Lamivudine-artesunate co-administration affects glucose metabolism in healthy and diseased wistar rats

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Abstract: HIV-Malaria co morbidity frequently requires the co administration of Lamivudine and Artesunate, in Malaria endemic areas where HIV is also a problem. This situation is a frequent occurrence in developing countries of the tropics, like Nigeria where the burden of Malaria and HIV is heavy. The co administration of these drugs may result in interactions with possible physiologic and/or therapeutic consequences. This study investigated the effect of Lamivudine-Artesunate co administration on body weight, relative organ weight and glucose tolerance in healthy and diseased (*Plasmodium berghei* infected and cyclophosphamide immunosuppressed) wistar rats. Animals received a cumulative 21 day treatment with Lamivudine (20 mg/kg) and/or 7 day Artesunate (10 mg/kg), with healthy or disease controls. Results showed that organ weights and body weights were not affected. Oral glucose was however affected in the combination and Artesunate groups in both disease and healthy rats. The study shows that glucose tolerance is altered with Lamivudine-Artesunate co administration, and may be beneficial, as hypoglycaemia is often a complication of Malaria therapy.

Keywords: Drug interaction, glucose tolerance, artesunate, lamivudine.

INTRODUCTION

Malaria and HIV are amongst the three biggest diseases of global importance alongside Tuberculosis, killing millions of people yearly (Hotez *et al.,* 2006), and also undermining development in resource poor countries. In sub-Saharan Africa, the overlap between Malaria and HIV has made an even greater health impact complicated by inadequate health facilities, poverty and ignorance which is very often associated with stigmatization in HIV. Data has also shown that viral RNA for HIV1 is increased in Malaria (Hoffman *et al.,* 1999). For life expectancy to be improved, the need for effective therapy in the co morbid state of Malaria and HIV is essential. With Malaria being an opportunistic infection in HIV, the occurrence of HIV-Malaria co morbid state increases the Malaria burden in Malaria endemic regions. Lamivudine is frequently used as a component of highly active anti-retroviral therapy (HAART) globally (John *et al.,* 2008). It is also used as a sole therapeutic agent in treatment of hepatitis B virus infection (Zhang, *et al.,* 2000) in the tropics including in Nigeria. Artesunate is the most widely used artemisinin derivative (Zhang *et al.,* 2001) and forms the backbone of many of the available artemisinin based combination therapies in *falciparum* Malaria. Artemisinin derivatives have a very rapid onset of action, resulting in rapid parasite clearance and also being effective against parasites that are resistant to other anti-Malarial drugs (Schwarz *et al.,* 2005), and is used in both uncomplicated and severe Malaria. Both Lamivudine and Artesunate often find use in non disease states when used either for post exposure prophylaxis, in the case of Lamivudine, or presumptive treatment in Malaria with Artesunate. This concurrent administration of Lamivudine and Artesunate presents possibilities for interactions that may affect various biochemical parameters and physiological functions, in addition to those that may be results of plasmodiasis and/or immunosuppression. The current study investigated the possible effect(s) of Lamivudine-Artesunate co administration on glucose tolerance, body and relative weights of the pancreas in healthy and diseased adult wistar rats.

MATERIALS AND METHODS

Animals

Wistar rats of either sex in-bred in the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria, weighing 180-245 g were used in this study. The animals were fed on Vital Feed (Bukuru, Jos-Nigeria) and water (from public supply) *ad libitum* except when oral glucose tolerance test was conducted. Animals were used in accordance with the NIH guideline for use of animals and were also approved by the Department.

Healthy animals

To investigate the effect of 3TC-AS co administration in apparently healthy animals, four groups of rats were used, with each group having five animals. Group I served as control. Group II received 3TC (20 mg/kg) for 21 days. In addition to 3TC, group III received AS 10 mg/kg from day 15-21. Group IV received only AS (10 mg/kg) from day 15-21.

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Disease-state animals

Disease state animals were animals that were immunosuppressed with cyclophosphamide, while simultaneously infected with *Plasmodium berghei* species of Malaria parasite obtained from the National Institute for Pharmaceutical Research and Development, Abuja-Nigeria.

