6

Variability of Plasma Methadone Concentration in Opiate Dependent Receiving Methadone: A Personalised Approach Towards Optimizing Dose

Nasir Mohamad[1,3], Nor Hidayah Abu Bakar[2], Tan Soo Choon[3], Sim Hann Liang[3], NIM Nazar[3], Ilya Irinaz Idrus[3] and Rusli Ismail[3]
[1]*Department of Emergency Medicine, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan,*
[2]*Department of Pathology, Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan,*
[3]*Pharmacogenetic Research Group, Institute for Research in Molecular Medicine (INFORMM), Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia*

1. Introduction

1.1 Methadone and methadone maintenance therapy (MMT): An overview

Methadone acts on the opioid receptors and produces many of the same effects of morphine and heroin. In the treatment of opioid dependence, methadone has cross-tolerance with other opioid, including heroin and morphine and a long duration of effect. Higher doses of methadone can block the euphoric effects of heroin, morphine, and similar drugs. As a result, properly dosed methadone patients can reduce or stop altogether their use of these substances.

Methadone is a misunderstood drug and ignorance about it is common. Even professionals, physicians and pharmacists who are supposed to be the "guardians" of MMT receive very little training about the very medication that they are responsible for. To compound the issue, addiction is mostly viewed not as a disease and its care is frequently relegated to the lay public, at least until very recently. In Malaysia, addiction has solely been under the charge of "*Agensi Anti Dadah Kebangsaan*" (AADK), an agency that has mainly adopted a criminal approach to addiction. However, this has recently changed in Malaysia. Addiction is now recognized as a medical illness, under the purview of the medical professionals.

Nevertheless, many in the medical profession only have a rudimentary understanding of addiction. Most physicians, pharmacists and nurses receive very little training about addiction and much less regarding methadone. Thus, generally, both medical and other caregivers have very limited knowledge about addiction and much less about methadone. They have generally been taught to approach addiction as a character disorder and administer methadone as a substitute.

1.2 Pharmacogenetic of methadone

Methadone has variable pharmacology. It binds to the μ-opioid receptor, the NMDA ionotropic glutamate receptor to exert its effects. Its metabolism is mediated by several enzymes including CYP3A4, CYP2B6 and CYP2D6, enzymes that are polymorphic and hence exhibit great variability. It is mainly administered through the oral route and adverse effects include hypoventilation, constipation and miosis, in addition to tolerance, dependence and withdrawal difficulties.

As a full μ-opioid agonist, methadone exhibits all the opiate-like effects. Furthermore, its binding to the glutamatergic NMDA (N-methyl-D-aspartate) receptor. This makes it a receptor antagonist against glutamate which is the primary excitatory neurotransmitter in the CNS. NMDA receptors modulate long term excitation and memory formation. NMDA antagonists such as dextromethorphan (DXM), ketamine, tiletamine and ibogaine have been studied for their role in decreasing the development of tolerance to opioids and as possible for eliminating addiction /tolerance /withdrawal. Its action on the NMDA has been proposed as a mechanism by which methadone decreases craving for opioids (Xiao *et al*, 2001).

Methadone is a lipophilic drug and requires biotransformation for elimination. It has a slow metabolism and is longer lasting than morphine-based drugs. Typically its elimination half-life ranges from 15 to 60 hours with a mean of around 22 hours. Due to the polymorphic nature of its metabolism, its metabolism rates vary greatly between individuals, up to a factor of 100. This variability is apparently due to genetic variability in the production of the associated enzymes CYP3A4, CYP2B6 and CYP2D6. Several studies have been conducted to explain the intra- as well as inter-individual variability in methadone's pharmacokinetic and clinical response. Typically, methadone is a substrate for several CYP450 enzymes as well as P-glycoprotein (PGP). Many Single Nucleotide Polymorphisms (SNPs) have been reported to contribute to its variability. Furthermore, as it binds to μ-receptors, SNPs in *OPRM* gene that encodes for these receptors may contribute to the clinical response in MMT patients. Thus, SNPs in *OPRM* gene, *CYP* gene and *ABCB1 (MDR1)* gene may contribute to determine the clinical outcomes of the MMT (Lötsch *et al*, 2009).

1.3 Pharmacokinetic of methadone

The pharmacokinetic parameters of methadone were first published in 1975 (Verebely *et al*, 1975). Methadone is a lipophilic basic drug with a pKa of 9.2, which is administered orally in a racemic mixture. There is strong evidence that the enantiomers differ in their distribution and elimination, though the majority of the studies were carried out on the racemic mixture. It has been suggested that methadone undergoes adaptive changes during chronic use according to the administered doses.

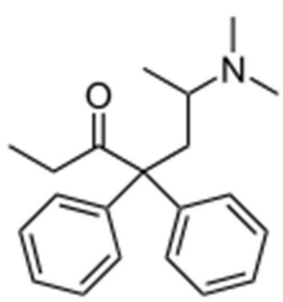


Fig. 1.1. Methadone, (*RS*)-6-(Dimethylamino)-4,4-diphenylheptan-3-one

Several attributes have been suggested such as clearance and *CYP3A4*. Accordingly, several pharmacokinetic studies have been carried out to investigate whether therapeutic drug monitoring (TDM) is effective as a clinical endpoint, on the one hand, and to study the methadone kinetic profile, on the other. There has been suggestive evidence to non-frequently monitor the kinetic of methadone to explain some unpredicted clinical response (Loimer and Schmid, 1992; Schmidt *et al*, 1993; Wolff and Hay, 1994; de Vos *et al*, 1996). It may be useful especially when all other measures have been taken adequately and a patient still cannot hold on methadone with high doses.

