PARA-AMINOSALICYLIC ACID – BIOPHARMACEUTICAL, PHARMACOLOGICAL, AND CLINICAL FEATURES AND RESURGENCE AS AN ANTITUBERCULOUS AGENT

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Summary: Para-aminosalicylic acid (INN aminosalicylic acid; PAS) is a bacteriostatic chemotherapeutic agent used in the therapy of all forms of tuberculosis, both pulmonary and extrapulmonary, caused by sensitive strains of the mycobacteria resistant to other antituberculotics or if the patient is intolerant towards other drugs. Since its clinical introduction in the late 1940s aminosalicylic acid (PAS) has been a mainstay in the treatment of TB into the 1960s. Along with isoniazid and streptomycin, it was a 'first-line' agent for tuberculosis. However, it was plagued by poor gastro-intestinal tolerance and rare but severe allergic reactions. Ethambutol was later shown to be approximately equivalent to PAS in potency, and generally better tolerated than PAS when ethambutol was used at dosages of 25 mg/kg/day or less. Therefore, PAS was replaced by ethambutol as a primary TB drug. However, because of the relative lack of use of PAS over the past 3 decades, most isolates of TB remain susceptible to it. Thus, PAS has experienced a renaissance in the management of patients with multi-drug resistant tuberculosis.

Key words: Aminosalicylic acid, Antituberculous agents, MDR-TB, XDR-TB

Introduction

In 1943 the Swedish chemist Jörgen Lehmann (1898-1989) addressed a letter to the managers of the pharmaceutical company Ferrosan, suggesting the elaboration of the para-amino derivative of salicylic acid as a potential antimycobaterial drug. He based his theory on available literature data regarding the avidity of tubercle bacilli to metabolize salicylic acid and hypothesized that by somewhat modifying the structure of the lead compound the new molecule would be taken up by the bacteria and would inhibit bacterial respiration and metabolism. Para-aminosalicylic acid (PAS) was produced and first tested as an oral therapy at the end of 1944. The first patient treated with PAS made a dramatic recovery; eventually PAS was shown to hinder the development of resistance to streptomycin in M. tuberculosis [1, 2].

Despite the dramatic efficacy established by the pioneering trials, initially the work of Lehmann found only small attendance [1, 2]. In 1950 in the British Medical Council the reduction of resistance of mycobacteria in case of combined application of PAS and streptomycin was published. Streptomycin cured in monotherapy approx. 20% of tuberculous meningitis, in combination with PAS and early initiation of treatment the cure rate improved up to 90%. The combination of both drugs reduced the selection of resistant strains tremendously [3]. In the 1950s of the past century isoniazid has been implemented into the treatment regimen [4]. The combination of isoniazid, PAS and streptomycin had been used until 1963 until ethambutol entered clinical testing. Ethambutol was shown to be approximately equivalent to PAS in potency, and generally better tolerated than PAS when ethambutol was used at dosages of 25 mg/kg/day or less. Therefore, PAS was replaced by ethambutol as a primary antituberculous drug. Eventually the introduction of other antituberculotics such as rifampicin and pyrazinamide further reduced the clinical utility of PAS [5].

Because of the relative lack of use of PAS over the past 3 decades, most isolates of tuberculosis remain susceptible to it. Thus, due to a more and more frequent occurrence of strains of Mycobacterium tuberculosis showing resistance to one or more of the first-line medicines PAS has experienced a resurgence in the management of patients with multi-drug-resistant tuberculosis (MDR-TB) which is evident from the survey of available retrospective trials, case reports, government and institutional guidelines [6-10]. Since

then and nowadays PAS is used primarily as a second-line drug to treat MDR-tuberculosis worldwide
At present, due to the low degree of initial resistance and on the basis of the clinical support (from retrospective cohort studies) PAS is considered a second-line agent for the chemotherapy of tuberculous disease originating from polyresistant strains. It has been implemented in the current WHO Guidelines for the programmed management of drug-resistant tuberculosis [8, 11] and in the respective recommendations of the representative authorities such as the German central committee for the fight against tuberculosis (DZK) [12], the British Thoracic Society (BTS) [13] the American Thoracic Society (ATS) [14] the Canadian Lung Association [15] and so on.

I. Biopharmaceutical and Pharmacokinetic Properties

For the treatment of tuberculosis aminosalicylic acid is administered orally (as a free acid or as sodium, potassium or calcium salts) or parenterally (as sodium aminosalicylate). More recently different dosage forms for pulmonary delivery have been described, as well [16-18]. The parenteral form of PAS is generally much better tolerated than the conventional oral forms, eben in excessive doses [19]. The oral dosage forms of aminosalicylic acid such as granules, tablets, oral solution etc. are available worldwide. The parenteral forms are available and widely used in Europe. The physicochemical and biopharmaceutical particulars of PAS and its sodium salt are summarized in table 1.

- **Absorption**

PAS and its salts are readily and completely absorbed from the gastrointestinal tract, whereby the bioavailability depends on the chemical species (free acid, salt, ester etc.) and on the dosage form (tablets, solution, granules) [20-25]. Representative absorption characteristics and pharmacokinetic parameters of different dosage forms of PAS are summarized in Table 2.

Table 1. Physicochemical and pharmaceutical characteristics of aminosalicylic acid and sodium aminosalicylate.

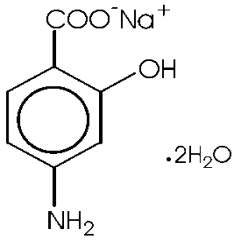
INN:	Aminosalicylic acid, Sodium aminosalicylate
Chemical name:	4-amino-2-hydroxybenzoic acid sodium salt dihydrate
CAS number:	Sodium PAS.2H_2O: 6018-19-5 PAS: 65-49-6
Molecular formula:	Sodium PAS.2H_2O: $C_7H_6NO_3Na.\ 2H_20$ PAS: $C_7H_7NO_3$
Structural formula:	
Molecular mass:	PAS: 153.14 Sodium PAS.2H_20: 211.2
Pharmacopoeias:	Ph. Eur.; BP;
Melting point (PAS):	Sodium PAS.2H_2O: 150.5°C PAS: 250-254°C
pKa (PAS)	3.25
Polarity (PAS)	Log P = 1.012
Properties	Sodium PAS.2H_2O: White or almost white crystalline powder or crystals, slightly hygroscopic. Freely soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride PAS: One gram dissolves in about 500 ml water, in 21ml alcohol. Slightly soluble in ether. Practically insoluble in benzene. Soluble in dilute nitric acid or dilute sodium hydroxide

Table 2. Summary of absorption characteristics and some pharmacokinetic parameters of different aminosalicylic acid preparations

PAS products	Dose (g)	C_{max} (μg/ml)	t_{max} (h)	$AUC_{0-\infty}$ (μg·h/ml)
PAS (p.o.)*	4.0	49.98	3.54	209.07
Na^+ PAS (p.o.)*	2.8	155.44	0.83	313.22
Na^+ PAS (i.v.)**	18.5	524[a]; 422[b]	—	—
Ca^{++} PAS (p.o.)*	2.6	139.51	1.02	326.22
K^+PAS (p.o.)*	2.6	121.09	1.10	313.22
PAS resin(p.o.)*	4.0	78.0	2.1	—
Paser® (p.o.)[c]*	4.0	20.23	7.95	107.92

[a]Means from two female patients; [b]Means from three male patients; [c]A proprietary oral formulation of PAS (sustained release granules) employed in the USA; References: *[23];**[25]

A single oral dose of 4 g of the free aminosalicylic acid produces maximal concentrations in plasma of 70-80 μg/ml [23]. The time to reach maximal plasma levels after oral ingestion is 1 to 2 hours. The sodium salt is absorbed even more rapidly [23]. Application of higher doses leads to higher plasma levels but the oral route generally fails to yield plasma levels exceeding 100 μg/ml; moreover single oral doses exceeding 12-18 g are practically intolerable due to the severe gastric irritation and the associated sequelae, produced by aminosalicylic acid [19, 26-28]. In contrast, when sodium aminosalicylate is applied as slow infusion patients are able to tolerate as mush as three fold higher doses than the maximally tolerated oral doses. Using intravenous infusion the initial plasma levels of aminosalicylic acid of 400 μg/ml or higher could be attained [25, 26].

- **Distribution**

Aminosalicylic acid is distributed throughout the total body water and reaches high concentrations in pleural fluid and caseous tissue [29]. The volume of distribution is estimated as approximately 16 L (~ 24% of the body weight) [26, 30]. The distribution half-life is *ca.* 1 h. The highest levels of aminosalicylic acid are attained in the kidney, adrenal glands and the spleen [26]. The concentrations in the lung and liver are high as well [19, 26]. The diffusion through the blood-brain barrier is poor because to the polarity of the drug and due to an active efflux transport [31]. In cases of meningeal inflammation however CSF levels as high as 50% of the plasma concentration could be reached. The co-administration of anti-inflammatory salicylates or of probenecid has been found to inhibit the transport of aminosalicylic acid across the arachnoidal plexus [19]. The drug is 50-73% bound to plasma proteins [23, 31]. Aminosalicylic acid crosses the blood brain barrier and is excreted into milk of lactating women [31, 32].

A specialized pharmacokinetic study has been conducted to assess the impact of hemodialysis on the biodisposition of different antituberculous agents, incl. aminosalicylic acid. The experimental results suggest that hemodialysis is capable of removing PAS and its major metabolite acetyl-PAS. Overall, recovery of acetyl-PAS in dialysate is greater than that of its parent compound, confirming that metabolism remains the primary mode of elimination. Due to the small magnitude of influence however the authors conclude that if PAS is used in a long-term hemodialysis patient, supplemental dosing to account for dialysis removal does not appear warranted [33]. PAS removal from circulation is not facilitated by peritoneal dialysis [19, 26].

- **Biotransformation**

Aminosalicylic acid is rapidly metabolized starting in the gastrointestinal tract and then in the liver. The main biotransformation pathway is N-acetylation to N-acetyl-PAS, which is devoid of bacteriostatic activity against *M. tuberculosis*. A glycine conjugate of aminosalicylic acid (aminosalicyluric acid) is also formed, which is also non-active against mycobacteria. Bang et al. have presented the metabolite-profile following a single application of 12 g aminosalicylic acid. Three hours post application the blood levels of

the drug and the metabolites have been presented as follows: approximately 67-80% of the dose occurs unchanged, 16-22% are metabolized to acetyl-PAS and 4-11% are converted into glycyl-PAS [34].
It is worth mentioning that both acetylation and glycine-conjugation of aminosalicylic acid are saturable processes [34]; thus 100 μg/mL unchanged aminosalicylic acid is found in the urine after a 4-g dose, and more than 500 μg/mL after an 8-g dose [23]. The saturation of conjugative metabolism is especially marked after intravenous application of high doses whereby a significant portion of the drug remains non-metabolized and hence the concentration of the free drug in plasma and tissues is significantly higher as compared to lower doses and especially to oral treatment [26]. Although N-acetyl-PAS and glycine-PAS are the chief biotransformation products there are also several minor inactive metabolites identified in the urine following administration of aminosalicylic acid, these include an sulphate metabolite of aminosalicylic acid, ester and ether glucuronides of the free drug, ester and ether glucuronides of N-acetyl-PAS [35, 36].

