Effects of Ivermectin, Chloroquine or Artemether on some serum biochemical constituents in zebu cattle naturally infected with *Onchocerca gutturosa* in the Sudan.

(With three tables)

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تأثير الايفرمكتين ، الكلوروكوين أو الارتيميثر علي مكونات مصل الدم الكيموحيوية في الأبقار المصابة طبيعيا بدودة كلابية الذنب الحلقية في السودان

حسنه محمد البشير، سعديه يونس، يوسف حسين عبد الله المنصوري، هشام إسماعيل سري، علي يوسف عثمان و علي محمد عبد الماجد

في هذه الدراسة تم تقصي بعض من مكونات مصل الدم الكيموحيوية في ثلاث مجموعات من العجول المصابة طبيعياً بدودة كلابية الذنب الحلقية(*Onchocerca gutturosa*). الحيوانات المصابة تم علاجها بجرعات متكررة من الايفرمكتين، الكلوروكوين أو جرعة قياسية من الارتيميثر. النتائج التي تم التوصل إليها أشارت إلي أن أي من الأدوية المستخدمة لم تحدث تغيرات معنوية في اختبارات وظائف الكبد و الكلي، و أن القيم التي تم الحصول عليها من قياس مستوي البروتين، الزلال، الجلوبيولينات المناعية، اليوريا، حمض اليوريك و الكرياتينين كانت في مستوي القياسات الطبيعية. الزيادات التي تم ملاحظتها في مستوي كل من الجلوبيولينات، اليوريا، حمض اليوريك عقب العلاج وجد أنها ذات علاقة مع الاختزال الملاحظ في عدد الخيطيات الجلدية الدقيقة. علي كل حال، هذه المؤيضات أظهرت ثبات في المستوي عقب الجرعات المتكررة من العقاقير التي استخدمت و اختفاء الخيطيات الجلدية الدقيقة. مما يؤكد الملاحظة التي تشير إلي أن الزيادة في مستوي المؤيضات ناجم عن موت هذا الطور من الطفيل و ما يترتب عليه من تكوين مركبات الجلوبيولينات و التي قد تؤدي إلي تغير وظيفي مؤقت للكبيبات.

SUMMARY

In the present study, serum biochemical constituents were investigated in three groups of calves naturally infected with *Onchocerca gutturosa*. They were medicated with Ivermectin, Chloroquine or Artemether. The results showed that administration of any of the aforementioned drugs did not significantly (P>0.05) affect liver or kidney functions of the treated calves and the obtained values of total protein, albumin, globulins, urea, uric acid and creatinine were found to be within the normal level. However, significant increases in serum globulins, urea, and uric acid post-treatment were observed to be associated with reduction in dermal microfilariae count. Furthermore, these metabolites showed sustainable levels following repeated doses with these drugs and prolonged clearance of skin microfilaria (mf) confirming the observation that they are due to death of microfilariae and its role in production of immunoglobulin complexes that may cause transient changes in functional glomeruli.

Keywords: Ivermectin, Chloroquine, Artemether, Cattle Onchocerciasis

INTRODUCTION

Onchocerca spp. of cattle namely *Onchocerca gibson; Onchocerca gutturosa; Onchocerca lienalis and Onchocerca ochengi* have been used for studies involving *in vivo* testing of potential new macrofilaricidal compounds (Trees *et al.,* 2000). High prevalence rate of *Onchocerca gutturosa* infection in cattle in the Sudan and its genetic similarity to *Onchocerca volvulus* may allow the utilization of *O. gutturosa* in evaluating some compounds against human onchocerciasis (Dafa'alla *et al.,* 1992).

