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Effect of Artesunate on Atherosclerosis in Experimentally Induced Nephrotic Syndrome in Rats

KEYWORDS: Artesunate , atherosclerosis , Nephrotic syndrome, Rat.

*** Amal A.Hassanin**
Clinical pharmacology department, Associate Prof. Of Clinical, Pharmacology Faculty of Medicine, Mansoura University, Egypt. * Correspondent author

Ahmed shata
Mansoura Faculty of medicine Lecturer, of Clinical Pharmacolog, , Faculty of Medicine, Mansoura University, Egypt

ABSTRACT *OBJECTIVES : This study tested the effect of Artesunate on atherosclerosis in experimentally Induced Nephrotic syndrome in rats.*

METHODS& RESULTS: 40 male Sprague-Dawley rats were randomly divided into two main groups: Group I: 10 rats served as control group and Group II: Nephrotic group in which nephrosis was induced by subcutaneous injection of HgCl2 (1mg/kg) on days 0, 2, 4, 7, 9 and 11.This group was further subdivided into 3 equal subgroups as follow: II-A: Nephrotic group Without treatment. II-B: Nephrotic group, received 10 mg/kg telmisartan daily intragastrically for 4 weeks II-C: Nephrotic group, received intraperitoneally administration of 5 mg/kg artesunate given every day for 5 days (5 injections), then every 2 day intervals (9 injections) for 4 weeks.

Artesunate decreased inflammatory markers of atherosclerosis in HgCl2-induced nephrotic syndrome rats; such as C-RP and TNF-α.

CONCLUSION: artesunate can ameliorate atherosclerosis through modulation of lipid disorders and inflammatory markers as C-RP and TNF-α , that culminate in improvement of nephritic syndrome. Howevere, telmisartan excerts its effect through its effect on lipid parameters and inflammatory markers as TNF-α only.

Introduction

Nephrotic syndrome is characterized by proteinuria, low serum albumin, edema and hyperlipidemia [1]. Hyperlipidemia is common in patients with the nephrotic syndrome [2]. It is characterized by increased total and low density lipoprotein cholesterol. Although total high density lipoprotein (HDL) values may be in the normal range, there is frequently an abnormality of HDL subclasses. The main cause is probably increased hepatic lipogenesis, a non-specific reaction to falling oncotic pressure secondary to hypoalbuminemia. So, cardiovascular morbidity and mortality are increased in patients with the nephrotic syndrome[1]. While these lipid changes may be considered a risk for atherosclerosis, they revert to normal with remission of the nephrotic syndrome. However, with chronic nephrotic range proteinuria, these abnormalities persist and may also be associated with increased levels of lipoprotein (a), increased levels of very light density lipoprotein and further reductions in HDL. These factors could all contribute to greater risk for atherosclerosis [3]. Endothelial-cell injury is the main stimulus for development of the atherosclerotic plaque: an inflammatory-fibroproliferative response results from various forms of insult to the endothelium [4].

$HgCl_2$ has been known to induce a systemic autoimmune disease, including membranous nephropathy with IgG deposits. This nephropathy is responsible for the development of high-range proteinuria and full-blown nephrotic syndrome associated with generalized edema and ascites. Multiple therapies such as statins were tried in amelioration of hyperlipidemia in nephrotic syndrome.

Artesunate is a semi-synthetic derivative of artemisinin extracted from the plant Artemisia annua . It is a safe and effective antimalarial drug [5]. Furthermore, Studies indicate that artemisinin and its derivatives may exert an anti-inflammatory effect [6]. Given these data, we reasoned that the broad spectrum antioxidant and immune modulating effects of artesunate might allow this agent to prevent the underlying oxidative processes that play an important role in the pathogenesis of nephrotic syndrome and ameliorate hyperlipidemia and atherosclerosis associated with it.

Thus, the aim of this work is to study the effect of Artesunate in atherosclerosis in experimentally Induced Nephrotic syndrome in rats.