Arylamine *N*-acetyltransferases which are involved in the most important biotransformation pathway of PAS, have been long associated with pharmacogenetics [37]. The ability to metabolize the anti-tubercular drug isoniazid was amongst the earliest genetic variation in drug metabolism to be identified. It has been established that the ability to inactivate isoniazid was under the control of a somatically inherited recessive trait leading to the slow acetylation phenotype [37, 38]. This peculiarity has been related to the ability to metabolize a range of drugs including some hydrazide (e.g. hydralazine, phenelzine, isoniazid) and also arylamine drugs (incl. procainamide and sulphamethazine) [37, 38]. Such substrates have been referred to polymorphic substrates. In contrast further series of arylamine drugs and arylamine compounds metabolized by *N*-acetylation have labeled as monomorphic e.g. PAS and p-aminobenzoic acid (PABA), because the ability to metabolize these compounds has showed a different population genetic pattern and is not influenced by the genetic polymorphism i.e. the acetylator status [37-39]. Now it is firmly established that the difference between the polymorphic and monomorphic substrates is conditioned by the abundance of two isoforms of acetyl-transferase in humans, designated NAT2 and NAT1 which have distinct albeit overlapping substrate profiles and also have specific substrates. Thus isoniazid and the other drugs showing the same interindividual variation in *N*-acetylation are metabolized by the human isoenzyme NAT2, whereas the monomorphic substrates such as aminosalicylic acid are metabolized by NAT1 [37, 39].

- **Elimination**

Following an oral dose aminosalicylic acid has a half-life of approximately 1 hour, and its concentrations in plasma are negligible within 4 to 5 hours after a single conventional dose; within 24 hours more than 80% of the dose is excreted. [19, 23, 31]. After intravenous application the elimination half life is generally shorter and is estimated to be approximately 26 min [19].
Over 80% of the drug is excreted in the urine; more than 50% is in the form of the acetylated compound; the largest portion of the remainder is made up of the free acid [29, 30]. Apart from glomerular filtration the half of the dose especially the metabolites are eliminated by tubular secretion [26]. Excretion of aminosalicylic acid is greatly retarded by renal dysfunction, and the use of the drug is not recommended in such patients. Probenecid decreases the renal excretion of aminosalicylic acid and especially of its major metabolites [31].

As already mentioned the conjugative metabolism of aminosalicylic acid is a saturable process and hence with high doses greater amounts of non-metabolized drug will be present in the urine. Thus when doses higher than 12 g/daily are applied crystalluria may ensue [19, 26]. Crystalluria may be avoided by maintaining the urine in an alkaline or neutral pH e.g. by application of sodium hydrogen carbonate which will enable the formation of sodium and potassium salts, which are more soluble than the free acid [30].
Minor amounts of an ingested dose (ca. 3%) are eliminated via feces and bile [30]. Aminosalicylic acid crosses the placenta and is excreted into human milk [19, 32].

- **Dosage and administration**

The guidelines for the oral application of the drug in adults suggest a dose of 12 g PAS per day. This dose should be divided into two donations throughout the day. The American Thoracic Society recommends a PAS dose of 150 mg/kg daily in adults and children, with a maximum daily dose of 12 g [14].
The parenteral dosage of PAS should take place intravenously. As solvent a volume of 500 ml of water for injections is recommended. A duration of infusion of 2-4 hours is proposed. The parenteral application of PAS is almost entirely used in Europe; Opl and Kuntz et al. have described the exemplary parenteral appli-

cation of aminosalicylic acid [40, 41].

The monograph of PAS published in the German Bundesanzeiger and the guidelines of the German central committee for the fight against tuberculosis recommend a daily dose in adults and children older than 14 years of 10-15 g of free PAS, usually applying a dose of 12 g [12, 19]. A maximal dose of 40 g free PAS as intravenous infusion should not be exceeded [19, 26].

Dosage in special populations

The elimination of aminosalicylic acid is slower in neonates and especially in premature newborns. Neonates and premature infants as well as children up to an age of 6 should be given 200-300 mg free PAS per kg bodyweight on a daily basis. Children in school age should receive 200 mg of free PAS per kg bodyweight daily [26].

Although aminosalicylic acid is mainly metabolized via the hepatic N-acetyltransferases subject to genetic polymorphism its half-life is not influenced by the acetylator status; it is thus considered a monomorphic substrate [37-39]. Nevertheless the possibility of clinically significant interactions with isoniazid is presumably higher in fast-acetylators. The clinical significance of this interaction is considered generally low [30].

Even though some reports show no alteration of the drug in patients with liver disease, compared with normal volunteers, aminosalicylic acid should be used with caution in patients with hepatic impairment, as these patients may not tolerate aminosalicylic acid as well as patients without liver disease [23].

Aminosalicylate sodium should be generally avoided in severe renal disease, unless no therapeutic alternative exists [19, 26, 31]. Although the serum half-life of the parent compound is not altered in renal disease, that of the metabolites is prolonged 6-fold. Because deacetylation is not significant, accumulation of the acetyl metabolite is likely. This may exacerbate the GI symptoms and platelet dysfunction associated with uremia and potentiate acidosis [23, 42]. Few reports are available regarding experience with PAS in patients with renal failure. Case reports involving five patients indicate that PAS has been used in patients with renal failure in doses ranging from 2 to 6 g after dialysis to 4.5 to 12 g/daily (the dosage forms have not been indicated). A patient who received 12 g/daily experienced upper gastro-intestinal bleeding attributed to drug-induced gastritis [33]. Aminosalicylic acid should be avoided in patients with a GFR below I0 mL/min, if possible [23]. Because both PAS and acetyl-PAS are removed by dialysis, the drug should be given after dialysis to avoid premature removal of the drug [14, 33].

Due to the high sodium content of the parenteral PAS-Na formulations and the propensity of the drug to cause electrolyte abnormalities it has to be applied with caution in patients with edematous states [19]. No specific recommendations for dose adjustments in obesity have been formulated. Aminosalicylic acid is a hydrophilic agent, and therefore, larger than usual doses may be required in patients with ascites or edema. This can be determined by measuring serum concentrations and therapeutic drug monitoring [23].

II. Pharmacodynamics

Although the drug is in clinical use for over 6 decades and despite the extensive research efforts, its precise mode of action remains elusive. There are two mechanisms responsible for aminosalicylic acid‘s bacteriostatic action against *Mycobacterium tuberculosis*. Firstly aminosalicylic acid inhibits folic acid synthesis; as mycobacteria are unable to use external sources of folic acid, cell growth and multiplication slows [23, 43-45]. Moreover aminosalicylic acid has been proven to inhibit the synthesis of mycobactins, which are crucial siderophores for tuberculous bacilli, and hence the drug disrupts the iron intake of susceptible acid-fast bacteria [46] (See below).

- **Antifolate effects**

Aminosalicylic acid is a bio-isostere of *para*-aminobenzoic acid, and its mechanism of action appears to be analogous to that of the sulfonamides. Nevertheless the sulfonamides are ineffective against *M. tuberculosis,* and aminosalicylic acid is inactive against sulfonamide-susceptible bacteria. This differential sensitivity presumably reflects differences in the enzymes responsible for folate biosynthesis in the various microorganisms [26, 29, 31]. Sulfonamides are also structural analogues of para-aminobenzoic acid, the substrate of dihydropteroate synthase (DHPS) (encoded by *folP1*/*folP2* in Mycobacteria), and function as competitive inhibitors of this enzyme. FolP1 and its putative homologue FolP2 catalyze the condensation of para-aminobenzoic acid and 6-hydroxymethyl-7,8-dihydropterein pyrophosphate to 7,8-dihydropteroate, which in turn is converted to dihydrofolate and reduced to generate the tetrahydrofolate (THF) by the enzyme dihydrofolate reductase (encoded by *dfrA*) [47]. THF is an important co-factor needed for 1C fragment transfer and the synthesis of purines, thymidine and methionine [47].

Surprisingly, unlike the sulfonamides the PAS-inhibitory activity of DHPS has proven to be unexpectedly poor *in vitro*. The inability to demonstrate inhibition in vitro, however, does not rule out the role of DHPS as the in vivo target of PAS. It is possible that PAS accumulates to high levels within bacterial cells, leading to inhibition of DHPS activity in vivo. Alternatively, analogous to the mode of action of other antituberculosis drugs such as isoniazid and pyrazinamide, PAS might be converted to a more potent 'active' form inside bacterial cells [47], as recently corroborated by experimental findings [43, 44]. Zheng et al. have recently shown that PAS is a prodrug targeting dihydrofolate reductase (DHFR) through an unusual and novel mechanism of action. They have provided evidences that PAS is incorporated into the folate pathway by dihydropteroate synthase (DHPS) and dihydrofolate synthase (DHFS) to generate a hydroxyl dihydrofolate antimetabolite, which in turn inhibits DHFR enzymatic activity. Interestingly, PAS is recognized by DHPS as efficiently as its natural substrate para-amino benzoic acid [43].

- **Inhibition of mycobactin biosynthesis and disruption of iron homeostasis in mycobacteria**

Salicylate is precursor for the biosynthesis of mycobactins and carboxymycobactins, which are important siderophores in mycobacteria [48, 49]. Aminosalicylic acid is a salicylate isostere and has been proven to inhibit the biosynthesis of both mycobactin and carboxymycobactins thus hampering the iron transport and trafficking in Mycobacteria [46, 48, 49]. Treatment of *M. smegmatis* and *M. bovis* with PAS has been found to be always more effective when the cells are growing with a sufficiency of iron than with a deficiency of iron. Under high iron conditions the genes

Table 3. Minimum inhibitory concentration (MIC) of aminosalicylic acid for different mycobacteria [50].

Species/strain identifier	Medium	Inoculum	Incubation time (days)	MIC (μg/ml)
M. tuberculosis H37RV	YS	0.01 mg ww	N.S.	1.5
	D	N.S.	N.S.	1.2
	D	0.001 mg dw	14	0.49
	HO	N.S.	21	4.0
	HO	0.01 mg ww	28 + 42	1.0
	KT	—	—	0.25
	TAM	N.S.	21	1.0
	KI	N.S.	21	0.5
	YS	1 mg ww	21 + 28	0.32
M. tuberculosis	—	—	—	0.48
M. tuberculosis (14 strains)	S	N.S.	20	0.25 – 0.5
	GD	N.S.	N.S.	1.53
	LJ	—	21	3.3
M.bovis (3 strains)	S	N.S.	20	25
M. avium	S	N.S.	20	500
M. kansasii	Sula	10^4-10^6 cells	24	1-10
M. kansasii	YS	1 mg ww	21 + 28	19.0
M. smegmatis	KTA	—	—	125
M. battei	YS	1 mg ww	21 + 28	250

Abbreviations of media: LJ=Löwenstein-Jensen, HO=Hohn, D=Dubos; KI=Kirchner; KT=Kirchner with Tween: KTA=Kirchner with Tween and albumine; TAM=Tween-albumine-medium; YS=Youmans with serum; ww – wet weight; dw – dry weght.

for mycobactin and carboxymycobactin biosynthesis are repressed and thus the few copies of the various enzymes that remain are adequately inhibited by PAS [48]. However when the cells are iron deficient, PAS is less effective because its intracellular concentration is, in effect, diluted by the greater abundance of the target enzyme(s). Nevertheless under these conditions the formation of mycobactin is strongly inhibited by PAS though that of carboxymycobactin is less effected and may even be increased, and the concentration of salicylic acid is increased considerably, in some cases by over 15-fold, by the action of PAS. These inhibitions though do not lead to the same degree of cell killing as occurs with the iron sufficiently grown mycobacteria [48]. These data point out that PAS at least partly exerts its pharmacological action in the conversion of salicylate to mycobactin; thus its effectiveness as an anti-tuberculous compound is mediated by preventing the bacteria from acquiring iron from the host [46, 48].

- **Antimicrobial activity/Spectrum**

Aminosalicylic acid is a bacteriostatic drug with a remarkably narrow spectrum of clinically-relevant activity. It is specifically active only against acid-fast organisms, with clinically useful activity primarily against *M. tuberculosis* and *M.* bovis [29, 50]. Aminosalicylic acid does not have sufficient *in vitro activity* against *M. avium* complex and most other atypical mycobacteria to warrant its use, although some strains appear to be susceptible [23, 50, 51]. The minimal inhibitory concentrations (MICs) of PAS against *M. tub*erculosis vary, depending upon both the media and the inoculum size. Generally in the susceptible strains MICs of 0.5 to 2.0 μg/mL or 1 up to 10 μg/mL have been reported (See Table 3). It is important to note that MICs reported with PAS often do not represent complete (>90%) inhibition [23].

