PYRAZINAMIDE – PHARMACEUTICAL, BIOCHEMICAL AND PHARMACOLOGICAL PROPERTIES AND REAPPRAISAL OF ITS ROLE IN THE CHEMOTHERAPY OF TUBERCULOSIS

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Abstract. Pyrazinamide is an important antimycobacterial drug used in the contemporary short-course therapy of tuberculosis. Pyrazinamide is similar to isoniazid in its narrow spectrum of clinically-relevant antibacterial activity, which essentially includes only M. tuberculosis. The drug is bactericidal to slowly metabolizing organisms located within the acidic environment of the phagocyte or caseous granuloma; it is active only at a pH of ca. 6.0. Pyrazinamide is considered a prodrug and is converted by the tubercle bacillus to the active form pyrazinoic acid. The clinical recognition that the inclusion of pyrazinamide (PZA) allowed a reduction in the duration required to achieve predictable cures has revolutionized tuberculosis chemotherapy. PZA has been found to accelerate the time required to achieve culture negativity and to yield ca. 95% cure rates in 6 months when combined with isoniazide and rifampicin. Thus the short-course regimens have been implemented worldwide as the golden standard for management of tuberculosis and remained unchanged for decades. The most frequently recommended and effective combination is isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months. The presented review gives a concise outline of the biochemical, pharmacological and clinical features of pyrazinamide as a first line antituberculous drug.

Key Words: Pyrazinamide, Tuberculosis, Antituberculous agents, Chemotherapy, MDR-TB, XDR-TB

Introduction

It is hard to establish the first mention of PZA as an antituberculous drug. Its chemical synthesis was reported by Hall and Spoerri in 1936, although it was not discussed as an antimicrobial agent [1]. Yeager et al. were the first to use PZA clinically in a study which began in 1949 and was reported in 1952 [2, 3]. Its antimicrobial activity in comparison to other related pyrazine compounds was tested in a murine model by Kushner et al. in 1952. In any event, it was found to be promising from the outset in studies of murine and guinea pig tuberculosis [2].

Yeager's early clinical work, in which PZA was given as a single agent, demonstrated sputum conversion in 19 of 21 patients within four months, but later relapse with the development of drug resistance was a problem [2]. Other early clinical studies showed encouraging results using combination chemotherapy with streptomycin [4], isoniazid [2, 5, 6] or streptomycin + aminosalicylic acid [7]. However, these reports described also an unacceptable incidence of hepatotoxicity, including some fatalities. These early reports of toxicity, together with a discouraging statement by the American Trudeau Society, led to its early abandonment as a first-line agent for the treatment of tuberculosis and its relegation to use for brief periods for "coverage" of drug-resistant cases subjected to pulmonary resection, and as a component of second-line multiple-drug salvage therapy in advanced drug-resistant cases. It should be emphasized, however, that the doses used then were higher (40-70 mg/kg) than those presently employed (25 - 35 mg/kg), and the duration of therapy longer [2].

The resurgence or pyrazinamide as a first-line

agent and its worldwide implementation as an inevitable component in the initial phase of modern 6 month regimens was made on the basis of further advances in the late 1960s and the 1970s [8]. The discovery of rifamycins, with their introduction in the armamentarium of antituberculous drugs in 1966 prompted renewed investigation on the reduction of treatment duration: eventually, the 6 months short-course chemotherapy, with combinations of rifampicin, isoniazid and pyrazinamide as back-bone of the regimen became standard [9].

On the basis of extensive clinical trials with short course regimens these have been implemented worldwide as the golden standard for management of tuberculosis [10, 11]. Moreover this conventional short-course therapy has remained unchanged for decades [1, 12]. The most frequently recommended and effective combination is isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months [13]. This regimen is very effective for treatment of patients with tuberculosis and is recommended for all new cases of tuberculosis by WHO, ATC/CDC/IDSA, BTC and other authorities worldwide [13, 14]. Besides pyrazinamide has important clinical role for the treatment of HIV-infected individuals with tuberculosis co-infection [15, 16] and since the 1990's for the treatment of multi-drug resistant and extensively drug resistant tuberculosis [17-21].

The presented review article gives a concise outline of the biochemical, biopharmaceutical, pharmacological, safety and clinical features of pyrazinamide as a first line antituberculous drug.

List of abbreviations and terms

Abbreviated names of antituberculous agents: TB-tuberculosis, MDR-TB multidrug resistant tyberculosis, extensively drug resistant tuberculosis. In order to facilitate readability and due to space limitations in the following text the standard three-letter or the international single-letter abbreviations are used, instead of the full INNs of the antituberculous agents, as follows: i) first line agents: pyrazinamide- Z, PZA; ethambutol – E,EMB; Isoniazid – H,INH; streptomycin – S,SM; rifampicin – R,RMP; ii) second line agents and MDR-TB drugs: aminosalicylic acid – P,PAS; amikacin – Am; kanamycin – Ka; cycloserine – CYC, Cy, CS; ethionamide – ETH, ciprofloxacin – Ci; levofloxacin – Lfx; ofloxacin – Of; thiacetazone – TB1, T; Terizidone – Te (See below).

Regimen designation: Any number at the start of the regimen abbreviation indicates the duration of the initial phase/ followed by the continuation phase. Subscript numbers indicate the number of doses per week, i.e., 1 = once weekly; 3 = 3 times weekly; no number or 7 = daily; e.g. $2H_7R_7Z_7E_7/4H_7R_7$ – 2 month initial phase H+R+Z+E, each agent given daily, followed by a 4 months continuation phase with H+R in daily doses.

Medicinal chemistry and structure-activity relationships (SAR)

Pyrazinamide (Fig. 1) has been elaborated on the basis of the observed inhibitory activity of nicotinamide on mycobacteria. In studies of chemical modifications of the nicotinamide structure, other heterocyclic nuclei have been investigated, and the 2-carboxamidopyrazine (pyrazinamide) was tested for antituberculous activity [11]. The *in vitro* activity of pyrazinamide against *M. tuberculosis* was found to be negligible at neutral pH and of the order of 5-20 μg/ml at pH 5.5. The best activity of pyrazinamide is against intracellular mycobacteria in monocytes, probably because of the low intracellular pH, which favors its activity [1].

Compounds with other substitutions on the pyrazine nucleus or other carboxamido heterocycles were found to be inactive or less active than pyrazinamide. The only active analog developed because of its potential superiority over pyrazinamide was morphazinamide (N-morpholinomethylamide of pyrazinoic acid) [1, 8]. Interest in this drug however ceased when it was ascertained that the activity and toxicity parallel those of pyrazinamide, to which morphazinamide is converted *in vivo* [11]. Some pyrazinoic acid esters, PZA-Mannich bases and some N-pyrazinylthyoureas were found to be active in vitro against *M. tuberculosis* [1, 11, 22]. A 5-chloroderivative has been shown to be a potent inhibitor of Fas-I, the putative pharmacological target of pyrazinamide [23].

Mechanism of action

PZA as a prodrug enters mycobacteria by passive diffusion and possibly active transport, and then is converted by pyrazinamidase (PZase) into the active antibacterial moiety POA (an acid of intermediate strength with a pKa of 2.9). In mycobacteria the natural substrate of POA is nicotinamide (Figure 1).

POA exits from the mycobacterial cell by passive diffusion and via an efflux mechanism that appears to be deficient in M. tuberculosis which explains the sensitivity of these species to pyrazinamide. If the extracellular pH is acidic (e.g., 5.5), a proportion of the POA outside the bacterial cell membrane will take an uncharged conjugate acid form, HPOA, which

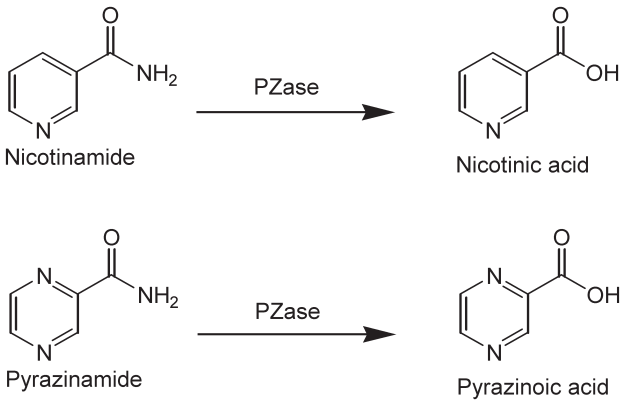

Fig. 1. *Convertion of nicotinamide and PZA by the nicotinamidase/pyrazinamidase (PZase), to their carboxylic-acid forms.*

permeates through the membrane easily [24, 25]. The theory of HPOA re-entering the bacilli best explains the role of acid pH in PZA action, and is based on the following observations [24]: i) POA is found outside the cells in the culture supernatant when 14C-PZA is added to M. tuberculosis cultures; ii) POA is accumulated inside the bacilli at acidic pH but not neutral pH; iii) PZA-resistant M. tuberculosis lacking PZase does not take up PZA, but takes up POA at acid pH.

As the MIC is little altered by changes in the size of small inocula, it is probable that POA is trapped to some extent between the cell membrane and the cell wall. The acid-facilitated POA influx is apparently stronger than the weak or deficient POA efflux, so that there is an accumulation of POA in *M. tuberculosis* cells [24]. The protonated POA brings protons into the cell, which could eventually cause cytoplasmic acidification such that vital enzymes can be inhibited [1, 25]. In addition, protonated POA could potentially de-energise the membrane by collapsing the proton motive force and affecting membrane transport as a possible mechanism of POA [23, 26]. At neutral or alkaline pH, there is little POA found in the tubercle bacilli because over 99.9% of POA will be in charged anion form, which does not enter cells easily. This observation explains why PZA is active at acidic pH but has an MIC > 250 μg/ml at neutral pH, and also explains the correlation between PZA MIC and acidic pH values [23, 27].

While the process of acid-facilitated uptake of weak acid is a non-specific process, the specificity of PZA for M. *tuberculosis* is clearly conferred at the stage of POA transport, where *M. tuberculosis* has a deficient POA efflux mechanism that is unable to counteract the effect of influx. The non-specificity of the proposed action also helps to explain the greater activity of PZA against old, non-replicating bacilli. Drugs that bind to specific sites interrupt specific, vital metabolic steps and are always more actively bactericidal against actively multiplying bacilli than against dormant bacilli. However, the active efflux mechanism for POA would be down-regulated in old, dormant bacilli whereas its passive uptake would remain unchanged, leading to a greater accumulation of POA [23].

In recent studies it has been proposed that the target of PZA or POA is fatty acid synthase-I (Fas-I) [1, 25, 28]. Nevertheless, so far, no mutations in Fas-I have been found in PZA-resistant M. tuberculosis strains. [23]. The paradigm that Fas-I is the ultimate pharmacological target of PZA in *M. tuberculosis* has been questioned by Boshoff et al. They found that while Fas-I is the target a derivative of the drug - 5-Cl-PZA, it is not the target of either PZA or POA [29]. Another study however indicates that both PZA and POA inhibit the activity of the enzyme [22]. Further lines of circumstantial evidence appear to argue against the presence of a specific cellular target for POA [1]. First, attempts to isolate mutants resistant to POA have been unsuccessful [30]. Furthermore, among clinical isolates of *M. tuberculosis* resistant to PZA, none have been found to be resistant to POA [23]. It is unclear whether the inability to obtain a POA-resistant mutant is because a target mutant is non-viable or because POA affects so many targets in the cell that no mutant can be selected. Moreover, ^{14}C-POA does not appear to bind to any proteins in an *M. tuberculosis* cellular extract. Thus taken together, the current data do not favor a specific cellular target for POA, although this possibility cannot be completely ruled out. The possibility that POA, as an analogue of nicotinic acid, might incorporate into the NAD molecule and affect NAD function to inhibit *M. tuberculosis* remains to be tested [23].

In a recent report it has been shown that pyrazinoic acid, the active moiety of pyrazinamide, disrupts membrane energetics and inhibits the membrane transport function in *Mycobacterium tuberculosis*. The preferential activity of pyrazinamide against old non-replicating bacilli correlated with their low membrane potential and the disruption of membrane potential by pyrazinoic acid and acid pH. Inhibitors of membrane energetics increased the antituberculous activity of pyrazinamide [26].

The above considerations have been summarized in figure 2.

