Analysis of Effects of Radiographic Contrast Media (RCM) on Blood Cells of Albino Wistar Rat

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Abstract:
The purpose of this study was to analyze the effects of Radiographic Contrast Media (RCM) on the status of blood cells of albino Wistar rat. Thirty (30) Wistar rats weighing between 182 – 212 kg were used for the experiment. The animals were grouped into five, consisting of 6 animals per group. 1 group was used as control, while 4 served as experimental receiving low and high dosage of RCM [urografin (diatrizoate) and ultravist (iopromide)]. Collection of blood samples was carried out on the experimental groups at the intervals of 30 minutes, 1 hour, 12 hours and 24 hours. Blood sampling on the experimental groups and control group was carried out simultaneously. Results showed that RCM affect blood cells generally with urografin (ionic) showing sustained effect on platelet and ultravist (non-ionic) with un-pronounced effect on packed cells volume.

Keywords: Urografin, Ultravist, Blood cells, Radiographic contrast media

1. Introduction

Radiographic contrast media (RCM) are substances introduced into the body in order to make an organ, or the surface of an organ, or materials within the lumen of an organ visible on imaging (Ikamaise *et al*, 2015; Ikamaise *et al*, 2016).

The use of RCM to improve visualization of anatomic structures and track physiological processes is universal in medical imaging(Speck, 1993; Thomsen & Morcos, 2000; Morcos, 2003). These media, apart from enhancing visualization, which is key to medical diagnosis, have various adverse effects on the tissues.(Marshal, 2006[a]; Ikamaise 2015; Ikamaise *et al*, 2016).

Solutions which have sufficient iodine to be useful as RCM are all significantly hypertonic to blood serum. The osmolality of a solution is proportional to the number of particles in the solution. Therefore, high-osmolar compounds are ionic monomers and are not tolerated well by the body, giving rise to a high incidence of adverse effects while ionic dimmers and non-ionic monomers are better tolerated by the body with fewer adverse effects (Katayama *et al*, 1990; Hoffmann *et al*, 2000). It is established that adverse effects from the use of RCM stem from the osmolality of the contrast, the injection rate, type, formulation, the dose and the viscosity of the agent (Marshall, 2006[a& b]).

Studies carried out to investigate the safety and risk of contrast media are mostly surveys based on observation and classification of adverse effects on patients who had RCM administered on them, and some are trials on patients to establish physiological manifestations of effects of RCM. These studies reveal the occurrence of adverse effects but observe that there is no test that can predict a patient's reaction to contrast media. There is no correlation with cutaneous iodine patch test. Testing by subcutaneous injection is as likely to be risky as the full diagnostic study. Major reactions are unpredictable and intravenous injection carries a much higher risk than intra-arterial injection. Others observed that injection of a contrast medium into the blood vessel creates a sudden rise in plasma osmolality and may cause osmotic movement of extravascular fluid into the vascular space leading to hypervolaemia with attendant complications. However, some reactions are unpredictable in a giving patient because they are unrelated to the concentration or dose of contrast medium that is injected since even small quantity can elicit a reaction (Burns *et al*, 1981;Caro*et al*, 1991; Hoffmann *et al*,

2000;Esplugas*et al*, 2002;Tepel & Zidek, 2002;Person & Tepel, 2006; Meth and Maibach, 2006; Becker, 2007; Caca, *et al*, 2007; Cutroneo*et al*, 2007).

Via role extension Medical Imaging experts are performing designated medical tasks such as intravenous injections of contrast agents. Consequently, there is the need for Medical Imaging experts to receive more information regarding these agents in order to fully understand the characteristics and the possible effects of these drugs. It is necessary to carry out specific investigations of the effects of contrast media at the cellular level and this will serve as a step towards full understanding of aetiology which will enhance predictability of expected effects following administration of RCM. This study therefore, was designed to investigate effects of RCM on the blood cells of Wistar rats using an ionic RCM (urografin) and a non-ionic RCM (ultravist). This study establishes the histological pattern of post RCM injection on blood cells of the Wistar rats.