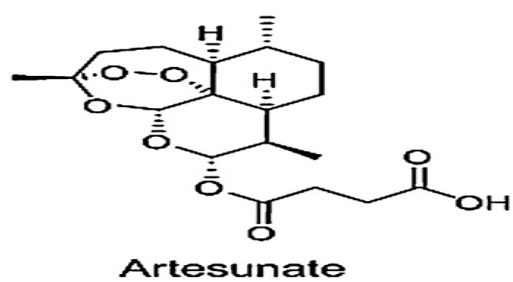

Fig. 1. Chemical structure of artesunate

Materials and Methods

Drugs investigated:

- **Artesunate** 5 mg/kg [7] was supplied by Guilin Pharmaceutical Co. Ltd, Guilin, Guangxi, China in the form of Artesor®, 60 mg/vial with one ampoule (1 ml) of 5% sodium bicarbonate solution.

- **Telmisartan** 10 mg/kg [8] was supplied by Sigma in the form of Micardis® 80 mg tablets.

Animals:

Forty adult male Sprague-Dawley rats (200–250 g) were obtained from the animal house of Mansoura Faculty of pharmacy, Mansoura University. Animals were handled with the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health and the approval from Animal Ethic Committee of the institution (Egypt).

Induction of nephrotic syndrome

$HgCl_2$ has been known to induce a systemic autoimmune disease, including membranous nephropathy with IgG deposits. This nephropathy is responsible for the development of high-range proteinuria and full-blown nephrotic syndrome associ-

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ated with generalized edema and ascites [9].

In our work, nephrosis was induced by subcutaneous injection of HgCl2 (1mg/kg) on days 0, 2, 4, 7, 9 and 11 [10]. At the end of the study period, rats in each group were weighed and individually housed in metabolic cage (Nalgene; Nalge Company, Rochester, NY, USA) for 24h urine collection. Total urine volume was measured; one ml was collected from the 24 h urine sample, and used for measurement of total proteinuria, creatinine clearance. Animals were anesthetized with pentobarbital sodium (50 mg/kg body weight; intraperitoneal) and ascites volume was measured by moistening and weighting an absorbent paper. The kidneys were removed and weighed.

Experimental design:
40 male Sprague-Dawley rats were randomly divided into two main groups:

- Group I: 10 rats served as control group, received distilled water.
- Group II: 30 rats served as HgCl2-induced nephrotic group; received 1 mg/kg, HgCl2 subcutaneous as mentioned above[10];further subdivided into 3 equal subgroups as follow:

o II-A: Nephrotic group Without treatment.
o II-B: Nephrotic group, received 10 mg/kg telmisartan daily intragastrically for 4 weeks [8].
o II-C: Nephrotic group, received intraperitoneally administration of 5 mg/kg artesunate given every day for 5 days (5 injections), then every 2 day intervals (9 injections) for 4 weeks [7].

All protocols were approved by our local committee of Animal Care and Use Committee.

Collection of Blood Sample
Blood was collected by heart puncture when rats were sacrificed. The blood samples were then centrifuged at 1000 rpm and sera stored at -20°C till biochemical analysis.

The following biochemical parameters were investigated:

Determination of serum creatinine.
Serum creatinine was estimated according to the alkaline picrate method [11].

Determination of plasma albumin
Plasma albumin was estimated according to method of Doumas et al.,[12].

Estimation of total cholesterol and triglyceride (TG)
Serum TG was estimated according to the method of Fassati [13]; while enzymatic determination of serum total cholesterol was determined according to method of Richmond [14]. They were measured spectrophotometrically with the use of Spinreact kits.

Determination of TNF-α
TNF-α was measured by method of aderka et al., [15], using kits of TNF-α enzyme immunoassay kit.

Determination of uric acid
Quantitative determination of uric acid according to colorimetric (590nm) method [16] Using ELISA-based test kits.