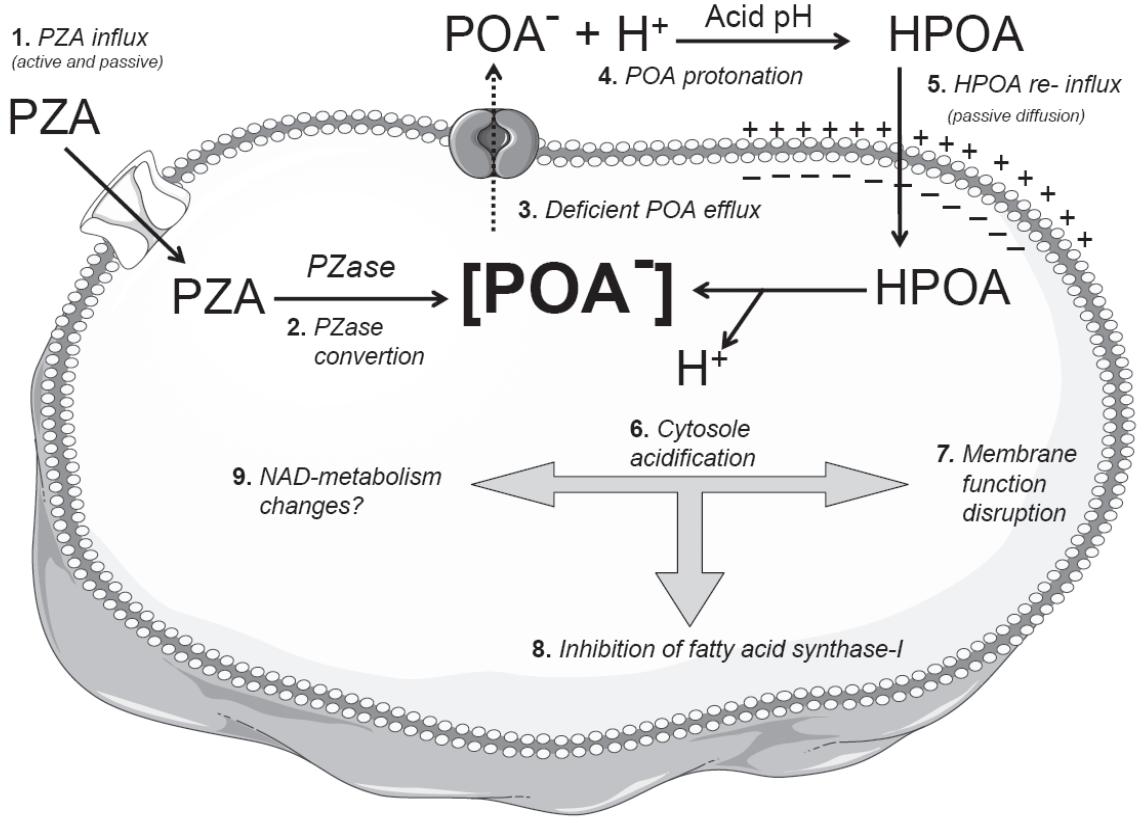

Fig. 2. *Schematic representation of pyrazinamide's mode of action (For the sake of clarity the cell wall of the pathogene has been omitted from the scheme). PZA enters Mycobacterium tuberculosis by passive diffusion, is converted to pyrazinoic acid (POA) by nicotinamidase/pyrazinamidase (PZase) and is then excreted by a weak efflux pump. Protonated POA (HPOA) is reabsorbed into the bacilli under acid conditions and accumulates because the efflux pump is inefficient, causing cellular damage via modification of several putative vital functions of the pathogene (See text for details)*

Antimicrobial activity in vitro/Spectrum

Pyrazinamide is an antimycobacterial agent with a remarkably narrow spectrum of clinically-relevant activity. PZA is active only against *M. tuberculosis* complex organisms (*M. tuberculosis*, *M. africanum* and *M. microti*), but not against *M. bovis* or *M. bovis BCG* [2, 23, 28]. The minimal inhibitory concentration (MIC) of PZA for *M. tuberculosis* has been reported as in the range of 6.25–50 μg/ml at pH 5.5 whereas non-tuberculous bacteria such as *Escherichia coli* and *M. smegmatis* are resistant to at least 2000 μg/ml PZA at pH 5.5 [23]. In experimental work with liquid media, there is a narrow pH range bounded on the alkaline side by no activity of PZA and on the acid side by complete inhibition of growth in drug-free medium. As growth in this pH range is usually very slow in the absence of PZA, it often appears to exert only slow or no bactericidal activity. Thus, using the BACTEC 7H12B medium (pH 5.6), Heifets and Lindholm-Levy found that PZA killed 0, 33, 60, 68, 57, and 72% of the bacterial population at 31, 62, 125, 250, 500 and 1000 μg/ml during a 2-week drug exposure period. The MBC (minimum bactericidal concentration, defined as killing 99% bacterial population) could not be determined since even at the highest concentration used (1000 μg/ml) PZA killed no more than 72% of the bacterial population. This concentration is at least 20 times greater than the MIC (50 μg/ml) and the peak concentration attainable in humans (30–60 μg/ml). After exposure of small inocula of tubercle bacilli to 50 μg/ml PZA for 96 hours, re-growth of the bacilli did not occur within 76 days, suggesting a long post-antibiotic lag [23].

- **Resistance**

It is well established that PZA-resistant clinical isolates of M. tuberculosis are usually defective in PZase activity; moreover there is good correlation between PZA resistance and loss of this enzyme

activity [31, 32]. PZA-resistant *M. tuberculosis* strains lack both nicotinamidase and PZase and are cross-resistant to nicotinamide, but not to other antituberculosis drugs. This suggests that the antitubercular activity of these agents relies on the activity of the same enzyme nicotinamidase/PZase [23].

Zhang et al. have identified the PZase gene (*pncA*) from *M. tuberculosis* and showed that transformation of PZA-resistant BCG with *M. tuberculosis pncA* conferred PZase activity and PZA susceptibility to BCG. In addition, the same group has shown that *pncA* mutations are a major mechanism of PZA resistance [23]. Other studies have confirmed these findings [23, 33-40].

Differences in the frequency of *pncA* mutations (72–98%) found in clinical isolates reportedly resistant to PZA may in part reflect incorrect PZA susceptibility testing, a common problem of the current PZA susceptibility testing, and in part imply other undefined gene(s) involved in PZA resistance [23]. PZA-resistant *M. tuberculosis* strains including *M. bovis* are still susceptible to POA, indicating that the PZA resistance is caused by changes in PZase activity due to mutations in pncA gene or, in rare cases, to mutations in the *pncA* regulatory gene. Repeated attempts have failed to isolate any POA-resistant mutants [23, 30].

Identified *pncA* mutations are largely missense, causing amino acid substitutions, and in some cases nucleotide insertions or deletions, nonsense mutations in the *pncA* structural gene, or in the putative promoter region of *pncA*. It is interesting to note that the *pncA* mutations are highly diverse and scattered along the gene. However, some degree of clustering does appear to occur at three regions of the PncA, 3–17, 61–85 and 132–142 [23, 30]. A recent study that determined the crystal structure of the P. Horikoshii PncA (37% identity with *M. tuberculosis* PncA) has provided some structural basis for the *pncA* mutations in *M. tuberculosis* that cause PZA resistance [23].

The three regions where *pncA* mutations appear to cluster correspond to three of the four loops that contribute most to the scaffold of the active site. Mutations at C138, D8, K96, D49, H51 and H71 modify the active site triad and metal binding site. Residues F13, L19, H57 (position of characteristic mutation of H57D in *M. bovis*), W68, G97, Y103, I113, A134 and H137 line up the active site, and mutations at these positions are also predicted to cause loss of enzyme activity. Mutations at Q10, D12, S104 and T142 are predicted to disrupt the hydrogen-bonding interactions between the side chain and main chain atoms. Loss of PZase activity due to mutations at other sites can be attributed to potential perturbation of the active site, or to disruption of the protein core [23].

These predictions need to be confirmed when the structure of the *M. tuberculosis* PncA has been determined. The high diversity of *pncA* mutations is unique to PZA resistance; mutations in other drug resistance genes do not usually show so much diversity. Although the basis for this is unclear, it is likely that since PZase is a non-essential enzyme there is no selective pressure on the type of mutations that can occur, and thus all types of mutations in the *pncA* gene are tolerated. Consistent with this hypothesis, PZase enzyme is dispensable and PZA-resistant strains without PZase are still fully virululent and capable of causing active transmission and disease [35].

While *pncA* mutation is the main mechanism of PZA resistance, it appears that a small proportion of PZA-resistant strains do not have mutations in the *pncA* gene or its putative promoter region. One group of such strains is PZase-negative suggesting mutations in its promoter region or in an unknown *pncA* regulatory gene that are involved in PZA resistance [23, 35]. Another group of such strains is typically PZase-positive without *pncA* mutations, indicating a possible alternative mechanism of PZA resistance. Such strains are rare, and have low level resistance [30] with an MIC of 200 μg/ml by the BACTEC method [35]. It is of interest to note that a low level PZA-resistant strain DHM444 (MIC > 200 μg/ml), which does not have *pncA* mutation [30], still responded to PZA treatment in a murine model at a dose of 150 mg/kg [41]. Thus, whether such low level resistant strains still respond to PZA treatment is unclear. One has to be careful in categorizing low level resistant strains and rule out the following possibilities: 1) false resistance by repeated MIC testing, 2) contamination with other naturally PZA-resistant non-tuberculous mycobacteria, and 3) mixed populations, which may contain a significant portion of PZA-resistant mutants in a population of susceptible tubercle bacilli [23].

While acquired PZA resistance in *M. tuberculosis* occurs by mutations in the *pncA* gene, the mechanism of natural PZA resistance in other mycobacteria is more complex. *M. bovis* strains including BCG are naturally resistant to PZA [23]. Their resistance is due to a single point mutation (C6G) at nucleotide position 169 in *pncA*, causing amino acid substitution of histidine at position 57 in *M. tuberculosis* to aspartic acid in *M. bovis*. Thus, *M. bovis* can be considered a special case of PZA-resistant *M. tuberculosis*. The natural PZA resistance (MIC = 250 μg/ml) in

M. kansasii is also due to very weak PZase activity, while it has apparently normal nicotinamidase activity[23]. PZA susceptibility of *M. kansasii* is increased upon transformation with the pncA gene from M. tuberculosis or *M. avium* [42]. *M. smegmatis* is highly resistant to PZA (MIC > 2000 μg/ml) at pH 5.5. The natural resistance is due to a very active POA efflux mechanism such that POA is much more efficiently removed from *M. smegmatis* than from the susceptible *M. tuberculosis* [24, 43]. Similarly, *M. avium* has high PZase activity, and its natural resistance to PZA is most likely due to POA efflux mechanism [23]. The active POA efflux mechanism is also likely to be the cause of the intrinsic PZA resistance in many other non-susceptible bacterial species. The efflux pumps involved in POA extrusion in mycobacteria are yet to be identified [23].

In a search for the PZase enzyme that is involved in PZA conversion and resistance in *M. tuberculosis*, Boshoff et al. identified the *M. smegmatis pzaA*, a broad-spectrum amidase that cleaves a wide range of amides, including PZA, nicotinamide and benzamide [23]. PzaA is a 50 kD enzyme with no apparent homology to the 20 kD *M. tuberculosis* nicotinamidase/ PZase. Inactivation of *pzaA* in *M. smegmatis* revealed that the mutant still had residual PZase activity, which led to the identification of *pncA* gene in this organism [23]. Transformation of BCG (a natural PZA-resistant mutant due to a *pncA* mutation) with M. smegmatis pncA or pzaA gene restored sensitivity to PZA. Similarly, transformation of an *M. tuberculosis pncA* mutant with the *pzaA* of *M. smegmatis* conferred susceptibility to both PZA and benzamide [23]. It will be of interest to understand how PZA is activated by PZase and how *pncA* and *pzaA*, with no apparent sequence homology, both have PZase activity. Transformation of PZA-resistant BCG with the *M. smegmatis pncA* or *pzaA* or *M. avium pncA* all conferred a PZA-sensitive phenotype [23].

- **Early bactericidal activity**

Studies regarding the early bactericidal effects[1] (EBA; defined as the fall in colony-forming unit counts/mL sputum/day during the first 2 days of treatment) of pyrazinamide have been carried out in Nairobi and Cape Town [8, 44]. The EBA of PZA is particularly interesting as PZA contributes significantly to sterilization of lesions and thus treatment shortening. The EBA_{0-2} of PZA in Nairobi was 0.044 and 0.004 $\log_{10}$CFU/mL sputum/day, respectivelly in Cape Town. Despite this negligible EBA_{0-2} the Nairobi EBA_{0-14} was 0.110. The individual PZA EBA_{0-14} results appear in a figure and it is interesting that in 4 of the 9 patients evaluated there appears to be a fairly rapid fall in counts, while in the remaining 5 patients the counts increase or remain static, falling only at day 4 or later. The effects of streptomycin and PZA in combination (EBA_{0-2}, 0.118 $\log_{10}$CFU/mL sputum/day and EBA_{2-14}, 0.176 $\log_{10}$CFU/mL sputum/day) appear to be complementary [44].