It should be noted that methadone Cp cannot be used directly to describe the clinical response, as a certain time is required for the drug to distribute adequately in the nervous system. Thus, some researchers have suggested the use of an effect-compartment or link-model to describe the effect appropriately (Ekblom *et al*, 1993). So far, only four studies have modeled methadone by this approach and only one among them for MMT patients (Dyer *et al*, 1999). It was noticed that there is an inverse relationship between plasma concentrations and withdrawal scores and pupil diameters. On the other hand, there was a direct relationship between plasma concentrations and pain threshold in the same patients. The area under the curve did not differ between those who reported withdrawal symptoms and those who did not. The study suggested that there is correlation between methadone clinical responses and changes in the plasma levels for methadone racemic mixture.

1.3.1 Absorption

The absorption of methadone following oral administration is fast and almost complete. The mean time to achieve peak concentration ranges from 2.5 - 30 hours depending on the formulation (Wolff *et al*, 1991). Oral bioavailability of methadone may range from as little as 45 percent up to 90 percent following a single dose (Meresaar *et al*, 1981). As methadone is a basic drug, acid secretions may contribute to such huge variability (Kukanich *et al*, 2005).

1.3.2 Distribution

Being a lipo-soluble drug, methadone distributes widely in body tissues such as: liver, lung, kidney, gut, brain, and muscle with different distribution coefficients (Sawe, 1986). In opioid addicts, the volume of distribution at a steady state (Vss) ranged from 0.2 to 9.2 L/kg. On the other hand, in patients with chronic pain, Vss ranged from 1.71 to 5.34 L/kg (Inturrisi *et al*, 1990), though higher doses are usually given in such situations.

Methadone pharmacokinetic is described as a two-compartment model. Although there are wide differences in the reported clearance, the reported terminal half-life was estimated to range from 23-26 hours. Half-life depends also on the volume of distribution, making the explanations much more complicated and inconclusive (Eap *et al*, 2002; Li *et al*, 2008)

Methadone binds to plasma protein to a high degree of 86 percent, predominantly to acute α-glycoprotein (AAG) (Romach *et al*, 1981; Eap *et al*, 1990). AAG is an acute phase protein that exhibits significant variations in its plasma levels according to the physiological and/or pathological situation of the patient (Fournier *et al*, 2000; Yang *et al*, 2006; Mestriner *et al*, 2007). AAG levels are significantly increased in stress, leading to very low concentrations in the free fraction (fu) of methadone in cancer patients compared to healthy participants (Abramson, 1982; Gómez *et al*, 1995). Therefore, some studies have measured the concentration of AAG itself to study the impact of their concentration on methadone concentration and / or clinical outcomes. Rowland and Tozer (1995) have stated that 'after a

rapid input of methadone, a decrease in fu will be indicated by an increase in Cp, because Vss is proportional to fu. On the other hand, Cu levels remain unchanged. So, if the Cu is the pharmacologically active concentration, a decrease in fu will not modify the maximum response. Thus, it has been suggested that AAG is significantly higher in patients exhibiting abstinence syndrome compared to those who are stable (Garrido *et al*, 2000) and AAG may contribute to the variations in methadone plasma levels.

Other factors that may contribute to variability include age and sex. It has been suggested that these factors may explain about 33 percent of the inter-individual variations in Vss. These parameters are found to be higher in females and they are directly related to weight (Wolff *et al*, 2000).

Furthermore, it has also been suggested that a time-dependent increase in methadone clearance may result from auto-induction of its own metabolism by *CYP3A4*, and the change in Vss may be due to up or down-regulation of AAG (Rostami-Hodjegan *et al*, 1999). Therefore, a time-dependent decrease in Vss may be associated with the observed time-dependent increase in AAG.

1.3.3 Elimination

Generally, there is a huge inter-individual variability in methadone clearance that can reach up to 20 -100 folds in magnitude (Eap *et al*, 2002; Li *et al*, 2008). Methadone is eliminated by hepatic metabolism and renal excretion. It has been shown that at urinary pH of six and above, renal clearance accounts for four percent only. However, when urinary pH was lower than 6, the clearance of unchanged drug will be increased by 33 percent (Rostami-Hodjegan *et al*, 1999). It was concluded that, about 20-50 percent of the inter-individual variability can be explained by urinary excretion (KuKanich and Borum, 2008). With regard to hepatic clearance, methadone can be recognized as a drug with a low extraction ratio, 0.16 in MMT patients.

2. Objective of clinical study

2.1 General objective

To investigate factors that influence successful MMT in opiate-dependent individuals,

2.2 Specific objective

To investigate the impact of daily clinical methadone dose on plasma concentration of methadone.

3. Clinical study

The study involves opiate-dependent individuals who consented, met our study criteria and were invited to participate in the study. The study involved them taking prescribed doses of daily methadone according to Methadone Maintenance Therapy (MMT) guidelines prepared by the Malaysian Ministry of Health and be monitored regularly based on our study protocols. They were followed up for 12 months during the study period. At follow up, 5 ml of venous blood were drawn for the determination plasma methadone level using in-house methadone ELISA kit.

However, at 12th month follow up, 88 out of the 128 participants fail to meet the inclusion criteria. Thus, in order to assess the efficacy of low dose methadone on the withdrawal effect

and sleeping quality, a subset of only 40 patients was further selected to participate. They were given a fixed 40 mg daily dose of methadone. Their withdrawal score and sleeping quality were assessed during the fourth week of the study.