Determination of C-reactive protein(CRP)
CRP was measured by method of Elisa [17,18]using ELISA-based test kits.

Statistical analysis
All statistical calculations were performed with SPSS version 20 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean±S.D. Statistical significance between means was done by one-way Anova, followed by Dunnett's post hoc tests. P< 0.05 was assumed to denote a significant difference. All the P values are presented.

Results
Effect of HgCl2-induced nephrotic syndrome on tested parameters:
As shown in table (1): There were insignificant changes in body weight and kidney as regards HgCl2-induced nephrotic syndrome group as compared to control group. Also, There was a significant increase in ascitic fluid of HgCl2-induced nephrotic syndrome group as compared to control ($p<0.05$).

There were a significant reduction of creatinine clearance ,albumin in HgCl2-induced nephrotic syndrome group as compared to control group ($p<0.05$). Also, There was a significant elevation of cholesterol in HgCl2-induced nephrotic syndrome group compared to control group. Table2 showed a significant increase in C-RP,TNF- α and uric acid in HgCl2-induced nephrotic syndrome group compared to control group.

Effect of tested drugs on tested parameters in HgCl2-induced nephrotic syndrome group.
There was a significant decrease of ascitic fluid in telmisartan treated group as compared to HgCl2-induced nephrotic syndrome group ($p<0.05$). On the other hand, there was no significant change in artesunate treated group as compared HgCl2-induced nephrotic syndrome group.

Both treated groups exert a significant decrease of total urinary proteins as compared to HgCl2-induced nephrotic syndrome group ($p<0.05$). No significant changes were recorded in plasma creatinine and creatinine clearance as compared HgCl2-induced nephrotic syndrome group. Also, there was a significant difference of albumin in artesunate treated group as compared to control group ($p<0.05$).

Both treated group exert a significant decrease of cholesterol as compared to HgCl2-induced nephrotic syndrome group ($p<0.05$), on other hand there were a significant elevation of cholesterol in treated group versus control group. Artesunate treated group exert a significant reduction of TG on comparison to HgCl2-induced nephrotic syndrome ($p<0.05$).But there were a significant difference as compared to control group ($p<0.05$).

Effect of tested drugs on tested markers of atherosclerosis
Artesunate treated group exerts a significant reduction of c-RP on comparison to HgCl2-induced nephrotic syndrome ($p<0.05$). However, Telmisartan exerts a non significant effect on comparison to HgCl2-induced nephrotic syndrome ($p>0.05$).

Both treated group exert a significant decrease of TNF-α when compared to HgCl2-induced nephrotic syndrome group ($p<0.05$).

No changes were recorded as regard uric acid levels when comparing both treated group versus HgCl2-induced nephrotic syndrome.

Table 1 Effect of Artesunate (5mg/kg) and Telmisartan (10 mg/kg) on kidney functions and lipid profile in HgCl2-induced nephrotic syndrome rats

	Control group	HgCl2-induced nephrotic syndrome group	Telmisartan treated nephrotic syndrome group	artesunate treated nephrotic syndrome group
Body weight (g)	282±4.8	269±4.6	275.6±5.99	270.2±5.6
Kidney eight (g)	2.44±0.05	2.7±0.09	2.6±0.07	2.63±0.1
Ascitic fluid (g)	0.61±0.02	0.72±0.0[2]3b	0.63±0.014[a]	0.65±0.009

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Urinary proteins (mg/24 h)	13.3±0.86	271.6±6.9b	166.3±4.5[a,b]	180.7±2.6[a,b]
Plasma creatinine (mg/dl)	0.49±0.02	0.56±0.014	0.49±0.015	0.48±0.02
Creatinine clearance (ml/min)	1.72±0.06	1.45±0.07[b]	1.56±0.08	1.58±0.06
Plasma albumin (g/L)	22.7±0.9	15.7±0.58[b]	19.23±1.03	18.8±0.93[b]
Plasma cholesterol (mg/dl)	61.3±1.54	136.7±3.34[b]	113.1±2.64[a,b]	109±2.4[a,b]
Triglyceride (mg/dl)	85.6±2.4	193.5±3.6[b]	183.6±2.9[b]	174.4±3.2[a,b]

aP < 0.05; when compared to HgCl2-induced nephrotic syndrome group
bP < 0.05 ;when compared to control
cP < 0.05; when compared to Telmisartan treated