2. Methodology

An experimental study was carried out using a total number of Thirty (30) Wister rats and two types of RCM, namely; ultravist (iopromide) and urografin (diatrizoate). RCMwere chosen based on the fact that ultravist is a non-ionic while urografin is ionic for the purpose of comparing effects. The sourcing, breeding, and sacrificing of the experimental animals was carried out under standardized laboratory conditions following recommendations of the institute of Laboratory Animal Resources (Cheesbrough, 2000; Ikamaise *et al*, 2015; Ikamaise *et al*)

The rats were grouped into Six with 5 rats in each of the groups.Four groups served as experimental groups, while the remaining two groups were control groups. Each group was kept in a separate cage throughout the experimental and sampling period. Numbers were used to match rats with their body weights for the purpose of dose calculation for each rat. From the experimental and controlgroups collection of bloodsamples was carried out on ratsat intervals of 30 minutes, 60 minutes, 2 hours, 12 hours and 24 hours after administration of RCM on the experimental groups, the sampling from experimental and control groups was carried out based on the use of two different contrast media and administration of low and high doses of these RCM.

The dose of RCM for each rat was calculated using equation (1) below. (Ikamaise *et al,* 2015and Ikamaise *et al*, 2016)

Dosage $\qquad$ = $\dfrac{V_c \times W_r}{W_{man}}$ $\qquad$ Eqn (1)

Where V_c = Volume of contrast medium
W_r = Weight of rat
W_{man} = Weight of a standard physiological man (70kg)

Rats were sacrificed by cervical dislocation method and place on a dissecting board in the supine position for laparotomy. A cardiac puncture was made and 4 ml of blood obtained using a 5 ml syringe. The blood sample obtained was infused immediately into EDT bottles to prevent coagulation while in transit to the haematology laboratory where blood analysis was carried out.

Haematological assessment of Red Blood Cell (RBC) employs Haemacytometer Formol- citrate was prepared and used as the diluting reagent (Baker and Silverton, 1990).Twenty (20) µl blood samples was taken using automatic pipette and added to 4ml of the diluting reagent in a kahn test tube to obtain a dilution of 1 in 200. The diluted sample was mixed and using a Pasteur pipette put into a counting chamber consisting of a heavy glass slide with four troughs. When the cells were settled out of suspension, the number lying on 4 of the 0.04mm^2 areas and those in the central 1mm^2 areas were counted using light microscope. The final result was expressed as the number of cells per litre using equation (2) (Cheesbrough, 2000).

$RedCellCount$ = $\dfrac{N \ X \ DF \ X \ 10^6}{AXD} Perlitre$ - - - - -Eqn (2)

Where N = *Number of cells counted*
DF = *Dilution factor*
10^6 = *Converts to cells per litre*
A = *The area of chamber counted*

For White Blood Cells (WBC), 0.38 ml (380µl) of turks fluid was measured using plain pipette and dispensed into khan tube.20µl (0.02ml) of blood sample was measured using a pipette into the test tube containing the diluting reagent to give a final dilution of 1 in 20. The haemocytometer was assembled as for RBC count. The diluted blood sample was remixed and loaded into the counting chamber. Using the improved Neubuer chamber, WBCs present in the four (4) corners 1mm^2 area and those in the central 1mm^2 areas was counted using the light microscope. The final white cell count for the whole blood sample was calculated using equation (2).

For platelet count, 1 in 20 dilution of the blood sample was made in 1% ammonium oxalate. The diluted sample was mixed for several minutes after which it was loaded into an improved Neubauer counting chamber. The chamber was allowed to stand in a petri-dish containing a piece of moist filter paper for 20 minutes to allow the cells to settle. The cells lying on the line of the 0.04mm^2 area were counted using light microscope. For final count of the platelet, equation (2) was used to calculate.

The packed cell volume or haematocrit is a measure of the relative mass of red cells present in a sample of whole blood. The packed cell volume was obtained using the centrifugation method because of its simplicity, speed and overall reproducibility.