4. Results

One hundred and twenty eight patients were enrolled for this pilot study. Their doses were titrated appropriately as tolerated. However, at 12th month follow up, 88 patients out of the 128 participants fail to meet the inclusion criteria. Thus, in order to assess the efficacy of low dose methadone on the withdrawal effect and sleeping quality, a subset of only 40 patients was further selected to participate where they were given a fixed 40 mg daily dose of methadone. Daily dose averaged 57.2 mg (SD ± 22.7) (Table 4.1) and ranged from 20 to 160 mg per day (Figure 4.1). The corresponding plasma methadone concentration averaged 281.3 ng/ml (SD ± 567.9) (Table 4.1) and ranged from 0 to 4634 ng/ml (Figure 4.2, Figure 4.3)

	Daily Dose,(mg)	Plasma Concentration, (ng/ml)
Mean	57.19828	299.842
Standard Error	2.110416	57.0856
Median	50	180.8249
Standard Deviation	22.72988	582.1612
Sample Variance	516.6473	338911.6
Kurtosis	2.263482	39.11501
Skewness	1.040586	5.905204

Table 4.1. The Summary of Statistics, Daily Methadone Dose (mg) and Plasma Methadone Concentration (ng/ml)

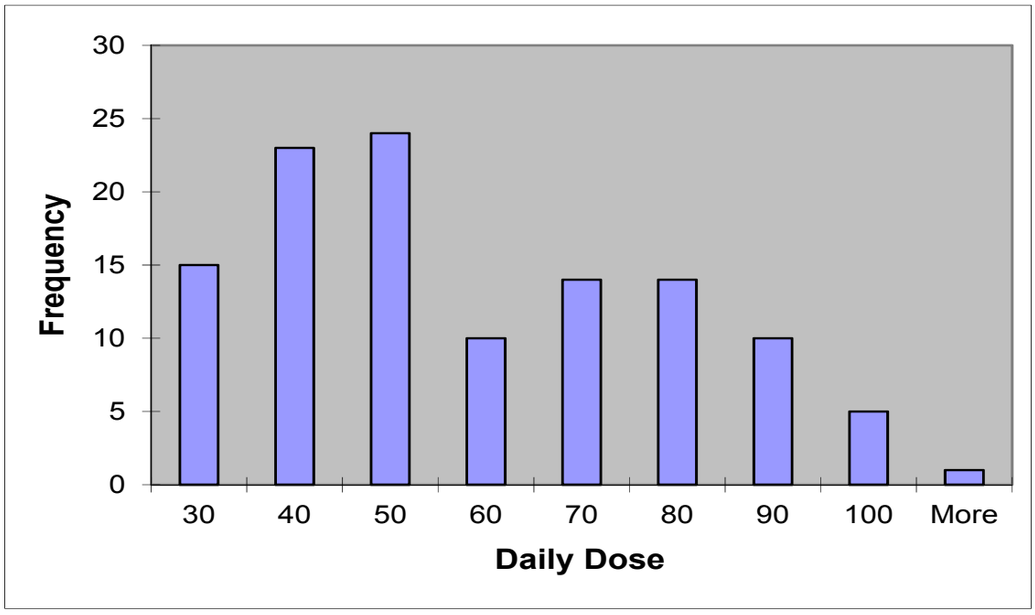


Fig. 4.1. Daily Methadone Dose in the Study Patients

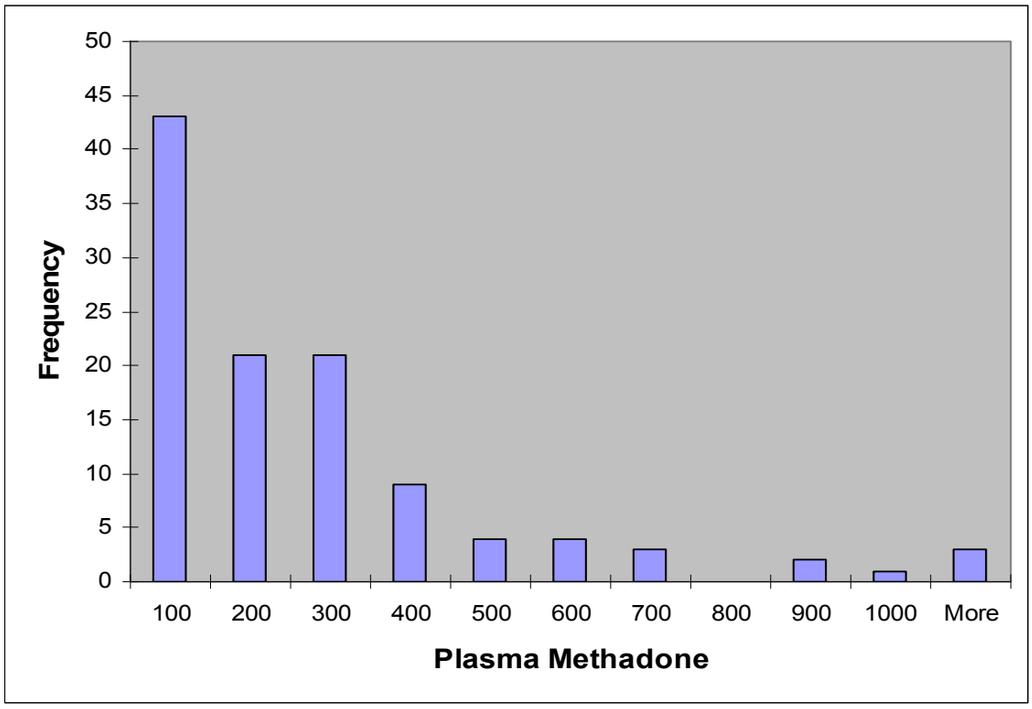


Fig. 4.2. Plasma Methadone Concentrations (ng/ml) as a function of daily methadone dose in the studied patients (outlying concentrations were removed).

