabolic degradation of rifampicin and prolonged plasma levels of the drug. Concurrent administration of clofazimine with dapsone does not exert any significant influence on plasma half-life or urinary excretion of dapsone [71,113].

3.3.2. Methods for determination of clofazimine in biological fluids

The simple method for determination clofazimine in biological samples is colorimetric method [108]. However, the technique is not sensitive enough for routine determination of clofazimine levels in plasma and urine samples. Peters *et al* [114] developed high-performance liquid chromatographic (HPLC) technique for determination of clofazimine in plasma samples with a sensitivity 10 ng/ml after extraction into organic solvents. Later, rapid and simple HPLC methods were developed for accurate measurement of clofazimine in patients samples [115,116]. Recently, various analytical methods including spectrophotometry, different chromatographic methods, and mass spectrometry for estimation of clofazimine in biological samples and pharmaceutical formulations were critically reviewed [117].

4. Multidrug regimen for leprosy

Since treatment of leprosy with only one antileprosy drug (monotherapy) could always result in development of drug resistance, multidrug therapy (MDT) was introduced by World Health Organization (WHO) for the treatment of leprosy in all endemic countries since 1995. Multidrug therapy has the advantage of targeting *M. leprae* in multiple ways for a quick response. The drugs used in multidrug therapy are combination of dapsone, rifampicin, and clofazimine for multibacillary cases and dapsone and rifampicin for paucibacillary patients. Since rifampicin is the most potent antileprosy drug, it is included for the treatment of both types of leprosy. The standard multidrug regimen for multibacillary leprosy is dapsone 100 mg daily, rifampicin 600 mg once in a month, and clofazimine 50 mg daily and 300 mg once a month for a period of 12 months. The standard multidrug regimen for paucibacillary leprosy is dapsone 100 mg daily and rifampicin 600 mg once a month for a period of six months. The above treatment regimen and

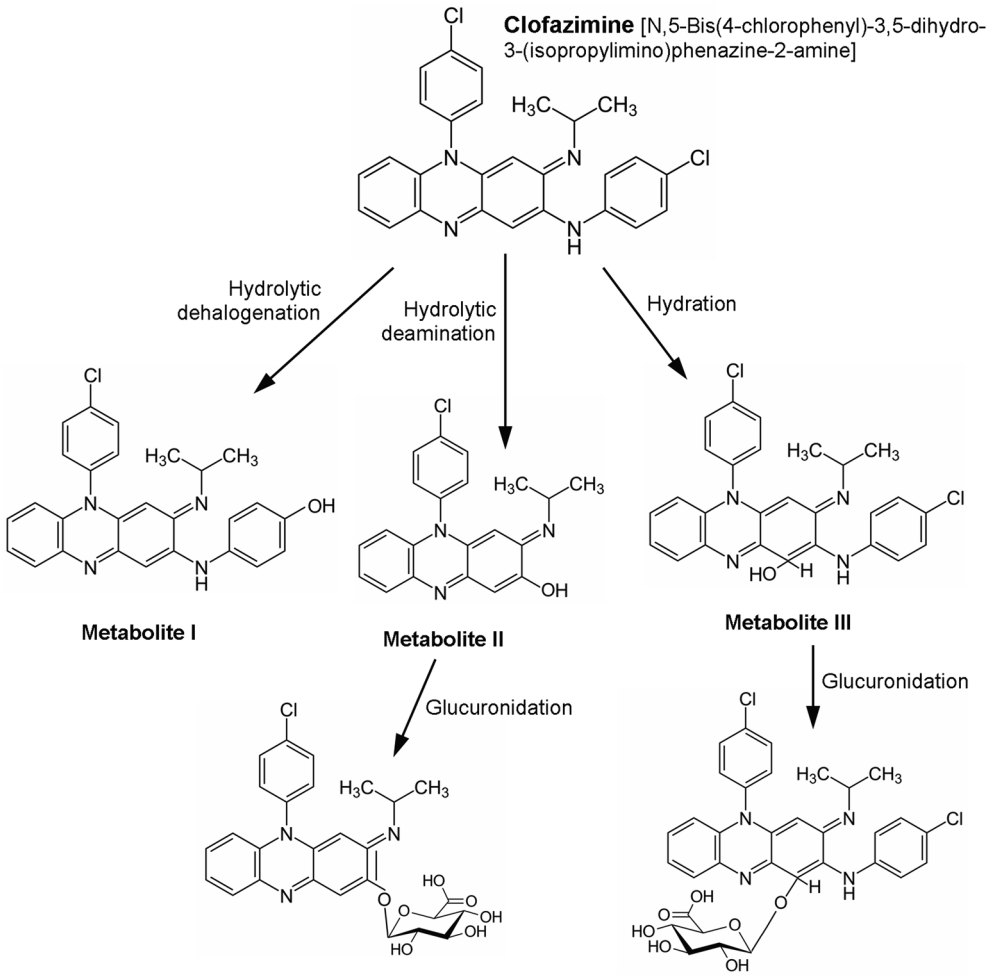

Fig. 3. The three major urinary metabolites of clofazimine in human. Metabolite I: 3-(*p*-hydroxyanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-isopropyl-iminophenazine. Metabolite II: 3-(hydroxy)-10-(*p*-chlorophenyl)-2,10-dihydro-2-isopropyl-iminophenazine. Metabolite III: 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-4,10-dihydro-4-hydorxy-2-isopropyl-iminophenazine. Metabolite I is excreted in urine in free form, while metabolites II and III are excreted after conjugation to β-D-glucopyranosiduronic acid.

duration is enough to cure multibacillary and paucibacillary cases, respectively. In periods before 1995, the multidrug treatment regimen for multibacillary leprosy patients continued for several years that caused various side effects. It was observed that multibacillary leprosy patients who had undergone treatment with multidrug regimens viz. dapsone, rifampicin, and clofazimine for a period of 2–5 years developed thyroglobulin autoantibodies in serum [118,119].