Immunosuppression and plasmodium infection

Immunosuppression was induced using cyclophosphamide, with an intraperitoneal stat dose of 100 mg/kg on day 1 (Huang *et al.,* 2005), with a booster 50 mg/kg on day 8. *Plasmodium berghei* infection was achieved by intraperitoneal injection of a 0.2 ml inoculum containing about $1x10^7$ parasites (Okokon *et al.,* 2006) seventy-two hours before the commencement of AS administration for groups that received AS. Animals for this study were grouped into 5 with group I serving as healthy controls. All other groups were infected with *Plasmodium berghei* and received immunosuppressive treatment with cyclophosphamide. Animals in the remaining four groups (n=5) were treated as follows; the second group served as diseased control. The third and fourth groups received Lamivudine (Evans) 20 mg/kg (dissolved in distilled water) from days 1-14. The fifth group received Artesunate (Tuyil) 10 mg/kg (dissolved in saline with sodium bicarbonate aided dissolution, from day 15 to 21, while the third group added Artesunate from day 15 to 21, the drugs being all administered intraperitoneally.

In both healthy and diseased animals, the weights of the animals were recorded weekly. On the 20^{th} day, the animals were subjected to overnight fast (18 hours) after which they were subjected to the oral glucose tolerance test by receiving 2 g/kg oral glucose (Evans) in solution following the method previously described (Saravanan and Pari, 2008). Glucose levels were determined by snipping the terminal 3 mm of the rats' tails, using glucose oxidase method, with the aid of a digital glucometer with compatible strips (On-call-Now, USA) at times 0, 30, 60 and 120 minutes. On the 21^{st} day, the animals were sacrificed following light chloroform anaesthesia and the animals were dissected and their pancreas removed, weighed using a Denver Balance and the relative pancreas weights were calculated.

STATISTICAL ANALYSIS

Data obtained from the study are presented as mean ± SEM and analysed using One Way ANOVA followed by Dunnett's pos hoc test. P values less than 0.05 were considered statistically significant.

RESULTS

Following the 21 day treatment including one week of co-administration of Lamivudine and Artesunate, there was no statistically significant variation in the oral glucose tolerance test result for all the groups in the healthy animal model when compared with vehicle control. However, there was a slight alteration in glucose tolerance observed in rats that received Artesunate (table 1). Body weight was also not significantly altered comparing the controls with the test through the 21 day study period (table 2). Oral glucose tolerance was also not significantly altered in rats that were immunosuppressed with cyclophosphamide while also simultaneously infected with *Plasmodium berghei* (table 3), with groups that received Artesunate however showing elevated glucose levels in the OGGT. Table 4 shows that in the diseased model, there were statistically significant differences in body weights, particularly observed with animals that received only Lamivudine or vehicle with immunosuppression ($p<0.01$), while the loss in body weight was less significant with animals that received Artesunate alone; or in combination with Lamivudine ($p<0.05$). In both healthy and diseased rats, there were no statistically significant differences in the relative weights of the pancreas (table 5).

DISCUSSION

In both healthy and disease state models, the Artesunate, and the combination groups showed increase in the blood glucose levels in the oral glucose tolerance test, even though not significant (see tables 1 and 3). The maintenance of glucose homeostasis is essential for daily functioning (La Fluer *et al.,* 2001) and several antimalarial drugs have been shown to have effects on glucose metabolism. Quinine and quinidine have been reported to cause hypoglycaemia during Malaria therapy, and mefloquine, even when used as a prophylactic agent has also been shown to produce a similar hypoglycaemic effect (Davis, 1997). Although the fasting plasma glucose appears to have become favoured in the diagnosis of diabetes mellitus, data have shown that the oral glucose tolerance test is able to detect diabetic patients that may have as low as 109 mg/dl fasting blood sugar (Wiener and Roberts, 1998). Patients apparently not showing clearly impaired fasting glucose may have impaired glucose tolerance with insulin resistance as reported from a study in children in Europe (Wiegand *et al.,* 2004) which may signal pre diabetes. The values that were obtained were even greater in the disease model showing that there may be a higher propensity for the development of more serious alteration in glucose tolerance in Malaria and immunosuppression. With Artesunate resulting in altered glucose tolerance, this combination which showed altered glucose tolerance requires close observation during therapy. This therefore would also pre suppose that patients that are already diabetic during the course of HAART and Malaria therapy may need to be monitored more closely in terms of their glucose metabolism irrespective of concurrent drug therapy. This may be of

