

To Study the Chemotherapeutic Effect of Doxorubicin When Combined With Aspirin and Clopidogrel in Ehrlich Ascites Carcinoma Induced Adult Wistar Rats

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Research Article

Abstract: Objective: To determine whether there is an enhanced chemotherapeutic effect of Doxorubicin when combined with Aspirin and Clopidogrel (anti platelet drugs) in Ehrlich Ascites Carcinoma induced adult wistar rats. **Methodology:** The adult male wistar rats selected for the experiment were divided into 5 groups with 6 rats in each group. Rats bearing Ehrlich Ascites Carcinoma were used for the study. After 48 hours of inoculation of the tumor cells the rats in the control group were administered normal saline as i.p. injection on day 5. The toxic control group were administered Doxorubicin (15mg/kg stat dose i.p.) on day 5. Then Aspirin and Clopidogrel were administered orally for 7 days in different combinations and Doxorubicin was given as i.p. on the 5th day. On the 7th day rats were sacrificed with pentobarbitone overdose and the ascitic fluid was removed and volume was measured. The viability of the tumor cells from the ascitic fluid was measured by trypan blue exclusion technique. **Result:** The results obtained shows that Aspirin enhances the chemotherapeutic effect of Doxorubicin by reducing the tumor cell count. But Clopidogrel didn't show any effect. **Conclusion:** Hence the enhanced chemotherapeutic effect of Doxorubicin with Aspirin was not due to anti-platelet activity. But there is some underlying mechanism by which Aspirin showed synergism and enhanced the chemotherapeutic effect of Doxorubicin, which has to be found out in future studies. Hence after further clinical trials Aspirin can be used as a novel combination with Doxorubicin in chemotherapy. **Keywords:** Ehrlich Ascites Carcinoma, Chemotherapy, Antiplatelet drugs, Doxorubicin HCl, Platelet aggregation, Apoptosis.

Introduction

Combined therapy with multiple drugs is a common practice in the treatment of cancer, which can achieve better therapeutic effects than a single drug, and can reduce the side effects as well as drug resistance¹. The anthracycline antibiotic, doxorubicin (adriamycin) is

effective in the treatment of a broad range of haematogenous and solid human malignancies, but its clinical use is limited by its dose-dependent side effects^{2,3}. Hence a combined therapy can be tried. A study has shown that Aspirin enhances doxorubicin-induced apoptosis and reduces tumor growth¹. It has also been established that ascorbic acid administration inhibited platelet aggregation, reduced thromboxane B2 levels and enhanced the susceptibility of the tumor cells to natural killer cells⁴. Thereby the role of platelet aggregation in chemotherapy was put forth. Hence in this study we planned to establish the synergistic activity of anti-platelet drugs (namely Aspirin and Clopidogrel), with Doxorubicin.

Objectives

To determine whether there is an enhanced chemotherapeutic effect of Doxorubicin when combined with Aspirin and Clopidogrel (anti platelet drugs) in Ehrlich Ascites Carcinoma induced adult wistar rats.

Materials and Methods

Animals: 30 adult male Albino Wistar rats weighing 180-250gm were used for this experiment and were equally divided into 5 groups and maintained at 24 ± 5°C with 12 hours light and dark cycle with free access to food and water

Drugs & Chemicals

Doxorubicin HCl (DOX), Aspirin (ASP), Clopidogrel (CLOP) and other chemicals were obtained for the study. Ehrlich ascites tumor cells are obtained from national centre for cell science (NCCS), Pune. All chemicals were of analytical grade and purchased from Hi Media, and SD Fine Chemicals.

Methodology

The adult male wistar rats selected for the experiment were divided into 5 groups with 6 rats in each group. To

closely model the clinical scenario only rats bearing Ehrlich Ascites Carcinoma⁵ were used for the study. After 48 hours of inoculation of the tumor cells the rats in the control group were administered normal saline as i.p.injection on day 5. The second group was administered with Doxorubicin (15mg/kg stat dose i.p.) on day 5. Then Aspirin and Clopidogrel were administered orally for 7 days in different combinations and Doxorubicin was given as i.p. on the 5th day.