- **Post-antibiotic effect (PAE)**

Chan et al. have investigated the postantibiotic effects (PAEs) of pyrazinamide and other antimycobacterial agents, tested at their respective peak concentrations in serum alone and in different combinations, against *Mycobacterium tuberculosis* ATCC 27294 (See Table 1). The tests were carried with a radiometric culture system in parallel with the viable count method. Pyrazinamide alone had insignificant PAE of 1.9 h comparable to that of ethambutol. In contrast rifampicin gave the longest PAE (67.8 h) among the drugs used alone. The combinations of first-line drugs, however, including two pyrazinamide-based regimens (HRSZ and HRZM) generally gave PAEs longer than 120 h [45].

- **Persistence of clinical efficacy**

It is very important to stress out that pyrazinamide is recommended only for the initial 2-month phase of short-course regimens and has no place in the continuation regimens as its activity wanes out after 2 months of application [13, 14, 46]. The following hypothesis is compatible with many aspects of drug action during the chemotherapy of pulmonary tuberculosis and the role of pyrazinamide. Three stages are postulated. As shown by the EBA studies, in phase I, which only lasts for 2–3 days, rapidly growing extra-cellular tubercle bacilli in the cavity walls, comprising at least 90% of the bacterial population, are quickly killed by isoniazid (INH) [44]. In phase II, lasting for perhaps 2 months, surviving bacilli are killed more slowly. At this stage there is sufficient acute inflammation to create local acidity necessary for the extra-cellular sterilizing activity of pyrazinamide [23]. At the end of this phase, caseous material has been coughed up, allowing close contact of the bacilli with macrophages, so that many are now intra-cellular. In phase III, lasting about 4 months, semi-dormant, intra-cellular bacilli (persisters) are killed very slowly, mainly by rifampicin. As inflammation has subsided, there is insufficient acidity for pyrazinamide to be bactericidal. At the end of treatment there is still a population of persisters that are held from multiplication by host immunity, but may rarely give rise to a relapse [8].

Table 1. *PAEs against M. tuberculosis H37Rv after a 2-h exposure to single drugs and combinations of drugs, determined by the BACTEC T100 method [45]*

Drug(s)[a]	*MIC (mg/liter)*	*PAE (h)*[b]	*%CV*
Isoniazid	0.06	18.1 (5.7–31.2)	46
Rifampicin	0.5	67.8 (11.9–134.9)	70
Streptomycin	1.0	32.2 (0.7–60.2)	93
Pyrazinamide	≤50c	1.9 (0.0–5.2)	153
Ethambutol	2.0	1.8 (0.0–5.6)	139
HR	–	159.8 (114.4–220.0)	–
HRS	–	160.6 (97.0–275.1)	–
HRM	–	155.3 (89.7–215.6)	–
HRSZ	–	167.9 (72.6–286.9)	–
HRZM	–	125.9 (90.0–176.1)	–

[a]*Abbreviations used for combinations: H, isoniazid; R, rifampicin; S, streptomycin; Z, pyrazinamide; M, ethambutol;*
[b]*Values are means (ranges);*
[c]*The breakpoint concentration used to determine susceptibility to pyrazinamide by the absolute concentration method.*

- **Pharmacokinetic/pharmacodynamic relationships**

Although use of pharmacokinetic-pharmacodynamic (PK-PD) dosing is not a new concept in infectious diseases, little information was available that defined the PK-PD index and target values of antituberculosis drugs and pyrazinamide in particular. Microbial PK-PD concepts relate to the shape of the concentration-time profile that optimizes efficacy, as well as the particular antibiotic exposure that is associated with optimal kill of the pathogen. These concepts and their utility in treatment of many infectious diseases have been summarized recently. In general, antibiotics segregate into one of three patterns: those whose effect is best explained by the area under the concentration-time curve (AUC):minimum inhibitory concentration (MIC) ratio, those whose effect is best explained by peak concentration (C_{max}):MIC ratio, and those whose effect is best optimized by keeping the percentage of time that the concentration is above MIC (T>MIC) high. Drugs whose effect is linked to the C_{max}:MIC ratio should have doses combined together and administered as high intermittent doses. Drugs whose effect is linked to T>MIC should have their doses divided into smaller doses that are administered more frequently, unless the antibiotic already has a long half-life. The effect of the shape of the concentration-time profile on toxicity will also help determine whether this is feasible in patients. The actual drug concentrations that drive the PK-PD indexes are subject to pharmacokinetic variability [47].

Pyrazinamide population pharmacokinetics was recently evaluated in 227 patients with tuberculosis. The results demonstrated that patients were either slow or fast absorbers. These investigators also found that serum clearance and volume of distribution were mostly dependent on weight. Pyrazinamide serum clearance increased by 0.545 L/hour^{-1} for every 10-kg increase in weight over 48 kg. Similarly, pyrazinamide's volume of distribution increased by 4.3 L for every 10-kg weight increase above 48 kg [47]. A recent hollow fiber PK-PD study demonstrated that pyrazinamide's sterilizing effect was best explained by the AUC:MIC ratio, whereas resistance suppression was linked to T>MIC. Monte Carlo simulations revealed that doses higher than the currently recommended 2 g/day would have a better likelihood of achieving the AUC:MIC ratio associated with 90% of maximal effect. However, the safety of higher doses is unclear [48]. Additional clinical studies are needed to explore higher-dose pyrazinamide therapy, as well as dosing based on weight, so that different weight categories will receive different pyrazinamide doses (in mg/kg) [47].

The therapeutic plasma concentrations for pyrazinamide range between 30-75 μg/ml [49]. The therapeutic drug monitoring approaches to optimization of pyrazinamide-based chemotherapy have been summarized by Peloquin. Pyrazinamide and its biological effects can be used as markers of compliance with therapy. Patients who are taking the prescribed pyrazinamide-based regimen, will have elevated uric acid serum concentrations and will have pyrazinamide in the serum throughout a 24-hour dose administration interval. Concentrations will be easily measurable from 2 to 12 hours post-dose, and usually

can be measured at 24 hours. If pyrazinamide is not present, and if the uric acid is not elevated, then it is very unlikely that the patient is taking the medications. The targeted C_{max} is 20 to 40 μg /ml after a 25 mg/kg daily dose and 40 to 60 μg/ml after a 50 mg/kg biweekly dose; a dose increase if the peak is less than 75% of the desired range is generally recommended [50].

Secondary pharmacodynamics of pyrazinamide

Pyrazinamide is a narrow spectrum anti-mycobacterial agent. Besides its extensive use in tuberculosis management this agent has no appreciable secondary pharmacological properties. In a recent study pyrazinamide displayed activity against intracellular *Leishmania major* (MIC = 8.2 μM) and reduced lesion sizes in *L. major*-infected C57BL/6 mice when given orally at 150 mg/kg in fifteen doses [51]. Another study evaluated the in vitro interactions of pyrazinamide and other antituberculous drugs (ATDs) with antifungals against *Coccidioides posadasii*. Eighteen drug combinations, formed by an ATD (isoniazid, pyrazinamide or ethambutol) plus an antifungal (amphotericin B, ketoconazole, itraconazole, fluconazole, voriconazole or caspofungin), were tested using the checkerboard method. All the antimicrobial combinations inhibited *C. posadasii* strains and synergistic interactions were observed for 10 combinations. Antagonism between the tested drugs was not observed. Stronger synergistic interactions were seen in the combinations formed by triazoles plus ethambutol as well as itraconazole plus pyrazinamide. Further studies in animal models are needed to confirm the usefulness of these combinations [52]. These findings are at this stage with no clinical significance.

Pharmacokinetics and biopharmaceutical characteristics of pyrazinamide

Pyrazinamide is administered orally only; there are no commercially available formulations for parenteral application. The literature data relevant to waiver of in vivo bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing pyrazinamide (PZA) as the only active pharmaceutical ingredient (API) have been reviewed. Pyrazinamide is a borderline BCS Class III/I, with linear absorption over a wide dosing range [53, 54]. The risk of bioinequivalence is estimated to be low. Thus a biowaiver-status has been recommended for IR solid oral dosage forma of pyrazinamide [54].

No absolute oral availability of pyrazinamide formulations could be identified in the literature. However, a generic product Pyrazinamide marketed in Germany, has been approved on the basis of a bioequivalence study in 8 volunteers. 500 mg of the generic pyrazinamide product and 500 mg of the reference product were administered in a crossover design. At a 0.05 significance level, AUC and C_{max} of the tested pyrazinamide formulations were found not to be significantly different ($p>0.05$) [54].

Several in vivo and in vitro BE studies have compared pyrazinamide fixed dose combination (FDC) formulations to formulations containing pyrazinamide as the only active pharmaceutical ingredient at the same dose level. The bioequivalence of the product under investigation was confirmed in each case [55-58].

The pharmacokinetic parameters of the drug in healthy subjects and tuberculosis patients with or without HIV-infection are summarized in Table 2.

- **Absorption**

Pyrazinamide is perhaps the most reliably absorbed of the antituberculous drugs. After oral intake pyrazinamide usually reaches C_{max} 1 to 2 hours post-dose and, with its long half-life, is present in the serum for many hours [50, 59]. Following a single 500-mg oral dose in healthy adults, the peak plasma concentrations of pyrazinamide (attained within 2 hours) range 9-12 μg/mL; plasma concentrations of the drug average 7 μg/mL at 8 hours and 2 μg/mL at 24 hours. Plasma concentrations following doses of 20-25 mg/kg reportedly range 30-50 μg/mL [46, 50]. The blood levels of pyrazinoic acid, the major active metabolite of pyrazinamide, generally are greater than those of the parent drug and peak within 4-8 hours after an oral dose of the drug [2, 46]. Concomitant food has been found to not interfere with the absorption of pyrazinamide; there was no difference in C_{max} and only a slight 4-12% decrease in the AUC_{0-8} values [60].

In a single-dose study in healthy fasting males, the extent of absorption (as measured by area under the plasma concentration-time curve) of isoniazid, rifampicin, or pyrazinamide in dosages of 250, 600, or 1500 mg, respectively, was similar whether the drugs were administered individually as capsules (rifampicin) and tablets (isoniazid and pyrazinamide) or as a fixed combination containing isoniazid 50 mg, rifampicin 120 mg, and pyrazinamide 300 mg per tablet [46].

- **Distribution**

Pyrazinamide is widely distributed into body tissues and fluids including the liver, lungs, and CSF [46]. In a limited number of adults with tuberculous meningitis, mean serum and CSF concentrations of pyrazinamide 2 hours after an oral dose of approximately 41 mg/kg were 52 and 39 μg/mL respectively. Within 5 hours after an oral dose, CSF concentrations of pyrazinamide are reported to be approximately equal to concurrent plasma concentrations of the drug [61]. An experimental study demonstrated similar rates of penetration of PZA, INH and RMP into the sciatic nerve [8].

Generally, the extend of protein binding is estimated as low – 10-20%. For instance, plasma protein binding of pyrazinamide (determined by ultrafiltration) in a limited number of healthy men averaged approximately 17% at a pyrazinamide concentration of 20 μg/mL [46]. Nevertheless a recent literature report indicating a plasma protein binding of about 50% without specifying the assay conditions. Woo et al. determined the plasma protein binding of the drug to α1 glycoprotein (15%), albumin and whole plasma (40%) *in vitro* [54].

It is not known if pyrazinamide crosses the placenta. The extent of distribution of the drug into milk is very low [46, 62, 63].

- **Biotransformation**

The metabolism of pyrazinamide consists of two major reaction sequences in humans - deamidation of pyrazinamide to pyrazinoic acid, followed by oxidation to 5-hydroxypyrazinoic acid by xanthine oxidase (XO). The other is the direct oxidation of pyrazinamide to 5-hydroxypyrazinamide by xanthine oxidase and other non-identified enzymes [28, 64, 65].

The limiting factor has been found to be the activity of a microsomal deamidase (pyrazinoic acid formation from pyrazinamide and 5-hydroxy-pyrazinoic acid formation from 5-hydroxy-pyrazinamide) [64, 65]. In contrast, oxidation by xanthine oxidase has been found to occur very rapidly (5-hydroxy-pyrazinamide formation and pyrazinoic acid catabolism to 5-hydroxy-pyrazinoic acid) [65]. The existence of the other metabolite, produced by the direct action of xanthine oxidase on PZA has been postulated as early as in 1972 by Weiner and Tinker and eventually identified as 5-hydroxy pyrazinamide (5-OH-PZA) [65]. The 5-OH-PZA is also metabolized on by an deamidase. Finally, 5-OH-PA is formed at the junction of two metabolic pathways. Additionally Yamamoto et al. have shown that a second oxidizing enzyme operates jointly with XO in the biotransformation of PZA to 5-OH-PZA [64, 65]. Through a minor route, PA combines with glycine to form pyrazinuric acid (PUA) [66]. These considerations suggest the biotransformation scheme depicted in figure 3.