5. Drug interactions during multidrug regimens

It is well established that there is drug-drug interactions during multidrug regimens for the treatment of leprosy [120,121]. Rifampicin, the most potent antileprosy drug is the culprit. This is one of the reasons that rifampicin is administered only once in a month under the WHO-MDT program for the treatment of leprosy. Rifampicin is a potent inducer of hepatic drug metabolizing enzymes, as evidenced by proliferation of smooth endoplasmic reticulum and increase of cytochrome P450 content in the liver [122]. We have noticed that during concurrent administration of dapsone and rifampicin for the treatment of leprosy, the metabolism of dapsone is enhanced by rifampicin, which resulted in decreased half-life of dapsone [71]. When 100 mg dapsone and 600 mg rifampicin were administered daily for 15 days on a cohort of 30 patients, the mean plasma level of dapsone at 6 h after administration of the drugs was reduced from 2.45 μg/ml on day 2 to 1.15 μg/ml on day 15. Similarly, the mean dapsone level at 24 h was 1.43 μg/ml and 0.44 μg/ml, respectively. A comparative increased urinary excretion of dapsone metabolites was also observed [71,121]. There was no such influence on the plasma levels of dapsone during combination treatment with clofazimine [71,121]. Potent drug interaction was not reported during concurrent administration of rifampicin and clofazimine for the treatment of leprosy.

6. Molecular mechanism of reduced half-life of dapsone during concurrent administration with rifampicin

It was demonstrated that rifampicin is the most potent inducer of cytochrome P450 (CYP) family of hepatic microsomal drug metabolizing enzymes, including isoenzymes CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 [123,124]. Among these, CYP3A4 depicted the highest level of induction up to 55 fold [124,125]. Cytochrome P450 3A4 (CYP3A4) catalyses the metabolism of more than 30% of all clinically used small molecule drugs [126]. Rifampicin induces both intestinal and hepatic CYP3A4 and the induction of intestinal CYP3A4 was almost double than that of hepatic CYP3A4 [126]. Oral daily administration of 600 mg rifampicin for more than 10 days resulted in maximum induction of hepatic CYP3A4 [126]. This is the reason that rifampicin induces its own metabolism during rifampicin monotherapy [120,127,128]. The plasma half-life of rifampicin will reduce gradually and significantly after daily oral administration of the drug for 10 days. Dapsone is also metabolized by CYP subfamily of drug

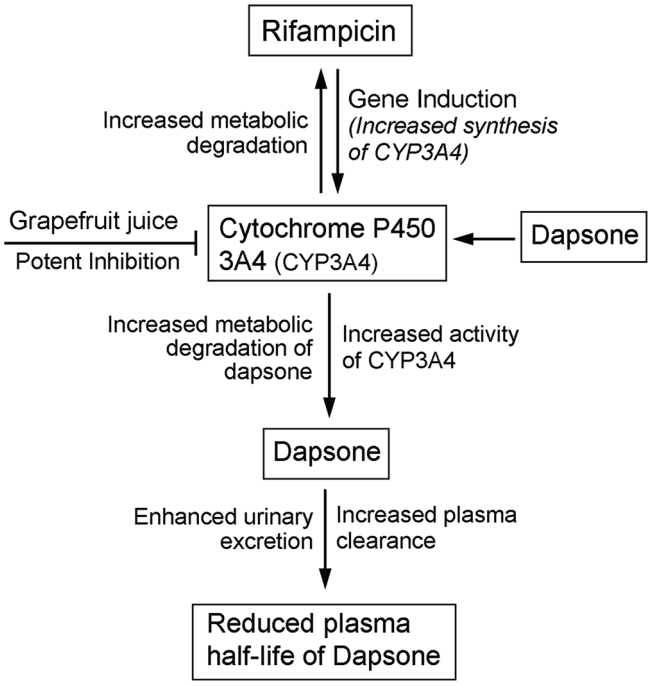

Fig. 4. Schematic representation of the mechanism of reduced plasma half-life of dapsone during concurrent administration with rifampicin. Rifampicin dramatically upregulates the major drug metabolizing enzyme cytochrome P450 3A4 (CYP3A4). Both Rifampicin and dapsone are metabolized by CYP3A4. This causes rapid metabolism and reduced plasma half-life of dapsone during concurrent administration with rifampicin. Simultaneously, the increased activity of CYP3A4 results in enhanced metabolic degradation of rifampicin. Dapsone does not induce the activity of CYP3A4. Grapefruit juice is a potent inhibitor in CYP3A4 and may lead to adverse effects with various drugs.

metabolizing enzymes, specifically isozymes CYP2D6, CYP2B6, CYP3A4, and CYP2C19 [63,129]. However, dapsone does not induce or upregulates any of these enzymes and thus do not affect the metabolic degradation or half-life of dapsone during monotherapy. We have observed that the plasma half-life of dapsone has been reduced more than 3-fold after combined treatment of 100 mg dapsone and 600 mg rifampicin for 15 consecutive days [71].

Fig. 4 depicts schematic presentation of the mechanism of decreased plasma half-life of dapsone during concurrent administration with rifampicin and the auto-induction of rifampicin metabolism. Upon absorption, rifampicin binds to the nuclear pregnane X receptor (PXR). The activated PXR complex forms a heterodimer with the retinoid X receptor (RXR) which in turn binds to the proximal promoter regions of CYP3A4 gene, resulting in increased transcription and expression of CYP3A4. The 10–50 fold increased synthesis of the enzyme leads to enhanced metabolic degradation of dapsone since CYP3A4 is a major enzyme that metabolizes dapsone. This in turn cause increased plasma clearance and enhanced urinary excretion of dapsone metabolites resulting reduced plasma half-life of dapsone (Fig. 4). The dramatically elevated levels of CYP3A4 also results in enhanced metabolic clearance of rifampicin. Grapefruit juice and grapefruit in general is a potent of inhibitor of CYP3A4 [130,131], which could affect the metabolism of both rifampicin and dapsone. Grapefruit juice has the greatest effect when taken an hour prior to administration of the drug and it lasts for 3–7 days [131].