Efficacy of Ivermectin against *Onchocerca* spp. is well established and its annual regimen in man showed that there is approximately 30% reduction in microfilariae production (Plaisier *et al.,* 1995). Repeated standard doses of Ivermectin every 6 or 12 months affect the proportional recovery of living

adult *Onchocerca volvulus* (Duke *et al*., 1990) or result in mild to moderate macrofilarial effects (Awadzi *et al*., 1999). The repeating administration of Ivermectin 6 or 3 monthly was recommended by Gardon *et al.* (2002). This would greatly increase the rate of elimination of the parasite in the long-term control, and reduce the microfilarial load in infected individuals, thereby reducing the transmission of the parasite.

This study was undertaken to examine the effects of repeated doses of Ivermectin, Chloroquine or standard dose Artemether on some serum biochemical parameters in calves naturally infected with *O. gutturosa* for possible changes that may occur following medication.

MATERIALS and METHODS

Experimental animals: Fifteen male zebu calves, 2-4 years old, naturally infected with microfilariae of *Onchocerca gutturosa*, were used in this study. The animals were housed at the Central Veterinary Research Laboratories (CVRL) animal premises. They had free access to water and straw (*Sorghum bicolar*) *ad libitum*. Prior to experiment the calves were weighed and divided into three equal groups each of five and they were allotted to the following regimens: Group I (5 animals) received daily I/M injection of Chloroquine (Chloroquine – Phosphate Base - France Lab) at dose rate of 200 mg/kg daily for 7 days; then repeated weekly for 14 weeks. Calves in group II (5 animals) received weekly subcutaneous injection of Ivermectin (Ivomec®, Merck Sharp and Dohme, New Jersy1986, USA) at dose rate of 150 µg/kg body weight for 14 weeks; whereas calves in group III (5 animals) were injected with 160 mg Artemether I/M (Artemedine, Kunming Pharmaceutical Corp - China) for three successive days.

Determination of Biochemical Parameters: blood samples were taken from each calf at day 0, 1, 2, 3, then week 1, 2, 3, 4 during treatment. About five ml of blood were collected in a plain vacutainer tubes. The blood samples were

allowed to clot and the serum was harvested in containers and kept at -20ºC until analysis.

Commercial kits (Randox Laboratories Ltd., Diamond Road, Crumlin, Co. Antrim, U. K., and BT29) were used for serum metabolites analysis. The optical density of the resulting reactions in each parameter was read using spectrophotometer (Jenway, 6105 U.V. spectrophotometer / U.K.).

Statistical analysis: Changes in the values of the serum biochemical constituents were evaluated statistically by ANOVA test using the computer programme SPSS 10.0 for Windows. Values of $P < 0.05$ were considered significant.

RESULTS and DISCUSSION

Injection of Artemether and Ivermectin showed a rapid and almost 100% clearance of skin microfilariae of *O. gutturosa* in calves within the first 2-3 days of treatment as shown in Tables (2 and 3). In Chloroquine – treated group a consistent reduction in skin mf was obtained later and until week 4 the mf count was only 4.64% of their initial count. No adverse physical reactions were observed post-treatment indicating that drug associated toxicity or adverse side effects did not occur. To our knowledge, the use of Artemether in treatment of helminth infections is meager, the present study showed a comparatively high efficacy against mf of *O. gutturosa*, which may provide baseline data for its filaricidal effects on Onchocerca spp.

Pretreatment mean values of total protein, albumin and globulins were similar in all groups; however, the observed increases post treatment in total protein and globulins were not significant ($P>0.05$) and were within the normal range reported for zebu cattle.

The pretreatment values of total protein ranged between 70.6 and 78.6 g/l (Tables 1-3); nevertheless, it showed non-significant increases ($P>0.05$) at day 1 0r week 2 of treatment with either Ivermectin or Artemether. On the other hand, Chloroquine-treated group showed a delayed peak in total protein