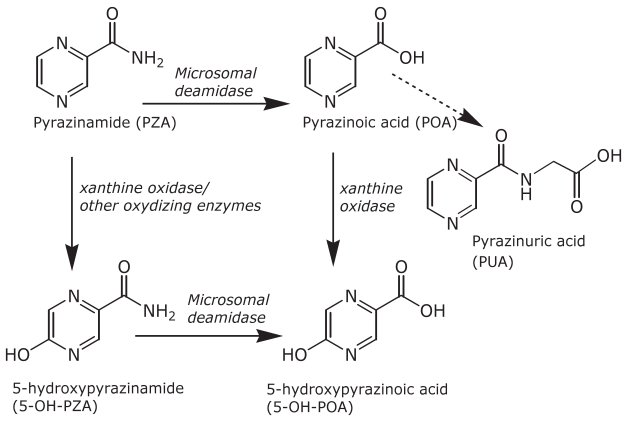

Fig. 3. *Biotransformation of pyrazinamide (PZA) with formation of its active metabolite POA and hydroxylated excretory metabolites with no pharmacological activity. Formation of pyrazinuric acid is a minor pathway.*

- **Elimination**

The plasma half-life of pyrazinamide is 9-10 hours in patients with normal renal and hepatic function. The elimination half-life of the drug may be prolonged in patients with impaired renal or hepatic function [46, 50]. Pyrazinamide is hydroxylated to 5-hydroxypyrazinoic acid, the major excretory product. Within 24 hours, approximately 70% of an oral dose of pyrazinamide is excreted in urine, mainly by glomerular filtration. About 4-14% of the dose is excreted as unchanged drug; the remainder is excreted as metabolites [65, 67].

Clinical pharmacology and efficacy studies

Pyrazinamide is indicated in combination with other antituberculosis agents for the treatment of all forms of tuberculosis (pulmonary/extrapulmonary) caused by sensitive strains of *Mycobacterium tuberculosis*. The drug has to be always applied in combination with other antituberculous drugs.

- **Classical studies and establishment of short-course regimens**

The first relevant clinical study to this end was carried out in East Africa and Zambia in association with the British Medical Research Council. In this study, four 6-month daily regimens with streptomycin, isoniazid and rifampicin (SHR); streptomycin,

Table 2. *Comparative clinical pharmacokinetics of pyrazinamide – synopsis of representative pharmacokinetic studies in healthy subjects [65, 67], tuberculosis patients [68] and tuberculosis patients co-infected with HIV-1 [69].*

Subjects	*Comp.**	*PZA Dose (mg/kg)*	t_{max} *(hours)*	C_{max} *(μg/mL)*	$t_{1/2\alpha}$ *(min)*	$t_{1/2\beta}$ *(min)*	*AUC (μg/ml).h*	Vd_{ss} *(L/kg)*	*CL (ml/min/kg)*	**Urine *(% dose)***
Healthy volunteers[a]	PZA	38.7 ± 1.1	0.8 ± 0.2	50.1 ± 2.7	3.2 ± 0.4	23 ± 5.4	760.4 ± 14.8	1.65 ± 0.35	55.4 ± 1.96	1.6 ± 0.1
Healthy volunteers[a]	POA	38.7 ± 1.1	4.7 ± 0.8	66.6 ± 9.0	2.7	12.3 ± 1.9	1209.5 ± 128.0	–	–	36.5 ± 4.5
Healthy volunteers[b]	PZA	27 ± 3.4	1.0	38.7 ± 5.9	1.6 ± 0.8	9.6 ± 1.8	520 ± 101	0.71 ± 0.07	61.0 ± 11.0	3.2 ± 1.6
Healthy volunteers[b]	POA	27 ± 3.4	4.9 ± 1.7	4.5 ± 0.9	–	11.8 ± 1.6	100 ± 21	–	–	33.2 ± 4.5
TB patients[c]	PZA	35.7 (25.2–47.3)	2.0 (1.1-2.5)	52.7 (46.0-61.4)	–	5.9 (4.9-7.1)	499.7 (406.2–632.3)	–	–	–
HIV-1/TB patients[d]	PZA	1.5-2 g/day	2.31 ± 0.98	42.2 ± 11.3	–	9.05 ± 4.1	290.76 ± 86.56	–	–	–
HIV-1/TB patients[d]	PZA	2-2.5 g/intermittent	2.73 ± 1.51	52.8 ± 17.6	–	9.04 ± 3.9	366.64 ± 107.25	–	–	–

**In studies with healthy subjects the pharmacokinetic parameters have been reported for both the parent drug and its active metabolite; abbr: PZA=Pyrazinamide; POA=pyrazinoic acid; References: [a][67]; [b][65]; [c] [68]; [d] [69].*

isoniazid and pyrazinamide (SHZ); streptomycin, isoniazid and thiacetazone (SHT) and streptomycin plus isoniazid (SH) were compared with a standard 18-month streptomycin, isoniazide and thiacetazone (STH for 2 months and then HT for 16 months) regimen. The study demonstrated the efficacy of the 6 months regimens and the superiority of the rifampicin or pyrazinamide containing regimens, as the 6 month SHT and SH regimen had the highest relapse rates at 30 month follow up (22–32%), with the SHR regimen producing the lowest one (2%), that was not significantly different from that obtained with the 18 month standard treatment (3%) [8]. Thereafter Fox and Mitchison, with the Medical Research Council, developed a pharmacokinetic rationale from clinical and laboratory studies, whereby isoniazid and rifampicin were considered as complete bactericidal drugs, being capable of killing bacteria in all environments, while streptomycin and pyrazinamide were ‘‘half’’ bactericidal drugs, being the former active in the more alkaline extracellular milieu and the latter active on the more acidic intracellular environment. Interestingly, streptomycin and pyrazinamide were perfectly complementary drug which, together, made a very powerful bactericidal combination [8, 9]. Following this reasoning, the East African SHR short-course regimen contained two and a half bactericidal drugs and the almost equally powerful SHZ regimen contained two full killers. In contrast, the SHT and SH less powerful 6-month regimens contained only one and a half killer. The East African trial so suggested that at least two full bactericidal drugs were required to successfully cut treatment duration from 12–18 months down to a 6-month short course [8, 9].

A number of randomized trials followed suit, in East Africa, Madras, Hong Kong, Singapore and Algeria [8, 9, 70]. In a second East African study, the 6-month SHR daily regimen, the best arm of the previous study, was compared with a similar regimen without streptomycin (HR daily for 6 months) and with two regimens where rifampicin was given only during the first 2 of the 6-month course, with the ad-

dition of pyrazinamide (SHRZ daily for 2 months plus TH daily for 4 months and SHRZ daily for 2 months plus SHZ twice per week for 4 months) in order to explore the efficacy of limiting rifampicin administration to the first 2 months of treatment. Interestingly, the study showed that 2SHRZ, the regimen with three full bactericidal drugs, produced better bacteriological results at 2 months, thereby suggesting that the use of such a combination in the initiation phase of treatment could lead to complete elimination of all susceptible organisms. After a subsequent East African study of 4- month, short-course therapy using the same initiation scheme (2SHRZ) followed by three different, 2- month continuation schemes using either isoniazid, isoniazid and rifampicin, isoniazid and pyrazinamide or the three drugs, was interrupted for an unacceptably high number of relapses, a fifth study was undertaken with which the role of pyrazinamide in short-course therapy was to be established. This study tested the same 2-month initiation therapy (2SHRZ) followed by four different continuation regimens of 4 months i.e., 4HR, 4HZ, 4H or 6H. The study showed that, after the common initiation phase with 2SHRZ, the 4HR continuation regimen was significantly better than all others, with only 2% relapses at 24 months after the completion of treatment. The 4HZ continuation regimen, with 8% relapses, was no better than the 4H with 10%. This, together with the finding that the 6H regimen resulted in only 3% relapses, suggested that the contribution of pyrazinamide was essential during the 2-month initiation phase of the short-course combination regimens, but not thereafter [9].

Similar results were obtained in a study of the Research Committee of the British Thoracic Association that found that streptomycin or ethambutol, with pyrazinamide added to isoniazid and rifampicin during the 2-month initiation phase (2SHRZ or 2EHRZ) followed by 4 HR equalled the standard 9HR with negligible relapse rates and by the Algerian working group's study which found that a similar 6-month regimen had better results than a 12-month standard treatment [8]. The Singapore Tuberculosis Service/ British Medical Research Council study also confirmed the efficacy of short-course regimens using streptomycin, isoniazid, rifampicin and pyrazinamide in the initiation phase with isoniazid plus rifampicin in the continuation phase: In addition, it confirmed that a 4 months course was ineffective. In this study the 2SHRZ, 4HRZ short-course regimen produced no relapses at 24 months after treatment cessation and the 2SHRZ, 4HR regimen 2.5%, while with the shorter regimens (2SHRZ, 2HRZ or 2HR) 8% and 11.8% relapses were observed, respectively [8].

The high cost of rifampicin and pyrazinamide, have forced tuberculosis services throughout the developing world to establish cost-effective short-course intermittent regimens that could be given to outpatients under supervision. Two important studies were carried out at the Hong Kong Chest Service with the collaboration of the British Medical Research Council to determine the best regimen for fully supervised outpatient treatment. In the first study, a fully intermittent 6-month regimen of streptomycin, isoniazid, rifampicin and pyrazinamide three times weekly for 4 months ($4S_3H_3R_3Z_3$), followed by streptomycin, isoniazid and pyrazinamide ($2S_3H_3Z_3$), resulted in a bacteriological relapse rate of 6% at 18 months in patients with fully drug-sensitive bacteria, with an even higher relapse rate with isoniazid-resistant strains. The second study, evaluated four three-times weekly intermittent short-course regimens containing isoniazid and rifampicin together with (i) streptomycin, pyrazinamide, and ethambutol ($6SH_3R_3Z_3E_3$), (ii) streptomycin and pyrazinamide ($6S_3H_3R_3Z_3$), (iii) streptomycin and ethambutol ($6S_3H_3R_3E_3$), or (iv) pyrazinamide and ethambutol ($6H_3R_3Z_3E_3$). As a control, a daily regimen of isoniazid, rifampicin, pyrazinamide and ethambutol ($6H_3R_3Z_3E$) was used. At 24 months after the completion of treatment, the bacteriological relapse rate was 1% for the three-weekly regimens with isoniazid and rifampicin plus streptomycin and pyrazinamide and for the daily regimen versus an almost equally low 2% for the regimen with pyrazinamide but no streptomycin. Strikingly, relapse the rate of the regimen not containing pyrazinamide was up to 8% [8]. Very importantly, the 5-year follow up of the same study showed that among patients with drug-susceptible pre-treatment strains there were 3.4% relapses in the pyrazinamide- containing regimens, compared to 10.3% in the non-pyrazinamide series. In addition, the pyrazinamide containing regimens were also very effective on isoniazid and streptomycin resistant strains as less than 4% relapses were observed [70].

This study was important for it confirmed the sterilizing role of pyrazinamide, in the association with isoniazid and rifampicin, in the induction phase of short-course chemotherapy. Further, it determined that the role of streptomycin was limited to the prevention of drug resistance, or further drug resistance in isoniazid resistant strains. Collectively, the data indicated that an intermittent regimen based upon isoniazid and rifampicin, with pyrazinamide and streptomycin giv-

en for a shorter period would save costs and increase manageability of domiciliary treatments while reducing side effects at no bacteriological cost [9, 71].

Another important study was carried out by the Singapore Tuberculosis Service, also in collaboration with the British Medical Research Council, with which it was established the role of pyrazinamide and streptomycin in short course, intermittent regimens. This study examined three treatment protocols with streptomycin, isoniazid, rifampicin, and pyrazinamide for two months (2SHRZ), or for 1- month (1SHRZ), or for 2 months without streptomycin (2HRZ), followed by three-times-weekly isoniazid and rifampicin (H_3R_3) up to 6 months for all patients. The data showed that, in patients with drug sensitive strains, relapse rates were less than 1% and that, importantly, they were only 3% in the isoniazid and streptomycin resistant. A similar trial was carried out at the Tuberculosis Research Centre in Madras, India. In this study, three short-course chemotherapy regimens were evaluated in patients with newly diagnosed, bacteriologically positive pulmonary tuberculosis: patients were allocated to either rifampicin, streptomycin, isoniazid and pyrazinamide daily for 2 months, followed by streptomycin, isoniazid and pyrazinamide twice weekly for a total of 5 ($2SHRZ/3SHZ_2$) or 7 months ($2SHRZ/5SHZ_2$) or to the same 7 month regimen but without rifampicin ($2SHZ/ 5SHZ_2$). The result at 5 years indicated a slightly higher percentage of bacteriological relapses, compared to the Hong Kong and the Singapore studies, with 7% for the 5-month rifampicin regimen, 4% for the 7-month rifampicin regimen and 7% for the 7 month no-rifampicin regimen [8]. In addition, a 10% relapse rate was observed among isoniazid or streptomycin resistant patients. Eventually, the 1980s studies brought to the recommendations of the International Union Against Tuberculosis and Lung Disease (IUATLD), indicating isoniazid, rifampicin, and pyrazinamide for the 2-month induction phase followed by isoniazid and rifampicin for the 4-month continuation phase of the 6-month daily regimen. The same recommendations also indicated adding ethambutol or streptomycin in the initial phase of the three times weekly regimen [9].