- **Resistance**

Resistance to PAS may be natural or acquired. As with other antimycobacterial agents, resistance in previously susceptible organisms may develop rapidly both in vivo and in vitro when PAS is used as a single agent in the treatment of tuberculosis [23]. This appears to be due to naturally occurring resistant mutants present in a population of mycobacteria. In particular the spontaneous occurrence of aminosalicylic acid-resistant mutants in wild strains of *Mycobacterium tuberculosis* is as low as 10^{-6} [15]. Under the selective pressure of a single drug, all organisms are eliminated except the resistant subpopulation, which continues to multiply, eventually becoming the dominant population. It was found that 17.2% of patients previously treated with PAS acquired resistance; this was more profound in those receiving PAS monotherapy. Acquired resistance may be prevented by using PAS in combination with at least one or two other antimycobacterial drugs [23].

Although primary drug resistance to PAS is possible, it is much less common in developed countries. In 1963 Britain reported an incidence of 0.890 primary resistance while Australia reported 0.7 to 1.0% from 1972 to 1977. An incidence of primary resistance below 1% was reported from 1975 to 1982 in the United States, with some geographic and ethnic variance. The incidence is higher in developing countries, some Eastern European countries and in Southeast Asia [23].

Resistance to aminosalicylic acid in *Mycobacterium bovis* BCG is associated with insertions in the thymidylate synthase (thyA) gene, a critical determinant of intracellular folate levels. BCG thyA mutants have reduced thymidylate synthase activity and are resistant to known inhibitors of the folate pathway. Mutations in thyA and dihydrofolate synthase are associated with clinical PAS resistance as well [45, 47, 52, 53].

With the only exception of thiacetazone, there is no evidence of cross-resistance between aminosalicylic acid and other antituberculosis drugs. The cross-resistance appears only in PAS-highly-resistant mutants, which are 5 times as resistant to thiacetazone as parent sensitive organisms. PAS-low-resistant strains remain sensitive to thiacetazone. All thiacetazone-resistant mutants, on the other hand are sensitive to PAS [50]. This cross-resistance would not be predicted on the basis of structure similarity of known mechanism of action, and its nature is not known [23]. Although p-aminobenzoic acid antagonizes both PAS and sulphonamides, no cross-resistance between PAS and the sulphonamides has been detected [50].

Acquired resistance to PAS can be prevented by using the drug with at least two additional active agents. Higher doses of PAS as monotherapy do not prevent the emergence of resistant microorganisms. In vitro studies have indicted that sub-inhibitory concentrations facilitate selection of resistant mutants. It is not known if this also occurs in vivo, although the possibility exists. Thus, it appears desirable to achieve serum concentrations above the MIC of the isolate [23, 27].

• **PK/PD relationships, Therapeutic drug monitoring**

Aminosalicylic acid, like the other antimicrobial antifolates in clinical use is not bactericidal. It does not penetrate well into mammalian cells, and it does not appear to inhibit the growth of *M. tuberculosis* within macrophages, based on current data. PAS solutions may be light sensitive, and heat (>80°C) can rapidly destroy the drug in solution [23, 50]. Para-aminobenzoic acid (PABA), biotin and certain purines competitively antagonize the action of PAS *in vitro*, but neither folic acid nor folinic acid appears to do so [23, 50, 54].

No post-antibiotic effect has been reported with aminosalicylic acid. Moreover sub-inhibitory concentrations of aminosalicylic acid do not seem to exert a significant beneficial effect; in contrast they may be associated with selection of resistant isolates *in vitro*. It is not known if sub-inhibitory concentrations of aminosalicylic acid contribute to multidrug therapy *in vivo* [23].

There appears to be a linear relationship between aminosalicylic acid concentration *in vitro* and growth of mycobacteria, producing 50 to 95% inhibition [50]. PAS is fairly slow to act taking more than 24 h to show inhibition, even at high concentrations. There also appears to be an significant inoculum effect with PAS; thus large inocula yield nonlinear decreases in the in vitro activity of the antituberculosis drug [23, 50]. Studies regarding the early bactericidal effects (EBA; defined as the fall in colony-forming unit counts/mL sputum/day during the first 2 days of treatment) of aminosalicylic acid has been carried out in 4 patients. Although considered a weak drug, mainly useful for preventing the development of resistance to companion drugs, a moderate EBA within days 0-2 post treatment of 0.259 $\log_{10}$CFU/mL sputum/day was recorded for PAS, although this is far less prominent than the EBAs for the first-line agents and especially isoniazid [55].

The issue of whether PAS works optimally with continuous serum concentrations or intermittent high serum concentrations has been raised. A controlled, prospective study compared the outcomes of patients receiving self-administered isoniazid (INH) (4 mg/kg/day) plus PAS (0.2 g/kg) every day, each as two equally divided doses, with directly observed INH (15 mg/kg/day) and PAS (0.2 g/kg) twice weekly, each as single oral doses. All patients received 14 days of an initial intensive regimen or daily directly observed INH, streptomycin, and PAS. Of the evaluable patients, favorable bacteriologic responses were obtained in 79 of 90 (88%) twice-weekly patients and in 72 of 83 (87%) of the daily patients. Exceptions to the favorable responses in the twice-weekly group included those resistant to INH and those with advanced disease. The twice-weekly regimen was better tolerated. Although the differences were not large, they do indicate that future studies into varying dosing regimens of PAS may be warranted [23].

III. Clinical efficacy

At present, due to the low degree of initial resistance and on the basis of the clinical support (from retrospective cohort studies) PAS is considered a second-line agent for the chemotherapy of tuberculous disease originating from polyresistant strains. The following section deals with a concise representation of most important pioneering trials as well as with the more recent investigations of the drug in the MDR/XDR-setting.

• **Pioneering trials - use as a monotherapy**

At present tuberculosis is treated with combined chemotherapeutic regimens. The following section is given for the sake of comprehensiveness. Lehmann has reviewed in depth the pioneering clinical experiences with PAS monotherapy [1, 2]. The results of 378 cases of pulmonary tuberculosis treated with PAS alone, were presented in the reports of six Swedish sanatoria. Two-hundred and five of these cases were selected and compared with 223 cases treated with streptomycin alone, reported by the Veterans Administration in the United States. The patients treated with PAS alone showed a somewhat slower regression of the pulmonary infiltration but a better effect on temperature and the erythrocyte sedimentation rate. The Swedish National Association against tuberculosis published a report of 94 patients treated with PAS who were compared with 82 patients given a placebo. Statistically significant, more rapid improvement was seen in the PAS group [2]. DeJanney et al. have published some of the first clinical efficacy data with PAS (a non-controlled evaluation) for the treatment of pulmonary tuberculosis and tuberculosis meningitis (see below). The presented series comprises 40 patients with pulmonary tuberculosis. In these pulmonary cases there were five who also had endobronchial lesions which failed to respond to prolonged streptomycin therapy. Regarding the clinical safety aspects, the results for the study show that 64.3% of the patients had evidence of some degree of toxicity. Fifteen grams per day were found to be intolerable when given as the free acid with few exceptions. There was fairly good

tolerance on a schedule of 9.6 to 10.5 g/day and this dosage was found to be therapeutically effective. On the other hand 22 cases (52.4%) showed symptomatic benefit. Of the remainder who exhibited no symptomatic improvement 11.9% were asymptomatic before PAS was given. In this series PAS was given to patients with severe disease; 88% of the patients were streptomycin failures and 90% had bilateral disease. Cavitation was present in all cases except six. Of the 40 patients with pulmonary tuberculosis 42.5% had mixed or productive disease, and definite clinical and x-ray improvement directly attributable to PAS was seen in 27 or 67.5%. Taken together the results of this study indicate that PAS is highly effective in endobronchial tuberculosis; moreover pulmonary tuberculosis which has failed to respond to streptomycin does not preclude the use of PAS. Of such cases in the present series 67.5% showed improvement directly attributable to the latter drug [56].

Phillips et al have presented an interesting dose escalation study of PAS monotherapy. This study was an attempt to determine whether the administration of large doses of para-aminosalicylic acid, 18 to 24 g/day, was feasible and advantageous in the treatment of pulmonary tuberculosis. Patients for this study were not especially selected and were treated with gram quantities of PAS daily. In addition, six patients whose sputum investigation showed organisms definitely resistant to streptomycin were added to the study. This made a total of 28 patients who were started on the proposed therapy. In six of the patients, collapse measures were added sometime during the course of treatment. The 28 included PAS was given in the form of sodium salt. The equivalent of 6 g of PAS was administered four times daily with food for a proposed period of 120 days. Of the 28 begun on 24 grams of PAS, only 12 (42.8%) completed the course of 120 days. However, six others were able to complete the course after the daily dose was reduced to 18 grams. This makes a total of 18 (64.2%) who were able to tolerate high doses of PAS for 120 days. Almost all developed some symptom of drug toxicity, mainly anorexia, vomiting, or diarrhea. In most, however, the omission of the drug for a day or two was sufficient to alleviate the symptoms and permit the prompt resumption of therapy. On the other hand, in three, these symptoms were so severe as to warrant the cessation of therapy within the first month. Weight loss occurred in 54.2% of our group, probably as a result of the gastro-intestinal dysfunction produced by the large doses of PAS and moreover marked decrease in prothrombin levels was associated with the high dose regimen. These findings indicate that albeit high dosages of PAS are possible in most patients able to take smaller doses, the side-effects are more common and more troublesome. Most importantly the results obtained with the use of high doses of PAS, are not impressive as compared to the use of combined therapy and anyway the use of PAS alone gives rise to strains of resistant mycobacteria [27].

- **Efficacy in combination with other drugs**

What follows is a brief outline of the controlled clinical trial, follow-up studies, retrospective cohort studies and illustrative case reports to demonstrate the clinical efficacy of PAS as part of combined chemotherapeutic regimens.

In order to facilitate readability and due to space limitations in this section the following standard abbreviations are used, instead of the full INNs of the antituberculous agents, as follows: i) first line agents: ethambutol – EMB; Isoniazid – INH; streptomycin – SM; rifampicin – RMP; ii) second line agents: aminosalicylic acid – PAS, cycloserine - CS , ethionamide – ETH, levofloxacin - Lfx, kanamycin – KM, and prothionamide – PTH.

PAS vs. SM vs. PAS + SM

In a randomized clinical trial 166 patients with acute progressive bilateral pulmonary tuberculosis were treated with PAS and/or streptomycin. Of these 59 patients were treated with PAS (P group), 54 with streptomycin (S group), and 53 with both streptomycin and PAS (SP group). Patients were assigned to a treatment group by random selection. PAS was given in the form of the sodium salt, 20 g/day. The daily dose of streptomycin was 1 g. Chemotherapy was given for three months, and observation for the trial continued for further three months. Patients treated only with PAS showed better response than patients with similar disease treated without chemotherapy, reported in earlier streptomycin trials in pulmonary tuberculosis. PAS was less effective than streptomycin. In 34% of the P group there was no appreciable radiological change, compared with 6% of the S group. Marked radiological improvement (2- or 3-plus) was seen in 22% of the P group and in 56% of the S group. The difference was greatest in febrile patients. Improvement was somewhat greater in the SP group than in the S group, but the differences were small. The proportion of cases becoming bacteriologically negative was highest in the SP group. The outstanding effect of combined therapy was on emergence of drug resistance. Strains with a streptomycin

resistance ratio above 8 were isolated in 33 of 49 S cases, and in only 5 of 48 SP cases; in 2 of these 5 there was only a single resistant culture. Deterioration in the S group was related to the emergence of streptomycin resistance [3].