7. Conclusions

The metabolic interaction of antileprosy drugs, rifampicin and dapsone due to the gene induction of the major drug-metabolizing enzyme CYP3A4 is a potent problem for the treatment of leprosy. However, it has overcome through appropriate treatment strategy introduced by WHO during multidrug therapy. The important aspect of therapy to leprosy is regular and uninterrupted consumption of the prescribed antileprosy drugs. Early diagnosis of the disease, especially the asymptomatic infection is critical for the successful treatment of leprosy without deformities.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] D.M. Scollard, C.M. Martelli, M.M. Stefani, F. Maroja Mde, L. Villahermosa, F. Pardillo, K.B. Tamang, Risk factors for leprosy reactions in three endemic countries, Am. J. Trop. Med. Hyg. 92 (2015) 108–114 PMID: 25448239.
[2] L.M. Irgens, The discovery of the leprosy bacillus, Tidsskr. Nor. Laegeforen. 122 (2002) 708–709 PMID: 11998735.
[3] J.C. Lastória, M.A. Abreu, Leprosy: review of the epidemiological, clinical, and etiopathogenic aspects - part 1, An Bras Dermatol. 89 (2014) 205–218 PMID: 24770495.
[4] C.K. Job, J. Jayakumar, M. Kearney, T.P. Gillis, Transmission of leprosy: a study of skin and nasal secretions of household contacts of leprosy patients using PCR, Am. J. Trop. Med. Hyg. 78 (2008) 518–521 PMID: 18337353.
[5] F.M. Amorim, M.L. Nobre, L.C. Ferreira, L.S. Nascimento, A.M. Miranda, G.R. Monteiro, K.M. Dupnik, M.S. Duthie, S.G. Reed, S.M. Jeronimo, Identifying Leprosy and Those at Risk of Developing Leprosy by Detection of Antibodies against LID-1 and LID-NDO, PLoS Negl. Trop. Dis. 10 (2016) e0004934PMID: 27658042.
[6] P. Singh, A. Benjak, V.J. Schuenemann, A. Herbig, C. Avanzi, P. Busso, K. Nieselt, J. Krause, L. Vera-Cabrera, S.T. Cole, Insight into the evolution and origin of leprosy bacilli from the genome sequence of Mycobacterium lepromatosis, PNAS 112 (2015) 4459–4464 PMID: 25831531.
[7] X.Y. Han, F.M. Aung, S.E. Choon, B. Werner, Analysis of the leprosy agents Mycobacterium leprae and Mycobacterium lepromatosis in four countries, Am. J. Clin. Pathol. 142 (2014) 524–532 PMID: 25239420.
[8] X.Y. Han, Y.H. Seo, K.C. Sizer, T. Schoberle, G.S. May, J.S. Spencer, W. Li, R.G. Nair, A new Mycobacterium species causing diffuse lepromatous leprosy, Am. J. Clin. Pathol. 130 (2008) 856–864 PMID:19019760.
[9] X.Y. Han, K.C. Sizer, J.S. Velarde-Félix, L.O. Frias-Castro, F. Vargas-Ocampo, The leprosy agents Mycobacterium lepromatosis and Mycobacterium leprae in Mexico, Int. J. Dermatol. 51 (2012) 952–959 PMID: 22788812.
[10] S. Suvirya, S. Pathania, K.P. Malhotra, A. Jain, P. Verma, P. Kumari, A case of diffuse lepromatous leprosy with Lucio phenomenon, QJM 113 (2020) 138–139 PMID: 31198950.
[11] E.A. Fischer, S.J. de Vlas, J.D. Habbema, J.H. Richardus, The long-term effect of current and new interventions on the new case detection of leprosy: a modeling study, PLoS Negl. Trop. Dis. 5 (2011) e1330PMID: 21949895.
[12] K. Suzuki, T. Akama, A. Kawashima, A. Yoshihara, R.R. Yotsu, N. Ishii, Current status of leprosy: epidemiology, basic science and clinical perspectives, J. Dermatol. 39 (2012) 121–129 PMID: 21973237.
[13] S. Bhattacharya, N. Vijayalakshmi, S.C. Parija, Uncultivable bacteria: implications and recent trends towards identification, Indian J. Med. Microbiol. 20 (2002) 174–177 PMID: 17657065.
[14] S.T. Cole, K. Eiglmeier, J. Parkhill, K.D. James, N.R. Thomson, P.R. Wheeler, et al., Massive gene decay in the leprosy bacillus, Nature 409 (2001) 1007–1011 PMID: 11234002.
[15] N. Nakata, M. Matsuoka, Y. Kashiwabara, N. Okada, C. Sasakawa, Nucleotide sequence of the *Mycobacterium leprae katG* region, J. Bacteriol. 179 (1997) 3053–3057 PMID: 9139928.
[16] M. Madan Babu, Did the loss of sigma factors initiate pseudogene accumulation in M. leprae? Trends Microbiol. 11 (2003) 59–61, https://doi.org/10.1016/s0966-842x(02)00031-8 PMID: 12598124.
[17] R. Sharma, R. Lahiri, D.M. Scollard, M. Pena, D.L. Williams, L.B. Adams, J. Figarola, R.W. Truman, The armadillo: a model for the neuropathy of leprosy and potentially other neurodegenerative diseases, Dis. Model Mech. 6 (2013) 19–24 PMID: 23223615.
[18] L. Levy, B. Ji, The mouse foot-pad technique for cultivation of Mycobacterium leprae, Lepr. Rev. 77 (2006) 5–24 PMID: 16715686.
[19] R.C. Medeiros, K.D. Girardi, F.K. Cardoso, B.S. Mietto, T.G. Pinto, L.S. Gomez, et al., Subversion of schwann cell glucose metabolism by mycobacterium leprae, J.