at week three of treatment and returned to pretreatment level in week 4. Ivermectin treated animals expressed non-significant increase (P>0.05) from day 1 to the week 3 and returned to pretreatment level in week 4 of the experiment. On the other hand, Artemether treated animals showed only non significant increase in day 1 and then the values decreased none significantly till the end of the experiment. The increases in the three groups in total protein were coincided with the time of reduction in skin mf counts. The profile of total protein is associated with an increase in the globulins level while the values of albumin remained unchanged, suggesting that this profile may reflect an increase in immunoglobulins molecules in the serum parallel to the clearance in skin mf of treated calves and ultimately release of antigens due to their massive death. These observations are in line with Seri *et. al*., (2006) who reported similar increases in serum total protein and globulins 16-28 days in donkeys naturally infected with *Onchocerca railleti* being treated with a single dose of Doramectin (Dectomax, Pfizer, France). None significant increase in serum total proteins and globulins following Ivermectin injection were reported in camels (Ibrahim *et.al*., 1981; Hisham, 1999); in sheep (Shaddad, 1997) and in horses (Herd and Kociba, 1985).

In human, on the other hand results obtained by Burchard *et.al.* (1999) showed that a single dose of Ivermectin caused a significantly higher proteinuria 5 days post treatment in patients with high *O. volvulus* mf densities.

The pretreatment values of urea, uric acid and creatinine varied between the treated groups. Following treatment the values of urea increased significantly in the treated groups ($P < 0.05$) Table (1-3), whereas uric acid and creatinine showed inconsistency in their obtained values. The urea level increased significantly after treatment and reached its maximum values (30 – 38 mmol/L) in Chloroquine -treated group within 3 days and at week 1-2 in both Ivermectin and Artemether treated groups. Such increases coincided with

mf death until the level subsides later towards the end of the experiment. The obtained results were in agreement with our earlier study (Seri *et. al.*, 2006) where blood urea increased significantly in donkeys 20-24 days post treatment with Ivermectin. Similar increase in urea nitrogen was reported in horses by Herd and Kociba (1985) on day 8 of treatment with Ivermectin. This increase in urea was attributed to the fact that the drug induced immobilization of mf into urine and their induced immune complexes may cause mechanical damage to the glomeruli resulting in renal disturbances following massive death of mf (Langhammer *et al.*, 1997). Transient non - significant renal disturbances reported by Burchard *et.al*. (1999) suggest that Ivermectin seems to cause minor damage to the glomeruli and affects the re-absorption of low molecular weight proteins in the tubular system.

In a conclusion our findings showed that the use of repeated weekly doses of Chloroquine or Ivermectin or a standard dose of Artemether did not adversely affect the normal liver and kidney functions of the treated calves. However, further research is warranted before a final conclusion could be drawn.

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Table 1: Changes in serum biochemical constituents following repeated doses of Chloroquine in calves naturally infected with *O. gutturosa*.

Time	Total protein (g/l)	Albumin (g/l)	Globulins (g/l)	Urea (mmol/L)	Uric acid (mg/dL)	Creatinine (mg/dL)	Mf count %
Day 0	78.61± 6 .54	33.78 ± 3.04	44.83 ± 6.84	15.59 ± 2.99	1.02 ± 0.32	1.67 ± 0.29	100
Day1	81.23 ± 3.26	33.74 ± 4.58	47.48 ± 3.08	21.19 ± 14.24*	1.12 ± 0.34	1.58 ± 0.26	272.47
Day2	79.10 ± 5.09	35.46 ± 7.26	43.64 ± 5.64	30.36 ± 10.26*	1.16 ± 0.20	1.47 ± 0.33	117.51
Day3	81.33 ± 5.12	34.07 ± 3.51	47.26 ± 4.53	23.54 ± 8.20*	1.18 ± 0.19	1.52 ± 0.11	33.04
W1	81.04± 4.96	32.30 ± 5.20	48.74 ± 2.84	10.72 ± 4.96	1.08 ± 0.20	1.50 ± 0.33	8.08
W2	80.36 ± 2.25	33.46 ± 3.59	46.89 ± 3.54	7.90 ± 4.63	0.97 ± 0.27	1.57 ± 0.38	29.18
W3	84.28 ± 6.09	32.73 ± 5.23	51.55 ± 4.51*	12.61 ± 4.80	1.24 ± 0.30*	1.70 ± 0.30	12.02
W4	78.67 ± 6.79	38.20 ± 2.64	40.47 ± 4.94	9.91 ± 3.73	0.99 ± 0.45	1.67 ± 0.57	4.64