Three quarter of plain capillary tubes was filled with well mixed DTA anti-coagulated blood sample. The distal parts of the capillary tube were flame-sealed using the Bunsen burner. The slots of the micro-haematocrite rotor with the sealed end against the rim gasket and was placed in haematospin machine. The machine was set to spin at 12,000 revolution/minutes for 5minutes and the PCV was estimated by measuring the height of the erythrocyte column in the capillary tubes using the micro-haematocrite reader.

Descriptive statistics and Student t-test statistical tool was used to analyse the data obtained from control group and experimental groups on blood counts. A probability level of 0.05 was considered statistically significant.

4. Results and Discussion

Results for RBC showed differences between the control and experimental groups for low dose of urografin and, for low and high doses of ultravist ($P<0.05$).No differences were recorded for high dose of urografin at .5, 1, 2 and 12 hours post injection (Table 1). After the initial reduction in RBC counts, rats injected with high doses of ultravist recorded increase in red blood cells count from 12 hours after injection and the counts were restored to normal at 24 hours after injection. Reduction from the high dose of urografin persisted beyond 24 hours

The result means that the RBC is affected significantly by RCM. Effect of urografin is sustained more than that of ultravist. Agwu, *et al* (2007) reported that 70% of the erythrocytes were morphologically altered from their approximate 100% normocytes shape in a study investigating the effect of in-vivo intravenous administration of sodium meglumine diatrizoate on some haematological parameters. Hardeman, *et al* (1991) reported that iopamidol affects RBC by producing echinocyte morphology and irregular RBC aggregates have been associated with the presence of echinocytes. The findings of this study will probably stem from the ability of the RCM to alter or induce some damages on the shape of the cells and thereby likely to affect its physiological functions.

Statistical significant differences($P<0.05$) were observed in values for WBC between the control and the experimental groups at all time intervals after injection of both RCM (Table 2).RCM administration on WBC caused increase in the count irrespective of ionic or non-ionic status of RCM or quantity injected. Primarily, WBC serves to protect the system by various mechanisms. The observed increase in WBC count signals alterations in the physiological state of the system. This effect is probably more on the neutrophils because neutrophil make up 50 – 80% of WBC and increase mean count of neutrophil was reported by Agwu *et al* (2007) after RCM injection.

Plateletscount were different significantly($P<0.05$) in all experimental groups compared the control group except for low dose of both RCM at .5 hour after injection (Table 3).This result suggests that RCM show signs of delayed effect on platelets. On the other hand, Chronos *et al* (1993) reported that platelets degranulation depends on osmolality of the RCM, it does not matter whether the RCM is ionic or non-ionic, and that a non-ionic low osmolar contrast medium (omnipaque) caused nearly 80% degranulation of platelets compared with 25% from urografin which is an ionic high osmolar contrast medium.

PCV values were significantly different ($P<0.05$) in rats that urografin was injected, while most of the rats injected with ultravist showed no difference (Table 4).Injection of urografin was observed to bring down the PCV values for 12 hours irrespective of whether it is low dose or high dose compared to ultravist that showed a stable and unaffected pattern in PCV. PCV is a strong indicator for the status of all the cells in the blood as well as other constituents of the blood.

Considering the effects of RCM on RBC and WBC these results suggest that the effect of urografin on RBC and WBC and the body system being persistent will probably be more harmful when high dosage of RCM is considered as is applicable for victims of road traffic accident when referred for radiographic contrast studies and other specialized examinations requiring high doses of contrast as is applicable to Computerized tomography and angiography.

Comparatively, the results obtained for ultravist and urografin in figures1 and 2, which showed similar pattern in the response of platelets to both low and high doses indicate that platelets may be affected in similar manner by ionic and non-ionic contrast medium.