Biol. Chem. 291 (2016) 21375–21387 PMID: 27555322.
[20] A. Takade, A. Umeda, M. Matsuoka, S. Yoshida, M. Nakamura, Amako K. Comparative studies of the cell structures of Mycobacterium leprae and M. tuberculosis using the electron microscopy freeze-substitution technique, Microbiol. Immunol. 47 (2003) 265–270 PMID: 12801063.
[21] S. Mahapatra, D.C. Crick, M.R. McNeil, P.J. Brennan, Unique structural features of the peptidoglycan of Mycobacterium leprae, J. Bacteriol. 190 (2008) 655–661 PMID: 18024514.
[22] F.E. Pardillo, T.T. Fajardo, R.M. Abalos, D. Scollard, R.H. Gelber, Methods for the classification of leprosy for treatment purposes, Clin. Infect. Dis. 44 (2007) 1096–1099 PMID: 17366457.
[23] R. Gupta, H.K. Kar, M. Bharadwaj, Revalidation of various clinical criteria for the classification of leprosy–a clinic-pathological study, Lepr. Rev. 83 (2012) 354–362 PMID: 23614253.
[24] D.S. Ridley, W.H. Jopling, Classification of leprosy according to immunity. A five-group system, Int. J. Lepr. Other Mycobact. Dis. 34 (1966) 255–273 PMID: 5950347.
[25] G. Currie, Macular leprosy in Central Africa with special reference to the "maculoid" (dimorphous) form, Int J Lepr 29 (1961) 473–487 PMID: 13882729.
[26] V.N. Sehgal, Joginder., Slit-skin smear in leprosy, Int. J. Dermatol. 29 (1990) 9–16 PMID: 1691740.
[27] M.L. de Alvarenga Lira, C. Pagliari, A.A. de Lima Silva, H.F. de Andrade, Duarte MI. Jr, Dermal dendrocytes FXIIIa+ are essential antigen-presenting cells in indeterminate leprosy, Am. J. Dermatopathol. 37 (2015) 269–273 PMID: 25365500.
[28] V.P. Silva, H.H. Fonseca, M.M. Sens, A.T. Bender, Indeterminate leprosy and lepromatous index case: four cases in the same family, An. Bras. Dermatol. 88 (6 Suppl 1) (2013) 105–108 PMID: 24346893.
[29] L.S. Brandão, G.F. Marques, J.A. Barreto, A.P. Coelho, A.P. Serrano, Tuberculoid leprosy presenting as a "racket" lesion, An. Bras. Dermatol. 90 (2015) 420–422 PMID: 26131879.
[30] C. Talhari, S. Talhari, G.O. Penna, Clinical aspects of leprosy, Clin. Dermatol. 33 (2015) 26–37 PMID: 25432808.
[31] M. Ramos-e-Silva, Rebello PF. Leprosy, Recognition and treatment, Am. J. Clin. Dermatol. 2 (2001) 203–211 PMID: 11705247.
[32] C. Matsuo, C. Talhari, L. Nogueira, R.F. Rabelo, M.N. Santos, S. Talhari, Borderline lepromatous leprosy, An. Bras. Dermatol. 85 (2010) 921–922 PMID: 21308324.
[33] V.N. Sehgal, Reactions in leprosy. Clinical aspects, Int. J. Dermatol. 26 (1987) 278–285 PMID: 3301703.
[34] C. Saini, V. Ramesh, I. Nath, Increase in TGF-β secreting $CD4^+CD25^+$ $FOXP3^+$ T regulatory cells in anergic lepromatous leprosy patients, PLoS Negl. Trop Dis. 16 (8) (2014) e2639PMID: 24454972.
[35] C. Lakshmi, C.R. Srinivas, Lepromatous leprosy treated with combined chemotherapy and immunotherapy (injection BCG): three case reports, Int. J. Dermatol. 53 (2014) 61–65 PMID: 23675902.
[36] S. Mohanan, A.S. Devi, R. Kumari, D.M. Thappa, R.N. Ganesh, Novel presentation of lepromatous leprosy in an erythema gyratum repens-like pattern, Int. J. Dermatol. 53 (2014) 210–212 PMID: 24320626.
[37] A.S. Kourosh, J.B. Cohen, D.M. Scollard, Nations SP, Leprosy of Lucio and Latapí with extremity livedoid vascular changes, Int. J. Dermatol. 52 (2013) 1245–1247 PMID: 24073907.
[38] E. Nunzie, L.V. Ortega Cabrera, F.M. Macanchi Moncayo, P.F. Ortega Espinosa, A. Clapasson, C. Massone, Lucio Leprosy with Lucio's phenomenon, digital gangrene and anticardiolipin antibodies, Lepr. Rev. 85 (2014) 194–200 PMID: 25509720.
[39] P. Sharma, A. Kumar, A. Tuknayat, G.P. Thami, R. Kundu, Lucio phenomenon: a rare presentation of Hansen's Disease, J. Clin. Aesthet Dermatol. 12 (2019) 35–38 PMID: 32038763.
