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Original article

HAEMATOLOGICAL CHANGES ACCOMPANYING PROLONGED OCULAR CHLORAMPHENICOL ADMINISTRATION IN LABORATORY RABBITS.

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The toxic effect of ocular chloramphenicol on haematological parameters was studied in laboratory rabbits; Oryctolagus cuniculus while the haemotoxic effect of oral chloramphenicol provided the basis for comparison. 20 adult male rabbits were randomly but equally divided into two main groups based on the route of administration of the drug (i.e ocular or oral). In each group of ten rabbits equal number of rabbits were randomly divided into test (n=5) and control (n=5) subgroups. Oral chloramphenicol was administered at a dosage of 500mg twice daily for 21 days. Drops of ocular chloramphenicol were administered on the conjunctiva of the animals thrice daily over the same period of time. The control animals were administered with 0.9% physiological saline orally and distilled water administered ocularly. Ocular chloramphenicol produced no significant changes in the haematological parameters evaluated on the 11th and 22nd days. Conversely oral chloramphenicol was observed to significantly (P<0.05) reduced the mean total erythrocyte count, PCV, mean corpuscular haemoglobin, and neutrophils progressively by the 11th and 22nd days. Ocular chloramphenicol was confirmed to have no dose-dependent haemotoxic effect however the possibility of idiosyncratic aplastic anaemia is highlighted in this study.

Key words: Ocular chloramphenicol, Haemotoxicity, Rabbits.

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INTRODUCTION

The clinical use of systemic chloramphenicol has been plagued by the established cases of haemotoxic effect of the drug. It has been reported that of the entire drug that may be responsible for pancytopaenia, chloramphenicol is the most common cause (Wallerstain: Kasper; Brown and Morrison, 1969) Investigations showed that chloramphenicol – induced toxicity is traceable to bone marrow depression (Robbana – Barnat, 1997., Holt, 1998). The clinical pictures include reticulocytopaenia (within 5 to 7 days of initiation of therapy). This is followed by decrease in hemoglobin, an increase in plasma iron, cytoplasm vacoulation of early erythroid forms and granulocytes forms. The dose-related bone marrow depression by chloramphenicol has been reported to progress to fatal aplasia (Daum, 1979). As a result of this toxic effect chloramphenicol is used with a lot of caution. It is only employed in treatment of certain diseases where the risk benefit of the drug out weighs the risk of the potential toxicities especially in case of typhoid fever.

The aforementioned effects of systemic chloramphenicol is widely accepted, however the degree of safety of the ocular use of this same drug is enmeshed in controversy. While some workers believe that ocular chloramphenicol could be as toxic as systemic chloramphenicol, others believe the contrary. Lazarov and Amichai (1996) reported adverse skin reactions due to eye drops of chloramphenicol. Laporte *et al* (1998) also confirmed an association between ocular chloramphenicol and aplastic anaemia. At present, topical ocular chloramphenicol is widely used in the U.K for the treatment of conjunctivitis, whereas it is very rarely prescribed for this indication in the U.S. Reyner and Buckley (1996) reported that the possible haemotoxic effect of chloramphenicol led

to the prohibition of the use this drug in the U.S.

We present in this study an attempt to investigate the effect of ophthalmic chloramphenicol on haematological parameters using animal model-(laboratory rabbits). The effect of oral chloramphenicol on the haematological parameters is intended to provide a basis for comparison between ocular and systemic chloramphenicol.

MATERIALS AND METHODS

Experimental Animals: 20 adult male rabbits kept on commercially prepared rabbits grower's mash (Guinea feeds, Nigeria) *ad lib* and allowed to fresh water without restriction. The twenty rabbits were randomly but equally divided into two groups based on the route of administration of the drug; ocular or oral (i.e Groups I and II respectively). In each group (I or II) 10 rabbits were randomly, but equally selected into test and control subgroups. In other words, ocularly administered rabbits consist of the test I animals (n=5), control I animals (n=5) and orally administered rabbits consist of the test II animals (n=5) and control II animals (n=5). The animals were allowed to stabilize for four weeks during which they were dewormed with levamisole hydrochloride (Levaject^{R}, Pantex. Holland).

Administration of Drug: In group I, the conjunctiva of the five rabbits in the test subgroup were on each occasion exposed and generally flooded with ocular chloramphenicol (0.5% chloramphenicol eye drop U.S.P Mubai, India) three times daily for a period of 21 days while drops of distilled water were similarly instilled on the eye of other five rabbits in the control subgroup thrice daily for the same period of time.

In group II, oral dose of 500mg of chloramphenicol (chloramphenicol palmitate oral suspension, vardhman, Bombay, India) was administered twice daily to each rabbit in the test subgroup for a period of 21 days while the rabbits in the control subgroup were administered orally with 0.9% physiological saline for the same period of time.

Collection of Blood Samples: Blood samples were collected from all rabbits from both groups (I and II) on day zero, 11th day and 22nd day; a day after the last administration of the drug. This was achieved by first anaesthetizing the animals with ether. Capillary tubes were introduced into the orbital sinus systematically in a way that allowed blood to collect in the lithium-heparinised tubes.