Table 2 Effect of Artesunate (5 mg/kg) and Telmisartan (10 mg/kg) on markers of atherosclerosis

	Control group	HgCl2-induced nephrotic syndrome group	Telmisartan- treated nephrotic syndrome group	Artesunate- treated nephrotic syndrome group
C-RP (mg/dl)	0.21± 0.01	0.28± 0.008[b]	0.25± 0.009[b]	0.24± 0.009[a,b]
TNF-α (ng/ml)	6.1± .23	9.5± 0.27[b]	6.9± 0.24[a]	6.8± 0.24[a]
Uric acid (mg/dl)	4.5± 0.16	6.5± 0.21[b]	6.2± 0.17[b]	6.12± 0.18[b]

aP < 0.05; when compared to HgCl2-induced nephrotic syndrome group
bP < 0.05 ;when compared to control;
cP < 0.05; when compared to Telmisartan treated

Discussion

The nephrotic syndrome is a consequence of urinary loss of plasma proteins and the resulting homeostatic responses to those losses. Hyperlipidemia is common in patients with the nephrotic syndrome [1]. So, cardiovascular morbidity and mortality are increased in patients with the nephrotic syndrome [2]. Endothelial-cell injury is the main stimulus for development of the atherosclerotic plaque: an inflammatory-fibroproliferative response results from various forms of insult to the endothelium [4]. Artesunate which is a semi-synthetic derivative of artemisinin extracted from the plant Artemisia annua is a safe and effective antimalarial drug [5]. Studies indicate that artemisinin and its derivatives may exert an anti-inflammatory effect [6].

Thus, the aim of this work is to study the effect of Artesunate in atherosclerosis in experimentally Induced Nephrotic syndrome.

In the present study, there were reductions of creatinine clearance and albumin in HgCl2-induced nephrotic syndrome rats. Also, there was elevation of urinary proteins, cholesterol and triglyceride in HgCl2-induced nephrotic syndrome rats.

This is in consistent with that of Tovar et al., [19,] Besse-Eschmann et al., [20].

Also, Vaziri& Liang [21] showed that Untreated nephrotic syndrome rats showed heavy proteinuria; hypoalbuminemia; elevated plasma cholesterol, triglyceride, LDL, VLDL. This could be explained by understanding pathological changes that occure in nephrotic syndrome as the following. Plasma protein composition is changed greatly. The synthesis of many other proteins secreted by the liver is also increased, causing an elevation in plasma levels of several large proteins, including lipoproteins and elements of the coagulation cascade. This results in hyperlipidemia and, in conjunction with the urinary loss of smaller proteins that impede coagulation. Lipoprotein catabolism is also reduced as a consequence of proteinuria contributing to increased lipid levels [22].

In the present study Artesunate produced a significant decrease of total urinary proteins ,cholesterol and TGin HgCl2-induced nephrotic syndrome rats.

This is in consistence with that of Razavi et al., [7] whose data suggested that artesunate (ART) therapy can ameliorate proteinuria, and suppress the progression of glomerular lesions in experimental model of nephrotic syndrome; it may also be recommended as a lipid-lowering drug. Furthermore, ART which has been accepted as the most effective and safe drug for treating severe and chloroquine-resistant malaria [23], Also ,has immunomodulatory properties that might be useful for treating autoimmune disease [24 , 25].

In the present study Telmisartan produced a significant decrease of total urinary proteins ,cholesterol and TG in HgCl2-induced nephrotic syndrome rats. This is like study of Villa et al., [8] who concluded that telmisartan ameliorates glomerular and tubulointerstitial damage in rat model glomerulonephritis.