[40] M. Thapa, M. Sendhil Kumaran, T. Narang, U.N. Saikia, G.U. Sawatkar, Dogra S.A prospective study to validate various clinical criteria used in classification of leprosy: a study from a tertiary care center in India, Int. J. Dermatol. 57 (2018) 1107–1113 PMID: 29809278.
[41] O. Parkash, Classification of leprosy into multibacillary and paucibacillary groups: an analysis, FEMS Immunol. Med. Microbiol. 55 (2009) 1–5 PMID: 19040664.
[42] P.V. Prasad, P.K. Kaviarasan, Leprosy therapy, past and present: can we hope to eliminate it? Indian J Dermatol 55 (2010) 316–324 PMID: 21430881.
[43] E. Fromm, J. Wittmann, Derivatives of p-Nitrothiophenols, Reports of the German Chem. Soc. 41 (1908) 2264–2273, https://doi.org/10.1002/cber.190804102131.
[44] Jopling WH, McDougall AC. Handbook of Leprosy 5th Ed. 2008 pp. 1-182. CBC Publishers, New Delhi, India.
[45] G.A. Ellard, P.T. Gammon, R.J. Rees, M.F. Waters, Studies on the determination of the minimal inhibitory concentration of 4,4'-diamino-diphenyl-sulphone (Dapsone, DDS) against Mycobacterium leprae, Lepr. Rev. 42 (1971) 101–117 PMID: 4948480.
[46] J.K. Seydel, M. Richter, E. Wempe, Mechanism of action of the folate blocker diaminodiphenylsulfone (dapsone, DDS) studied in E. coli cell-free enzyme extracts in comparison to sulfonamides (SA), Int J Lepr 48 (1980) 18–29 PMID: 6988345.
[47] J.H. Pettit, R.J. Rees, Sulphone resistance in leprosy. An experimental and clinical study, Lancet 2 (7361) (1964) 673–674 PMID: 14188912.
[48] J.M. Pearson, R.J. Rees, M.F. Waters, Sulphone resistance in leprosy. A review of one hundred proven clinical cases, Lancet 2 (7924) (1975) 69–72 PMID: 49662.
[49] M. Kai, M. Matsuoka, N. Nakata, S. Maeda, M. Gidoh, Y. Maeda, K. Hashimoto, K. Kobayashi, Y. Kashiwabara, Diaminodiphenylsulfone resistance of Mycobacterium leprae due to mutations in the dihydropteroate synthase gene, FEMS Microbiol. Lett. 177 (1999) 231–235 PMID:10474189.
[50] D.L. Williams, L. Spring, E. Harris, P. Roche, T.P. Gillis, Dihydropteroate synthase of Mycobacterium leprae and dapsone resistance, Antimicrob. Agents Chemother. 44 (2000) 1530–1537, https://doi.org/10.1128/aac.44.6.1530-1537.2000 PMID: 10817704.
[51] C.C. Shepard, The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice, J. Exp. Med. 112 (1960) 445–454 PMID: 19867175.
[52] E. Fourneau, J. Tréfouël, F. Nitti, D. Bovet, Chimiothérapie de infection pneumococcique par la di-(p-acétylaminophényl)-sulfone (1399 F), Proc Acad Sci, Paris 205 (1937) 299–300.
[53] G. Bolla, S. Mittapalli, A. Nangia, Pentamorphs of acedapsone, Cryst. Growth Des. 14 (2014) 5260–5274, https://doi.org/10.1021/cg5010424.
[54] J.H. Peters, J.F. Murray Jr, G.R. Gordon, L. Levy, D.A. Russell, G.C. Scott, D.R. Vincin, C.C. Shepard, Acedapsone treatment of leprosy patients: response versus drug disposition, Am. J. Trop. Med. Hyg. 26 (1977) 127–136 PMID: 842774.
[55] C.C. Shepard, Activity of repository sulfones against Mycobacterium leprae in mice, Proc. Soc. Exp. Biol. Med. 124 (1967) 430–433 PMID: 5335647.
[56] C.C. Shepard, J.G. Tolentino, D.H. McRae, The therapeutic effect of 4,4'-diacetyldiamino- diphenylsulfone (DADDS) in leprosy, Am. J. Trop. Med. Hyg. 17 (1968) 192–201 PMID: 4870563.
[57] J. George, S. Balakrishnan, Blood dapsone levels in leprosy patients treated with acedapsone, Indian J. Lepr. 58 (1986) 401–406 PMID: 3794408.
[58] Wallace RJ, Philley JV, Griffith DE. Antimycobacterial Agents. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (8th Ed.) Edited by Bennett EJ, Dolin R, Blaser MJ. 2015; Vol 1, pp. 463-478.
[59] D.A. Russell, C.C. Shepard, D.H. McRae, G.C. Scott, D.R. Vincin, Acedapsone (DADDS) treatment of leprosy patients in the Karimui of Papua New Guinea: status at six years, Am. J. Trop. Med. Hyg. 24 (1975) 485–495 PMID: 1098496.