Table 2: Changes in serum biochemical constituents following repeated doses of Ivermectin in calves naturally infected with *O. gutturosa.*

Time	Total protein (g/L)	Albumin (g/L)	Globulins (g/L)	Urea (mmol/L)	Uric acid (mg/dL)	Creatinine (mg/dL)	Mf count%
Day 0	78.19 ± 8.46	34.79 ± 3.87	43.40 ± 9.80	4.86 ± 2.34	1.40 ± 0.14	1.64 ± 0.34	100
Day1	84.19 ± 13.65	32.75 ± 2.31	51.44 ± 12.70*	10.41 ± 3.04	1.43 ± 0.17	1.67 ± 0.15	14.41
Day2	81.77 ± 2.09	34.91 ± 2.15	45.77 ± 2.37	9.53 ± 3.56	1.28 ± 0.25	1.56 ± 0.23	1.38
Day3	78.73 ± 7.41	34.31 ± 2.06	43.32 ± 6.96	10.86 ± 4.07	1.13 ± 0.50	1.53 ± 0.19	1.41
W1	77.59 ± 10.01	35.51 ± 8.05	42.07 ± 8.51	38.21 ± 3.46*	1.40 ± 0.14	1.54 ± 0.23	0.9
W2	84.17 ± 12.44	37.15 ± 5.28	47.02 ± 7.70*	32.40 ± 1.58*	0.88 ± 0.20*	1.73 ± 0.45	0.0
W3	80.24 ± 10.98	37.13 ± 3.80	43.12 ± 8.18	2.04 ± 1.18	1.00 ± 0.27*	1.58 ±0.23	0.0
W4	78.79 ± 12.46	33.99 ± 2.17	44.81 ± 11.26	2.52 ± 1.46	1.05 ± 0.16	1.64 ± 0.16	0.0

Table 3. Changes in serum biochemical constituents following standard dose of Artemether in calves naturally infected with *O. gutturosa.*

Time	Total protein (g/l)	Albumin (g/l)	Globulins (g/l)	Urea (mmol/L)	Uric acid (mg/dL)	Creatinine (mg/dL)	Mf count %
Day 0	70.68 ± 15.69	28.43 ± 5.08	42.27 ± 14.65	22.26 ± 8.34	1.42 ± 0.33	1.26 ± 0.06	100
Day1	73.59 ± 12.61	34.59 ± 7.35	37.20 ± 10.71	18.17 ± 5.62	0.94 ± 0.36	0.98 ± 0.22	15.45
Day2	68.39 ± 9.48	31.26 ± 3.88	38.53 ± 10.04	16.57 ± 3.83	1.01 ± 0.57	1.02 ± 0.23	0.0
Day3	64.94 ± 11.59	31.25 ± 2.93	33.69 ± 10.69	26.53 ± 4.86*	0.68 ± 0.20	0.82 ± 0.17	0.0
W1	67.02 ± 13.17	31.05 ±2.75	35.97 ± 11.47	32.23 ± 9.95*	0.99 ± 0.72	0.98 ± 0.12	0.0
W2	68.48 ± 9.68	26.86 ± 5.45	41.62 ± 6.34	30.13 ± 5.32*	1.25 ± 0.45	1.21 ±0.11	0.0
W3	69.66 ± 5.44	27.83 ± 0.86	41.81 ± 4.85	29.96 ± 9.11*	1.42 ± 0.33	0.86 ± 0.14	0.0
W4	66.53 ± 6.68	28.61 ± 1.17	37.84 ± 5.41	24.69 ± 1.40*	1.22 ± 0.18	1.02 ± 0.32	0.0