Urografin brought down the PCV values for 12 hours after injection, while ultravist did not affect the PCV values markedly. The implication is that urografin, an ionic contrast medium affects PCV markedly while ultravist, a non-ionic contrast medium does not. This finding may guide the Radiographer on the choice of RCM as means of protecting vulnerable patients from adverse effects of RCM.

Generally, patients referred for Radiographic contrast studies present with various clinical conditions that may lead to mild or fatal complications following injection of RCM. These clinical conditions may include allergic reactions, blood or bone marrow disorder, renal problems, asthmatic condition among others (Marshal, 2006$_a$). Considerations of findings reached by this study before choosing a contrast and deciding on the dosage may prevent some adverse effects.

5. Conclusion

RCM (ultravist and urografin) cause reduction in mean values of RBC and increase in mean values of WBC and delayed effects platelets.

Utravist and urografin affect haematological parameters in a similar manner except that urografin injection decreases the packed cell volume of Wistar rat while ultravist do not.

Findings of this study are useful as a guide to choice of type of contrast (ionic or non-ionic) and decision on dosage for injection for a particular patient depending on the prevailing clinical condition of the patient.

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Appendix

Time Interval (Hours)	Control Group	Urografin (Diatrizoate-Ionic)				Ultravist (Iopromide–Non-Ionic)			
		LD	P_{Value}	HD	P_{Value}	LD	P_{Value}	HD	P_{Value}
.50	10.70±.37	9.63±.05	.038*	10.60±.16	.709	9.70±.14	.048*	8.70±.27	.005*
1.0	11.10±.45	8.70±.43	.007*	10.67±.05	.242	8.76±.12	.013*	9.20±.16	.020*
2.0	14.60±2.15	8.57±.12	.040*	10.2±.22	.072	9.06±.05	.047*	9.20±.08	.049*
12	11.20±.16	8.60±.16	.000*	9.77±.21	.003*	9.20±.00	.002*	11.37±.12	.237
24	9.70±.08	9.13±.12	.006*	9.63±.05	.289	9.50±.00	.049*	13.57±.12	.000*

Table 1: Mean Values for Red Blood Cells Count At Time Intervals After Injectionof Contrast Medium (Values Are ×109/L Of Blood).
LD = Low Dose   HD = High Dose   (T- Stat. =P<0.05)   * = Significant

Time Interval (Hours)	Control Group	Urografin (Diatrizoate-Ionic)				Ultravist (Iopromide–Non-Ionic)			
		LD	P_{Value}	HD	P_{Value}	LD	P_{Value}	HD	P_{Value}
.50	158±.12	171± .50	.001*	314± 2.87	.000*	171±1.63	.005*	366±1.63	.010*
1.0	157±.21	230±.33	.000*	417±2.45	.000*	163±2.05	.034*	562±1.63	.000*
2.0	149±.13	232±.33	.000*	592±2.16	.000*	151±.81	.052	367±2.05	.000*
12	158±.22	233±.47	.000*	549±1.25	.000*	218±1.63	.000*	361±.94	.000*
24	158±.17	240±.47	.014*	302±.47	.005*	222±.47	.022*	185±4.50	.115

Table 2: Mean Values for White Blood Cells Count at Time Intervals after Injection of Contrast Medium (Values Are ×109/L of Blood)
LD = Low Dose   HD = High Dose   (T- Stat. = P<0.05)   * = Significant

Time Interval (Hours)	Control Group.	Urografin (Diatrizoate-Ionic)				Ultravist (Iopromide–Non-Ionic)			
		LD	P_{Value}	HD	P_{Value}	LD	P_{Value}	HD	P_{Value}
.50	77.3±2.21	79±1.25	.915	669±.94	.001*	76±3.40	.960	677±1.25	.001*
1.0	98±1.63	415±.2.2	.000*	802±2.16	.000*	312±1.70	.000*	740±.47	.000*
2.0	98±2.16	4.3±2.56	.000*	810±1.70	.000*	321±.47	.000*	757±2.62	.000*
12	99±.94	331±5.05	.001*	841±1.63	.000*	269±1.24	.000*	774±3.40	.000*
24	100±1.25	339±2.86	.000*	840±.50	.000*	246±0.81	.000*	826±3.27	.000*