In another trial to compare PAS has been evaluated in 30 patients five of whom received only this drug, 12 concurrently with streptomycin and 13 after streptomycin had been discontinued. The daily dose of PAS was 9 g in four divided doses. The total dose averaged 683 g; the smallest being 297 g and the largest 1288 g. The clinical end-points of the study, i.e. the criteria for improvement included changes in general condition, serial x-ray examinations of the chest, blood counts and sedimentation rates. Toxic effects were judged by frequent liver function tests and blood counts. The results from the study have shown that PAS is not as effective as streptomycin. Best results are obtained when it is used in conjunction with the latter. 40% of the patients improved who received only PAS, 72.7% when it was utilized in conjunction with streptomycin and 61.6% when it followed the use of streptomycin. Transient nausea and one case of transient leukopenia and granulocytopenia were the only toxic effects observed [57].

INH + PAS vs. INH alone

Devadatta et al. from Tuberculosis Chemotherapy Centre in Madras (nowadays Chennai, India) have presented a study to assess the response of patients infected with isoniazid-resistant tubercle bacilli to treatment with isoniazid plus PAS or isoniazid alone. Twenty (5.9%) patients excreting isoniazid-resistant mycobacteria were encountered among 338 previously untreated patients with active pulmonary tuberculosis who were admitted to a chemotherapeutic study. 315 of the patients infected with isoniazid-sensitive tubercle bacilli (the S patients) have been compared with the 20 patients infected with resistant organisms (the R patients). There was little difference in terms of the extent of the radiographic lesion, the extent of cavitation or the bacterial content of the sputum before the start of treatment. In all, 90 S patients and six R patients were treated with isoniazid plus PAS, while 225 S patients and 14 R patients received isoniazid alone in three different dosage schedules. The treatment was allocated for a year. By the end of 12 months of treatment eight (9%) of 86 S patients on isoniazid plus PAS showed an unfavorable bacteriological response, as compared with five (83%) of the six R patients. Correspondingly, 93 (43%.) of 216 S patients on isoniazid alone showed an unfavorable response, as compared with 12 (86%) of the 14 R patients. The differences attain statistical significance in both the treatment series. Evidence has been adduced to show that the response of the R patients was due to the studied chemotherapeutic intervention [58].

Another early study has been conducted to provide a controlled comparison of four chemotherapeutic interventions of PAS + isoniazide vs. various regimens of isoniazide administered alone. The chemotherapeutic regimens were as follows: (a) 3.9-5.5 mg/kg body-weight of isoniazid plus 0.2-0.3 g/kg body-weight of PAS (sodium salt) daily in two doses (the standard combined chemotherapy); (b) 7.8-9.6 mg/kg body-weight of isoniazid alone daily in one dose; (c) 7.8-9.6 mg/kg body-weight of isoniazid alone daily in two doses; (d) 3.9-5.5 mg/kg body-weight of isoniazid alone daily in two doses. The results from this trial unambiguously point out that isoniazid plus PAS has proved to be the most satisfactory regimen; it was clinically effective and there were very few toxic manifestations [59].

Ramakrishnan et al. reported a study on the prevalence and attack rate of tuberculosis among close family contacts of tuberculous patients in South India undergoing domiciliary chemotherapy either with isoniazid plus PAS or with one of three regimens of isoniazid alone. The report gives (a) the prevalence of tuberculosis among the contacts at the time of diagnosis of the disease in the patients and (b) the incidence of tuberculosis in the contacts during the first year of treatment of the patients. The contacts were divided into four series, corresponding to the four chemotherapeutic regimens of the patients. The prevalence of active tuberculosis was found to be particularly high among children less than five years of age, being 12.0% as compared with 7.6% for all age-groups combined. The incidence of active tuberculosis during the year of treatment of the patients was also found to be highest in the under five years' age-group-a further indication that child contacts are especially vulnerable to infection. The incidence was considerably higher in the first quarter of the year, and it was lowest in the last quarter. This finding, together with the fact that the attack rates in the four contact series were not related either to the duration of bacteriological positivity in the patients or to the period of excretion of isoniazid-resistant organisms by the patients, suggests that the major risk to contacts in the first year results from exposure to the patient before treatment rather than from exposure during treatment. These results thus confirm the findings in an earlier study by the Centre of the contacts of patients in a

controlled comparison of chemotherapy with isoniazid plus PAS at home and in sanatorium [60].

PAS + INH (daily) vs. PAS + INH (intermittent)

A large controlled clinical trial with PAS in combination with other anti-tuberculosis agents had been conducted in order to assess the efficacy of daily vs. intermittent application. In total the study enrolled 247 patients of an age of 12 years or older with newly diagnosed tuberculosis and at least two sputum cultures positive for *Mycobacterium tuberculosis*. For the first two weeks all the patients attended the outpatient clinic daily and received under supervision streptomycin 1 g, sodium PAS 6 g, and isoniazid 400 mg. For the next 50 weeks the patients received on an outpatient basis either a twice-weekly regimen (group 1) or a daily regimen (group 2). The twice weekly regimen comprised sodium PAS 0.2 g/kg body weight plus isoniazid 15 mg/kg body weight, both drugs being given at the same time in a single oral dose twice a week. The daily regimen comprised PAS 0.2 g/kg body weight plus isoniazid 4.7 mg/kg body weight, both drugs to be self-administered daily in two divided doses by mouth. The main analysis of the study finally enrolled 173 patients, 90 in group 1 and 83 in group 2. Radiologically the response to treatment was similar in the two series of patients. Eighty-five individuals (94%) in group 1 and 80 patients (96%) in group 2 showed improvement over the 12-month period. Cavitation was present initially in 80 patients in group 1 and 77 in group 2. It had disappeared after 12 months in 41 (51%) and 35 (45%), respectively and became less in 34 (42%) and 39 (51%), respectively. Cavitation was observed at 12 months in 3 out of 10 group 1- patients and two of six group 2-patients in whom it had not been apparent on admission. At least one PAS-resistant culture was observed before treatment from 21 of the 173 patients. The other 152 patients had PAS-sensitive cultures on admission to treatment. Eight patients on the twice-weekly regimen and six on the daily regimen had PAS-sensitive cultures before treatment and either produced positive cultures at 10, 11, and 12 months or had chemotherapy changed during the year on account of deterioration. In four patients in each group at least two of the last three cultures tested were PAS-resistant. On the basis of the clinical and bacteriological end-point assessment the authors have graded the findings of this trial with the fully oral twice-weekly regimen of isoniazid and PAS as encouraging [61].

INH + PAS vs. INH + SM

Another study conducted at Tuberculosis Chemotherapy Centre, Madras, has been aimed at establishing the value of intermittent dosing of a combination of isoniazid and PAS for the domiciliary treatment of pulmonary tuberculosis. In this controlled study a fully supervised intermittent regimen of isoniazid (12.5-16.1 mg/kg body-weight, orally) plus streptomycin (injected in a uniform dose of 1 g), given together twice weekly, compared with a standard, unsupervised, daily, oral regimen of isoniazid (3.7-6.3 mg/kg body-weight) plus sodium PAS (0.2-0.3 g/kg body-weight), given in two doses. The findings indicate that intermittent regimen was at least as effective as the standard oral regimen, and although the incidence of temporary giddiness in patients receiving this regimen was rather high, this did not appear to have any long-term importance nor did it appear unduly to affect the co-operation of the patients. These encouraging early findings has suggested a possible change in drug-administration patterns for tuberculosis in developing countries [62]. Nowadays however, intermittent dosing of PAS is considered as non justified and prone to developing acquired resistance to the drug [23].

INH + PAS vs. SM + PAS

A controlled trial was assigned to compare the efficacy of isoniazide + PAS vs. two regimens of streptomycin + PAS in the treatment of pulmonary tuberculosis. 391 patients were studied in 50 hospitals: 119 were treated with streptomycin (1 g/day) plus isoniazid (100 mg twice a day), 100 with streptomycin (1 g. twice a week) plus isoniazid (100 mg twice a day), 101 with PAS (sodium salt, 5 g. four times a day) plus isoniazid (100 mg twice a day), and 71 with PAS (sodium salt, 5 g. twice a day) plus isoniazid (100 mg twice a day). When submitting a case the physician did not know which treatment the patient would receive, this being determined by random allocation. The presented literature report analyses results at the end of three months‘ treatment. Three main groups were observed: Group 1, acute rapidly progressive disease of recent origin; Group 2, other forms considered suitable for chemotherapy; Group 3, chronic disease considered unlikely to respond to chemotherapy. The principal comparison in the trial report is between the patients in Groups 1 and 2 on streptomycin 1 g/day plus isoniazid 200 mg daily (SH) and those on PAS (sodium) 20 g/day plus isoniazid 200 mg daily (20 PH). On admission, these two treatment series had a similar distribution of patients

with severe and less severe illness. At the end of three months the general condition had improved in 89% of the SH patients and 88% of the 20 PH patients. The average gain in weight during the period was 5.8 kg for the SH patients and 4.9 kg for the 20 PH patients. The temperature fell to normal in 79% of febrile SH patients and 85% of febrile 20 PH patients. In patients with an erythrocyte sedimentation rate of 21 or more before treatment the rate fell to 10 or less in 37% of those on SH, compared with 46% of those on 20 PH. Changes in radiographic appearances were independently assessed by a radiologist unaware of the treatment of any patient. Two-plus or three-plus improvement was seen in 54% of SH and 42% of 20 PH patients. There were two radiographic deteriorations and one death on each treatment. None of the above differences however is statistically significant. The proportion of patients bacteriologically negative, both on direct examination and on culture, at a single examination at three months was, as follows: 65% for the SH series and 66% for the 20 PH series. Mycobacterial resistance to isoniazid was found in 2 of 39 culture-positive SH patients tested at three months, compared with 0 of 29 similar 20 PH patients. Mycobacterial resistance to streptomycin was found in 1 of 38 culture-positive SH patients, and to PAS in 1 of 28 similar 20 PH patients. It is concluded, judging solely from the results at three months, that PAS (sodium) 20 g/day plus isoniazid 200 mg daily is a very effective combination of drugs, on the basis of both clinical and bacteriological end-point assessment; this regimen ranks with the most efficacious contemporary treatments, e.g. streptomycin 1 g/day plus isoniazid 200 mg daily and streptomycin 1 g/day plus PAS (sodium) 20 g/day. A supplementary clinical comparison of all the 219 patients on streptomycin plus isoniazid and all the 172 on PAS plus isoniazid confirms the clinical efficacy of combining PAS with isoniazid. Nevertheless, patients on streptomycin plus isoniazid gained a little more weight, and a higher proportion showed substantial radiographic improvement. A preliminary analysis of results of sensitivity tests on each of the four treatments shows that PAS (sodium) 10 g/day plus isoniazid 200 mg daily may prove to be a bacteriologically effective combination for at least three months, whereas on the other hand, treatment with streptomycin 1 g. twice a week plus isoniazid 200 mg daily is apparently less effective than streptomycin 1 g/day plus isoniazid 200 mg daily in preventing the development of bacterial resistance to isoniazid over a three-month period [63].