[60] F.R. Oliveira, M.C. Pessoa, R.F.V. Albuquerque, T.R. Schalcher, M.C. Monteiro, Clinical applications and methemoglobinemia induced by dapsone, J. Braz. Chem. Soc. 25 (2014) 1770–1779, https://doi.org/10.5935/0103-5053.20140168.
[61] P.P. Raj, M. Aschhoff, L. Lilly, S. Balakrishnan, Influence of acetylator phenotype of the leprosy patient on the emergence of dapsone resistant leprosy, Indian J. Lepr. 60 (1988) 400–406 PMID: 3058828.
[62] J. Zuidema, E.S. Hilbers-Modderman, F.W. Merkus, Clinical pharmacokinetics of dapsone, Clin. Pharmacokinet. 11 (1986) 299–315 PMID: 3530584.
[63] S. Ganesan, R. Sahu, L.A. Walker, B.L. Tekwani, Cytochrome P450-dependent toxicity of dapsone in human erythrocytes, J. Appl. Toxicol. 30 (2010) 271–275 PMID:19998329.
[64] D. Ezhilarasan, Dapsone-induced hepatic complications: it's time to think beyond methemoglobinemia, Drug Chem. Toxicol. (2019) 1–4, https://doi.org/10.1080/01480545.2019.1679829 PMID: 31631707.
[65] Y. Bian, K. Kim, G.J. An, T. Ngo, O.N. Bae, K.M. Lim, J.H. Chung, Dapsone Hydroxylamine, an Active Metabolite of Dapsone, Can Promote the Procoagulant Activity of Red Blood Cells and Thrombosis, Toxicol. Sci. 172 (2019) 435–444 PMID: 31428780.
[66] M.D. Tingle, R. Mahmud, J.L. Maggs, M. Pirmohamed, B.K. Park, Comparison of the metabolism and toxicity of dapsone in rat, mouse and man, J. Pharmacol. Exp. Ther. 283 (1997) 817–823 PMID:9353403.
[67] J. George, M. Rajendran, V.N. Bhatia, Serum beta-glucuronidase in subtypes of leprosy, Indian J. Med. Res. 91 (1990) 106–110 PMID: 2345017.
[68] M.A. Abuirjeie, Y.M. Irshaid, al-Hadidi HF, Rawashdeh NM., Simultaneous high performance liquid chromatographic determination of dapsone and monoacetyldapsone in human plasma and urine, J. Clin. Pharm. Ther. 16 (1991) 247–255 PMID: 1939403.
[69] G.A. Ellard, P.T. Gammon, A fluorometric method for the simultaneous determination of 4,4'-diaminodiphenyl sulfone (DDS), N-acetyl-DDS (MADDS) and N, N'-diacetyl-DDS (DADDS) in serum or urine, Int. J. Lepr. Other Mycobact. Dis. 37 (1969) 398–405 PMID: 5394260.
[70] J.H. Peters, G.R. Gordon, W.T. Colwell Jr., The fluorometric measurement of 4,4'-diaminodiphenyl sulfone and its acetylated derivatives in plasma and urine, J. Lab. Clin. Med. 76 (1970) 338–348 PMID: 5434010.
[71] J. George, S. Balakrishnan, V.N. Bhatia, Drug interaction during multidrug regimens for treatment of leprosy, Indian J. Med. Res. 87 (1988) 151–156 PMID: 3397146.
[72] P.P. Raj, M. Aschhoff, M. De Wit, S. Balakrishnan, L. Lilly, Certain aspects of dapsone metabolism in leprosy patients as studied by high performance liquid chromatography (HPLC) and qualitative screening tests, Indian J. Lepr. 60 (1988) 215–224 PMID: 3192970.
[73] J. George, S. Balakrishnan, A comparative study of the haemagglutination inhibition (HI) test and spot test for detection of dapsone in urine, Indian J. Lepr. 57 (1985) 601–606 PMID: 3831099.
[74] A. Saxena R. Kumari U. Mukherjee P. Singh Lal R. Draft Genome Sequence of the Rifamycin producer Amycolatopsis rifamycinica DSM 46095 Genome Announc. 2, 2014, 00662-14, https://doi.org/10.1128/genomeA.00662-14. pii: e00662–14 PMID: 24994803.
[75] W. Wehrli, Rifampin: mechanisms of action and resistance, Rev. Infect. Dis. 5 (Suppl 3) (1983) S407–S411 PMID: 6356275.
[76] E.A. Campbell, N. Korzheva, A. Mustaev, K. Murakami, S. Nair, A. Goldfarb, S.A. Darst, Structural mechanism for rifampicin inhibition of bacterial rna polymerase, Cell 104 (2001) 901–912 PMID: 11290327.
[77] J.H. Grosset, C.C. Guelpa-Lauras, Activity of rifampin in infections of normal mice with Mycobacterium leprae, Int. J. Lepr. Other Mycobact. Dis. 55 (4 Suppl) (1987) 847–851 PMID: 3437168.
[78] Walker SL, Withington SG, Lockwood DNJ. 41- Leprosy In: Manson's Tropical Infectious Diseases (23rd Ed) Edited by Farrar J, Hotez PJ, Junghanss T, Kang, G,