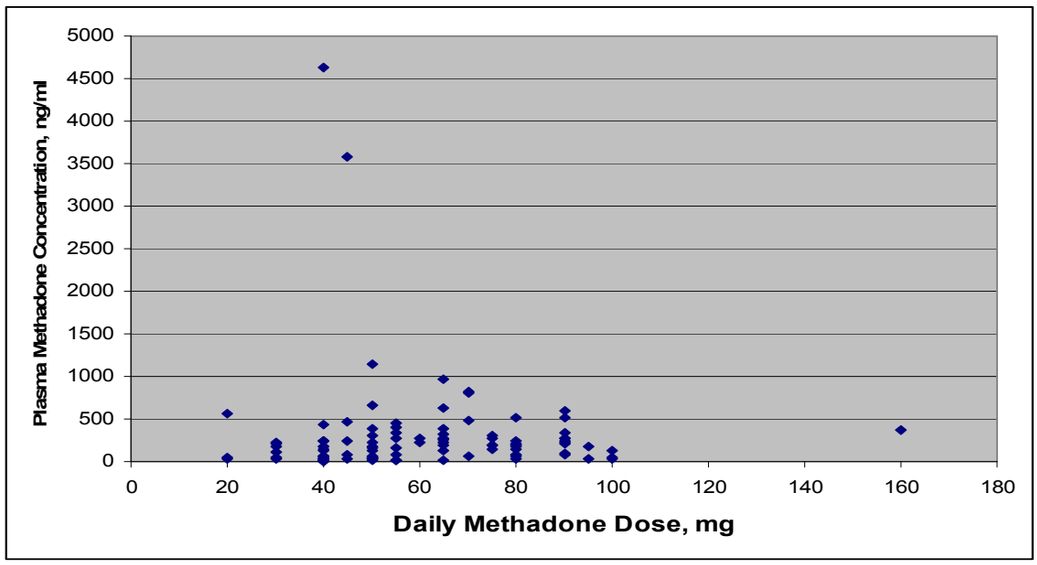


Fig. 4.3. Plasma Methadone Concentrations (ng/ml) as a Function of Daily Methadone Dose (mg) in the Studied Patients

Both the daily doses and the resulting plasma concentrations showed a non-normal distribution, more so for the plasma concentrations compared to the daily dose. Thus, although the daily dose averaged 57 mg, its median was lower at 50 mg. Similarly, although

plasma concentration averaged 300 ng/ml, its median was only 181 ng/ml. A closer look revealed that 33% of patients had doses of 40 mg/day or lower, 54% received 40 – 80 mg/day dose and only 13% had doses 80 mg or more per day. In terms of plasma methadone, most, 84%, had concentrations of 400 ng/ml and 16% had 400 ng/ml and above, 400 ng/ml being the proposed minimum concentration for effectiveness. Six percent of patients on the other hand, had potentially toxic concentrations of more than 700 ng/ml. (Table 4.2 and Table 4.3)

Statistics	Day 1	Day 7	Day 14	Day 21
Mean	136.25	242.91	196.94	216.52
Standard Error	13.49	21.13	18.27	19.66
Median	135.06	194.12	162.05	190.25
Standard Deviation	80.92	126.79	109.65	117.96
Sample Variance	6548.10	16075.43	12022.56	13913.47
Skewness	0.56	1.08	2.14	2.35
Range	317.65	463.53	573.99	584.88
Minimum	14.09	92.36	60.66	81.16
Maximum	331.74	555.89	634.65	666.04

Table 4.2. Plasma Methadone Concentrations (ng/ml) on Days 1, 7, 14 and 21 While Patients Received MMT 40 mg Daily

Plasma Methadone, up to mg/ml	Day 1		Day 7		Day 14		Day 21	
	N	Cumulative %	N	Cumulative %	N	Cumulative %	N	Cumulative %
100.00	10	32.26%	2	6.06%	0	0.00%	1	3.85%
200.00	10	64.52%	16	54.55%	17	56.67%	11	46.15%
300.00	10	96.77%	7	75.76%	5	73.33%	8	76.92%
400.00	1	100.00%	3	84.85%	7	96.67%	4	92.31%
500.00	0	100.00%	1	87.88%	0	96.67%	1	96.15%
600.00	0	100.00%	4	100.00%	0	96.67%	0	96.15%
700.00	0	100.00%	0	100.00%	1	100.00%	1	100.00%
800.00	0	100.00%	0	100.00%	0	100.00%	0	100.00%

Table 4.3. Plasma Methadone Concentrations (ng/ml) on Days 1, 7, 14 and 21 While Patients Received MMT 40 mg Daily

The Subjective and Objective Withdrawal Score from patient taking MMT 40 mg daily was poorly manifested (Figure 4.4 and Figure 4.5). It showed that methadone at 40 mg a day was not adequate to suppress the withdrawal from opiate dependence.

Subjective withdrawal scores (SOW) were determined at four weeks for patients given 40 mg daily dose of methadone. Scores averaged 32 (SD ±10.4). The lowest score was 11 and the highest 51. Objective withdrawal scores (OOW) were also determined at four weeks for patients given 40 mg daily dose of methadone. Scores averaged 8.2 (SD ±1.5).