Thus, with these trial-validated, intermittent short-course chemotherapy regimens, directly observed therapy (DOT) became feasible in the tuberculosis services of high prevalence countries. With the DOT strategy was developed by the IUATLD together with national tuberculosis programmes, the WHO recommended directly supervised treatment consisting of a 2-month daily regimen of isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin ($2H_7R_7Z_7E_7$ or $2SHRZE_7$) followed by 4-month continuation with either daily or intermittent isoniazid plus rifampicin ($4HR_7$ or $4HR_3$), for patients with newly diagnosed smear positive disease [9]. As a second option, an entirely intermittent 6-month treatment was recommended ($2HRZE_3/4HR_3$) . An 8-month regimen with the same induction phase ($2HRZE_7$ or $2HRZE_3$) and isoniazid plus thioacetazone in the continuation phase (6HT) was recommended for use in countries with limited financial resources. However, with the advent of the Human Immunodeficiency Virus (HIV) epidemic, being HIV-infected patients at increased risk of severe, in some cases fatal, dermatological toxic effects from thioacetazone, the WHO recommendations were modified and in the 8-month regimen above, ethambutol (6HE) was substituted for thioacetazone in the 6-month daily isoniazid plus thiacetazone [9].

On the basis of these extensive clinical trials with short course regimens these have been implemented worldwide as the golden standard for management of tuberculosis. Moreover this conventional short-course therapy has remained unchanged for decades. The most frequently recommended and effective combination is isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months. This regimen is very effective for treatment of patients with tuberculosis, including patients with HIV infection [13, 14].

- **Clinical rationale for antituberculous chemotherapy**

The current treatment for initial or never treated TB (Table 3) is governed by bacteriological bases and numerous controlled therapeutic trials conducted over 30 years by various scientific societies and international health organizations [13, 14, 16, 46, 71]. It is considered that a therapeutic regime is eligible to be used when it cures over 95% of patients and causes less than 5% serious intolerance requiring modification. The best treatment will be made up of drugs with a high bactericidal and sterilizing power, with a low number of recurrences, few side effects, well accepted and tolerated. This will facilitate compliance and prevent failures and the development of resistance [16].

Treatment regimens that meet all favorable requirements and have been universally recommended are for 6 months: two months with R, M, Z and E, followed by four months with R and H (2RHZE/4RH) [14]. When it is identified that isolation is sensitive to these

drugs E may be withdrawn. In exceptional low bacillary cases, treatment with three drugs (RHZ) can be used in the initial stage, but omission of ethambutol is not recommended by WHO [16].

Children often have a good tolerance to drugs. Their treatment needs to be the same as in adults, with weight adjusted doses. The regimen should also not be altered during pregnancy or lactation, because there is significant body of evidence that HRZE components are not teratogenic [46, 72].

b) Special populations at risk

In liver disease patients treatment should be attempted with a standard regimen, with close clinical and analytical monitoring of liver function. In cases of advanced liver disease, one of the three drugs with hepatotoxic potential (H, R, Z) will be removed. This will preferably be Z, while trying to keep R (Table 3). Only in cases of acute hepatitis or terminal stages of chronic liver disease will a drug treatment with no potential liver toxicity be opted for. This will include a quinolone, an injectable (aminoglycosides or capreomycin) and E or cycloserine. The duration of treatment must conform to the regime used [16, 46].

There is no need to modify the standard treatment in chronic renal failure. Only for patients with creatinine clearance below 30 mL/min or patients on hemodialysis is treatment recommended to be 3 times a week (always after hemodialysis), while maintaining the same daily dose intensity. Generally speaking, measuring the drug plasma levels should be considered. The H, E and, above all, Z are all drugs that are dialysed, so for patients undergoing hemodialysis it is recommended that drug administration be done after this [14, 16].

Evidence on the duration of treatment in the extrapulmonary locations is not sufficiently clear, and the recommendations of various guidelines are not unanimous. However, it is recommended to use the same regimen as in the pulmonary TB and prolong the duration of treatment in some situations, especially in tuberculous meningitis and tuberculous spondylitis with neurologic involvement (**Table 3**) [16, 73].

It is currently recommended that treatment of TB in HIV-infected patients needing antiretroviral treatment should be done with the standard HRZE regimen, provided that two nucleoside analogues are administered in combination with efavirenz, nevirapine or enfuvirtide (full-dose administration of ritonavir is not currently a practical option). If these combinations cannot be used, R can be replaced by rifabutin, whose interaction with protease inhibitors is lower and which can be co-administered with all boosted protease inhibitors, with the appropriate dose adjustment for rifabutin [16].

- **Bacteriological rationale – scientific basis of short course chemotherapy, role of individual agents**

Antituberculous drugs are theoretically described by their action in three areas: prevention of drug resistance rapidity of improvement prevention of relapse. The efficacy of the first-line anti-TB drugs in these actions is summarized in **Table 4**, in which a strong effect is reported as 3+ and no effect as 0. One of the goals of effective chemotherapy is to prevent acquired drug resistance. Acquired resistance occurs during therapy when resistance to one or more drugs develops in organisms that were originally susceptible to the drug(s). Drug resistance is prevented by

Table 3. *Initial tuberculosis treatments regimens [16]*

Condition	*Treatment*
Pulmonary and extrapulmonary	2HRZE/4HR */**
Alternative initial treatments	2HRZS/4HR 2HRE/7HR
Special situations (gout, severe chronic liver disease)	2HRE/7HR
Meningitis, tuberculomas	2HRZE/10HR
Silicosis, spondylitis TB with neurological affectation	2HRZE/7HR

*E: ethambutol; H: isoniazid; R: rifampicin; S: Streptomycin; TB: tuberculosis;Z: pyrazinamide.; * When the antibiogram shows sensitivity to all drugs, E may be withdrawn.; ** If it is not possible to use E, it can be replaced by S (2HRZS/4HR).*

using drugs that eliminate all mycobacterial populations and thus do not allow the emergence of resistant organisms. The best protection against acquired drug resistance is the use of at least two bactericidal drugs to which the organisms are sensitive [14, 74].

Bactericidal activity is the ability of a drug to kill rapidly replicating bacteria. In therapeutic doses, the bactericidal first-line drugs are isoniazid, rifampicin and pyrazinamide [74]. The bactericidal activity of a drug is dependent upon factors such as oxygen tension. In extracellular areas of high oxygen tension, the mycobacteria grow rapidly and reach high numbers. In these populations, the drugs with the most prominent bactericidal activity are isoniazid and rifampicin followed by high-dose ethambutol. Pyrazinamide has little activity in this population and therefore generally will not protect against the development of resistance (**Table 4**) [74]. Conversely, in areas of low oxygen tension, such as inside cells (acid pH) and in areas of fibrosis (neutral pH), the mycobacteria grow more slowly. In intracellular populations, the drug with the least bactericidal activity is isoniazid, followed in order of increasing activity by by rifampicin and pyrazinamide. Low-dose ethambutol is bacteriostatic. In areas of fibrosis, where organisms are thought to grow intermittently, rifampicin is the only drug that has bactericidal activity [74].

Sterilizing activity is the ability of a drug to kill the last viable, often semidormant, bacterium inside the host. The best measure of sterilizing activity is the proportion of patients with negative cultures after 2 months of treatment and the proportion who relapse within 2 years following completion of treatment [75]. Rifampicin and pyrazinamide are the most effective sterilizing drugs (pyrazinamide has optimal activity at low pH, so it acts on a sub-set or semidormant organisms in slightly acidic environments); isoniazid is intermediate, and ethambutol is the least effective (Table 4) [2, 14, 23, 74].

Table 4. *Activity of first line antituberculous agents [74].*

Drug	*Resistance Effect**	*Bactericidal effect*		*Sterilizing Effect*
		Rapid Replication Rate	Slow Replication Rate	
INH	++	++	+	++
RMP	++	++	+	+++
EMB	+/-	+/-	+/-	0
PZA	0	0	++	+++

Table 5. *Host and mycobacterial biomarkers of bactericidal and sterilizing activity [75]*

Surrogate markers:	*Informative value regarding:*	
	Bactericidal activity	Sterilizing activity
• **Host markers**		
Clinical	No	No
Radiological	No	No
Immunological	No	No
• **Mycobacterial markers**		
Sputum smear	Yes	No
Early bactericidal activity (EBA) studies	Yes	No
2-month sputum culture conversion	No	Yes

- **Surrogate markers/clinical endpoints for assessment of efficacy of ant-TB trials**

The available surrogate markers and their value for evaluation of bactericidal and sterilizing activity of drugs are summarized in Table 5.

Place in therapy

- **Pulmonary tuberculosis**

The currently recommended combined chemotherapy regimens are in large part, based on evidence from clinical trials. There are several recommended regimens (proposed by WHO and other international and national authorities; See **Table 6**) for treating patients with tuberculosis caused by drug-susceptible organisms [14, 46, 71, 76]. Although these regimens are broadly applicable, there are modifications that should be made under specified circumstances, described subsequently. Each regimen has an initial phase of 2 months followed by a choice of several options for the continuation phase of either 4 or 7 months. Because of the relatively high proportion of adult patients with tuberculosis caused by organisms that are resistant to isoniazid, four drugs are necessary in the initial phase for the 6-month regimen to be maximally effective. Thus, in most circumstances, the treatment regimen for all adults with previously untreated tuberculosis should consist of a 2-month initial phase of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB), recommended by WHO, ATC/CDC/IDSA, and other National Authorities, worldwide. If (when) drug susceptibility test results are

known and the organisms are fully susceptible, EMB need not be included [46], but WHO no longer recommends omission of ethambutol during the intensive phase of treatment for patients with non-cavitary, smear-negative pulmonary TB or extrapulmonary disease who are known to be HIV-negative [71, 72].

The WHO revised international guidelines for the treatment of tuberculosis in 2010, specifically responding to the growing evidence base and escalating problem of drug-resistant disease worldwide [13]. Earlier guidelines emphasized the use of two main standardized treatment regimens, one for new (previously untreated) cases and one for patients with sputum smear-positive disease who had previously received treatment (re-treatment regimen). The drug combinations used in these two regimens differed only by the addition of a single drug—a far from optimum situation with regard to prevention of emergence of drug resistance [72].

The key recommendations from the renewed guidelines are as follows [13]: i) First-line 6 month treatment regimen. New patients with pulmonary tuberculosis should receive a regimen containing 6 months of rifampicin: 2HRZE/4HR (high grade of evidence); ii) Alternative first-line continuation phase; In populations with known or suspected high levels of isoniazid resistance, new tuberculosis patients can receive HRE as treatment in the continuation phase as an acceptable alternative to HR (weak evidence) Wherever feasible, the optimum dose frequency for new patients with pulmonary tuberculosis is daily throughout the course (high grade of evidence). Alternatively, new patients with pulmonary tuberculosis can receive a daily intensive phase and then three-times weekly continuation phase provided that each dose is directly observed: 2HRZE/4(HR)3 (high/moderate grade of evidence). Alternatively, new patients with pulmonary tuberculosis can receive three-times weekly dosing throughout treatment, provided that every dose is directly observed and the patient is not living with HIV or living in a high HIV prevalence setting: $2(HRZE)_3/4(HR)_3$ (high/moderate grade of evidence); iii) Re-treatment regimens and detection and treatment of drug resistance. Ideally, DST is done for all patients at the start of treatment, so that the most appropriate treatment for each individual can be established. Specimens for culture and DST should be obtained from all previously treated patients with tuberculosis at or before the start of treatment; DST should be done at least for isoniazid and rifampicin. In settings where rapid molecular DST is available, the results should guide the choice of regimen. In settings where rapid molecular-based DST results are not routinely available to guide the management of individual patients, empirical treatment should be started as follows. Tuberculosis patients whose treatment has failed or other patient groups with high likelihood of MDR tuberculosis should be started on an empirical MDR regimen. Tuberculosis patients returning after defaulting or relapsing from their first treatment course can receive the re-treatment regimen containing first-line drugs: HRZES/1HRZE/5HRE if country-specific data show low or medium levels of MDR in these patients or if such data are not available . In settings where DST results are not yet routinely available to guide the management of individual patients, the empirical regimens will continue throughout the course of treatment.

There are slight differences in the recommendations of the US Centers for Disease Control and Prevention and the American Thoracic Society (CDC/ATS) [14], and the UK Joint Tuberculosis Committee of the British Thoracic Society (BTS) [76] and WHO and IUATLD [13]. WHO, IUATLD, and the BTS do not recommend twice-weekly dosing, although this is one recommendation in the USA. The UK and US guidelines recommend use of the same 6-month rifampicin-based regimens for both smear-positive and smear-negative pulmonary tuberculosis [15]. The differences however concern only the continuation (PZA-free) phase of treatment.