Sample size

30 rats divided into 5 groups of 6 each

G1: Normal saline- (control)

G2: DOX⁶ (15mg/kg i.p.stat dose)

G3: ASP³ 100mg/kg/day p.o. + DOX

G4: CLOP⁷ 10 mg/kg/day p.o.+ DOX

G5: ASP + CLOP + DOX

On the 7th day rats were sacrificed with pentobarbitone overdose and the ascitic fluid was removed and volume was measured. The viability of the tumor cells from the ascitic fluid was measured by trypan blue exclusion technique⁸. The cells that did not take up the dye were viable, and those that took up the dye were nonviable. These viable and nonviable cells were counted by using the following formula;

$$\text{Cell count} = \frac{\text{Number of cells} \times \text{dilution factor}}{\text{Area} \times \text{thickness of liquid film}}$$

Statistical analysis: One-way Analysis of Variance (ANOVA) using Epi.info3.4.3.

Results



Figure 1: Shows the live tumor cells in the Control group (which has not taken up the trypan blue stain)



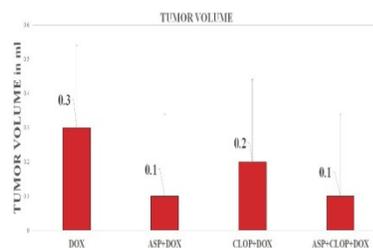
Figure 2: Shows the Dead tumor cells after treatment with Doxorubicin (Dead EAC cells have taken up the trypan blue stain)

Table 1:

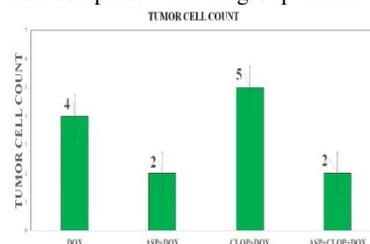
Group	Tumor volume in ml	Tumor cell count	% Tumor growth	% Tumor inhibition
G1	1.2±0.24	84±5.6	100	0
G2	0.3±0.13	4±1.3	4.76	95.24
G3	0.1±0.12*	2±1.3*	2.38	97.62
G4	0.2±0.06	5±0.8	5.95	94.05
G5	0.1±0.06*	2±0.8*	2.38	97.62

The values in the table are expressed as S.D.±SEM.

*P value < 0.05 and is significant when compared with the standard group(G2)



The above figure represents the variations in the tumor volume in ml in different groups. It shows that the Aspirin treated groups (group 3 and 5) shows significant reduction in tumor volume ($p < 0.05$) when compared with the group treated with DOX.



The above figure represents the variations in the tumor cell count in different groups. It shows that the aspirin treated Groups shows significant reduction in tumor cell count ($p < 0.05$) when compared with the group treated with DOX.

Discussion

This experimental animal study compares the effect of Doxorubicin alone and in combination with aspirin and clopidogrel. As from table 1, Control group (Normal saline, n=6) showed a tumor cell count of 84 ± 5.6 and a tumor cell volume of 1.2 ± 0.24 . With doxorubicin alone there is an appreciable decrease in the tumor cell count and volume (4 ± 1.3 & 0.3 ± 0.13 respectively with a $p = 0.001$ for both) and it shows a high tumor cell inhibition of 95.24 %. As with the combination regimen, doxorubicin when given alone with Aspirin shows an increase in the inhibition of tumor growth by 2.38 % (97.62 % compared to doxorubicin alone which produces 95.24 % of tumor inhibition) Tumor cell volume has decreased to 0.1 ± 0.12 ($p=0.023$) and the tumor cell count was 2 ± 1.3 ($p=0.023$). Similar results were obtained with Aspirin + Clopidogrel + Doxorubicin which also shows a tumor cell inhibition

of 97.62 % and a similar tumor cell count and volume. But when clopidogrel was given alone with Doxorubicin, the tumor cell inhibitory effect of doxorubicin tends to get decreased. As table 1, The tumor cell volume was 0.2 ± 0.06 ($p=0.12$) and the tumor cell count was very high compared to the other test groups (5 ± 0.52 , $p=0.14$). The tumor cell inhibition was 94.05 % which is lesser as compared with doxorubicin alone. The results obtained shows that Aspirin enhances the chemotherapeutic effect of Doxorubicin by reducing the tumor cell count. But Clopidogrel decreases the tumor cell inhibitory effect of Doxorubicin.

Conclusions

Hence the enhanced chemotherapeutic effect of Doxorubicin with Aspirin was not due to anti-platelet activity. But there is some underlying mechanism by which Aspirin showed synergism and enhanced the chemotherapeutic effect of Doxorubicin, which has to be found out in future studies. Hence after further clinical trials Aspirin can be used as a novel combination with Doxorubicin in chemotherapy.

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