In the final stage of the aforementioned large-scale trial of isoniazid combined with either PAS or streptomycin in the treatment of pulmonary tuberculosis a total of 588 patients were studied in 51 hospitals. Of these 182 were treated with streptomycin 1 g/day plus isoniazid 100 mg twice a day (SH); 142 with streptomycin 1 g twice a week plus isoniazid 100 mg twice a day (S2H); 159 with PAS, sodium salt, 5 g four times a day plus isoniazid 100 mg twice a day (20 PH); and 105 with PAS, sodium salt, 5 g. twice a day plus isoniazid 100 mg twice a day (10 PH). The study report analyses both clinical and bacteriological results at the end of three months‘ treatment. Bacteriological results are also studied for 241 patients over a six-month treatment period. The types of disease studied ranged from acute rapidly progressive disease to very chronic disease. Most of the cases were newly diagnosed; less than 5% had received previous courses of chemotherapy. All patients included in the analysis had organisms sensitive at the start of treatment to both of the drugs they were receiving. Cases in an important subgroup (acute rapidly progressive bilateral disease of recent origin, in patients aged 15-30) were allocated only to treatment with SH (67 patients) or with 20 PH (59 patients). The report contains a comparison of the SH, S2H, 20 PH, and 10 PH treatments, excluding the patients in this subgroup, and a further comparison of the SH and 20 PH treatments, including these patients. On admission, the four treatment series had similar distributions of patients with severe and less severe illness. At the end of three months‘ treatment the majority of patients had improved. In terms of general clinical condition, resolution of pyrexia, and improvement in the sedimentation rate, the differences between the four treatment series were small. The average weight gains were 5.1 kg for the SH, 6.7 kg for the S2H, 5.1 kg for the 20 PH, and 6.3 kg for the 10 PH patients. Changes in radiographic appearances were independently assessed by a radiologist; two-plus or three-plus improvement was seen in 54% of SH, 44% of S2H, 33% of 20 PH, and 38% of 10 PH patients. The differences between this result for the SH treatment and those for the 20 PH and 10 PH treatments attain statistical significance. There were no radiographic deteriorations and 2 deaths in the SH series, 6 deteriorations and no deaths in the S2H series, 2 deteriorations and 2 deaths in the 20 PH series, and 4 deteriorations and 2 deaths in the 10 PH series. The proportions of patients bacteriologically negative, both on direct examination and on culture, at a single examination at three months were 75% in the SH, 74% in the S2H, 73% in the 20 PH, and 75% in the 10 PH series. At three months bacterio-

logical resistance to isoniazid was found in 2 of 22 culture-positive SH patients, compared with 12 of 30 similar S2H patients, 0 of 24 20 PH patients, and 2 of 25 10 PH patients. Bacillary resistance to streptomycin was found at three months in 0 of 22 culture-positive SH patients and 3 of 31 S2H patients, and to PAS in 1 of 22 culture-positive 20 PH patients and 1 of 23 10 PH patients. It is concluded, judging solely from the results at three months, that streptomycin 1 g daily plus isoniazid 200 mg daily is not only the most effective of the four treatments but also represents the most effective drug combination studied at any stage of the trial. Streptomycin 1 g twice a week plus isoniazid 200 mg daily is less satisfactory in preventing the emergence of isoniazid-resistant organisms, and its use as a primary chemotherapeutic measure cannot be recommended. PAS plus isoniazid has proven to be a very effective combination of drugs, although it is not quite so powerful as daily streptomycin plus isoniazid. There is little to choose between the clinical and bacteriological efficacy of 20 g and of 10 g PAS (sodium salt) daily plus isoniazid 200 mg daily. Either combination of PAS with isoniazid is a most valuable oral form of combined chemotherapy in the treatment of pulmonary tuberculosis. A proportion of patients in each of the treatment series continued on the same combination of drugs beyond three months. For reasons given the analysis of bacterial sensitivity for these patients during the second three months may be regarded as a continuation of the analysis in the first three months. The analysis, based on a total of 241 patients, confirms, for a period of six months, the adequacy of the SH, 20 PH, and 10 PH treatments, and the inadequacy of the S2H treatment, in preventing the development of bacterial resistance to isoniazid. Combining the figures at four, five, and six months, 2 of 12 positive cultures from SH patients, 2 of 9 from 20 PH patients, and 0 of 6 from 10 PH patients were found to be isoniazid-resistant. On the other hand, 9 of 15 strains from S2H patients were resistant to isoniazid at four months, 5 of 9 at five months, and 8 of 9 at six months. A small subgroup of patients with PAS-resistant organisms on entry to the trial was not protected from the risk of development of isoniazid resistance during treatment with PAS plus isoniazid [64].

PAS (daily) + SM (intermittent) vs. PAS (daily) + INH (daily) + SM (intermittent)

Capon et al compared two combinations of PAS with streptomycin and isoniazid in the treatment of pulmonary tuberculosis. The three intervention groups were as follows: a) combined intermittent streptomycin-daily PAS, and b) combined intermittent streptomycin-daily PAS and daily isoniazid. There were 100 patients in each group. Over a 180 day period of therapy there was a statistically significant difference in favor of the „3 drug“ group in terms of x-ray improvement, cavity closure and sputum conversion. The results of the study suggest that combined streptomycin, PAS and isoniazid is favored in the treatment of pulmonary tuberculosis, particularly when compared to the alternative regimen [65].

INH + PAS vs. INH alone vs. INH + ethionamide

Le Hir et al. have described an comparative study of the clinical efficacy of three types of oral therapy: i) daily administration of 300 mg of isoniazid plus 15 g of PAS (sodium salt); ii) daily administration of 400 mg of isoniazid alone; iii) daily administration of 3-5 tablets of a mixture of isoniazid and ethionamide, designated „2127 Th“ and containing, per tablet, 100 mg of isoniazid and 166 mg of ethionamide. Both before and during treatment, cultures from sputum specimens were subjected to rigorous drug-sensitivity tests. The pretreatment radiographic examination revealed most of the patients to be seriously ill on admission to the study: 97.2% showed cavitation, and 68% and 11%, respectively, had lesions classified as „extensive“ and „very extensive“. After 4 months of treatment 76% patients treated with isoniazid alone were still sputum-positive, as compared with 32% of those treated with isoniazid plus PAS and only 23% of the patients treated with 2127 Th. Drug-sensitivity tests showed that isoniazid resistance had developed in 93% of the positive patients in the isoniazid-alone series, in 45% in the isoniazid plus PAS series, and in only 9% in the 2127 Th series. After 8 months‘ treatment, the overall rates for conversion to sputum negativity (excluding the patients with resistant organisms at 4 months) were: 77% in the 2127 Th series, 76% in the isoniazid plus PAS series, and 27% in the isoniazid-alone series. The drug-sensitivity tests at 8 months showed that isoniazid-resistance had appeared in only 2% of the total number of patients in the 2127 Th series, as compared with 20% in the isoniazid plus PAS series and 68% in the isoniazid-alone series. As to the radiographic response to treatment, moderate or greater improvement was shown after 4 months by 41% of the 2127 Th series and by 43% of the isoniazid plus PAS series, but by only 29% of the isoniazid-alone series. At 8 months, 57% of the patients receiving 2127 Th and 64% of those receiving isoniazid plus PAS showed a favorable or very favor-

able response. In view of the severity of the patients' disease before treatment these results were considered by the authors as encouraging [66].

SM + INH + PAS vs. SM + INH + EMB vs. SM + INH + RMP vs. SM + INH + PAS/SM + high-dosage INH (+ vit B6)

A controlled clinical trial has been conducted by the British Medical research Council to compare the efficacy of 3 alternative regimens to a regimen of isoniazid plus PAS with an initial streptomycin supplement are presented. Adults with newly-diagnosed sputum-positive tuberculosis were treated in hospital for 3 months followed by continuation chemotherapy for 9 months usually as outpatients. They were submitted from 51 chest clinics in Britain and were allocated at random to either: i) SM + INH + PAS daily, followed by daily self-administered INH + PAS (the P series); ii) SM + INH + EMB daily, followed by daily self-administered INH + EMB (the E series); iii) STM + INH + RMP daily, followed by daily self-administered INH + RMP (the R series); iv) SM + INH + PAS daily, followed by twice-weekly SM + high-dosage INH (+ pyridoxine) given together under the direct supervision of the staff (the S_2H_2 series). The triple combination was given for 3 months in all 4 regimens. A total of 481 patients were admitted to the study. Of these, 412 (109 P, 97 E, 103 R and 103 S_2H_2) patients were included in a main comparison of the 4 regimens even if they had had changes or interruptions of chemotherapy for drug toxicity or uncooperativeness. At 12 months, only a small minority of patients had an unfavorable bacteriological response. In a subsidiary comparison of patients who either continued on their allocated regimen throughout or had chemotherapy changed for bacteriological relapse, 3 of 82 P, 0 of 73 E, 0 of 85 R and 1 of 57 S_2H_2 patients had an unfavorable bacteriological outcome at 12 months. The speed of elimination of acid-fast bacilli on sputum smear examination was similar in the 4 series but in the cultures was significantly more rapid in the R patients than in the other 3 series. Overall the clinical efficacy did not differ significantly among the treatment arms of the study. The analysis of the side effects profile has indicated that generally the PAS-containing regimens had higher incidence of adverse effects, predominantly gastric and allergic events. The other most important side effects, most notably vestibular disorders were associated with streptomycin. [67].

PAS+RMP vs. PAS+INH vs. RMP+INH

The efficacy of PAS plus rifampicin, PAS plus isoniazid (INH), and rifampicin plus INH were compared within a clinical trial. Eighty-eight patients (mean 41 years) received PAS 10 to 12 grams/day for 6 months. Clinical evaluations performed at 3 and 6 months indicated that PAS+INH was the least successful drug group determined via x-ray. The incidence of adverse reactions was greater in the PAS drug groups than in the RMP-INH group [30].

PAS + INH + SM vs. RMP + INH

Aminosalicylic acid plus isoniazid plus streptomycin were compared with rifampicin plus isoniazide. In this study twenty-nine patients (mean 49 years) with tuberculosis received PAS 0.2 gram/kilogram/day for 4 to 51 weeks (mean 20). A moderate to marked x-ray improvement at 4 months was seen in 85% of patients in both groups of drugs. Sputum conversion judged by microscopy and culture was not significantly different in either group. However, the incidence and severity of adverse reactions were greater in the PAS-containing regimen as compared to RMP+INH drug group. These included gastrointestinal disturbances, abnormal liver function tests, allergic reactions, and dizziness [30].

Viomycin + PAS vs. viomycin + streptomycin vs. viomycin alone

An evaluation of 80 patients with pulmonary tuberculosis who received intermittent viomycin, two grams every third day, singly or combined with intermittent streptomycin or daily para-aminosalicylic acid for 120 days suggests that this agent is capable of exerting a favorable effect on the clinical course of the disease. The therapeutic effectiveness of this drug was considerably less than streptomycin, but approximated that of PAS. Viomycin combined with either streptomycin or para-aminosalicylic acid was superior to any of the drugs employed alone [68].

Efficacy in multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB)

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 [8-

10]. This has led to the increased use of second-line anti-tuberculosis drugs for treatment failure cases. The second-line anti-TB drugs are members of fluoroquinoline group, injectable agents, such as aminoglycosides (kanamycin, amikacin) and polypeptide (capreomycin), para-aminosalicyclic acid, cycloserine, prothionamide and thiacetazone. The increasing use of second- line anti-TB drugs gave rise to extremely drug-resistant tuberculosis (XDR-TB), where the MDR-TB organism was resistant to fluoroquinoline and one of the injectable agents [9, 69].

Several trials have assessed the efficacy of PAS as a component of multi-drug regimens for the treatment of resistant tuberculosis.

