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In vitro and *In vivo* Study of Effect of α-Adrenergic Agonist-Methyldopa on the Serum Biochemical Laboratory Findings

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Abstract

Objectives: The effects of the antihypertensive drug methyldopa on biochemical laboratory findings were monitored both *in vitro* and *in vivo* particularly those of metabolites and enzymes which are routinely requested by physicians.

Methods: *In vitro* and *in vivo* studies were performed. For the *in vitro* study, solutions of methyldopa concentrations were prepared according to its maximum serum concentration as reported in the literature and were added to blank, normal serum. The samples were then analyzed in parallel with a standard test using the same laboratory techniques. For the *in vivo* study, blood was collected before starting and two weeks after starting methyldopa therapy from 40 subjects that were newly diagnosed with essential hypertension. The control sera were collected from 30 healthy volunteers of comparable ages. The samples were analyzed for glucose, Total Protein (TP), urea, creatinine, Total Cholesterol (TC), Triglyceride (TG), Aspartate Transaminase (AST), Alanine Transaminase (ALT), Lactate Dehydrogenase (LDH) and Creatine Kinase (CK).

Results: In the *in vitro* study, methyldopa induced a decrease in the readings of serum glucose, TP, urea, TC, AST, ALT, and CK, whereas the LDH levels recorded an increase. *In vivo* study of methyldopa leads to increase the levels of serum glucose, TP, urea, TC, TG, AST, ALT and LDH.

Conclusions: Methyldopa induced significant alterations in the *in vitro* as well as in the *in vivo* measurements. These alterations are required to be taken seriously by physicians to avoid misinterpretations of data generated during routine practice. All the *in vitro* changes in biochemical parameters are a result of chemical or physical reactions, whereas the *in vivo* changes resulted mostly from physiological or metabolic factors.

Keywords: Methyldopa; Serum biochemical parameters; Physiological or Metabolic factors

Introduction

Methyldopa is an α-adrenergic agonist (selective for α_2–adrenergic receptors) psychoactive drug used as a central sympatholytic agent that is used to reduce hypertension [1,2]. It is L-α-Methyl-3,4-dihydroxyphenylalanine ($C_{10}H_{13}NO_4$) [3]. It has been produced under a number of commercial names including Aldomet, Aldoril, Dopamet and Dopegyt. Methyldopa is colorless or almost colorless crystals or a white to yellowish-white colorless fine powder, which may contain friable lumps [4]. Methyldopa is slightly soluble in water, very slightly soluble in ethanol (96%); practically insoluble in chloroform and ether. It is freely soluble in dilute mineral acids [3]. The drug crosses the placental barrier, appears in cord blood, and breast milk. The maximum serum concentration (C_{max}) after an oral administration of 250 mg of methyldopa is 975 ng/ml. It is given as an oral suspension of 50 mg/ml, or in tablets of 125 mg, 250 mg or 500 mg, in addition to 50 mg/ml injection [5,6].

Methyldopa is decarboxylated in the Central Nervous System (CNS) to α-methyl-noradrenaline, which stimulates α_2-adrenoceptors resulting in a reduction in sympathetic tone and a fall in blood pressure. Methyldopa reduces tissue concentrations of dopamine, nor adrenaline, adrenaline, and serotonin [4,6].

Although the use of methyldopa has declined because of its side effects, and especially following the introduction of safer drugs, it is still in use in cases of untreatable hypertension and in gestational hypertension, known as Pregnancy-Induced Hypertension (PIH). A single oral dose can produce a maximum effect within 4-6 hours although the maximum hypotensive effect may not occur until the second day of continuous treatment [4].

Methyldopa is used in the treatment of moderate to severe hypertension usually in combination with a diuretic or a beta-blocking agent. It reduces both the standing and the supine blood pressure. The initial oral dose is equivalent to 250 mg of anhydrous methyldopa two or three times daily for two days [4].

The direct effects of *in vivo* methyldopa administration exhibited a 25% reduction in intrasynaptosomal 5-HT and a 15% reduction in 5-HT synthesis when compared to synaptosomes from saline-treated animals. In addition a 15% reduction in synaptosomal tryptophan levels was observed [7]. According to Chatelain et al. [8] α-methydopa treatment increases the number of β-adrenoceptors by 20–32% and the maximal response of adenylate cyclase activity to β-blockers. Methyldopa treatment significantly affects clinical chemistry tests at five times the upper end of their therapeutic ranges on the following

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tests: glucose, bilirubin, carbon dioxide, calcium, cholesterol, uric acid and aspartate aminotransferase [9].

The present study was undertaken to evaluate the effects of the antihypertensive drug methyldopa on biochemical laboratory findings that were monitored both *in vitro* and *in vivo*, particularly those of blood metabolites and enzymes, which are usually requested by physicians for professional diagnosis and for better understanding of their clinical importance.

Materials and Methods

In vitro serum simulation test

A stock solution was prepared by dissolving 30 mg of methyldopa in 100 ml of 0.1 N HCl: 6.5 µl of this solution represent 1950 ng/ml. The stock solution was diluted to produce final concentrations of 1950, 975 and 487.5 ng/ml, which represent the C_{max} of the drug in serum following the administration of 500 mg, 250 mg and 125 mg, respectively. The negative control was mixed with solvent alone (0.1 N HCl). Serum were obtained from the methyldopa treated subjects and used to compare to control group.

Measured biochemical parameters included glucose, Total Protein (TP), urea, creatinine, Total Cholesterol (TC), Triglycerides (TG), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH) and Creatine Kinase (CK). These parameters were measured by using Randox kits (Randox Laboratories Ltd, United Kingdom), and read by spectrophotometry.

Net effect of drug on laboratory test = Value (Serum + Drug) − Value (Serum + Solvent)

In vivo test

An ethical approval was granted during the meeting of the Heads of Departments of the Pharmacy School in October 2002; and all patients were informed of the procedures and objectives of the study in advance.

Serum samples from 30 healthy subjects were used as controls. These control subjects were without evidence of hypertension and diabetes mellitus, and their ages ranged from 41 to 63 years to match with the test subjects. Similar biochemical parameters were measured and similar methods were utilized as those in the *in vitro* tests.

The study was conducted on sera from 40 patients (21 males and 19 females) aged 45-66 years, with Non-Insulin-Dependent Diabetes Mellitus (NIDDM) and newly diagnosed with essential hypertension at Dr. Abdul Majeed Hospital, Baghdad. They were given a dosage of 250 mg methyldopa daily for two weeks, when the post-treatment blood samples were collected. Pre- and post-treatment venous blood samples were collected from each patient. Serum was aspirated from clotted blood after centrifugation.

	Control	500 mg	250 mg	125 mg
Glucose mg/dl	94.33 ± 16.5	37.66 ± 23.15*	52.33 ± 26.1*	73 ± 22.91*
TP g/l	72 ± 8.88	51.33 ± 6.5*	57.33 ± 6.02*	66 ± 6.55*
Urea mg/dl	34.0 ± 9.8	15.0 ± 4.5*	22.6 ± 3.5*	27.0 ± 4.3*
Creatinine mg/dl	1.21 ± 0.265	1.13 ± 0.253	1.2 ± 0.05	1.29 ± 0.274
TC mg/dl	175.66 ± 51.54	61.33 ± 39.2*	76.33 ± 42.19*	91 ± 45.29*
TG mg/dl	154.33 ± 17.75	36 ± 19.97*	54.33 ± 17.78*	69.33 ± 13.31*
AST U/l	16.33 ± 6.5	14.33 ± 5.5	12.56 ± 3.66	10.46 ± 2.33
ALT U/l	12.66 ± 8.5	9.16 ± 8.75	11.5 ± 9.26	14.16 ± 11.003
CK IU/l	117 ± 46.60	85.66 ± 38.21	89.66 ± 38.37	92.66 ± 39.14
LDH U/l	168 ± 22.6	196 ± 4.58	187 ± 9.16	157.33 ± 18.03

*significant (p<0.05)
TP: Total Protein; TC: Total Cholesterol; TG: Triglyceride; AST: Aspartate Transaminase; ALT: Alanine Transaminase; LDH: Lactate Dehydrogenase; CK: Creatine Kinase

Table 1: *In vitro* effect of methyldopa on laboratory findings.

Three readings were taken, one for the serum from controls and two serum samples from the study subjects, one was taken before the start of the treatment (pre-treatment) and the other one was taken two weeks after (post-treatment). These three readings were compared with each other controls with pre-treatment, pre- with post-treatment and controls with post-treatment values to monitor the altered readings and evaluate their significance.

Statistical analyses

The readings of the biochemical tests were expressed as the mean ± SD. The differences in the mean of tested parameters were examined by Student's T-test and P values less than 0.05 were taken as significant. The effects of different concentrations of methyldopa on biochemical parameters were evaluated by ANOVA.

Results

Effects of methyldopa on *in vitro* biochemical laboratory findings

Methyldopa, *in vitro*, has significantly reduced (P<0.05) the concentrations of glucose, TP, urea, TC and TG. These reductions appeared to be dose dependent. CK was also reduced in a similar pattern, but the reductions were not significant (P >0.05). ALT readings were also reduced, non-significantly (P>0.05) though there was nonsignificant increase (P>0.05) above the control, in the readings obtained with sera treated with a low dose of 125 mg methyldopa. While there was nonsignifant (P>0.05) reduction in AST values in a dose-dependent manner with a nonsignificant rise in the readings above the control for lower dose of 125 mg. Creatinine readings were not significantly altered. LDH showed a dose-dependent pattern of reduction. The LDH readings were elevated with the higher doses of 500 and 250 mg, and slightly reduced dose of 125 mg (Table 1).

Effects of methyldopa on *in vivo* biochemical laboratory findings

The *in vivo* effects of methyldopa were measured in a different way. Some alterations were obtained in the readings of the biochemical parameters following the methyldopa therapy. Glucose was increased in both the pre- and post-treatment sera, though the increases were not significant (P>0.05). Urea, TC, AST and ALT all had the readings increased in the post-treatment sera only the increase of the AST was significant (P<0.05). Furthermore, there were significant (P<0.05) elevations in the readings of TG and LDH in the post-treatment sera. Creatinine was unaltered. The readings of TP were reduced in the pre-treatment sera whereas they were elevated in the post-treatment sera. The readings were reduced following the methyldopa treatment, although in the pre-treatment sera the reductions appeared to be significant (P<0.05) (Table 2).

Discussion

The *in vitro* study of methyldopa has yielded some important findings whereby alterations in the readings of panel of tests have been obtained. Except for creatinine and LDH, all the other parameters tested gave reduced readings, most of which were significant (glucose,

	Control	Pre-treatment	Post-treatment
Glucose mg/dl	93.562 ± 18.402	102.70 ± 19.562	112.600 ± 39.044
TP g/l	75.75 ± 8.434	69.40 ± 7.904	79.30 ± 11.005[b]
Urea mg/dl	32.6 ± 10.9	32.9 ± 9.3	40.6 ± 13.0
Creatinine mg/dl	1.113 ± 0.255	1.110 ± 0.218	1.253 ± 0.143
TC mg/dl	191.437 ± 34.298	189.00 ± 70.63	215.40 ± 84.417
TG mg/dl	123.06 ± 32.947	167.70 ± 49.349[a]	189.80 ± 41.651[c]
AST U/l	22.937 ± 6.884	23.40 ± 9.395	30.00 ± 8.856[c]
ALT U/l	18.375 ± 6.365	16.20 ± 8.495	23.20 ± 14.195
LDH U/l	130.50 ± 33.091	142.70 ± 31.478	169.20 ± 28.389[c]
CK IU/l	131.75 ± 33.914	100.50 ± 36.427[a]	120.50 ± 53.435

Non-significant ($p>0.05$), significant ($p<0.05$)
[a]Control with pre-treatment
[b]Pre-treatment with post-treatment
[c]Control with post-treatment
TP: Total Protein; TC: Total Cholesterol; TG: Triglyceride; AST: Aspartate Transaminase; ALT: Alanine Transaminase; LDH: Lactate Dehydrogenase; CK: Creatine Kinase

Table 2: *In vivo* effect of methyldopa on laboratory findings.

TP, urea, TC, TG, AST, ALT and CK). Creatinine was not affected, and LDH was with elevated readings with high drug doses.

The *in vivo* work measures the drug interferences with the quantity to be measured under physiological conditions. The proposed mechanism can be more diverse, especially with the knowledge that some drugs can cause induction of hepatic microsomal enzymes, a normal pharmacological response of a normally functioning liver. Hence, the *in-vivo* response to a drug depends on the patient, on the drug type and dosage and on whether other medications are being taken concurrently. The experimental design compared the concentrations of the biochemical parameters in patients before and after treatment. That set-up should enable the researcher to measure for the effects of the drugs on the biochemical parameters. Consequently, and following methyldopa consumption for two weeks, the only significant alteration obtained was that with Total Protein (TP) when comparing the pre- with the post-treatment measurements. There are two explanations for this. The first direct explanation would be that methyldopa is interfering, chemically or physically with the assay. Alternatively, it may be explained on the basis of a genuine rise in the TP following the control of the elevated blood pressure, as that may allow a rise in the intra-vascular oncotic pressure. Moreover, on comparing the post-treatment sera with the controls, their readings of TC, TG and AST and in the LDH were significantly higher. The enzymes elevations may be due to hepatic enzyme induction. Yet the rise of the TC and TG rise may be genuine, since the pre-treatment levels had been high compared with the controls. Such elevated TC and TG levels would be rather expected in the sera of hypertensive patients as a normal physiological effect or may due to a high oxidative stress in hypertensive patients [10,11].

The glucose findings have been in agreement with previously published data on the rise of glucose with methyldopa therapy, with a possible relation with the methods used [12,13]. With standardized methods, the effects of drugs on glucose level may decrease *in vitro* assays, whereas they would increase during the *in vivo* study. Such a controversy would probably be due to metabolic effects or due to interaction of the drugs or their metabolites.

Creatinine and urea levels were insignificantly increased with methyldopa therapy, in agreement with previous records [14,15]. Creatinine increment due to methodological effects such as being readily oxidized in the Alkaline picrate method and Fuller's earth procedures [16-19], indicate that methyldopa may interfere with the Jaffe reaction. Hence, these analytical interferences are due to chemical interferences of the drug with the analytical methods used [20].

Serum AST, ALT, LDH and CK levels were not significantly increased with methyldopa therapy as compared to the pre-treated patients and are generally in agreement with previous reports [21,22]. These alterations in the serum AST and ALT concentrations may be due to cholestatic effects [14], or due to some hepatotoxicity [19]. However, elevations in the CK levels can be expected since myocarditis has previously been associated with methyldopa therapy in several patients, with concurrent rises in CK [22,23].

With more drugs becoming available every year, many of which are extremely potent, the chances of more adverse drug reactions are multiplied. Knowledge of these alterations may be necessary for assessing the findings in patients receiving these medicines. The effects of drugs on laboratory tests are not negligible, especially that a great number of drugs' effects have been reported [24,25], and that approximately 12% of patients have their laboratory results affected by drugs in the general medical field [25]. Ultimately, the effects of drug additives cannot be completely excluded.

Knowledge of any aberrations in the results of laboratory determinations caused by drugs should be made clear to avoid misinterpretation of data and any possible unwanted consequences in the management and/ or outcomes of diagnoses and therapies.

In conclusions, physicians need to be alerted to alterations induced by drugs and clinical biochemists, as well as clinical pharmacists to be able to consider proper interpretations of laboratory results, particularly when more than one drug is used. The results presented in this study demonstrated clearly that laboratory findings are altered by methyldopa. They may be due to chemical and physical interferences. In the *in vivo* findings, the changes in concentration of metabolites and enzymes may be explained, in part, by the pharmacodynamic characteristics of the drug, in addition to physical and chemical factors.

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