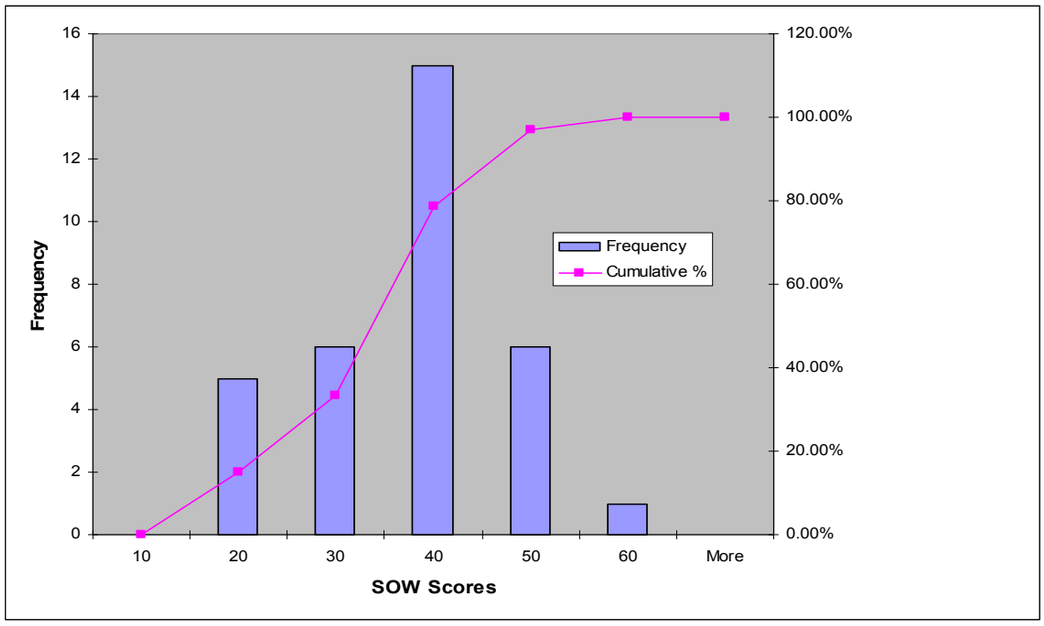


Fig. 4.4. Subjective Objective Withdrawal Scores from Patients Taking MMT 40 mg daily

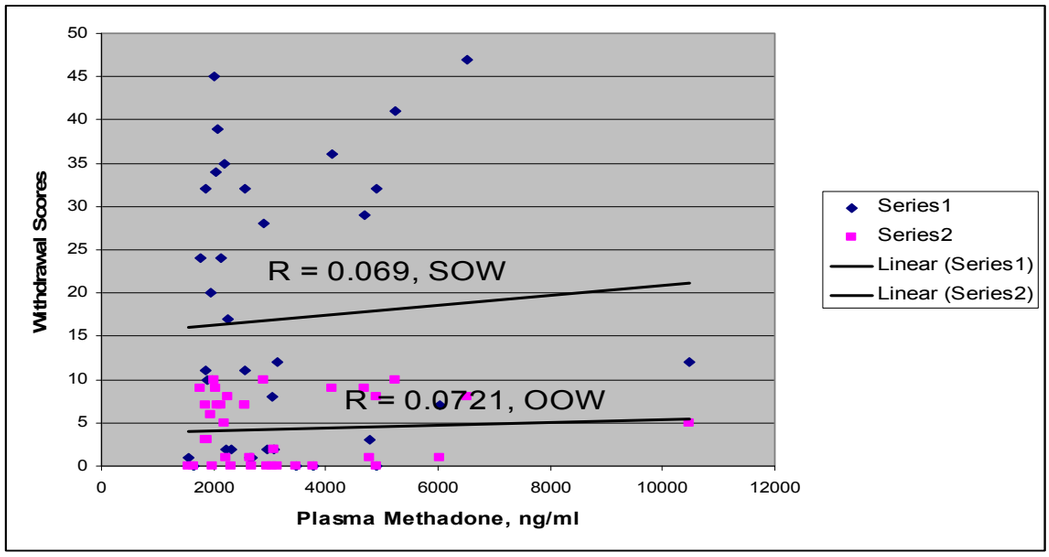


Fig. 4.5. Withdrawal Scores as a Function of Plasma Methadone Concentrations

(Series 1 = SOW; Series 2 = OOW)

5. Discussion

Methadone has a complex pharmacology. There is widespread "opiophobia" and it is frequently perceived negatively by physicians, patients and the society so much so that many just accept it as a necessary evil. The complex pharmacology and "opiophobia" present a great challenge to patients, physician and programs in terms of finding the most appropriate dose to

achieve the desired results. This study was an attempt to comprehensively look at MMT. Among our notable findings included the variable ages of our patients, the male predominance, the variable daily methadone doses used and the importance of high daily maintenance dose, the variable plasma methadone obtained and its poor correlation with daily doses. Eighty eight patients were excluded from plasma concentration of methadone because they did not comply with the protocol. We investigated only 40 patients for the outcomes of MMT when the daily dose was fixed at 40 mg. We found that this daily dose was associated with high withdrawal scores implying failure of therapy.

In our clinical study, initially we enrolled 128 patients. They comprised of heroin/opiate dependent individuals receiving MMT in our clinics. As have been observed in many previous studies with MMT, patients enrolled in this study were mostly males with most in the productive age group. The youngest was 20, the oldest 56 years old. They were also mainly Malays. This fact underscores the importance of proper management of drug use disorder. These young and otherwise healthy males, if not successfully managed, are lost to the society and may lead a criminal life to feed their habits, given the difficulties, stigma and discrimination they face to be employed. Thus, instead of becoming the work force of the country needed to generate economic activity, these youngsters in turn add the burden of the country. There will be added burdens in terms of law enforcement costs, judiciary costs and other related costs. This would be over and above other costs like the society and health-care costs.

Of note was a high prevalence of HIV positivity at 36%. In most countries with good harm reduction programs for injecting drug users, the prevalence of HIV positivity is generally 1-2% (Central Intelligence Agency). The high prevalence seen in our cohort underscores the need for urgent effective measures. As there is no cure for HIV/ AIDS, this high prevalence would mean that many young Malay males in Malaysia would eventually succumb to the disease. This would reduce the pool of available males for population growth and if this is allowed to go on unabated, this will impact on the demography of the Malaysian population. Ethnic proportions can change and population growth in some communities may be halted. They may face troubles to obtain gainful employment and may resort to crimes to feed their habits, themselves and may be even their families. Co-morbid conditions like psychiatric illnesses and stigma and discrimination may make them dangerous to self, family and the society.