Thus, according to current clinical guidelines pyrazinamide is an inevitable component of the initial intensive phase of tuberculosis treatment. If PZA cannot be included in the initial phase of treatment, or if the isolate is resistant to PZA alone (an exceptionally unusual circumstance), the ATC/CDC guidelines state that initial phase should consist of INH, RIF, and EMB given daily for 2 months (Regimen 4). Examples of circumstances in which PZA may be withheld include severe liver disease, gout, and initial resistance to the drug (a rare occasion) [14, 46].

- **Tuberculosis in HIV-infected patients**

Recommendations for the treatment of tuberculosis in HIV-infected adults are, with a few exceptions, the same as those for HIV-uninfected adults [13, 14, 76, 78]. Patients with *HIV* related tuberculosis should be given the standard four drug regimen unless multidrug resistant tuberculosis is suspected. It has been recommended that antituberculosis treatment should be continued for longer in patients with HIV infection than in those without, bur there is little evidence

Table 6. *Drug regimens for culture-positive pulmonary tuberculosis, caused by drug susceptible strains of M. tuberculosis as proposed by WHO [13], ATC/CDC/IDSA [14], BTC [76] and DZK (Deutshes Zentralkomitee zur Bekämpfung der Tuberkulose) [77].*

Initial Phase			*Continuation Phase*					
Regimen	*Drugs*[1]	*Interval and minimal duration*	*Regimen*	*Drugs*	*Interval and minimal duration*	*Total minimal duration*	*Level of evidence rating*[2]	
							HIV(-)	HIV(+)
World Health Organization								
1	INH+RIF+PZA+EMB	Daily dosing recommended; 2 months	1	INH+RIF	Daily dosing recommended; 4 months	6 months	N.S.	N.S.
American Thoracic Society, CDC, and Infectious Diseases Society of America								
1	INH+RIF+PZA+EMB	Seven days per week for 56 doses (8 wk) or 5 d/week for 40 doses (8 wk)[4]	1a	INH+RIF	Seven days per week for 126 doses (18 wk) or 5 d/wk for 90 doses (18 wk) [4]	182-130 (26 wk)	A (I)	A (11)
			1b	INH+RIF	Twice weekly for 36 doses (18 wk)	92-76 (26 wk)	A (I)	A (II)[3]
			1c[5]	INH+RPT	Once weekly for 18 doses (18 wk)	74-58 (26 wk)	B (I)	E (I)
2	INH+RIF+PZA+EMB	Seven days per week for 14 days (2 wk) then twice weekly for 12 doses (6 wk) or 5 d/wk for 10 doses (2 wk)4; then twice weekly for 12 doses (6 wk)	2a	INH+RIF	Twice weekly for 36 doses (18 wk)	62-58 (26 wk)	A(II)	B(II)[3]
			2b[5]	INH+RPT	Once weekly for 18 doses (18 wk)	44-40 (26 wk)	B(I)	E(I)
3	INH+RIF+PZA+EMB	Three times weekly for 24 days (8 wk)	3a	INH+RIF	Three times weekly for 54 doses (18 wk)	78 (26 wk)	B(II)	B(II)
4	INH+RIF+PZA+EMB	Seven days per week for 56 doses (8 wk) or 5 d/wk for 40 doses (8 wk)[4]	4a	INH+RIF	Seven days per week for 217 doses (31 wk) or 5 d/wk for 155 doses (31 wk)[4]	273-195 (39 wk)	C(I)	C(II)
			4b	INH+RIF	Twice weekly for 62 doses (31 wk)	118-102 (39 wk)	C(I)	C(II)
British Thoracic Society								
1	INH+RIF+PZA+EMB	Daily dosing recommended; 2 months	1	INH+RIF	Daily or intermittent dosing recommended; 4 months	6 months	N.S.	N.S.
Deutshes Zentralkomitee zur Bekampfung der Tuberkulose								
1	INH+RIF+PZA+EMB	Daily dosing recommended; 2 months	1	INH+RIF	Daily dosing recommended; 4 months	6 months	N.S.	N.S.

[1]Deffinition of abbreviations: EMB = Ethambutol; INH = isoniazid; PZA = pyrazinamide: RIF = rifampicin; RPT = rifapentin;

[2]Deffinition of evidence rating: A = preferred; B = acceptable alternative; C = one, when A and B cannot be given: E = should never be given; levels of evidence rating: I = randomized clinical trial; II = data from clinical trials that were not randomized or were conducted in other populations; III =expert opinion.

[3] Not recommended for HIV-infected patients CD4 cell counts < 100 cells/µl;*

[4]Five-day-a-week administration is always given by DOT. Rating for 5 day/week regimens is A(III);

[5]Regimens 1c and 2b should be used only in HIV-negative patients who have negative sputum smear at the time of completion of 2 months of therapy and who do not have cavitation on the initial chest radiograph. For patients started on this regimen and found to have a positive culture from the 2-month specimen, treatment should be extended an extra 3 months;

to support this for those with fully sensitive organisms. If cultures remain positive after three months, compliance and drug absorption need detailed assessments. Tuberculosis treatment should be under the management of an appropriately qualified specialist.

- **Tuberculosis in children**

Because of the high risk of disseminated tuberculosis in infants and children younger than 4 years of age, treatment should be started as soon as the diagnosis of tuberculosis is suspected. In general, the regimens recommended for adults are also the regimens of choice for infants, children, and adolescents with tuberculosis, with the exception that ethambutol is not used routinely in children [13, 79].

Most studies of treatment in children have used 6 months of INH and RIF supplemented during the first 2 months with PZA. This three-drug combination has a success rate of greater than 95% and an adverse drug reaction rate of less than 2%. Most treatment studies of intermittent dosing in children have used daily drug administration for the first 2 weeks to 2 months. DOT should always be used in treating children [14].

Because there is a lower bacillary burden in childhood-type tuberculosis there is less concern with the development of acquired drug resistance. However, children and adolescents may develop "adult-type" tuberculosis with upper lobe infiltration, cavitation, and sputum production. In such situations an initial phase of four drugs should be given until susceptibility is proven. When clinical or epidemiologic circumstances suggest an increased probability of INH resistance, EMB can be used safely at a dose of 15-20 mg/kg per day, even in children too young for routine eye testing. Streptomycin, kanamycin, or amikacin also can be used as the fourth drug, when necessary [14].

- **Extrapulmonary tuberculosis**

The basic principles that underlie the treatment of pulmonary tuberculosis also apply to extrapulmonary forms of the disease. Although relatively few studies have examined treatment of extrapulmonary tuberculosis, increasing evidence suggests that 6- to 9-month regimens that include INH ,PZA and RIF are effective. Thus, a 6-month course of therapy is recommended for treating tuberculosis involving any site with the exception of the meninges, for which a 9- 12-month regimen is recommended. Prolongation of therapy also should be considered for patients with tuberculosis in any site that is slow to respond. The addition of corticosteroids is recommended for patients with tuberculous pericarditis and tuberculous meningitis [10, 14, 16].

- **Culture-negative pulmonary tuberculosis and radiographic evidence of prior pulmonary tuberculosis**

Failure to isolate *M. tuberculosis* from persons suspected of having pulmonary tuberculosis on the basis of clinical features and chest radiographic examination does not exclude a diagnosis of active tuberculosis. If either clinical or radiographic improvement is noted and no other etiology is identified, treatment should be continued for active tuberculosis. Treatment regimens in this circumstance include one of the standard 6-month chemotherapy regimens or INH, RIF, PZA, and EMB for 2 months followed by INH and RIF for an additional 2 months (4 months total). However, HIV-infected patients with culture-negative pulmonary tuberculosis should be treated for a minimum of 6 months. Persons with a positive tuberculin skin test who have radiographic evidence of prior tuberculosis (e.g., upper lobe fibronodular infiltrations) but who have not received adequate therapy are at increased risk for the subsequent development of tuberculosis. Unless previous radiographs are available showing that the abnormality is stable, it is recommended that sputum examination (using sputum induction if necessary) be performed to assess the possibility of active tuberculosis being present. Also, if the patient has symptoms of tuberculosis related to an extrapulmonary site, an appropriate evaluation should be undertaken. Once active tuberculosis has been excluded (i.e., by negative cultures and a stable chest radiograph), the treatment regimens are those used for latent tuberculosis infection: INH for 9 months, RIF (with or without INH) for 4 months, or RIF and PZA for 2 months (for patients who are unlikely to complete a longer course and who can be monitored closely) [14].

- **Treatment of tuberculosis in patients with pre-existing comorbidities**

INH, RIF, and PZA all can cause hepatitis that may result in additional liver damage in patients with preexisting liver disease. However, because of the effectiveness of these drugs (particularly INH and RIF), they should be used if at all possible, even in the presence of preexisting liver disease [16]. If serum AST is more than three times normal before the initiation of treatment (and the abnormalities are not thought to be caused by tuberculosis), several treat-

ment options exist. One option is to treat with RIF, EMB, and PZA for 6 months, avoiding INH. A second option is to treat with INH and RIF for 9 months, supplemented by EMB until INH and RIF susceptibility are demonstrated, thereby avoiding PZA. For patients with severe liver disease a regimen with only one hepatotoxic agent, generally RIF plus EMB, could be given for 12 months, preferably with another agent, such as a fluoroquinolone, for the first 2 months; however, there are no data to support this recommendation. In all patients with preexisting liver disease, frequent clinical and laboratory monitoring should be performed to detect drug-induced hepatic injury [16, 46].

In renal failure there is usually no need to modify the standard HRZE treatment. Only for patients with creatinine clearance below 30mL/min or patients on hemodialysis is treatment recommended to be 3 times a week (always after hemodialysis), while maintaining the same daily dose regimen. Generally speaking, measuring the drug plasma levels should be considered. The H, E and, above all, Z are all drugs that are dialysed, so for patients undergoing hemodialysis it is recommended that drug administration be done after this [14, 16].

- **Treatment of tuberculosis in pregnancy and breastfeeding**

Because of the risk of tuberculosis to the fetus, treatment of tuberculosis in pregnant women should be initiated whenever the probability of maternal disease is moderate to high. The initial treatment regimen should consist of INH, RIF, EMB and PZA, which albeit cross the placenta, do not appear to have teratogenic effects. PZA in particular can be used safely during pregnancy and is recommended by the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) [13]. If PZA is not included in the initial treatment regimen, the minimum duration of therapy is 9 months [46]. Breastfeeding should not be discouraged for women being treated with the first-line antituberculosis agents because the small concentrations of these drugs in breast milk do not produce toxicity in the nursing newborn. Conversely, drugs in breast milk should not be considered to serve as effective treatment for tuberculosis or for latent tuberculosis infection in a nursing infant [13, 80].

- **Multi-drug resistant and extensively drug resistant tuberculosis**

The term drug-resistant TB encompasses three categories of resistance. Mono resistance — where the *M. tuberculosis* is resistant to one of the members of first-line antitubercular drugs and multi-drug resistance which confer the M. tuberculosis resistant to more than one anti-tuberculosis drug. MDR-TB denotes *M. tuberculosis* resistant to two main drugs of the first-line regimen—isoniazid and rifampicin [12, 17, 81]. This has led to the increased use of second-line anti-tuberculosis drugs for treatment failure cases (fluoroquinolones; kanamycin, amikacin, capreomycin, aminosalicylic acid, cycloserine, prothionamide and thiacetazone), which in turn gave rise to extremely drug-resistant tuberculosis (XDR-TB), where the MDR-TB organism was resistant to fluoroquinolone and one of the injectable agents [81, 82]. There are several basic rules for the management of patients with MDR or XDR tuberculosis. The recommended regimen is a combination of at least four drugs to which the *M tuberculosis* isolate is likely to be susceptible, although more than four might be necessary. Drugs are chosen with a stepwise selection process through five groups of antituberculosis drugs (summarized in **Table 7**), on the basis of efficacy, safety, and cost. An WHO guideline for the management of drug-resistant tuberculosis is summarized in **Table 8**. The duration of the intensive phase of treatment (when an injectable drug is given) should be at least 6 months (or 4 months after culture conversion). The continuation phase (without the injectable drug) should last until 18 months after culture conversion. Surgery could be considered under specific conditions (e.g., when less than four second-line drugs are available, when the patient has localized lesions, and when the patient has enough respiratory reserve to tolerate surgery). These principles are the same for the treatment of both MDR and XDR tuberculosis, but the treatment of patients with XDR tuberculosis is much more difficult, because more patients need group five drugs and surgery [12, 17, 21].