Table 1: Effect of lamivudine-artesunate co-administration on oral glucose tolerance test in healthy wistar rats

Treatment Group	Mean glucose concentration (mmoles/litre)			
	0 Minutes	30 Minutes	60 Minutes	120 Minutes
VEH	3.60 ± 0.26	5.25 ± 0.42	5.32 ± 0.13	4.60 ± 0.27
3TC	3.72 ± 0.02	5.34 ± 0.26	5.20 ± 0.17	4.96 ± 0.39
3TC+AS	4.10 ± 0.28	5.50 ± 0. 42	6.36 ± 0.60	5.42 ± 0.57
AS	4.00 ± 0.15	5.90 ± 0.45	5.86 ± 0.18	5.04 ± 0.51

VEH=vehicle; 3TC=Lamivudine; 3TC+AS=Lamivudine+ Artesunate; AS=Artesunate.
Data shown in the table are means ± SEM, (n=5). There were no statistically significant differences when data were subjected to ANOVA followed by Dunnett's post hoc test.

Table 2: Effect of lamivudine-artesunate co-administration on body weight in healthy wistar rats

Treatment Group	Mean weight of animals in grams		
	Day 0	Day 15	Day 21
VEH	228.4 ± 11.84	242.2 ± 11.5	231.2 ± 11.7
3TC	221.0 ± 10.11	229.8 ± 8.8	238.2 ± 8.7
3TC+AS	223.4 ± 8.17	225.0 ± 4.27	237.6 ± 6.9
AS	228.6 ± 7.36	243.0 ± 6.7	237.6 ± 8.7

VEH=vehicle; 3TC=Lamivudine; 3TC+AS=Lamivudine+ Artesunate; AS=Artesunate.
Data shown in the table are means ± SEM, (n=5). There were no statistically significant differences when data were subjected to ANOVA followed by Dunnett's post hoc test.

Table 3: Effect of lamivudine-artesunate co-administration on oral glucose tolerance test in cyclophosphamide-immunosuppresed and *P. berghei* infected wistar rats

Treatment Group	Mean glucose concentration (mmoles/litre)			
	0 Minutes	30 Minutes	60 Minutes	120 Minutes
VEH	2.26 ± 0.20	3.32 ± 0.43	4.36 ± 0.38	2.94 ± 0.16
VIP	3.00 ± 0.40	3.65 ± 0.75	3.05 ± 0.75	2.85 ± 0.05
LIP	2.55 ± 0.65	3.90 ± 1.80	3.50 ± 1.70	2.80 ± 1.00
LAIP	2.76 ± 0.42	3.86 ± 0.36	5.40 ± 0.47	3.66 ± 0.13
AIP	2.96 ± 0.13	3.90 ± 0.40	4.63 ± 0.28	3.73 ±0. 08

VEH=vehicle treatment alone, VIP=vehicle treatment with immunosuppression and parasitaemia, LIP=Lamivudine with immunosuppression and parasitaemia, LAIP=Lamivudine+Artesunate with immunuosuppression and parasitaemia, AIP=Artesunate with immunosuppression and parasitaemia; 3TC=Lamivudine; AS=Artesunate
Data shown in the table are means ± SEM, (n=5). There were no statistically significant differences when data were subjected to ANOVA followed by Dunnett's post hoc test.

worthy note as the consequence of this in frank diabetes may be more significant. Considering the long period of use of Lamivudine and intermittent Malaria therapy, the already known possible effects of Lamivudine involvement in pancreatitis, there may be unpredictable manifestations on glucose tolerance, prediabetes and diabetes.