No age is however spared by the drug use disorder. The youngest of our patients was a 20 year-old. They began their drug habit as early as when they were 12 years. The oldest patient was 56 years of age and the oldest age a patient started with the habit was 32 years. Drug use disorder is a chronic relapsing disease. The duration of illness among our patients ranged from two years to 38 years and averaged 13 years. These have implications. For one, preventive measures for drug use disorder must begin early and should be continued through all ages. Patients afflicted with the disease should also have long follow ups as they evidently continue with their habits right through their golden years. The longer they continue on the habit, the greater is the chance for them to contract diseases like HIV, if they have not yet been infected. Being young and otherwise healthy, young addicts may find themselves constrained in various activities and this may lead them to many unhealthy practices.

Drug users do not live in isolation. They have sexual partners and families. Apart from transmission through the sharing of injection equipments, having the HIV reservoir, drug users can also transmit the disease to their sexual partners, through penetrative sex. Thus, what started as a concentrated epidemic among drug users is now showing evidence for a

more generalized epidemic into the community through sexual transmission. In the beginning, less than one percent of HIV victims were females. Now it stands at about 20% and this clearly demonstrates the generalization of the HIV epidemic in Malaysia that began as a concentrated epidemic among drug users. Most of the afflicted females are also wives and spouses of drug users who are themselves HIV positive and not sex workers as many would have expected. There is however evidence for a growing epidemic among sex workers and this again has the potential to generalize into the community.

For the forty patients studied, their daily dose averaged 57.2 mg and ranged from 20 to 160 mg per day. Median dose was 50 mg per day. The corresponding plasma methadone averaged 281.3 ng/ml. It ranged from 0 to 4634 ng/ml. Daily methadone doses poorly predicted resulting plasma methadone concentrations, a hallmark for a drug metabolized by genetically polymorphic enzymes. Indeed when we measured plasma methadone concentrations in patients who received a fixed 40 mg daily methadone , they varied from 14 ng/ml to 331 ng/ml, a 23-fold difference. It is thus evident that no one dose fits all. As with many drugs used in the management of chronic diseases, methadone doses should be individualized to optimum outcomes that must be determined objectively.

It is also interesting to note that, despite claims by many physicians that relatively lower doses of methadone would be sufficient for our Malaysian patients, our observation of high withdrawal scores among patients who were maintained at 40 mg daily of methadone would imply this was not so. Severe withdrawal would discourage patients from remaining on treatment and by inference, they will not be retained. Indeed it has consistently been found that a sufficiently high dose of substitution therapy was required for improved outcome (Brady *et al*, 2005). High doses of methadone were significantly more effective in suppressing illicit heroin use and in retaining patients in the program (Family Health International; Mattick *et al*, 2003) and in producing optimum outcomes (Farré *et al*, 2002).

Inadequate doses and premature termination are the greatest threats to a successful MMT program in Malaysia. Malaysian doctors may outwardly say that they use lower methadone doses because of their fear for ethnic difference that would put their patients at higher risks for toxicity if they were to use doses as high as those recommended by the Western literature. What they may not want to admit is the fact that, inwardly, they have fears with methadone (and all opiates actually!) just for the simple reason that methadone is an opiate, just like the dreaded heroin and morphine! Indeed Malaysian doctors are not alone in this. Many doctors everywhere share the same view. Thus, despite ample evidence for the need to maintain patients at a daily dose of 80 mg to 100 mg, most patients are maintained on much less, and many are encouraged early termination.

It is probably understandable that the lay public may not understand the scientific basis for MMT and could be disparaging and become critical of it. It is however less clear why many physicians and other health care providers have the same views. Even those directly involved with MMT programs frequently fail to adhere to the basic principles of MMT. Most have actually received clear information on the pharmacologic principles underlying MMT and their claim that they want to prescribe as few medications as possible sound hollow, as they frequently easily prescribe other mood altering drugs, such as the benzodiazepines that are often prescribed with abandon and can produce psychological and physiologic dependency. Even if they claim they fear adverse effects, the adverse, physiologic effects of MMT are minimal and methadone is probably associated with the least side effects of any drug in a physician's pharmacologic armamentarium, when used appropriately. The real reason is probably more to do with the general “opiophobias” as it is known that some

doctors even hesitate to use opiates even when indications are clear. Efforts should therefore be made urgently to reeducate these doctors. In their hands is the future of the nation. Their failure to prescribe adequate methadone doses will lead to therapeutic failure for MMT. This has dire consequences.

There is another problem. The expectation of the public, doctors and patients as regards treatment of addiction is to have a drug-free ending. This puts extra pressures on the doctor and patient alike and this will encourage doctors and patients to use low doses for the shortest possible time. This is despite the fact that maintenance therapy for at least two years with adequate doses is known to be associated with the maximum chance of remaining abstinent when methadone has been tapered. Many patients can thus receive less than two years of treatment with methadone with encouragements to discontinue maintenance frequently coming from health care providers working in maintenance programs. Most treating doctors also often do not try to discover reasons why patients started drug in the first place, or the existence of comorbid psychiatric illnesses. This less than holistic approach to MMT can result in increased anxiety among patients that can lead to the use of other psycho-active drugs, such as the benzodiazepines.

Notwithstanding the requirement for higher doses, as with any drugs, the dosing of methadone should be individualized (Latowsky, 2006). While low doses are associated with relapse and failure, too high a dose may lead to toxicities such as prolongation of QT interval and subsequent fatal polymorphic ventricular fibrillation (Fanoe *et al*, 2007). As regards plasma methadone concentrations, although we did not observe a clear correlation between plasma concentration and clinical effects, in the individual patients they may prove useful as illustrated in the cases we described above. Notwithstanding that, it is clear that a dose of 40 mg a day is generally inadequate. Subjective withdrawal scores (SOW) at four weeks for patients given 40 mg daily dose of methadone averaged 32 and the standard deviation was large at 10.4. The lowest score was 11 and the highest 51. Objective withdrawal scores (OOW) were also determined at four weeks for patients given 40 mg daily dose of methadone. Scores averaged 8.2 (SD ±1.5). It is evident that severe withdrawals occurred in patients maintained on 40 mg daily.