Dosing Recommendations

The dosing guidelines issued by WHO, ATC/CDC/IDSA and other authorities are summarized in (**Table 9**). Thus WHO guidelines recommend daily dosage of 25 mg/kg [13]. The American Thoracic Society (ATS), US Centers for Disease Control and Prevention (CDC), and the Infectious Diseases Society of America (IDSA) recommend that when pyrazinamide is used in a daily regimen, adults and children 15 years of age or older weighing 40-55 kg should receive a dosage of 1 g, those weighing 56-75 kg should receive 1.5 g, and those weighing 76-90 kg should receive 2 g. The maximum dosage

Table 7. *WHO Classification of antituberculous drugs (ATD) for treatment of drug-resistant disease [12].*

Group one: first-line oral antituberculosis drugs (use all possible drugs)
Isoniazid 5 mg/kg
Rifampicin 10 mg/kg
Ethambutol 15–25 mg/kg
Pyrazinamide 30 mg/kg
Group two: fluoroquinolones (use only one; they share genetic targets)
Ofloxacin 15 mg/kg
Levofloxacin 15 mg/kg
Moxifloxacin 7•5–10 mg/kg
Group three: injectable ATD (use only one; they share similar genetic targets)
Streptomycin 15 mg/kg
Kanamycin 15 mg/kg
Amikacin 15 mg/kg
Capreomycin 15 mg/kg
Group four: less-effective second-line ATD (use all possible drugs if necessary)
Ethionamide/Prothionamide 15 mg/kg
Cycloserine/Terizidone 15 mg/kg
P-aminosalicylic acid (acid salt) 150 mg/kg
Group five: less-effective ATD or drugs with sparse clinical data (use all necessary drugs if there are less than four from the other groups)
Clofazimine 100 mg
Amoxicillin with clavulanate 875/125 mg every 12 h
Linezolid 600 mg
Imipenem 500–1000 mg every 6 h
Clarithromycin 500 mg/12 h
High-dose isoniazid 10–15 mg/kg
Thioacetazone 150 mg

Table 8. *WHO Guidelines for the management of drug-resistant tuberculosis according to resistance patterns [12].*

Resistance pattern	*Regimen*
Pan-susceptible	INH+RMP+EMB+PZA
INH (+/- SM)	RMP - EMB - PZA (six to nine months)
Polyresistant but not MDR	Continue the empiric second-line regimen. Patient may require a combination of first and second-line drugs.
INH/RMP INH/RMP/EMB	PZA - SM - Lfx - ETH - CS - PAS
INH/RMP/EMB/PZA	SM - Lfx - ETH - CS - PAS
INH/RMP/SM INH/RMP/EMB/SM INH/RMP/EMB/PZA/SM	KM - Lfx - ETH - CS - PAS
Resistance to any second-line drug	Continue the empiric second-line regimen. Consult with a specialist.

Abbr.: INH – isoniazid; RMP – rifampicin; EMB – ethambutol; PZA – pyrazinamide; SM – streptomycin; Lfx – levofloxacin; ETH – ethionamide; CS – cycloserine; KM – kanamycin; PAS – aminosalicylic acid.

recommended by the U.S. authorities for a daily regimen is 2 g [14].

If a 3-times weekly regimen is used WHO recommends a 35 mg/kg as a single dose per week [13]. The ATC/CDC/IDSA guidelines for 3 times weekly intermittent dosing are as follows: adults weighing 40-55 kg should receive a pyrazinamide dosage of 1.5 g, those weighing 56-75 kg should receive 2.5 g, and those weighing 76-90 kg should receive 3 g; the maximum dosage for this regimen is 3 g [14].

The twice weekly regimens are not recommended by WHO [13] and the German DZK [77], but are considered effective by the U.S. and U.K. authorities [14, 76]. If pyrazinamide is used in an intermittent regimen that involves twice-weekly administration of antituberculosis agents, the ATS, CDC, and IDSA recommend that adults and children 15 years of age or older weighing 40-55 kg should receive a pyrazinamide dosage of 2 g, those weighing 56-75 kg should receive 3 g, and those weighing 76-90 kg should receive 4 g; the maximum pyrazinamide dosage for this regimen is 4 g regardless of weight [14].

Regarding the pediatric population the ATS, CDC, and IDSA recommend a standard dosage of 15-30 mg/kg (up to 2 g) daily [14], and the American Academy of Pediatrics (AAP) recommends a dosage of 20-40 mg/kg daily (up to 2 g) [46]. If an intermittent treatment regimen is used, these experts recommend a pediatric dosage of 50 mg/kg (up to 2 g) twice weekly; [46, 73] the latter regimen is not recommended by WHO. The revised WHO guidelines recommend a higher dose intensity for children i.e. 35 mg/kg (20-40 mg) daily; the previous guidelines of WHO have recommended a daily dosage of 25 mg/kg (range 20-30 mg/kg) and 35 mg/kg (range 30-40 mg/kg) in intermittent three times weekly administration [79]. The German DZK recommends a dose of 30 mg/kg daily or 50 mg/kg thrice weekly [77].

- **Clinical safety**

It is noteworthy that despite the immense clinical experience with pyrazinamide many of the adverse effects reported in literature have been noted infrequently, sometimes only once without verification. Also as far as combined chemotherapy is concerned toxicity ascribed to a certain drug could be actually caused by another making the causal relationships troublesome (especially in cas of hepatotoxicity, which is associated with all first-line agents except streptomycin). For this reason in the following discussion emphasis is placed on those adverse effects that are well documented and occur more than rarely. Significant rare reaction, however as well as ones that have not been verified are also quoted.

The adverse effects profile of pyrazinamide is summarized in **Table 10**.

The most frequent adverse effect of pyrazinamide is hepatotoxicity [83-85]. Transient increases in serum aminotransferase (transaminase) concentrations, jaundice, hepatitis, and a syndrome of fever, anorexia, malaise, liver tenderness, hepatomegaly, and splenomegaly have been reported in patients receiving pyrazinamide [46]. Rarely, acute yellow atrophy of the liver and death have occurred. Although there is some controversy regarding this issue [86] hepatotoxicity appears to be dose related and may occur at any time during therapy [46]. With a dosage of 3 g daily, hepatotoxicity occurs in approximately 15% of patients, and jaundice occurs in 2-3%. Studies in adults with active tuberculosis indicate that the incidence of drug-induced adverse hepatic effects in patients who receive 25-35 mg/kg of pyrazinamide daily in the initial phase (i.e., first 2 months) of isoniazid and rifampicin therapy is the same as that in patients who receive isoniazid and rifampicin therapy without pyrazinamide [2, 46]. Intermittent application of the drug is shown to decrease the incidence and severity of hepatotoxicity [87].

Severe liver injuries, including some fatalities, have been reported in patients receiving a 2-month daily regimen of pyrazinamide and rifampicin and for the treatment of latent tuberculosis infection. Between October 2000 and June 2003, the US Centers for Disease Control and Prevention (CDC) received a total of 48 reports of severe hepatic injury (i.e., hospitalization or death) in patients with latent tuberculosis infection receiving a pyrazinamide and rifampicin regimen; there were 11 fatalities [88-90]. In many fatal cases, onset of hepatic injury occurred during the second month of the 2-month regimen. Some pa-

Table 10. *Adverse drug reactions associated with pyrazinamide [76, 77]*

Common reactions	Uncommon reactions	Rare and very rare reactions
Anorexia	Hepatitis	Gout
Nausea	Vomiting	Photosensitization
Flushing	Arthralgia	Sideroblastic anemia
	Hyperuricemia (asymptomatic)	Thrombocytopenia
	Cutaneous hypersensitivity	

Table 9. *Dosage of pyrazinamide§ in daily or intermittent dosing regimens as proposed by representative international and national guidelines for programmed management of tuberculosis.*

Authority	Daily dosing			Intermittent dosing					
				Twice weekly dosing			Thrice weekly dosing		
	Children	Adults		Children	Adults		Children	Adults	
		Weight (kg)	Dose		Weight (kg)	Dose		Weight (kg)	Dose
WHO[1]	35 mg/kg (30-40 mg/kg)	–	25 mg/kg (20-30 mg/kg)	N.R.	–	N.R.	N.R.	–	35 mg/kg (30-40 mg/kg)
WHO[2]	25 mg/kg (20-30 mg/kg)	–	–	N.R.	–	N.R.	35 mg/kg (30-40 mg/kg)	–	–
ATC/CDC/IDSA[3]	15-30 mg/kg max. 2.0 g	40-55	1.0 g (18.2-25 mg/kg)	50 mg/kg max. 4.0 g	40-55	2.0 g (36.4-50.0 mg/kg)	–	40-55	1.5 g (27.3-37.5)
		56-75	1.5 g (20.0-26.8 mg/kg)		56-75	3.0 g (40.0-53.6 mg/kg)		56-75	2.5 g (33.3-44.6)
		76-90	2.0 g (22.2-26.3 mg/kg)		76-90	4.0 g (44.4-52.6 mg/kg)		76-90	3.0 g (33.3-39.5)
NYCTCP[4]	20-30 mg/kg	< 50	1.5 g	50 mg/kg (40-60 mg/kg)	< 50	2.5 g	35 mg/kg (30-40 mg/kg)	< 50	2.0 g
		51-74	2.0 g		51-74	3.0 g		51-74	2.5 g
		≥ 75	2.5 g		≥ 75	3.5 g		≥ 75	3.0 g
BTC[5]	35 mg/kg	< 50	1.5 g	75 mg/kg	< 50	3.0 g	50 mg/kg	< 50	2.0 g
		≥ 50	2.0 g		≥ 50	3.5 g		≥ 50	2.5 g
DZK[6]	30 mg/kg	–	25 mg/kg (20-30 mg/kg)	N.R.	–	50 mg/kg (40-60 mg/kg)	50 mg/kg	–	35 mg/kg (30-40 mg/kg)

§ Recommendations of dosing for this drug vary widely. WHO and CDC/ATS recommend dosing of pyrazinamide in adults on a weight basis, but dosing based on weight categories as recommended by BTS and by tuberculosis programmes is more useful in practice. Adults weighing <45 kg can have pediatric doses. The doses given here are based on the: 1Current WHO Guidelines [13, 79], 2Preceding 2003 WHO Guidelines for Children [79]; 3ATC/CDC/IDSA [14]; 4NYCTCP (New York City Tuberculosis Control Program) [15]; 5BTC [76]; and 6DZK (Deutshes Zentralkomitee zur Bekämpfung der Tuberkulose) [77].

tients who died were receiving the pyrazinamide and rifampicin regimen because they previously experienced isoniazid-associated hepatitis and some had risk factors for chronic liver disease (e.g., serologic evidence of previous hepatitis A or B infection, idiopathic nonalcoholic steatotic hepatitis, alcohol or parenteral drug abuse, concomitant use of other drugs associated with idiosyncratic hepatic injury) [46]. Although data are limited, there is no evidence to date that HIV-infected individuals receiving this regimen are at any increased risk for severe hepatitis. There is evidence that the rate of severe liver injury and death related to the use of pyrazinamide and rifampicin are higher than the rates reported for isoniazid-associated liver injury in the treatment of latent tuberculosis infection. Based on these reports, pyrazinamide and rifampicin regimens should be used for the treatment of *latent* tuberculosis only when the potential benefits outweigh the risk of liver injury and death [14, 88, 91].

Pyrazinamide inhibits renal excretion of urates, frequently resulting in hyperuricemia. This effect is usually asymptomatic, but acute gout has occurred in some patients. Nongouty polyarthralgia, which

appears to be related to increased serum uric acid concentrations, reportedly occurs in up to 40% of patients receiving pyrazinamide. Uricosuric agents administered concurrently may reduce pyrazinamide-induced hyperuricemia; however, if hyperuricemia is severe or is accompanied by acute gouty arthritis, pyrazinamide should be discontinued and not resumed [14].

Mild arthralgia and myalgia have been reported frequently with pyrazinamide therapy [46]. Maculopapular rash, fever, acne, porphyria, dysuria, interstitial nephritis, and photosensitivity with reddish-brown discoloration of exposed skin have been reported rarely with pyrazinamide therapy [14, 92, 93]. Hypersensitivity reactions, including rash, urticaria and pruritus, also have been reported [94]. GI disturbances including nausea, vomiting, and anorexia also have occurred in patients receiving the drug [46, 83].

Thrombocytopenia and sideroblastic anemia with erythroid hyperplasia, vacuolation of erythrocytes, and increased serum iron concentrations have occurred rarely with the drug. Adverse effects on blood clotting mechanisms also have been reported rarely [46, 83, 95].

Pyrazinamide is contraindicated in patients with severe hepatic damage, acute gout, porphyria and in patients with known hypersensitivity to the drug [13, 83, 96]. Pyrazinamide should be used only when close observation of the patient is possible. Serum AST (SGOT), ALT (SGPT), and uric acid concentrations should be determined prior to and every 2-4 weeks during pyrazinamide therapy. Patients should be advised about the initial symptoms of hepatitis (e.g., fatigue, nausea, abdominal pain, anorexia) and the importance of discontinuing therapy and contacting their clinician should such symptoms develop. Pyrazinamide should be used with caution in patients with renal failure or a history of gout. The drug should also be used with caution in diabetics because the management of diabetes mellitus may become more difficult during pyrazinamide therapy [14, 46].