*Haematology:*Haemoglobin concentration (HBC) was determined by cyanomethaemoglobin method, packed cell volume (PCV) by capillary tube method, total erythrocyte count (TEC) by the haemocytometer method, total leucocyte count (TLC) and it's differentials by Giemsa stains slides methods (Jain, 1986). The mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were calculated from the data obtained.

Statistical Analysis: Results are expressed as the mean $\pm$ standard error of mean. Differences between means of values of test and control animals were statistically determined by the student's t-test. Differences are considered significant at $p<0.05$ probability level (Bailey, 1992).

RESULTS

***The effect of ocular chloramphenicol on erythrocyte indices*:** The mean value of PCV and mean corpuscular hemoglobin of rabbits in the test group increased through the period of administration of ocular chloramphenicol. However the differences are not significant (Table 1) when compared with the control values. Conversely mean value of MCV and HBC of the same rabbits decreased insignificantly ($P>0.05$) (Table 1) through the same period of time. By the 11th day of ocular administration of the drug, the mean total

circulating erythrocyte count had decreased while MCHC increased. These various changes are not statistically significant (P>0.05) when compared with the control values at any of the days.

The effect of ocular chloramphenicol on total leukocyte count and its differentials: There was slight increase in mean of basophils, eosinophils and lymphocytes covering the period of drug administration in the test group. The differences are not significant (P>0.05) when compared with the control values (Table 2). By the 11th day of administration of ocular chloramphenicol, neutrophils was found to have decreased while the monocytes and total leucocytes count increased but the reverse was the case by the 22nd days. However, these changes are not statistically significant (P>0.05) when compared with the control values (Table 2).

The Effect of oral chloramphenicol on erythrocyte indices: The mean value of circulating erythrocytes, PCV and mean corpuscular haemoglobin decline consistently through the period of oral administration of chloramphenicol. The difference are statistically significant in TEC, PCV and MCH (P<0.05) when compared with the values obtained for control group (Table 1). MCHC and MCV were found to have decreased by the 11th day and by the 22nd day: these values have increased. The changes were not statistically significant (P>0.05) over the values obtained for the control group (Table1). HBC increased by the 11th day and was observed to have decreased by the 22nd day but these changes are not statistically significant (P>0.05) Table (1).

The effect of oral chloramphenicol on leucocyte count and it's differential: The total leucocyte count, neutrophils, eosinophils, monocytes and basophils decreased consistently through the period of oral administration of the drug. The changes in the mean values when compared with control values are only significant (P<0.05) for neutrophils. (Table 2).

Table 1. The mean (± S.E.M) of total erythrocyte counts and other haematological indices of rabbits before, during and after administration with ocular or oral chloramphenicol.

Parameter	Day	Ocular chloramphenicol (n=5)		Oral Chloramphenicol (n=5)	
		Test group I	Control group I	Test group II	Control group II
RBC 10^6/ul)	0	5.76 ±0.11	5.40±0.12	4.92±0.11	5.01±0.09
	11	4.90±±0.12	5.13±0.16	*4.50±0.16	4.98±0.11
	22	5.27±0.10	521±0.12	*4.45±0.05	5.04±0.03
PCV (%)	0	25.25±1.00	25.75±0.36	26.11±0.08	26.23±0.09
	11	25.53±0.29	26.50±0.43	*25.19±0.2	26.21±0.15
	22	26.00±0.18	24.50±0.74	*24.70±0.1	26.45±0.14
MCV (fl)	0	54.22±3.41	46.82±1.12	53.24±0.56	51.73±0.89
	11	52.28±0.93	52.18±1.18	51.72±0.64	49.66±0.92
	22	49.60±0.79	47.03±0.90	53.17±0.85	50.95±0.77
MCH(Pg)	0	7.93±0.36	8.10±0.29	7.57±0.14	7.63±0.11
	11	8.03±0.29	7.83±0.32	7.45±0.12	7.68±0.11
	22	8.05±0.14	7.45±0.23	*7.41±0.14	7.90±0.16
MCHC (g/dl)	0	31.27±0.45	30.90±0.44	31.27±0.67	31.12±0.64
	11	31.44±0.27	31.27±0.79	31.06±0.64	31.05±0.72
	22	31.06±0.49	30.47±0.54	31.24±0.70	31.15±0.74
HBC(g/dl)	0	20.82±0.39	21.00±0.33	20.72±0.33	20.99±0.35
	11	20.77±0.56	20.98±0.60	21.11±0.29	20.50±0.29
	22	20.69±0.47	20.70±0.49	20.60±0.36	21.00±0.30

*n=Number of animals in the group; *=Implies significant differences (P<0.001) exists between means values of test and control animals; **= Implies significant differences (P<0.05) exists between mean values of test and control animals.*

Table 2:

The mean (±S.EM) of total white blood cell count and the different cells of rabbits before, during and after administration with ocular or oral chloramphenicol