Furthermore, In the present study there was an elevation of C-RP,TNF-α and Uric acid in HgCl2-induced nephrotic syndrome rats.

Atherosclerosis is a process of gradual inflammation inside the artery wall. It begins with a change in the endothelium phenotype, followed by artery wall thickening, and finally, by the appearance of atherosclerotic plaque [26]

Markers related to inflammation can be divided into proinflammatory cytokines (e.g.TNF-α), inflammation markers associated with lipid peroxidation and prostaglandin synthesis (MCP-1) [27,] and inflammation markers synthesized by hepatocytes (e.g.CRP). Proinflammatory cytokines provide a systemic stimulus that leads to hepatic synthesis of inflammatory markers such as CRP. There may be benefit from measurement of multiple inflammatory markers including the proinflammatory cytokines. Cesari et al [28] showed that high incidence of cardiovascular events in the elderly was linked with 3 markers of inflammation (i.e., IL-6, TNF-α, and CRP).

Moreover, In the present study Artesunate produced a significant decrease of both C-RP and TNF-α in HgCl2-induced nephrotic syndrome rats. Howevere, telmisartan produced a significant decrease of TNF-α only.

This is similar to many studies . Tian et al., [29] suggested that telmisartan may attenuate inflammatory process induced by TNF-alpha. Also, Artesunate (30 mg/kg) demonstrated strong anti-inflammatory effects on pulmonary cell infiltration in ovalbumin (OVA)-challenged mice, especially eosinophil and neutrophil recruitment. The overall effects are comparable to those produced by 1 mg/kg dexamethasone, a very potent corticosteroid drug [30]. It acts by the suppression of NADPH oxidases and iNOS and by modulation of antioxidants such as catalase and SODs in the lungs, probably via promotion of the nuclear Nrf2 levels [31]. Nrf2 is a redox-sensi-

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tive transcription factor that is involved in the transcriptional regulation of many antioxidant genes [32]. Also, Wei et al., [33] found that the intragastrical administration of Dihydroartemisinin (DHA) at 30 mg/kg in the ovalbumin (OVA)-induced mouse asthma model, significantly decreased the number of infiltrating inflammatory cells, T-helper type 2 (Th2) cytokines, OVA-specific immunoglobulin E (IgE) and airway hyper-responsiveness. Moreover, Artesunate produced major alterations in the activities and expression of various antioxidants in experimental asthma model. SOD is responsible for the dismutation of reactive O2 into less potent H2O2 and has been shown to be induced in airway inflammation [34]. Artesunate and dexamethasone strongly suppressed total SOD activity to the same extent.

Also, The active moiety of artesunate is an endoperoxide bridge that generates carbon-centered free radicals and oxidative stress upon cleavage [35]. The endoperoxide pharmacophore is responsible for both antiproliferative and erythroid cell inhibitory effects. This is consistent with the antimalarial, anti-tumour, anti-angiogenic and neurotoxic activity [36, 37, 38].

Furthermore, evidence indicates that the antimalarial agent artesunate has immunomodulatory properties that may be useful for treating rheumatoid arthritis.

ART treatment significantly attenuated inflammation symptoms and prevented cartilage and bone destruction. ART decreased expression of the proinflammatory cytokines interleukin-1b, tumor necrosis factor-a, and interleukin-17a. In human RA fibroblast-like synoviocytes (FLS), ART could inhibit angiogenic factor expression, and TNF-a–induced production of proinflammatory cytokines via inhibition of NF-kB andPI3 kinase/Akt signaling pathway [39, 40].

In conclusion, artesunate can ameliorate atherosclerosis through modulation of lipid disorders as well as inflammatory markers as C-RP and TNF-α , that culminate in improvement of nephritic syndrome. Howevere, telmisartan excerts its effect through its effect on lipid parameters and inflammatory markers as TNF-α only.

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