Because of reports of liver injury (including fatalities) when regimens containing pyrazinamide and rifampicin were used in patients with latent tuberculosis infection, these regimens generally should not be offered to HIV-infected or HIV-negative patients. Regimens containing pyrazinamide and rifampicin should be considered for the treatment of latent tuberculosis infection only when the potential benefits outweigh the risk of liver injury and death; when the preferred or alternative regimens (i.e., 9-month isoniazid regimens, 6-month isoniazid regimens, 4-month daily rifampicin regimen) are judged unlikely to be completed; and when oversight can be provided by a clinician with expertise in the treatment of latent tuberculosis. A pyrazinamide-rifampicin regimen should never be offered to patients who are currently taking other drugs associated with liver injury, patients who drink excessive amounts of alcohol (even if alcohol is discontinued during treatment), or patients with underlying liver disease or a history of isoniazid-associated liver injury. An expert in the treatment of latent tuberculosis should be consulted before a regimen of pyrazinamide and rifampicin is offered. Individuals being considered for a 2-month pyrazinamide-rifampicin regimen should be informed of potential hepatotoxicity, questioned regarding prior liver disease or history of adverse effects during treatment with isoniazid or other drugs, and cautioned against the concurrent use of potentially hepatotoxic drugs (including OTC drugs such as paracetamol)[14, 46].

If a decision is made to use a pyrazinamide-rifampicin, serum AST and bilirubin concentrations should be measured at baseline and at 2, 4, 6, and 8 weeks and patients should be reassessed in person by a health-care provider at 2, 4, 6 and 8 weeks for adherence, tolerance, and adverse effects. To facilitate these periodic assessments, it is advisable that no more than a 2-week supply of the drugs should be dispensed at a time. Patients should be instructed to discontinue the pyrazinamide-rifampicin regimen immediately and seek clinical consultation if abdominal pain, emesis, jaundice, or other manifestations of hepatitis develop. The drugs should be discontinued and not reinitiated in asymptomatic patients who have an AST concentration exceeding 5 times the upper limit of normal, in patients with symptoms of hepatitis who have an AST concentration exceeding the upper limit of normal, and in patients who have serum bilirubin concentrations exceeding the upper limit of normal (regardless of the presence or absence of symptoms) [46].

Pyrazinamide appears to be well tolerated in children [79, 97]; the American Academy of Pediatrics (AAP) states that in dosages of 30 mg/kg daily or less, the drug is well-tolerated and seldom hepatotoxic [46]. Clinical studies of pyrazinamide did not include sufficient numbers of patients 65 years of age or older to determine whether they respond differently than younger adults. Other reported clinical experience has not identified differences in responses between geriatric and younger adults. In general,

dosage of pyrazinamide for geriatric patients should be selected carefully starting at the low end of the dosage range because these individuals frequently have decreased hepatic and/or renal function and concomitant disease and drug therapy [46].

Pyrazinamide was not mutagenic in the Ames microbial (*Salmonella*) mutagen test, but it did induce chromosomal aberrations in human lymphocyte cell cultures. Pyrazinamide reportedly was not carcinogenic in rats or male mice (as determined in lifetime bioassays) when administered in daily doses of approximately 10-40 times the maximum recommended human dose; results in female mice could not be determined because of insufficient numbers of surviving mice in the control group [46, 98].

Animal reproduction studies have not been performed with pyrazinamide, and it is also not known whether the drug can cause fetal harm when administered to pregnant women or whether it can affect reproduction capacity [80]. Pyrazinamide should be used during pregnancy only when clearly needed. The American Thoracic Society (ATS), US Centers for Disease Control and Prevention (CDC), and Infectious Diseases Society of America (IDSA) state that routine use of pyrazinamide in pregnant women is not recommended [14]. Nevertheless WHO considers first–line antituberculous drugs as non-teratogenic and warrants their use in pregnant women since the benefits of pyrazinamide may outweigh the unquantified risk in some pregnant women with tuberculosis, especially when resistance to other drugs but susceptibility to pyrazinamide is likely [13]. Because pyrazinamide is distributed into milk in very small amounts, there is generally no need to discontinue nursing or the drug [62].

Pyrazinamide is contraindicated in patients with severe hepatic damage, acute gout, porphyria, and in individuals with known hypersensitivity to the drug and the pharmaceutical excipients [14, 46]. Pyrazinamide should be used only when close observation of the patient is possible. Serum AST (SGOT), ALT (SGPT), and uric acid concentrations should be determined prior to and every 2-4 weeks during pyrazinamide therapy [46]. Patients should be advised about the initial symptoms of hepatitis (e.g., fatigue, nausea, abdominal pain, anorexia) and the importance of discontinuing therapy and contacting their clinician should such symptoms develop [46]. Pyrazinamide should be used with caution in patients with renal failure or a history of gout. The drug should also be used with caution in diabetics because the management of diabetes mellitus may become more difficult during pyrazinamide therapy [14, 46].

Drug Interactions

In contrast to most of the clinically useful antituberculous drugs and especially rifampicin and isoniazid, pyrazinamide is not an inducer of CYP-enzymes, has no toxic metabolites and is generally devoid of significant drug-to-drug interactions [77, 99].

There is a complex pharmacokinetic and pharmacodynamic two-way interaction between pyrazinamide and probenecid – decreased efficacy of the uricosuric agent and hampered elimination of the antimycobacterial drug and its metabolites. The appropriate dose of probenecid in co-treatment has not been established. Therefore, concomitant use should be avoided [100, 101].

Co-administration with allopurinol has been found to increase the AUC of the active metabolite of pyrazinamide, pyrazinoic acid, by approximately 70%. Vice versa pyrazinamide has been found to significantly lower the renal clearance of oxypurinol, the active metabolite of allopurinol. Since pyrazinoic acid inhibits urate elimination, allopurinol is not effective in treating pyrazinamide-associated hyperuricemia [100, 101].

Combined application poses risk of decreased effect of colchicine, as the active metabolite of pyrazinamide increases the serum levels of uric acid [100, 101].

Combined treatment with cyclosporin has been associated with decreased serum concentration of the immune suppresant [100].

Co-treatment with pyrazinamide and either ofloxacin or levofloxacin has been associated with a high levels of adverse events (e.g. hepatic, gastrointestinal, musculoskeletal) leading to discontinuation of therapy [102, 103]. On these grounds co-treatment is not recommended. Nevertheless, when deemed necessary, careful safety monitoring should be applied.

Co-treatment of pyrazinamide with other hepatotoxic drugs (e.g. rifampicin, isoniazid, ethionamide, ketoconazole): may potentiate hepatotoxicity [100]. Due to potential for liver toxicity alcohol intake should be limited during pyrazinamide treatment [1]. Moreover concurrent application of pyrazinamide with other drugs associated with iatrogenic hyperuricemia and/or gout and especially thiazides has to be avoided [104].

An issue of special concern is combining drug therapies for dual infection by *Mycobacterium tuberculosis* and HIV-1; it is made complex by high

pill burdens, shared drug toxicities, drug-drug and drug-disease interactions, immune reconstitution inflammatory syndrome, co-morbid diseases requiring drug therapy and so on [104]. Some of the shared drug toxicities of pyrazinamide, other anti-TB drugs and drugs commonly used in HIV-infected individuals, which pose risk for possible interactions are summarized in **Table 11**. Pyrazinamide co-administration with the antiretroviral drug nevirapine is associated with an increased risk of hepatotoxicity and dermatological reactions [105].

Pyrazinamide may interfere with urinary ketone determination tests that utilize the sodium nitroprusside method such as Ketostix® and Acetest®, and thereby cause difficulty in monitoring diabetic patients if these tests are used [101].

Pyrazinamide and its metabolites inhibit the secretion of uric acid in the kidneys. It also is possible that they inhibit the secretion of other drugs, although this has not been carefully studied. It is possible that the much higher than expected intolerance of pyrazinamide and levofloxacin as an empirical treatment for latent MDR-TB is due to such an interaction [50, 102].

Safety in Pregnancy and Lactation

The drug is classified as FDA pregnancy risk **Category C.**

The available data indicate that pyrazinamide as well as the other first line agents (except streptomycin), namely, isoniazid, rifampicin, and ethambutol are not teratogenic, and WHO recommends their use in women who are pregnant [15]. In the USA, pyrazinamide is not recommended for use during pregnancy except when alternative drugs are not available or are less effective [14]. Active tuberculosis in pregnancy must be treated, because untreated disease will harm the mother and the unborn child more than standard drugs would. [15, 106].

Limited information indicates that maternal pyrazinamide therapy produces low levels in milk and would not usually be expected to cause any adverse effects in breastfed infants, especially if the infant is older than 2 months [62]. One woman who was lactating, but not breastfeeding (time postpartum not stated) was given a single oral dose of 1 gram of pyrazinamide. A peak milk level of 1.5 mg/L occurred 3 hours after the dose. The half-life of the drug

Table 11. *Shared drug toxicities of pyrazinamide, other anti-TB drugs and antiretrovirals in the HIV/TB patient [104]*

Side-effect	*Anti-retrovirals*	*First-line TB drugs*	*MDR-TB drugs*	*Other drugs commonly used in HIV*	*Other HIV disease*
Nausea & vomiting	AZT, ddI, PI: IDV,amprenavir (other PIs)	**Z**, Rfm, H, E	Et, Of, Ci, (other FQ), PAS, Clf, Lin	TMP-SMX, Amphotericin B	OI, IRIS
Gastritis	–	E, **Z**	Et	–	–
Transaminitis	NVP, PI, EFZ	**Z**, R, H	Of, Ci (other FQ), Et, Cy, Te, PAS, Clr	TMP-SMX, Azoles	OI
Nephrotoxicity	TDF (including Fanconi syndrome)	S, R (interstitial nephritis & GN), **Z**+H also rarely cause interstitial nephritis	Ka, Am, Cpr, FQ – rarely cause IN, PAS causes crystalluria	Amphotericin B, TMP-SMX (interstitial nephritis)	HIVAN
Arthralgias and gout	–	E, **Z**, H, R	Of, Ci, (other FQ, PAS)	Thiazide (gout)	HIV itself
Skin rash	NNRTIs, ABC, PI	**Z**, R, H, S, E	FQ, Clf, PAS, Clr, Cpr, Et, Cy, Te	TMP-SMX	Folliculitis & asteatosis
Leucopenia, anaemia	AZT, 3TC	R, H, **Z** (sideroblastic anaemia), RH**Z**E rarely cause thrombocytopenia	FQ, strep, Cy (megaloblastic), PAS, Lin, Cpr, Clr (last 4 also thrombocytopenia)	Ganciclovir, TMP-SMX, Amphotericin B	HIV itself

in milk was estimated to be 9 hours Using these data, a fully breastfed infant would receive a maximum of about 1.4% of the maternal weight-adjusted dosage [62, 63, 80]. Pyrazinamide was used as part of multi-drug regimens to treat 2 pregnant women with multi-drug-resistant tuberculosis throughout pregnancy and postpartum. Their two infants were breastfed (extent and duration not stated). At age 1.25 and 5.1 years, the children were developing normally [62].

WHO, the Centers for Disease Control and Prevention and other authorities state that breastfeeding woman who has TB should receive a full course of TB treatment, because timely and properly applied chemotherapy is the best way to prevent transmission of tubercle bacilli to the baby; breastfeeding should not be discouraged in women taking pyrazinamide [13, 14, 80]. Exclusively breastfed infants should be monitored for rare cases of jaundice, hepatitis and arthralgia if this drug is used during lactation. The amount of pyrazinamide in milk is insufficient to treat tuberculosis in the breastfed infant [62].

Conclusions

Pyrazinamide is an essential antituberculous drug used as an integral component of the contemporary short-course therapy for tuberculosis. Pyrazinamide is similar to isoniazid in its narrow spectrum of clinically-relevant antibacterial activity, which essentially includes only *M. tuberculosis*. The drug is bactericidal to slowly metabolizing organisms located within the acidic environment of the phagocyte or caseous granuloma; it is active only at a pH of *ca*. 6.0. Pyrazinamide is considered a prodrug and is converted by the tubercle bacillus to the active form pyrazinoic acid. The clinical recognition that the inclusion of pyrazinamide (PZA) allowed a reduction in the duration required to achieve predictable cures has revolutionized tuberculosis chemotherapy. PZA has been found to accelerate the time required to achieve culture negativity and to yield ca. 95% cure rates in 6 months when combined with isoniazide and rifampicin. Thus the short-course regimens have been implemented worldwide as the golden standard for management of tuberculosis and remained unchanged for decades. The most frequently recommended and effective combination is isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months. This regimen is very effective for treatment of patients with tuberculosis and is recommended for all new cases of tuberculosis by WHO, ATC/CDC/IDSA, and other authorities worldwide. Besides pyrazinamide has an important clinical role for the treatment of HIV-infected individuals with tuberculosis co-infection and since the 1990's for the treatment of multi-drug resistant and extensively drug resistant tuberculosis.

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