Mukherjee has presented an in-depth analysis of treatment outcomes in MDR-TB and presented several cohort-studies. In one of these Viskum et al. examined the incidence, treatment and prognosis for patients with tuberculosis due to MDR *Mycobacterium tuberculosis;* all bacteriologically confirmed new cases of tuberculosis and treatment relapses in Denmark are examined for drug resistance. In the years 1993-1995, nine cases of multidrug-resistant tuberculosis (MDR-TB), all acquired outside Denmark, were identified among 1354 cases of tuberculosis. Multidrug resistance was present in less than one of patients with tuberculosis. One patient died from tuberculosis without revision of treatment, and eight patients responded favorably to a regimen of pyrazinamide, streptomycin or amikacin, ofloxacin and cycloserine. In two patients, this regimen was supplemented with aminosalicylic acid and thiacetazone respectively. The mean duration of chemotherapy was 13 months, with 100% cure rate and all patients needed prolonged hospitalization and had observed treatment. These findings suggest that using aggressive combined regimens allows curative treatment of MDR-TB [7]

Mitnick et al. evaluated the results of community-based therapy for multidrug-resistant tuberculosis in a poor section of Lima, Peru. They have conducted a retrospective review of the charts of all patients enrolled in the program for ambulatory treatment with individualized regimens for chronic multidrug-resistant tuberculosis between August 1, 1996, and February 1, 1999. The infecting strains of *Mycobacterium tuberculosis* were resistant to a median of six drugs. The 75 patients received treatment with 58 different regimens and lasting a median of 23 months (range, 0.4 to 35.9). PAS was included in the treatment of 90% of the patients. The records of 60 patients were reviewed systematically for adverse events; 44 patients (73 percent) had such events, and all events were managed without physician-directed discontinuation of therapy. Among the 66 patients who completed four or more months of therapy, 83% were probably cured at the completion of treatment. Five of these 66 patients (8 percent) died while receiving therapy. Only one patient continued to have positive cultures after six months of treatment. All patients in whom treatment failed or who died had extensive bilateral pulmonary disease. In a multiple Cox proportional-hazards regression model, the predictors of the time to treatment failure or death were a low hematocrit and a low body-mass index. Inclusion of pyrazinamide and ethambutol in the regimen (when susceptibility was confirmed) was associated with a favorable outcome. These finding indicate that with an early initiation of appropriate therapy, which can preserve susceptibility to first-line drugs community-based outpatient treatment of multidrug-resistant tuberculosis can yield high cure rates even in resource-poor settings [70].

Mitnick et al. have described the management of extensively drug-resistant tuberculosis and treatment outcomes among patients who were referred for individualized outpatient therapy in Peru. From a total of 810 patients who were referred for free individualized therapy 48 (7.4%) had extensively drug-resistant tuberculosis; the remaining 603 patients had multidrug-resistant tuberculosis. The patients with extensively drug-resistant tuberculosis had undergone more treatment than the other patients and had isolates that were resistant to more drugs. None of the patients with extensively drug-resistant tuberculosis were co-infected with the human immunodeficiency virus (HIV). Patients with extensively drug-resistant tuberculosis received daily, supervised therapy with an average of 5 drugs, including cycloserine, an injectable drug, and a fluoroquinolone. Several principles of management of highly resistant disease were concurrently applied to all patients in this program. Aggressive regimens with many drugs, at the highest tolerated dose level were used to maximize the chemotherapeutic benefit. Treatment was protracted, lasting more than 2 years in most patients. The results of drug-susceptibility testing were used to design (and adjust) regimens containing at least five drugs that were likely to be effective whenever possible. Regimens relied heavily on three agents with little prior use in Peru, namely aminosalicylic acid (in 96% of XDR-TB patients), capreomycin (in 53% of XDR-TB patients), and cycloserine (in 100% of XDR-TB patients). Twenty-nine of the XDR-TB

patients (60.4%) completed treatment or were cured, as compared with 400 patients (66.3%) with multidrug-resistant tuberculosis. These findings indicate that extensively drug-resistant tuberculosis can be cured in HIV-negative patients through outpatient treatment, even in those who have received multiple prior courses of therapy for tuberculosis [71].

Leimane presented the clinical outcomes of combined treatment using different regimens (incl. PAS among other agents) in Latvia, which has one of the highest rates of multidrug-resistant tuberculosis (MDR-TB) worldwide. The authors assessed the treatment outcomes for the first full cohort of MDR-TB patients treated under Latvia's DOTS (directly observed therapy short course) strategy following WHO guidelines. All civilian patients who began treatment with individualized treatment regimens for pulmonary MDR-TB in Latvia between Jan 1, and Dec 31, 2000 were analyzed. The 204 patients under investigation received initial treatment with 107 different initial empirical regimens. Of the drugs given for 3 months or more, patients were given a median of six drugs (range 3–8). The most common oral medications used were aminosalicylic acid, ofloxacin, protionamide, thioacetazone, pyrazinamide, ethambutol, and cycloserine. Kanamycin was the most commonly used injectable medication, followed by capreomycin. PAS was given to a total of 90 patients. The degree of drug resistance was high at the start of treatment. Resistance to second-line anti antituberculosis drugs in all patients was most common against kanamycin, protionamide, aminosalicylic acid, and thioacetazone. Initial resistance to PAS was encountered in 46% of the patients never treated for tuberculosis, in 45% of those previously treated and in 40% in those that have received previous treatment for MTR-TB. Of the 204 patients assessed, 55 (27%) had been newly diagnosed with MDR-TB, and 149 (73%) had earlier been treated with first-line or second-line drugs for this disease. Assessment of treatment outcomes showed that 66% patients were cured or completed therapy, 7% died, 13% defaulted, and treatment failed in 14%. Of the 178 adherent patients, 135 (76%) achieved cure or treatment completion. In a multivariate Cox proportional-hazards model of these patients, independent predictors of poor outcome (death and treatment failure) included having previously received treatment for MDR-TB, the use of five or fewer drugs for 3 months or more, resistance to ofloxacin, and body-mass index less than 18.5 at start of treatment [72].

Keshavjee et al. conducted a retrospective cohort study of 608 patients with multidrug resistant tuberculosis who had treatment in civilian or prison services, between Sept 10, 2000, and Nov 1, 2004, according to the treatment strategy recommended by WHO. The main outcome was the frequency of poor and favorable outcomes at the end of treatment. Of 608 patients with multidrug resistant tuberculosis, 29 (4.8%) patients had baseline XDR tuberculosis. The drugs employed have included rifampicin, pyrazinamide, ethambutol, kanamycin, capreomycin, ethionamide, cycloserine, aminosalicylic acid (used in the majority of both MDR and XDR cases), amikacin, ofloxacin and amoxicillin/clavulanate. Treatment failure was more common in patients with XDR tuberculosis than in those with non-XDR tuberculosis (31% vs. 8.5%). 48.3% of patients with XDR tuberculosis and 66.7% of patients with non-XDR tuberculosis had treatment cure or completion (p=0.04). The frequency and management of adverse events did not differ between patients with XDR and non-XDR tuberculosis. The chronic features of tuberculosis in these patients suggest that extensively drug-resistant tuberculosis may be acquired through previous treatments that include second-line drugs. Nevertheless the finding from this study suggest that aggressive management of this infectious disease is feasible and can prevent high mortality rates and further transmission of drug-resistant strains of *Mycobacterium tuberculosis* [73].

Dheda et al. have presented retrospective cohort study to assess the early treatment outcomes from antituberculosis chemotherapy in patients with extensively drug-resistant (XDR) with relation to their HIV status. The authors analysed the case records of patients (>16 years old) with XDR tuberculosis (culture-proven at diagnosis) between August, 2002, and February, 2008, at four provincial treatment facilities in South Africa. Treatment of XDR disease was given in hospital, and individualized with the use of capreomycin (in 92% of cases) and aminosalicylic acid (in 91% of cases) as the main drugs, with other first-line and second-line drugs used at the discretion of the attending clinician, or to which the microorganism showed susceptibility; patients were given a median of seven drugs per regimen. Highly active antiretroviral therapy was offered to all patients infected with HIV. 195 out form a total of 227 patients were analysed. 21 died before initiation of any treatment, and 174 patients (82 with HIV infection) were treated. 62 (36%) of these patients died during follow-up. Initiation of treatment resulted in culture conversion in 33 (19%) of 174 patients, and the probability of culture

conversion did not differ by HIV status. 23 (70%) of these patients converted by 6 months, 28 (85%) by 9 months, and 30 (91%) by 12 months. Before the date for censor follow-up, two (6%) of 33 patients with XDR tuberculosis who had converted had reverted back to culture-positive status. In a univariate Cox regression analysis, low weight (<50 kg) before treatment was associated with conversion failure. The number of deaths was not significantly different in patients with or without HIV infection. Treatment with moxifloxacin, previous culture-proven multidrug-resistant tuberculosis, and number of drugs used in a regimen were independent predictors of death. Fewer deaths occurred in patients with HIV infection given highly active antiretroviral therapy than in those who were not. The clinical data showed a high incidence of adverse events associated with drugs used for the treatment of XDR tuberculosis incl. gastrointestinal disturbances, renal and electrolyte abnormalities, hypothyroidism etc. Adverse drug reactions were reported in 58% of the patients. 36% of the adverse drug reactions required no intervention; 43% needed modification in the duration of treatment or frequency of administration of the drug, or prescription of an additional drug to treat the adverse event; the drug causing the adverse reaction was stopped in 16% reactions; and 4% of the patients died (five with rapidly deteriorating renal failure and one with hypokalemia) at a median of 14 days after starting capreomycin. Highly active antiretroviral therapy was generally well tolerated [74].

Lessnau and Qarah have presented valuable data regarding the clinical efficacy of PAS in combination with other drugs for the treatment of MDR-TB in pregnant women. The authors described a case report of a woman at 23 weeks‘ gestation who was previously treated with rifampicin, isoniazid, and ethambutol for cavitary tuberculosis. She did not respond within 3 weeks, and multidrug-resistant tuberculosis (MDR-TB) was suspected. Direct plating on susceptibility media was performed immediately. Treatment was initiated with venous capreomycin, levofloxacin, aminosalicylic acid, pyrazinamide, cycloserine, and high-dose vitamin B6 at 26 weeks‘ gestation. The patient delivered vaginally at week 35. The placenta appeared normal on pathology examination. The infant’s tuberculin skin test and three nasogastric aspiration cultures were negative for tuberculosis. A neurologic examination and electrophysiological hearing studies of the infant were normal. The baby continued to thrive, and therefore a lumbar puncture was not performed. Following delivery, ethionamide was added as a sixth drug, and levofloxacin was replaced with moxifloxacin. The patient‘s sputum became smear-negative and culture-negative for TB. All reported cases of MDR-TB during pregnancy are reviewed. The authors have also reviewed the clinical outcomes in other available cases of MDR-TB in pregnancy. In 1981, 16 pregnant women were reported to have isoniazid-resistant tuberculosis. All were treated with first-line medications and PAS during pregnancy. One patient had a spontaneous abortion after 4 months of pregnancy. After birth, one infant died of miliary tuberculosis at 3 months, and one infant died of meningeal tuberculosis. Another infant developed pulmonary tuberculosis from inhalation at 6 months. The 12 remaining pregnancies had positive outcomes [6].

Avendano et al. have reviewed the clinical management and long term outcome of 40 non-HIV-infected individuals with MDR-TB referred to the only specialized TB inpatient service in Ontario. The study enrolled 21 men and 19 women (mean age 41±18 years) who were admitted between January 1986 and June 1999 with MDR-TB and negative serology for HIV. Thirty-eight patients (95%) were born outside of Canada. Twenty-six patients (65%) had a history of previous TB. All were symptomatic with productive cough and positive sputum smears for acid-fast bacilli. There was a delay of 4.5 months between the initial diagnosis of TB and the recognition of the presence of MDR-TB. Four patients (10%) had tuberculosis resistant to isoniazid and rifampicin only. Over 50% of patients had TB with additional resistance to streptomycin, and over 40% had additional resistance to ethambutol. Coexisting resistance was also found in significant numbers for PAS, pyrazinamide, ethionamide, para- and cycloserine. If the resistance pattern was known the initial treatment included at least three bactericidal agents. Patients with tuberculosis resistant to all first-line drugs were treated with a regimen containing amikacin or capreomycin, a fluoroquinolone, clofazimin, with or without PAS, cycloserine and ethionamide. Bacteriological conversion was achieved in 34 patients (85%). Six patients underwent surgical resection for localized lung disease. Treatment was continued for 2 years following the bacteriological conversion. Twenty-four patients completed treatment and have remained free of disease for 33±25 months. All five patients (12%) who died had longstanding disease before their referral [75].