Lalloo D, White NJ. 2014, pp 506-518.e1.
[79] E. Andre, L. Goeminne, A. Cabibbe, P. Beckert, B. Kabamba Mukadi, V. Mathys, S. Gagneux, S. Niemann, J. Van Ingen, E. Cambau, Consensus numbering system for the rifampicin resistance-associated rpoB gene mutations in pathogenic mycobacteria, Clin. Microbiol. Infect. 23 (2017) 167–172 PMID: 27664776.
[80] S.C. Vedithi, S. Malhotra, M. Das, S. Daniel, N. Kishore, A. George, S. Arumugam, L. Rajan, M. Ebenezer, D.B. Ascher, E. Arnold, T.L. Blundell, Structural implications of mutations conferring rifampin resistance in mycobacterium leprae, Sci. Rep. 8 (2018) 5016, https://doi.org/10.1038/s41598-018-23423-1 PMID: 29567948.
[81] S. Bankar, R. Set, D. Sharma, D. Shah, J. Shastri, Diagnostic accuracy of Xpert MTB/RIF assay in extrapulmonary tuberculosis, Indian J. Med. Microbiol. 36 (2018) 357–363 PMID: 30429387.
[82] B.P. Goldstein, Resistance to rifampicin: a review, J. Antibiot. (Tokyo) 67 (2014) 625–630 PMID: 25118103.
[83] M.T. Zaw, N.A. Emran, Z. Lin, Mutations inside rifampicin-resistance determining region of rpoB gene associated with rifampicin-resistance in Mycobacterium tuberculosis, J Infect Public Health 11 (2018) 605–610, https://doi.org/10.1016/j.jiph.2018.04.005 PMID: 29706316.
[84] G. Acocella, Clinical pharmacokinetics of rifampicin, Clin. Pharmacokinet. 3 (1978) 108–127, https://doi.org/10.2165/00003088-197803020-00002 PMID: 346286.
[85] Hardman Joel G., Lee E. Limbird, and Alfred G. Gilman, eds. “Rifampin.” The Pharmacological Basis of Therapeutics. 10th ed. The McGraw-Hill Companies, USA 2001 pp. 1277–1279.
[86] U.M. Zanger, M. Schwab, Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation, Pharmacol. Ther. 138 (2013) 103–141 PMID: 23333322.
[87] M. Sousa, A. Pozniak, M. Boffito, Pharmacokinetics and pharmacodynamics of drug interactions involving rifampicin, rifabutin and antimalarial drugs, J. Antimicrob. Chemother. 62 (2008) 872–878 PMID: 18713760.
[88] M. Strolin Benedetti, P. Dostert, Induction and autoinduction properties of rifamycin derivatives: a review of animal and human studies, Environ. Health Perspect. 102 (Suppl 9) (1994) 101–105 PMID: 7698069.
[89] B. Williamson, K.E. Dooley, Y. Zhang, D.J. Back, A. Owen, Induction of influx and efflux transporters and cytochrome P450 3A4 in primary human hepatocytes by rifampin, rifabutin, and rifapentine, Antimicrob. Agents Chemother. 57 (2013) 6366–6369 PMID: 24060875.
[90] G. Acocella, Pharmacokinetics and metabolism of rifampin in humans, Rev. Infect. Dis. 5 (Suppl 3) (1983) S428–S432 PMID: 6356276.
[91] S. Sunahara, H. Nakagawa, Metabolic study and controlled clinical trials of rifampin, Chest 61 (1972) 526–532 PMID: 5032150.
[92] J.M. Dickinson, V.R. Aber, B.W. Allen, G.A. Ellard, D.A. Mitchison, Assay of rifampicin in serum, J. Clin. Pathol. 27 (1974) 457–462 PMID: 4212955.
[93] J. George, V.N. Bhatia, S. Balakrishnan, Microbiological assay versus spectrophotometry for determination of rifampicin in urine, Indian J. Lepr. 60 (1988) 47–52 PMID: 3060546.
[94] J.B. Lecaillon, N. Febvre, J.P. Metayer, C. Souppart, Quantitative assay of rifampicin and three of its metabolites in human plasma, urine and saliva by high-performance liquid chromatography, J. Chromatogr. 145 (1978) 319–324 PMID: 649727.
[95] J.S. Patil, S. Suresh, A.R. Sureshbabu, M.S. Rajesh, Development and validation of liquid chromatography-mass spectrometry method for the estimation of rifampicin in plasma, Indian J. Pharm. Sci. 73 (2011) 558–563 PMID: 22923869.
[96] T. Narang, A. Bishnoi, S. Dogra, U.N. Saikia, Kavita. Alternate anti-leprosy regimen for multidrug therapy refractory leprosy: A retrospective study from a tertiary care center in North India, Am. J. Trop. Med. Hyg. 100 (2019) 24–30 PMID: 30298809.
[97] M.C. Cholo, H.C. Steel, P.B. Fourie, W.A. Germishuizen, R. Anderson, Clofazimine: current status and future prospects, J. Antimicrob. Chemother. 67 (2012) 290–298 PMID: 22020137.
[98] J.L. Arbiser, S.L. Moschella, Clofazimine: a review of its medical uses and mechanisms of action, J. Am. Acad. Dermatol. 32 (2 Pt 1) (1995) 241–247 PMID: 7829710.
[99] N.E. Morrison, G.M. Marley, The mode of action of clofazimine DNA binding studies, Int. J. Lepr. Other Mycobact. Dis. 44 (1976) 133–134 PMID: 945233.
[100] M. Faouzi, J. Starkus, R. Penner, State-dependent blocking mechanism of Kv 1.3 channels by the antimycobacterial drug clofazimine, Br. J. Pharmacol. 172 (2015) 5161–5173 PMID: 26276903.
[101] R.H. Costa Queiroz, A.M. de Souza, S.V. Sampaio, E. Melchior Jr., Biochemical and hematological side effects of clofazimine in leprosy patients, Pharmacol. Res. 46 (2002) 191–194 PMID: 12220960.
[102] R. O'Connor, J.F. O'Sullivan, R. O'Kennedy, The pharmacology, metabolism, and chemistry of clofazimine, Drug Metab. Rev. 27 (1995) 591–614 PMID: 8925720.
[103] Z. Schaad-Lanyi, W. Dieterle, J.P. Dubois, W. Theobald, W. Vischer, Pharmacokinetics of clofazimine in healthy volunteers, Int. J. Lepr. Other Mycobact. Dis. 55 (1987) 9–15 PMID: 3559339.
[104] J. Baik, G.R. Rosania, Molecular imaging of intracellular drug-membrane aggregate formation, Mol. Pharm. 8 (2011) 1742–1749 PMID: 21800872.