6. Conclusion

We concluded that the variable plasma methadone obtained was poorly correlated with daily doses of methadone and low dose methadone was inadequate to suppress opiate withdrawal.

A daily dose of 40 mg was associated with a high incidence of opiate withdrawal. Thus, prescription of methadone dose should be individualised to achieve a higher success of MMT.

7. Acknowledgement

I would like to thank USM (Universiti Sains Malaysia) RU grant 1001/PSSP/812056 for sponsoring this project.

8. References

Abramson, F.P. Methadone plasma protein binding: alterations in cancer and displacement from alpha 1-acid glycoprotein. *Clin Pharmaco Ther*. 1982. 32(5): 652-658.

Brady, T.M., Salvucci, S., Sverdlov, L.S., Male, A., Kyeyune, H., Sikali, E., DeSale, S. and Yu, P. Methadone dosage and retention: an examination of the 60 mg/day threshold. *Journal of Addictive Diseases.* 2005. 24(3): 23-47.

de Vos, J.W., Ufkes, J.G.R., van Brussel, G.H.A. & van den Brink, W. Craving despite extremely high methadone dosage. *Drug and Alcohol Dependence.* 1996. 40(3): 181-184.

Dyer, K.R., Foster, D.J.R., White, J.M., Somogyi, A.A., Menelaou, A. & Bochner, F. Steady-state pharmacokinetics and pharmacodynamics in methadone maintenance patients: Comparison of those who do and do not experience withdrawal and concentration-effect relationships[ast]. *Clin Pharmacol Ther.* 1999. 65(6): 685-694.

Eap, C.B., Buclin, T. & Baumann, P. Interindividual variability of the clinical pharmacokinetics of methadone: implications for the treatment of opioid dependence. . *Clin. Pharmacokinet.* 2002. 41(1153-1193.

Eap, C.B., Cuendet, C. & Baumann, P. Binding of d-methadone, l-methadone, and dl-methadone to proteins in plasma of healthy volunteers: role of the variants of alpha 1-acid glycoprotein. *Clin Pharmaco Ther.* 1990. 47(3): 338-346.

Ekblom, M., Hammarlund-Udenaes, M. & Paalzow, L. Modeling of tolerance development and rebound effect during different intravenous administrations of morphine to rats. *Journal of Pharmacology and Experimental Therapeutics.* 1993. 266(1): 244-252.

Family Health International. Managing opioid dependence: treatment and care for HIV-positive injecting drug users Disember 27,2009, from http://www.fhi.org/training/en/HIVAIDS/IDUModules/pdf/Module_04_Treatment_Care_for_HIV_positive_IDUs.pdf

Fanoe, S., Hvidt, C., Ege, P. and Jensen, G.B. Syncope and QT prolongation among patients treated with methadone for heroin dependence in the city of Copenhagen. *Heart.* 2007. 93(9): 1051-1055.

Farré, M., Mas, A., Torrens, M., Moreno, V. and CamI, J. Retention rate and illicit opioid use during methadone maintenance interventions: a meta-analysis. *Drug and Alcohol Dependence.* 2002. 65(3): 283-290.

Fournier, T., Medjoubi-N, N. & Porquet, D. Alpha-1-acid glycoprotein. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology.* 2000. 1482(1-2): 157-171.

Garrido, M.J., Aguirre, C., Trocóniz, I.F., Marot, M., Valle, M., Zamacona, M.K. and Calvo, R. Alpha 1-acid glycoprotein (AAG) and serum protein binding of methadone in heroin addicts with abstinence syndrome. *Int J Clin pharmaco Ther.* 2000. 38(1): 35-40.

Gómez, E., Martinez-Jordá, R., Suárez, E., Garrido, M.J. & Calvo, R. Altered methadone analgesia due to changes in plasma protein binding: Role of the route of administration. *General Pharmacology: The Vascular System.* 1995. 26(6): 1273-1276.Xiao, Y., Smith, R.D., Caruso, F.S. & Kellar, K.J. Blockade of Rat α3β4 Nicotinic Receptor function by Methadone, its metabolites, and structural analogs. *JPET.* 2001. 299(1): 366-371.

Inturrisi, C.E., Portenoy, R.K., Max, M.B., Colburn, W.A. & Foley, K.M. Pharmacokinetic-pharmacodynamic relationships of methadone infusions in patients with cancer pain. *Clin Pharmaco Ther.* 1990. 47(5): 565-577.

KuKanich, B. & Borum, S.L. The disposition and behavioral effects of methadone in Greyhounds. *Veterinary Anaesthesia and Analgesia.* 2008. 35(3): 242-248.

Kukanich, B., Lascelles, B.D.X., Aman, A.M., Mealey, K.L. & Papich, M.G. The effects of inhibiting cytochrome P450 3A, p-glycoprotein, and gastric acid secretion on the oral bioavailability of methadone in dogs. *Journal of Veterinary Pharmacology and Therapeutics.* 2005. 28(5): 461-466.

Latowsky, M. Methadone death, dosage and torsade de pointes: risk-benefit policy implications. *J Psychoactive Drug.* 2006. 38(4): 513-519.

Liu, E., Liang, T., Shen, L., Zhong, H., Wang, B., Wu, Z. & Detels, R. Correlates of methadone client retention: a prospective cohort study in Guizhou province, China. *The International Journal on Drug Policy.* 2009. 20(4): 304-308.