Extra-pulmonary disease

PAS has been well documented to be effective in diverse types of extra-pulmonary disease incl. intestinal, meningeal, musculo-skeletal, skin, genitourinary infections etc [1, 2, 76-79]. The following section gives a concise outline of some of the more illustrative trials, non-controlled studies or case reports. A detailed description of the early reports with PAS monotherapy in extrapulmonary infections are generally omitted as currently PAS monotherapy is of no value for the treatment of active tuberculous disease.
The early clinical experience with the drug in tuberculous meningitis shows, that intravenous infusions of PAS in combination with streptomycin have shown definite advantages over streptomycin alone. Thus the full effect of PAS is first manifest after four to eight months, and resistance to it emerges later and more slowly than it does to streptomycin [1, 2]. This has been confirmed by the experience of DeJanney et al. who have found PAS highly valuable in the treatment of tuberculous meningitis that relapse after a favorable response to streptomycin therapy. In this series all relapse cases of tuberculous meningitis treated with streptomycin alone have died. The only survivals have been those treated with combined streptomycin and PAS or only with p-aminosalicylic acid [56].
Lehmann has described a study conducted by Carstensen and Sjölin on the treatment of 22 cases of secondary intestinal tuberculosis with PAS. The authors report a dramatic effect that there was no longer any doubt about the clinical efficacy of the drug. These results were later corroborated by the findings from a trial of Källquist with 22 other cases of intestinal tuberculosis which were verified and followed by X-ray examination [2].

The clinical efficacy of PAS-based combined regimens for the treatment of tuberculosis of the spine has been well-documented in clinical trials [76-79].
Konstam was the first to report an ambulatory, medical approach for tuberculous spondylitis. He reported treatment of 207 patients with isoniazid and PAS for at least 12 months (until there was radiographic improvement) and did not include bracing or immobilization as part of the therapy. Surgery was only performed in 27 patients who needed abscesses drained. Eighty-six percent of patients exhibited complete recovery. For the first time it was demonstrated that patients with vertebral tuberculosis could be cured with chemotherapy alone and without prolonged immobilization or a complicated surgical procedure [80].

A five year assessment of controlled trial in Korea has shown favorable therapeutic effects of PAS in combination with isoniazid or isoniazid + streptomycin. The analysis has enrolled cases from two centers in Korea. 350 patients with a diagnosis of tuberculosis of the thoracic and/or lumbar spine were allocated at random: in Masan to in-patient rest in bed (IP) for six months followed by out-patient treatment or to ambulatory out-patient treatment (OP) from the start; in Pusan to out-patient treatment with a plaster-of-Paris jacket (J) for nine months or to ambulatory treatment without any support (No J). All patients received chemotherapy with PAS with isoniazid for eighteen months, either supplemented with streptomycin for the first three months (SPH) or without this supplement (PH), by random allocation. The main analysis of this report concerns 299 patients (eighty-three IP, eighty-three OP, sixty-three J, seventy No J; 143 SPH, 156 PH). Pre-treatment factors were similar in both centers except that the patients in Pusan had, on average, less extensive lesions although in a greater proportion the disease was radiographically active. One patient (J/SPH) died with active spinal disease and three (all No J/SPH) with paraplegia. A fifth patient (IP/PH) who died from cardio respiratory failure also had pulmonary tuberculosis. Twenty-three patients required operation and/or additional chemotherapy for the spinal lesion. A sinus or clinically evident abscess was either present initially or developed during treatment in 41% of patients. Residual lesions persisted in ten patients at five years. Thirty-two patients had paraparesis on admission or developing later. Complete resolution occurred in twenty on the allocated regimen and in eight after operation or additional chemotherapy or both. Of the remaining four patients, all of whom had operation and additional chemotherapy, three died and one still had paraparesis at five years. Of 295 patients assessed at five years 89% had a favorable status. The proportions of the patients responding favorably were similar in the different series and sub-series, namely: IP (91%), OP (89%); J (90%), No J (84%); and in the SPH (86%) and PH (92%) series [77].

An extended follow-up of the preceding study confirmed the results and also showed additional evidence from the clinical experience in 150 cases of tuberculosis of the spine in Hong Kong. The patients were treated with PAS plus isoniazid plus streptomycin (for the first three months) over 18 months. All 150 patients were randomized to either radical excision of the spinal lesion with bone graft or open debridement. On average at 15 years the great majority of patients in achieved a favorable status, no evidence of CNS involvement, no radiological evidence of dis-

ease, no sinus or clinically evident abscess, and no restriction of normal physical activity. Most patients had already achieved a favorable status much earlier. The earlier results of these trials are confirmed by the long-term follow-up with no late relapse or late-onset paraplegia [78].

Another comparative study has enrolled 265 patients with tuberculosis of the thoracic and/or lumbar spine who were followed-up for three years from the start of treatment. The patients were randomly allocated to four daily regimens of chemotherapy, namely: i) isoniazid plus rifampicin for 6 months (6HR, 65); ii) the same drugs as in i) but for 9 months (9HR, 71); iii) isoniazid plus aminosalicylic acid (PAS) or ethambutol for 9 months (9P/EH, 62); or iv) the same drugs as in iii) but for 18 months (18P/EH, 67). All patients were ambulatory from the start of chemotherapy and no form of support or operation was used in any case. Over half (55%) the patients were children and one-third had sinuses or clinically evident abscesses. At three years a favorable status, defined as no sinus nor clinically evident abscess, no myelopathy with functional impairment, no surgery nor additional chemotherapy, full physical activity with disease quiescent clinically and radiographically, was achieved in 203 patients (77%) and in another 41 (15%) in all respects except radiographically. Only 20 patients (8%) had an unfavorable status the proportion being highest (19%) in the 9P/EH series. Thirteen of these were classified as unfavorable solely because they had needed additional chemotherapy; only seven still had an unfavorable status at three years. The clinical results at three years were thus excellent in all treatment regimens series except the 9P/EH, in which more patients had required additional chemotherapy. In the 88 patients with sinuses or abscesses on admission, the rate of resolution was similar in all the series, whereby most lesions (83%) had resolved by 12 months [79].

A case-report of primary tuberculosis of the conjunctiva successfully treated with streptomycin and PAS was presented. A female patient with tuberculosis conjunctivitis was admitted to hospital; throughout her stay she was afebrile. She was given streptomycin 750 mg/day, and PAS 15 g/day. One month later she was transferred to a sanatorium regime. Five months after initiation of chemotherapy the streptomycin dose was reduced to 750 mg twice weekly, and after an additional month when the conjunctiva was completely healed she was discharged from the hospital. Streptomycin and PAS were discontinued after nine months treatment. By this time the eye appeared normal, apart form a little scar at the site of biopsy [81].

Pauker et al. described the successful treatment of BCG osteomyelitis with a PAS-based regimen. A 3 year boy presented with an osteolytic lesion and periosteal reaction in the distal metaphysis of the right femur which failed to respond to immobilization and intensive antibiotic treatment. Since the infection seemed to be of low virulence and fewer than 4 years had elapsed since BCG vaccination BCG osteomyelitis was suspected. Because of the slow progression it was decided to carry out a trial treatment with tuberculostatic drugs alone. Sodium PAS 2 g/d, isoniazid 75 mg/d, and streptomycin intramuscularly twice weekly were given for a period of 2 months. 5 weeks after treatment began the boy still had a slight limp but did not complain of pain. X-ray examination showed that the periosteal reaction had disappeared. A month later the limp had also gone and treatment was stopped. When last seen 3 years later he had no symptoms or signs. A radiograph of the femur showed a normal bone structure, no periosteal reaction, and the epiphyseal plate undamaged [82].

- **Efficacy as a non-chemotherapeutic agent**

PAS has been used in the treatment of mild to moderate ulcerative colitis in patients who are intolerant of sulfasalazine and in the treatment of Crohn's disease; the drug is designated an orphan drug by the FDA for use in these conditions [31]. In an exemplary trial Ginsberg et al. have described a trial enrolling 40 patients with ulcerating colitis who had been treated with 4 g PAS daily or with placebo. After 12 weeks, 55% of the patients in the PAS-group showed clinical improvement but only 5% of the patients in the placebo group [83]. Schreiber et al who had tested the effect of different derivatives of the salicylic acid in Crohn's disease have found that the PAS substituted in the 4-position was approximately twice as effective as the 5-PAS [84].

PAS has been found to lower serum lipids, an effect which is not entirely due to the well established malabsorption syndrome and steatorrhea it induces [23, 26]. Clinical experience with PAS ascorbate (PAS-C) has shown that it significantly decreased very low-density lipoprotein-cholesterol and very low-density lipoprotein-triglyceride, but the low-density lipoprotein-cholesterol/light-density lipoprotein-cholesterol ratio did not change. Fourteen hyperlipidemic patients received PAS ascorbate at a dose of 6 to 8 grams/day in 3 divided doses for 3 months. Cholesterol and triglyceride levels were monitored monthly and were compared with baseline values. The response to the drug was erratic and diarrhea was

a significant adverse effect. These data indicate PAS is not as safe or effective as other agents for lowering cholesterol and triglyceride level and hence, at present its use in dyslipidemia is not warranted [30].

Both clinical and non-clinical findings have shown that PAS is of value for the management of manganese intoxications [85-87].

IV. Safety profile

- **Adverse drug reactions**

The incidence of untoward effects associated with the use of PAS is approximately 10% to 30%. Gastrointestinal problems following oral administration, including anorexia, nausea, epigastric pain, abdominal distress, and diarrhea—are predominant and often limit patient adherence [14, 30, 31]. Patients with peptic ulcers tolerate the drug especially poorly. After intravenous application the drug is far better tolerated and the most important gastrointestinal side effects are colics and cramps following high dose regimens. Nevertheless gastrointestinal irritation could not be ruled out in parenteral treatment, especially in high-dose regimens due to partial secretion of the drug into the GIT [19, 26].

While some gastrointestinal effects including mild irritation could be evident in up to 70% of the patients these are rarely severe enough to warrant discontinuation of the drug. Treatment cessation due to gastrointestinal discomfort is relatively rare and is obligatory in the rare occasions of gastric bleeding, peptic ulcer as well as in ca. 4% of affected patients due to nausea and vomiting as well as in 1-2% of patients with persisting diarrhea [19, 26].

Hypersensitivity reactions to PAS are seen in 5% to 10% of patients. High fever may develop abruptly, with intermittent spiking, or it may appear gradually and be low-grade. Generalized malaise, joint pains, and sore throat may be present at the same time. Skin eruptions of various types appear as isolated reactions or accompany the fever. At this time results form the laboratory test are usually within normal limits, but eosinophilia and other abnormalities could be present as well. If the offending PAS is discontinued the patient soon recovers, if not the reaction becomes progressively worse and is often accompanied by skin reactions, incl. exfoliative dermatitis that could be severe; hepatitis; renal abnormalities and occasionally severe blood dyscrasias and cardiologic abnormalities. Severe reactions can be fatal [14, 23, 31].

The hematological aberrations that have been observed are leukopenia, agranulocytosis, eosinophilia, lymphocytosis, an atypical mononucleosis syndrome, and thrombocytopenia (in some instances regarded as induced by a glycine conjugate rather than by the parent drug). Acute hemolytic anemia may appear in some instances [19, 31, 88].

In a review of 7,492 patients being treated for tuberculosis, 38 (0.5%) developed hepatitis, of which 28 cases (0.3%) were attributed at least in part to the administration of PAS [14]. A mortality rate as high as 21% for hepatotoxicity has occurred in patients continuing to receive PAS following signs of hepatitis. Signs and symptoms of hepatitis, which included pyrexia followed by jaundice, usually appear 4 weeks after initiating therapy. Elevated serum transaminases (alanine aminotransferase and aspartate aminotransferase) may be present even in the absence of jaundice [23, 89].