[105] L. Levy, Pharmacologic studies of clofazimine, Am. J. Trop. Med. Hyg. 23 (1974) 1097–1109 PMID: 4611255.
[106] M.R. Holdiness, Clinical pharmacokinetics of clofazimine, A review. Clin Pharmacokinet 16 (1989) 74–85 PMID: 2656045.
[107] R.V. Swanson, J. Adamson, C. Moodley, B. Ngcobo, N.C. Ammerman, A. Dorasamy, S. Moodley, Z. Mgaga, A. Tapley, L.A. Bester, S. Singh, J.H. Grosset, D.V. Almeida, Pharmacokinetics and pharmacodynamics of clofazimine in a mouse model of tuberculosis, Antimicrob. Agents Chemother. 59 (2015) 3042–3051 PMID: 25753644.
[108] K.V. Desikan, S. Balakrishnan, Tissue levels of clofazimine in a case of leprosy, Lepr. Rev. 47 (1976) 107–113 PMID: 948215.
[109] K. Venkatesan, A. Mathur, A. Girdhar, B.K. Girdhar, Excretion of clofazimine in human milk in leprosy patients, Lepr. Rev. 68 (1997) 242–246 PMID: 9364825.
[110] P.C. Feng, C.C. Fenselau, R.R. Jacobson, Metabolism of clofazimine in leprosy patients, Drug Metab. Dispos. 9 (1981) 521–524 PMID: 6120809.
[111] P.C. Feng, C.C. Fenselau, R.R. Jacobson, A new urinary metabolite of clofazimine in leprosy patients, Drug Metab. Dispos. 10 (1982) 286–288 PMID: 6125367.
[112] Y. Shimokawa, N. Yoda, S. Kondo, Y. Yamamura, Y. Takiguchi, K. Umehara, Inhibitory potential of twenty five anti-tuberculosis drugs on CYP activities in human liver microsomes, Biol. Pharm. Bull. 38 (2015) 1425–1429 PMID: 26094899.
[113] K. Venkatesan, V.P. Bharadwaj, G. Ramu, K.V. Desikan, Study on drug interactions, Lepr India 52 (1980) 229–325 PMID: 7453137.
[114] J.H. Peters, K.J. Hamme, G.R. Gordon, Determination of clofazimine in plasma by high- performance liquid chromatography, J. Chromatogr. 229 (1982) 503–508.
[115] T.R. Krishnan, I. Abraham, A rapid and sensitive high performance liquid chromatographic analysis of clofazimine in plasma, Int. J. Lepr. Other Mycobact. Dis. 60 (1992) 549–555 PMID: 1299710.
[116] R.H. Queiroz, R.C. Pereira, M.A. Gotardo, D.S. Cordeiro, E. Melchior Jr., Determination of clofazimine in leprosy patients by high-performance liquid chromatography, J. Anal. Toxicol. 27 (2003) 377–380 PMID: 14516492.
[117] T.S. Patil, A.S. Deshpande, S. Deshpande, Critical review on the analytical methods for the estimation of clofazimine in bulk, biological fluids and pharmaceutical formulations, Crit. Rev. Anal. Chem. 48 (2018) 492–502 PMID: 29621407.
[118] J. George, S. Balakrishnan, V.N. Bhatia, D. Anandan, S. Harikrishnan, Thyroglobulin autoantibodies in leprosy, Indian J. Lepr. 58 (1986) 191–195 PMID: 3805790.
[119] V.K. Sharma, K. Saha, V.N. Sehgal, Serum immunoglobulins an autoantibodies during and after erythema nodosum leprosum (ENL), Int J Lepr 50 (1982) 159–163 PMID: 6811448.
[120] K. Venkatesan, Clinical pharmacokinetic considerations in the treatment of patients with leprosy, Clin. Pharmacokinet. 16 (1989) 365–386 PMID: 2661102.
[121] S. Balakrishnan, P.S. Seshadri, Drug interactions - the influence of rifampicin and clofazimine on the urinary excretion of DDS, Lepr India 53 (1981) 17–22 PMID: 7218762.
[122] K. Venkatesan, Pharmacokinetic drug interactions with rifampicin, Clin. Pharmacokinet. 22 (1992) 47–65 PMID: 1559307.
[123] U.M. Zanger, M. Turpeinen, K. Klein, M. Schwab, Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation, Anal. Bioanal. Chem. 392 (2008) 1093–1108 PMID: 18695978.
[124] J.M. Rae, M.D. Johnson, M.E. Lippman, D.A. Flockhart, Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays, J. Pharmacol. Exp. Ther. 299 (2001) 849–857 PMID: 11714868.
[125] F. Yamashita, Y. Sasa, S. Yoshida, A. Hisaka, Y. Asai, H. Kitano, M. Hashida, H. Suzuki, Modeling of rifampicin-induced CYP3A4 activation dynamics for the prediction of clinical drug-drug interactions from in vitro data, PLoS ONE 8 (2013) e70330, https://doi.org/10.1371/journal.pone.0070330 PMID: 24086247.
[126] A.J. Kapetas, M.J. Sorich, A.D. Rodrigues, A. Rowland, Guidance for rifampin and midazolam dosing protocols to study intestinal and hepatic cytochrome P450 (CYP) 3A4 induction and de-induction, AAPS J. 21 (2019) 78 PMID: 31218462.
[127] U. Loos, E. Musch, J.C. Jensen, H.K. Schwabe, M. Eichelbaum, Influence of the enzyme induction by rifampicin on its presystemic metabolism, Pharmacol. Ther. 33 (1987) 201–204 PMID: 3628475.
[128] J. Chen, K. Raymond, Roles of rifampicin in drug-drug interactions: underlying molecular mechanisms involving the nuclear pregnane X receptor, Ann. Clin. Microbiol. Antimicrob. 5 (2006) 3, https://doi.org/10.1186/1476-0711-5-3 PMID: 16480505.
[129] C.M. Fleming, R.A. Branch, G.R. Wilkinson, F.P. Guengerich, Human liver microsomal N- hydroxylation of dapsone by cytochrome P-4503A4, Mol. Pharmacol. 41 (1992) 975–980 PMID: 1588928.
[130] M.L. Veronese, L.P. Gillen, J.P. Burke, E.P. Dorval, W.W. Hauck, E. Pequignot, S.A. Waldman, H.E. Greenberg, Exposure-dependent inhibition of intestinal and hepatic CYP3A4 in vivo by grapefruit juice, J. Clin. Pharmacol. 43 (2003) 831–839 PMID: 12953340.
[131] J.J. Lilja, K.T. Kivistö, P.J. Neuvonen, Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin, Clin. Pharmacol. Ther. 68 (2000) 384–390 PMID: 11061578.