Parameter	DAY	Ocular chloramphenicol		Oral Chloramphenicol	
		Test group I n=5	Control group I n=5	Test group II n=5	Control groupII n=5
Total WBC count 10^3/ul)	0	6.64±0.61	6.73±0.53	7.88±0.32	6.99±0.31
	11	6.82±0.80	6.92±0.51	6.24±0.57	7.16±0.33
	22	6.74±0.50	7.13±0.45	5.70±0.40	5.04±0.03
Neutrophils (%)	0	45.04±1.7	42.75±1.1	44.10±0.8	42.21±0.8
	11	43.07±0.5	44.26±.8	**40.22±0.5	42.73±0.6
	22	45.34±1.1	43.88±0.8	**40.13±0.5	42.50±0.7
Lymphocytes (fl)	0	40.36±0.6	41.12±0.6	41.42±0.36	41.93±0.3
	11	41.04±0.7	39.75±0.9	43.25±0.76	41.62±0.8
	22	42.03±0.7	42.91±0.7	41.76±0.54	42.54±0.7
Monocytes (Pg)	0	9.34±0.16	9.57 ±0.2	9.16 ±0.23	8.95±0.19
	11	9.72±0.28	9.26 ±0.5	8.79 ±0.34	9.23±0.26
	22	9.65±0.33	9.10 ±0.6	8.46 ±0.38	9.01±0.32
Eosinophils (%)	0	1.51±0.16	1.27±0.24	1.82±0.19	1.53±0.23
	11	1.53±0.20	1.93±0.28	1.67±0.21	1.55±0.23
	22	1.62±0.10	1.50±0.21	1.52±0.11	1.64±0.18
Basophils (%)	0	5.34±0.32	5.07±0.21	5.27±0.14	5.14±0.24
	11	5.65±0.22	5.33±0.28	5.25±0.33	5.36±0.18
	22	5.87±0.56	5.25±0.63	5.01±0.28	5.13±0.27

n = Number of rabbits in the group.

*** = Implies significant difference (P< 0.05) exists between means of test and control animal*

The mean circulating lymphocytes increased by the 11th day of drug administration and was found to have decreased by the 22nd day. Statistical comparism of these values with the control values shows that the differences are not significant (P>0.05). (Table 2).

DISCUSSION

There was no specific pattern of effect of ocular chloramphenicol on the erythrocyte indices, leucocyte count and it's differential cells. Much of the changes observed were not consistent or statistically significant thus creating an impression that these changes are merely due to chances. It will be right to affirm based on this study that ocular chloraphenicol may not have haemotoxic effects. This assertion is similar to the opinion of Field *et al* (1999) that ophthalmic chloramphenicol is demonstrably effective, safe, cost-effective treatment for most superficial eye infection. Walker (1998) had earlier reported that ocularly administered chloramphenicol failed to accumulate to detectable levels in the blood to warrant the drug to be a risk factor for inducing dose-related bone marrow toxicity.

On the other hand, systemic chloramphenicol had long been confirmed to exhibit haemotoxic effects. This was clearly observed in this study; by 11th day of administering Chloramphenical orally; the circulating RBC, WBC, neutrophilis, PCV and mean corpuscular haemoglobin levels reduced significantly and these reductions were progressively so observed by the 22nd day. The effect of systemic Chloramphenicol on the blood has been attributed to inhibition of mitochondrial protein synthesis in the myeloid tissue (Wakabayashi, 1999) or due to membrane stabilising effect of cells by

Chloraphenicol (Wuc, 1996).Holt, (1998) further observed that these toxic effects are exerted at the differentiation stage of the committed marrow progenitor cells rather than at the replicative sage of the stem cells. Haemotoxicty of systemic Chloramphenicol has also been reported to be presented in two forms, (I) dose-dependent bone marrow depression and (ii) fatal idiosyncratic aplastic anaemia (Holt *et al*. 1997). Even though ocular chloramphenicol may yield low detectable levels in the blood (Walker, 1998) which may not be significant to precipitate dose-dependent bone marrow depression, the incidence of aplastic anemia is a simple possibility. This is so when considering the fact that aplastic anaemia is a factor of hypersensivity or idiosyncrasy rather than dose-dependence. In this same line of argument, McGhee (1996) proved that the risk of chloramphenicol-induced idiosyncratic aplastic anaemia exists with topical ophthalmic therapy with the minimum risk of death (equalling that of systemic penicillin therapy being 1 in 50,000 to 90,000. This figure he stressed is comparable to the risk of fatal anaphylaxis resulting from any route (i.e 1 in 100,000). Laporte *et al* (1998) also gave credence to this fact when he confirmed a probable association between ocular chloramphenicol and fatal idiosyncratic aplastic anaemia. Field *et al* (1999) hinted that even in UK where ocular chloramphenicol is freely prescribed, the use of the drug is being reviewed starting from 1995.

In conclusion, it is very pertinent to establish that in the clinical use of ocular chloramphenicol the benefit of it's proved safety, tolerance, cost and efficacy must be weighed viz a viz the remote risk of serious adverse effect of drug induced aplastic anaemia. This risk-benefit assessment is therefore the duty of every prescribing physician.

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