Usually the hypersensitivity reactions appear between the 2-7 weeks of treatment and peak at the fifth week. If a drug or group of antituberculous drugs however is tolerated well for at least four months a full course of chemotherapy is usually feasible [26].
It is worth mentioning that patients who develop hypersensitivity to one antituberculous agent may be at greater than usual risk of reacting to others. In case of PAS cross-reactivity is especially prominent in patients allergic to salicylates. When such reactions occur all chemotherapy should be discontinued unless the tuberculosis is life-threatening, in which case drugs which are least likely to contribute to hypersensitivity reactions are continued. Desensitization to PAS as well as to streptomycin and other agents may be successful. Nevertheless in view with the number of effective alternative drugs available it is not advisable to risk continuing treatment with an agent that has caused such a severe reaction [14, 19, 26, 31].
PAS may cause Coombs'-positive hemolytic and hematuria in patients with glucose-6-phosphate dehydrogenase deficiency. Hypokalemia, acidosis, albuminuria, and crystalluria have occurred occasionally in patients receiving PAS. Crystalluria may be prevented by maintaining the urine at a neutral or alkaline pH [30, 31].

Goiter, with and without myxedema, has been reported in patients receiving prolonged high-dose therapy with some PAS products Goiter development can be prevented by administration of thyroxin but not iodide [14, 23].

Aminosalicylate sodium given intravenously poses risk of hypokalemia, acidosis, cation loss, and has been reported to induce local irritation and/or phlebitis. It has been occasionally associated with anaphylactic shock or other severe anaphylactic reactions [19, 26, 41].

It is noteworthy that despite the immense clinical experience with PAS 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. For this reason in the following section emphasis is placed on those adverse effects that are well documented and occur more than rarely. Significant rare reactions as well as ones that have not been verified are also quoted.

- **Safety in pregnancy and lactation**

No studies have been done in humans, but nevertheless PAS has been used safely in pregnancy. The drug should be used only if there are no alternatives for a pregnant woman who has multidrug-resistant tuberculosis [14, 31, 90]. In reproduction studies with rats at doses within the human dose range, occipital malformations were observed. Nevertheless no adverse effects on the fetus were observed in rabbits treated with 5 mg/kg/day throughout gestation [90]. No reports describing the placental transfer of PAS have been located. The molecular weight (approximately 153) is low enough, however, that passage to the fetus should be expected [90].

The Collaborative Perinatal Project monitored 50,282 mother-child pairs, 43 of whom had 1st trimester exposure to PAS. Congenital defects were found in five infants. This incidence (11.6%) was nearly twice the expected frequency. No major categories of malformations or individual defects were identified. An increased malformation rate for ear, limb, and hypospadias has been reported for 123 patients taking 7–14 g of PAS per day with other antitubercular drugs. An increased risk of congenital defects has not been found in other studies [90].

In an earlier survey it has been suggested that children born to women with pulmonary tuberculosis may show an excess of serious congenital malformations. Among a random sample of 3,295 pregnant women who were interviewed at their first antenatal visit and followed up to ascertain the outcome of their pregnancies, 61 gave a history of pulmonary tuberculosis and four of the children born to them were subsequently found to have major congenital defects (two were anencephalic, one had absent kidneys, and one a cardiac defect. Thus McDonald has suggested that infants born to women with a history of tuberculosis show an excess of serious congenital defects. An detailed study of the outcome of 253 pregnancies in women known to have been on the active register of the Cardiff Chest Clinic however has failed to substantiate this. During the first months of 74 of the pregnancies, various combinations of isoniazid, PAS, and streptomycin had been administered. There was nothing to suggest that any of the drugs was teratogenic. The data indicate that chemotherapy in women with respiratory tuberculosis no longer poses an increased risk of stillbirth in pregnancy or of infant death after delivery [91].

Marcus also has presented valuable clinical evidence regarding the safe utility of PAS during pregnancy. The report analyzed 19 children, delivered of 18 mothers (1 twin). Fifteen mothers were already being treated at the time of conception, one started treatment at 4 weeks and two at 8 of weeks gestation. Eleven were treated with streptomycin 1 g daily, and two with 1 g three times a week ; 14 with PAS 10 - 15 g daily; and 17 on isoniazid 500 - 700 mg daily. Six were also being treat with other antituberculous drugs, e.g. 2 with isoxyl 1.5 g and 5 g daily: 3 with pyrazinamide 2- 3 g daily: 3 with ethionamide 500 - 750 mg daily: and 2 with thiacetazone 120 mg/day. These drugs were used until at least the end of the first trimester. Of the 19 babies, one was born at 36 weeks. The babies were examined at ages ranging from week to one year with an average age of 3.8 months. The only abnormalities found were 2 umbilical hernias (common in the native Africans), with a slight divarication of the rectum, and 1 child whose left testis was smaller than the right, There were no stillbirths or neonatal deaths. 411 the children followed up eventually and were considered healthy and free of adverse effects [92].

Aminosalicylic acid is excreted into human breast milk. In one non-breast-feeding patient given an oral 4-g dose of the drug, a peak milk concentration of 1.1 μg/mL was measured at 3 hours with an elimination half-life of 2.5 hours [32]. The peak maternal plasma concentration, 70.1 μg/mL, occurred at 2 hours [90].

- **Drug interactions**

Pharmacokinetic interactions

Aminosalicylic acid may reduce the acetylation of isoniazid, especially in patients who are rapid acetylators, resulting in increased isoniazid serum levels. The severity of this interaction is moderate is usually of delayed onset and is considered clinically insignificant [19, 23, 26]. Nevertheless the magnitude of this interaction and its sequelae has not been studied with the presented parenteral formulation. It has to be stressed out however that at aminosalicylate serum

concentrations exceeding 100 μg/ml (e.g. in infusion therapy) some modest increase in serum isoniazid concentrations is possible due to displacement from plasma proteins as well [23]. On these grounds the patients should be monitored for symptoms of isoniazid toxicity (nausea, vomiting, dizziness, slurring of speech, blurred vision, and visual hallucinations) [30].

Older oral dosage forms of PAS, containing bentonite have been reported to reduce serum rifampicin concentrations but there is no such interaction in case of novel formulations free from this additive [23]. Such interaction is absolutely impossible and is clinically irrelevant in case of co-administration of rifampicin with sodium aminosalicylate infusion.

PAS has been documented to decrease digoxin bioavailability leading to reduced digoxin serum concentrations presumably due to an effect on gut wall functions [30]. In a literature report in two out of ten patients, digoxin levels were reduced by 40% eight hours after the last dose of PAS 2 grams four times daily. The mechanism of this effect is presumably due to inhibition of the function of intestinal absorbing cells by PAS [30, 31].

PAS appears to increase phenytoin plasma levels [19, 31]. In such occasions phenytoin toxicity could be expected mainly in patients receiving both PAS isoniazid (especially slow acetylators), the latter also known to increase plasma phenytoin levels. On these grounds tuberculosis patients receiving PAS plus isoniazid and phenytoin should be closely observed for signs of phenytoin intoxication [31].

Long-term therapy with PAS has been shown to reduce the absorption of vitamin B12 from the gastrointestinal tract, possibly resulting in cyanocobalamin deficiency. [30]. The mechanism of this interaction remains elusive; it may be related to an PAS-induced malabsorption syndrome. No abnormally in the production of intrinsic factor (IF) or its binding has been found, nor is malabsorption of vitamin B12 corrected by coadministration of IF [23]. Theoretically, higher doses of oral cyanocobalamin may be required in patients treated with PAS. However, this interaction is of doubtful clinical relevance concerning intravenous infusion with sodium aminosalicylate unless large doses are applied for prolonged periods; patients receiving PAS for more than one month may require supplemental cyanocobalamin [30].

The treatment with PAS is associated with reduced absorption of dietary folate in turn could increase the toxicity of antifolate antineoplastic agents such as methotrexate [19].

Coadministration with diphenhydramine is associated with reduced absorption of PAS [19]; this interaction is of no significance as far as the parenteral route of PAS application is considered.

With the only exception of diclofenac, the non-selective NSAIDs especially salicylates can increase the plasma concentrations and reduce the clearance of PAS leading to potentiation of the adverse effects of the antitubercular agent [19, 93]. This interaction is especially troublesome with phenylbutazone. The latter displaces PAS from albumin binding sites and also inhibits the renal tubular secretion of both PAS and its metabolites which could leas to increased plasma levels and toxicity [26].

Concomitant probenecid has been reported to increase serum concentrations of PAS, at least transiently, and it has been suggested that probenecid be used with caution in patients receiving PAS. However, limited data available to date suggest that concurrent administration of these drugs does not result in clinically important increases in PAS concentrations [31].

Ammonium chloride should not be used in patients receiving PAS because of the increased probability of crystalluria during therapy [31].

Pharmacodynamic Interactions

Co-administration of PAS and ethionamide may intensify the adverse effects of PAS in patients receiving this combination of agents especially its hepatotoxicity and antithyroid effects [23, 30, 93]. Adverse reactions may include jaundice, hepatitis, nausea, vomiting, diarrhea, abdominal pain, or anorexia [30]. The severity of the effects is moderate. Patients receiving combined PAS and ethionamide should be monitored for excessive side effects, especially hepatotoxicity, antithyroid effects and gastro-intestinal distress [30]. This combination of agents should only be administered to patients found to have normal baseline liver and thyroid function. Regular liver function tests should be performed in patients receiving this combination of agents [30]. Patients with diabetes mellitus are more likely to experience hepatotoxicity and have more difficulty with diabetic management; these patients need close monitoring. Ethionamide should be withdrawn if significant, intolerable adverse reactions develop [30].

Concomitant use of angiotensin-converting enzyme inhibitors and PAS can reduce the antihypertensive effect of the latter, and the use of calcium channel blockers can increase the anticoagulant effect of PAS [93].

Simultaneous utilization of PAS and carbonic anhydrase inhibitors may potentiate the adverse effects of both drugs [93], especially having into consideration recent data indicating that the antituberculosis agent has intrinsic inhibitory activity on carbonic anhydrase as well [94].

Co-administration of PAS and systemic corticosteroids can increase the number and severity of adverse effects, especially the gastrointestinal disturbances [93].

PAS can reduce the effect of loop diuretics, and, *vice versa*, loop diuretics can increase the serum levels of PAS [93].

PAS has been associated with hypoglycemia in diabetes patients [30, 95] and conversely can increase the hypoglycemic effects of sulfonylureas [93]. Moreover it has been shown to increase prothrombin time [96], which poses an increased risk of bleeding when administered in conjunction with oral anticoagulants, fibrinolytics, salicylates or anti-platelet drugs [93].

PAS has been reported to interfere technically with the serum determinations of albumin by dye-binding, SGOT by the azoene dye method and with qualitative urine tests for ketones, bilirubin, urobilinogen or porphobilinogen. PAS reportedly causes false-positive results with cupric sulfate solution (Benedict's reagent) for urine glucose determinations. It dose not interfere with the enzymatic test for blood glucose monitoring [26, 31].

Conclusion and prospectus

Para-aminosalicylic acid was historically the second medicine to be introduced for the treatment of tuberculosis, in 1946, and was an inevitable part of standard treatment regimens up-until the 1970s. Eventually its use recommenced in the 1990s with the emergence of multidrug-resistant tuberculosis. Noteworthy, approximately 450,000 cases of multidrug-resistant tuberculosis occur globally every year, which corresponds to roughly 5% of the world's annual burden of tuberculosis. In the European Union, however tuberculosis is an orphan indication. It was estimated in 2011 to occur in 2.3 out of 10,000 people. Nevertheless, multidrug-resistant tuberculosis is associated with a very high mortality rate and poses a significant public-health threat as patients infected with drug-resistant strains are unable to receive adequate curative chemotherapy and can potentially spread their infection.

To address the issue of growing resistance to antituberculous agents the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) has recommended an centralized authorization of para-aminosalicylic acid for use in combination with other medicines against multidrug-resistant tuberculosis. The Committee has recommended granting centralized marketing authorizations for para-aminosalicylic acid in EU against multidrug-resistant tuberculosis in adults and pediatric patients when an effective treatment regimen cannot otherwise be devised for reasons of resistance or tolerability.

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