Li, X. & Wei, W. Chinese materia medica: combinations and appication Hertfordshire: Donica Publishing. 2002. 75-76.

Li, Y., Kantelip, J.P., Gerritsen-van, S.P. & Davani, S. Interindividual variability of methadone response: impact of genetic polymorphism [Abstract]. *Mol Diagn Ther.* 2008. 12(2): 109-124.

Loimer, N. & Schmid, R. The use of plasma levels to optimize methadone maintenance treatment. *Drug and Alcohol Dependence.* 1992. 30(3): 241-246.

Lötsch, J., von Hentig, N., Freynhagen, R., Griessinger, N., Zimmermann, M., Doehring, A., Rohrbacher, M., Sittl, R. & Geisslinger, G. Cross-sectional analysis of the influence of currently known pharmacogenetic modulators on opioid therapy in outpatient pain centers. *Pharmacogenetics and Genomics.* 2009. 19(6):

Mattick, R.P., Breen, C. and Kimbler, J. Methadone maintenance therapy versus no opioid replacement therapy for opioid dependence. *Cochrane Database of Syst Rev.* 2003.

Meresaar, U., Nilsson, M.I., Holmstrand, J. & Änggård, E. Single dose pharmacokinetics and bioavailability of methadone in man studied with a stable isotope method. *European journal of clinical pharmacology.* 1981. 20(6): 473-478-478.

Mestriner, F.L.A.C., Spiller, F., Laure, H.J., Souto, F.O., Tavares-Murta, B.M., Rosa, J.C., Basile-Filho, A., Ferreira, S.H., Greene, L.J. & Cunha, F.Q. Acute-phase protein α-1-acid glycoprotein mediates neutrophil migration failure in sepsis by a nitric oxide-dependent mechanism. *Proceedings of the National Academy of Sciences.* 2007. 104(49): 19595-19600.

Romach, M.K., Piafsky, K.M., Abel, J.G., Khouw, V. & Sellers, E.M. Methadone binding to orosomucoid (alpha 1-acid glycoprotein): determinant of free fraction in plasma. *Clin Pharmaco Ther.* 1981. 29(2): 211-217.

Rostami-Hodjegan, A., Wolff, K., Hay, A.W.M., Raistrick, D., Calvert, R. & Tucker, G.T. Population pharmacokinetics of methadone in opiate users: characterization of time-dependent changes. *British Journal of Clinical Pharmacology.* 1999. 48(1): 43-52.

Rowland, M. & Tozer, T.N. Physiologic concepts and kinetics: distribution. In Rowland, M. and Tozer, T. N. (Eds.), *Clinical Pharmacokinetics. Concepts and Applications*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins. 1995. 137–155.

Sawe, J. High-dose morphine and methadone in cancer patients. Clinical pharmacokinetic considerations of oral treatment. *Clin Pharmacokinet.* 1986. 11(2): 87-106.

Schmidt, N., Sittl, R., Brune, K. & Geisslinger, G. Rapid Determination of Methadone in Plasma, Cerebrospinal Fluid, and Urine by Gas Chromatography and Its

Application to Routine Drug Monitoring. *Pharmaceutical Research.* 1993. 10(3): 441-444-444.

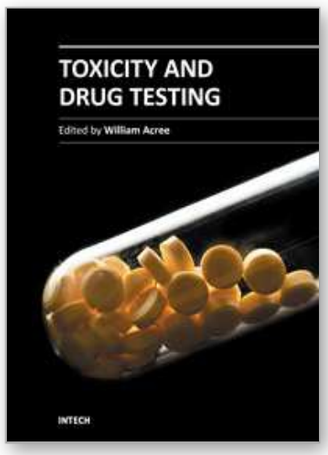
Wolff, K. & Hay, A.W. Plasma methadone monitoring with methadone maintenance treatment. *Drug Alcohol Depend.* 1994. 36(1): 69-71.

Wolff, K., Rostami-Hodjegan, A., Hay, A.W.M., Raistrick, D. and Tucker, G. Population-based pharmacokinetic approach for methadone monitoring of opiate addicts: potential clinical utility. *Addiction.* 2000. 95(12): 1771-1783.

Wolff, K., Sanderson, M., Hay, A.W. and Raistrick, D. Methadone concentrations in plasma and their relationship to drug dosage. *Clinical Chemistry.* 1991. 37(2): 205-209.

Verebely, K., Volavka, J., Mulé, S. & Resnick, R. Methadone in man: pharmacokinetic and excretion studies in acute and chronic treatment. *Clinical Pharmacology and Therapeutics.* 1975. 18(2): 180-190.

Yang, Y., Wan, C., Li, H., Zhu, H., La, Y., Xi, Z., Chen, Y., Jiang, L., Feng, G. & He, L. Altered Levels of Acute Phase Proteins in the Plasma of Patients with Schizophrenia. *Analytical Chemistry.* 2006. 78(11): 3571-3576.



Toxicity and Drug Testing
Edited by Prof. Bill Acree

ISBN 978-953-51-0004-1
Hard cover, 528 pages
Publisher InTech
Published online 10, February, 2012
Published in print edition February, 2012

Modern drug design and testing involves experimental in vivo and in vitro measurement of the drug candidate's ADMET (adsorption, distribution, metabolism, elimination and toxicity) properties in the early stages of drug discovery. Only a small percentage of the proposed drug candidates receive government approval and reach the market place. Unfavorable pharmacokinetic properties, poor bioavailability and efficacy, low solubility, adverse side effects and toxicity concerns account for many of the drug failures encountered in the pharmaceutical industry. Authors from several countries have contributed chapters detailing regulatory policies, pharmaceutical concerns and clinical practices in their respective countries with the expectation that the open exchange of scientific results and ideas presented in this book will lead to improved pharmaceutical products.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen