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Hydroxychloroquine Treatment Raises CK-MB and ACH Levels in the Mice

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Abstract

Recently, hydroxychloroquine (HCQ), the antimalarial drug, has been proposed as an effective treatment for Covid-19, But in April 2020, the Food and Drug Administration warned service providers against using it because of its serious heart rhythm effects. So this research aimed to demonstrate the impact of HCQ treatment on heart function by determined CK-MB and acetylcholine (ACH) levels in the mice. Thirty adult male albino Balb/C mice, aged 2-3 months, were used in this study. These mice were divided randomly into three groups (n=10): (T1) treated with the high dose of hydroxychloroquine (8.1mg/kg body weight, 2-times for 10days), (T2) treated with the low dose of hydroxychloroquine (6.4mg/kg body weight, 2-times in the first day and then 1-time for 4days), while (C) is left without treated and served as a control group. Mice were sacrificed in different time points by cervical distraction. The blood sample is collected from the eye and the Serum is separated to be used for CK-MB level determination Spanish Linear chemicals device using Roshe company's kits. Brain, spleen, and kidney were taken and homogenized to be used for Ach level determinations by ELISA technique using Mslin-China Ach kits. The results showed significantly higher CK-MB level in the T1 group compared to T2 and C groups and significantly higher ACH level in all studied tissues of both treated groups compared to control. These results indicate that HCQ could dangerously raise the heart rate, so caution and monitoring while using it are advised.

Keywords: Hydroxychloroquine, CK-MB, ACH.

Introduction

Hydroxychloroquine, 4-aminoquinolines, is widely used in the treatment of malaria, rheumatic diseases, SLE, and cutaneous and musculoskeletal lupus [1]. These drugs have been suggested as effective treatments for coronavirus disease 2019 (Covid-19) on the grounds of both anti-inflammatory and antiviral effects [2] by inhibiting the viral replication as found in SARS-CoV-2 [3]. However, it has many complications such as dyspepsia, nausea, occasional vomiting, visual disturbances (particularly transient accommodation difficulties), and headache. Orthostatic hypotension may be accentuated in febrile patients [4] Importantly, long-term CQ and HCQ use are associated with retinopathy, neuromyopathy, and cardiomyopathy [5]. In April 2020, FDA warned providers not to use the anti-malaria drugs CQ and HCQ to treat Covid-19 patients outside of clinical trials or hospital settings because the drug could cause serious heart rhythm problems[6]. Then according to WHO, On 17 June 2020, announced that the hydroxychloroquine (HCQ) arm of the Solidarity Trial to find an effective COVID-19 treatment was being stopped [6]. Data from Solidarity (including the French Discovery trial data) and the recently announced results from the UK's Recovery trial both showed that hydroxychloroquine does not result in the reduction of mortality of hospitalized COVID-19 patients when compared with standard of care[6]. The use of hydroxychloroquine and chloroquine, even with recommended doses, could result in heart rhythm problems [3] . So this research aims to study the effect of HCQ on heart rhythm by determining the levels of CK-MB, the most specific cardiac enzyme used for the clinical

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diagnosis of myocardial injury, and ACH, a neurotransmitter used at the neuromuscular junction.

Materials and methods

Thirty adult male albino Balb/C mice provided by the Iraqi Center for Cancer Research and Medical Genetic/ Ministry of Higher Education and Scientific Research were used in this work. These mice aged were between 2-3 months. Animals are socially housed in standard cages (10 in each cage) under conventional conditions, constant temperature, and 12h light-dark cycle. Animals were provided with mice pellet and tap water *ad liptum* for feeding and drinking. After two weeks of acclimatization, the mice were divided randomly into 3 groups (n=10). The first one was treated with a high dose of hydroxychloroquine drug (8.1mg/kg body weight), two doses per day for 10 days. The second was treated with a low dose of hydroxychloroquine drug (6.4mg/kg body weight), two doses on the first day and one dose on the other four days. The last one was not treated with the hydroxychloroquine drug and served as control group. Mice were sacrificed at the end of the experiment at different time points by cervical distraction. The blood sample is collected from the eye in the type of sterile gel tubes which allowed clotting. Serum is separated by centrifugation of blood sample at (10000) rpm for (10-15) min then were separated, aliquot, and frozen at -20C° until used to determine the levels of CK-MB by Spanish Linear chemicals device using and Acetylcholine. Then, the mouse body was dissected. Brain (as central nerve center), spleen (as a lymphatic organ), and kidney (as a non-lymphatic organ) were taken and frozen at -20C° until homogenized and used for Acetylcholine levels determinations. ACH levels were determined in the mice serum and tissue aliquot by sandwich enzyme-linked immunosorbent assay technique (ELISA) using commercially available murine ACH kits.

Results

Table 1 shows the comparison of the mean ± SE CK-MB among the two treated groups (high and low) and control mice group. In which there was no significant difference between the treated groups while the level of CK-MB was significantly (P<0.005) higher in the treated-high group (462.2±62.4 mg/dl) compared to treated-low and control groups (332.3±36.9 and 313.1±28.9 mg/dl, respectively).

Table 1: Mean±Stander error (mean±SE) of CK-MB levels in the serum of control and treated mice groups

Groups	CK-MB (mg/dl)
Control	313.1±28.9
Treated-high	462.2±62.4*
Treated-low	332.3±36.9
P. Value	C vs. T-h= 0.03 C vs. T-l= 0.8 T-h vs. T-l= 0.07

*Significantly different between control vs. treated groups.

The comparison of the mean ± SE ACH nanogram in microliter serum and gram tissues (brain, spleen, and kidney) among the two treated groups (high and low) and control mice group were shown in table 2. While there were no significant (P>0.05) differences among the levels of acetylcholine in the serum of the three groups, control and high and low treated groups (4.9±0.08, 4.6±0.2, and 4.7±0.02 ng/dl, respectively). Significantly (P>0.05) higher ACH level in brain, spleen, and kidney of

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treated groups; treated-high (202.3±25.2, 193.2±3.08, and 201.7±11.8 ng/g, respectively) and treated-low (180.5±16.9, 173.6±6.3, and 172.4±19.9 ng/g, respectively) compared to control (137.7±17.5, 140.4±14.7, and 143.4±13.2 ng/g, respectively). The difference between ACH levels in brain, spleen, and kidney of treated-high and low groups was also significant ($P<0.05$) since it was significantly ($P<0.05$) higher in the treated-high (202.3±25.2, 193.2±3.08, and 201.7±11.8 ng/g, respectively) compared to treated-low (180.5±16.9, 173.6±6.3, and 172.4±19.9 ng/g, respectively) (Table 4).

Table 2: Mean± Stander error (mean±SE) of acetylcholine (ACH) levels in the serum and tissues (brain, spleen, and kidney) of control and treated mice groups

Group	Serum Ach (ng/µl)	Ach in brain (ng/g)	Ach in kidney (ng/g)	Ach in spleen (ng/g)
Control	4.9±0.08	137.7±17.5	143.4±13.2	140.4±15.7
Treated-high	4.6±0.2	202.3±25.2*#	201.7±11.8*#	193.2±3.08*#
Treated-low	4.7±0.02	180.5±16.9*	172.4±19.9*	173.6±6.3*
P-Value	C vs. T-h= 0.19 C vs. T-l= 0.21 T-h vs. T-l= 0.94	C vs. T-h= 0.001 C vs. T-l= 0.020 T-h vs. T-l= 0.040	C vs. T-h= 0.004 C vs. T-l= 0.031 T-h vs. T-l= 0.037	C vs. T-h= 0.007 C vs. T-l= 0.03 T-h vs. T-l= 0.04

*significantly difference between control vs. treated groups. #significantly difference between high vs. low treated groups

Discussions

Recently, some studies indicate that using CQ and HCQ antimalarial drugs to treat Covid-19 patients may cause serious heart rhythm problems. This why the Food and Drug Administration warned service providers against using these drugs as treatment for Covid-19 patients [6] [3] [7] [8].CK-MB is the most specific cardiac enzyme found primarily in the outer plasma layer of cardiomyocytes and used for the clinical diagnosis of myocardial injury [9]. It is a bound mixture of two different types, homologous CKM and CKB, of the phosphocreatine kinase enzyme [10]. So This marker was determined in patients with covid-19 by Li et al who indicated that increased CK-MB levels were associated with a risk of severe COVID-19 due to pathogen toxicity and hypoxia resulting from excessive difficulty breathing, as in Covid-19 or pneumonia severe ones, resulting in varying degrees of myocardial injury [9]. Yükselmesi reported that risk factors for muscle toxicity are poorly understood but they could contain kidney failure, ethnicity, older age, female sex, and simultaneous use of other drugs that caused muscle toxicity [11]. Moreover, some recent studies found that a low dosage of HCQ used to treat Covid-19 increases the level of CK-MB gradually starting from the first day of treatment[12]. then, when HCQ treatment is stopped after the 6th dose, CK levels decreased 72 hours after cessation [13]. Now, the question is why could this drug increase the risk of cardiac disorders which in turn increased CK-MB levels?

Since lysosomes are one of the acidic organelles, this drug can enter the lysosomes by diffusion from the intracellular medium in the muscle tissue and accumulate there that inhibits lysosomal phospholipase and lead to cardiac and skeletal muscle cells being vacuolized Pereira, 2020 leading to rhythm disorders. On the other hand, HCQ has the potential that causes ventricular arrhythmias [14] because of the potassium-lowering effect of HQ [15], especially in patients with renal disorders [13]. Although there is no conclusive evidence to prove beyond any doubt that these drugs cause cardiotoxicity, it is still required further pharmacovigilance [7] . These pieces of

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evidence could explain the increase of CK-MB levels in the mice groups consumed low and high dosage of HCQ which was found in this work. When taking into consideration all these pieces of evidence presented, the reason for the negative effect of this drug on the heart of Covid-19 patients in contrast to patients with other diseases and used this drug also, is that covid-19 disease in itself increases CK-MB level which meets with its elevation due to HCQ consumption and leading to a significant change in this enzyme in covid-19 patients. On the other hand, Acute myocardial injury can be caused by many reasons but the most common cause is acute myocardial infarction (AMI) which is one of the most clinically significant forms. The most important step in effective AMI treatment is to restore insufficient coronary blood flow as quickly as possible to the heart muscle in the event of ischemia. Although that, reperfusion of blood into the ischemic area can lead to the death of heart muscle cells, a phenomenon called ischemic injury/reperfusion (I/R) [16]. As mention above, all body organs were innervations by ANS (SNS and PSNS) despite limited innervations of the parasympathetic nervous system in the ventricular regions of the heart, Parasympathetic nerves release ACh which regulates subtle changes in heart rate and contractility needed for proper cardiovascular function through muscarinic receptors, which reverses sympathetic nervous system activity [17]. Parasympathetic cardiac activity disorder is a common hallmark of different types of cardiovascular diseases. As have shown in some experimental studies, the elevation of Parasympathetic cardiac activity has a protective effect against myocardial I/R injury. Additionally, has been shown that ACh, the primary neurotransmitter of the heart, replicates the protective effects of ischemic heart case [16].

Recently studies reported that the cultured cardiomyocytes, just like lymphocytes and pancreatic alpha cells, can release ACH by express the required enzymatic machinery. Furthermore, the dependence of ACh secretion from cultured cardiomyocytes on transporter activity of vesicular acetylcholine (VAChT; ref. 12), [17] gives additional evidence regarding the signaling cascade's importance of non-neuronal cholinergic [16]. Although the information on the non-neuronal ACh contribution to autonomic regulation of other bodily functions in vivo is limited, this does not negate the fact that there is a non-neural source for ACh in the lymphoid and non-lymphoid organs. From the foregoing, the reason for the increase of ACh level in all studied organs in this work, namely the brain and lymphatic and non-lymphatic organs, can be attributed to it as a response to controlling heart injury arising from consumption HCQ which could lead to hyperactive of parasympathetic nervous activity. Unfortunately, however, it was not possible to measure ACH in cardiac muscle tissue due to its hardest to disrupted it and extract intracellular fluid from it.

Conclusion

The recently available evidence indicated the appearance of serious heart rhythm problems in the patients with COVID-19 after treated with HCQ which differs from other evidence in patients with other problems also treated with HCQ. The results reported here showed 1) an increase in CK-MB levels, the most specific cardiac enzyme used for the clinical diagnosis of myocardial injury, which raises the risk of heart rhythm problems, and 2) an increase in Ach level in the tissues may be as a response to controlling heart injury arising from consumption HCQ which could lead to hyperactive of parasympathetic nervous activity.

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