The combination of 3TC-AS did not have any significant effect on body weight in the healthy animals (table 2). This was however not the case in the diseased animals (table 4) where there was significant weight reduction between all immunosuppressed and parasitized group when compared with the group that relieved only the vehicle. However weight loss was not as much in the group that received Artesunate alone or Artesunate and Lamivudine. When compared with the immunosuppressed and parasitized vehicle group alone, weight variations were numerically different but not of statistical significance. This shows that the observed weight difference was due only to immunosuppression and parasitaemia. Weight loss is usually associated with HIV wasting syndrome. It has also been reported that in non HIV $CD4^+$ lymphocytopenia significant weight loss is observed (Kaczmarski *et al.,* 1994), and may therefore be attributed to the effects of immunosuppression. This thus supports the observed weight loss consequent on immunosuppression and also shows the importance of compliance with therapy in these disease conditions.

In both healthy and diseased animals, there were no significant differences in the relative weights of the pancreas (table 5). Thus it may be possible that the alteration in glucose metabolism owing to the co

Table 4: Effect of lamivudine-artesunate co-administration on body weight in CYC and mp treated wistar rats

Treatment Group	Mean Weight of Animals in grams		
	Day 1	Day 15	Day 21
VEH	177.8 ± 8.046	217.2± 7.519	217.2 ± 7.519
VIP	177.6 ± 8.177	148.3± 10.68**	148.3 ± 10.68*
LIP	176.2 ± 8.387	162.8± 6.047**	162.8 ± 6.047*
LAIP	177.0 ± 8.204	172.2± 13.62*	172.2 ± 13.62*
AIP	177.2 ± 8.206	183.5 ± 9.811	183.5 ± 9.811*

VEH=vehicle treatment alone, VIP=vehicle treatment with immunosuppression and parasitaemia, LIP=Lamivudine with immunosuppression and parasitaemia, LAIP=Lamivudine+Artesunate with immunuosuppression and parasitaemia, AIP=Artesunate with immunosuppression and parasitaemia; 3TC=Lamivudine; AS=Artesunate
Data shown in the table are means ± SEM, (n=5). *=p<0.05, **=p<0.01 compared with VEH. a=p<0.05 compared with VIP (ANOVA followed by Dunnett's post-hoc test).

Table 5: Effect of lamivudine-artesunate co-administration on relative weights of pancreas in healthy and diseased wistar rats

Healthy Wistar Rats				Diseased Wistar Rats			
Group	Treatment	Rel wt of pancreas	p-value	Group	Treatment	Rel wt of pancreas	p-value
1	VEH	0.32±0.02	p >0.05	1	VEH	0.17 ± 0.02	p >0.05
2	3TC	0.32±0.03	p >0.05	2	VIP	0.21 ± 0.08	p >0.05
3	3TC+AS	0.29±0.02	p >0.05	3	LIP	0.21 ± 0.01	p >0.05
4	AS	0.29±0.01	p >0.05	4	LIAP	0.21 ± 0.03	p >0.05
				5	AS	0.17 ± 0.02	p >0.05

VEH=Vehicle; 3TC=Lamivudine; AS=Artesunate; VIP=Vehicle treatment with immunosuppression and parasitaemia, LIP=Lamivudine with immunosuppression and parasitaemia, LAIP=Lamivudine+Artesunate with immunuosuppression and parasitaemia, AIP=Artesunate with immunosuppression and parasitaemia; 3TC=Lamivudine. Data shown in the table are means ± SEM, (n=5). There were no statistically significant differences when data were subjected to ANOVA followed by Dunnett's post hoc test.

administration of Lamivudine and Artesunate may not necessarily be consequent upon significant damage of the pancreas.

CONCLUSION

The current study has shown that glucose tolerance is affected by the co administration of Lamivudine and Artesunate in healthy rats as well as in parasitized and immunosuppressed rats. Relative weights of the pancreas were however unchanged and body weight in diseased rats were not altered significantly when diseased controls and treated animals were compared. Although these alterations were not statistically significant, this may signal a need for glucose monitoring with the concurrent use of both drugs. The impairment in glucose tolerance may also need to be followed for longer time for possibility of development of frank diabetes. The possible consequence of this interaction in clinical situations should be studied in order to make necessary therapeutic modifications if need be.

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