Table 3: Mean Values for Platelets at Time Intervals after Injection of Contrast Medium
(Values Are ×109/L Of Blood).
*LD = Low Dose   HD = High Dose   (T- Stat. = P<0.05) * = Significant*

Time Interval (Hours)	Control Group.	Urografin (Diatrizoate-Ionic)				Ultravist (Iopromide–Non-Ionic)			
		LD	P_{Value}	HD	P_{Value}	LD	P_{Value}	HD	P_{Value}
.50	45.16±.24	44.17±.24	.007*	44.17±2.32	.530	50.67±.47	.003*	43.33±.47	.027*
1.0	48.67±1.23	40.17±.62	.009*	37.83±1.64	.003*	47.0±.82	.145	41.67±2.36	.020*
2.0	47.67±1.70	39.5±2.04	.013*	43.38±.47	.052	45.0±.82	.145	45.33±1.70	.167
12	46.67±.47	39.17±.85	.001*	41.17±.24	.003*	46.67±1.25	1.000	42.33±2.05	.070
24	50.0±1.63	40.17±.24	.009*	40.33±1.24	.004*	46.67±1.25	.067	49.0±2.94	.642

Table 4: Mean Values for Packed Cell Volume at Time Intervals after Injection of Contrast Medium
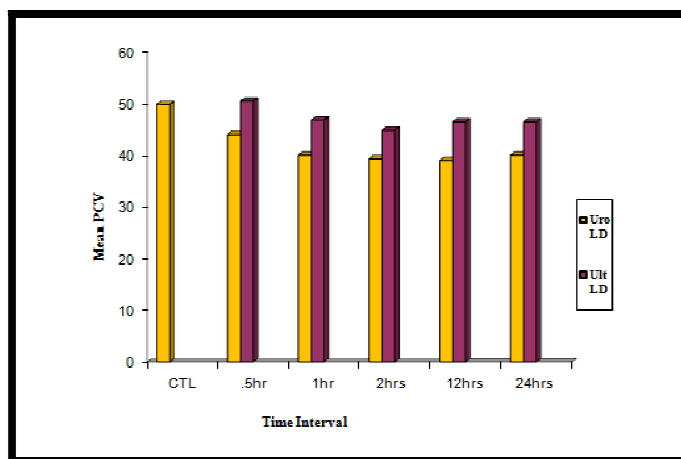*LD = Low Dose   HD = High Dose   (T- Stat. = P<0.05)   * = Significant*

Figure 1: Effects of Low Dose Radiographic Contrast
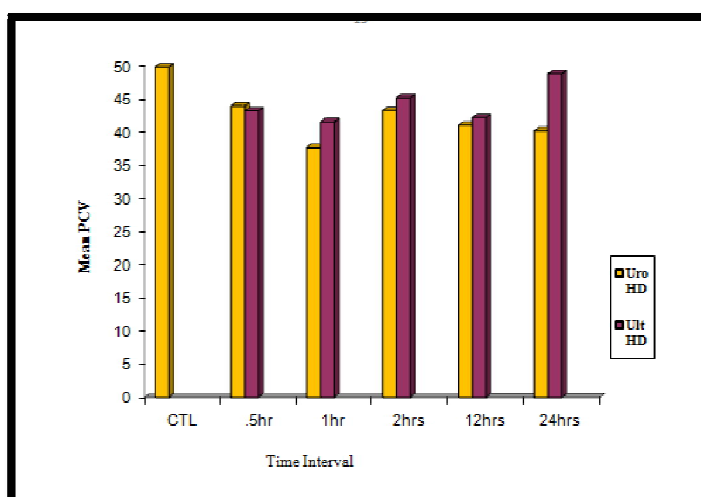*Medium on Packed Cells Volume*

Figure 2: Effects of High Dose Radiographic Contrast
Medium on Packed Cells Volume