ORIGINAL PROF-991

LAGENDORFF'S HEART PREPARATION;
EVALUATION OF MUSCARINIC RECEPTOR ACTIVITY OF DOXORUBIC

DR. FAREEDA ISLAM, MBBS, M.PHIL
Associate Prof & Head of Pharmacology and Therapeutics
Karachi Medical & Dental College
Block M North Nazimabad Karachi

DR. M. IQBAL AHSUN, MBBS, MCPS
Senior Registrar
Department of Anaesthesia
Karachi Medical & Dental College (ASH)
Karachi

PROF. DR. SYED MUHAMMAD SHAMIM
BSC, MBBS, MCPS, M PHIL, Ph.D
Department of Pharmacology and Therapeutics
Karachi Medical & Dental College
Block M North Nazimabad Karachi

ABSTRACT...Doxourbicin (Dox) is an effective anticancer chemotherapeutic agent, for the treatment of several different human cancers, causes an insidious and delayed cardiotoxicity. Its use is limited, as cardiac toxicity occurs above a cumulative dose 450 mgrrr2 and incidence of this toxicity increases markedly at dose2 greater than 550 mgrrr2. Pre-clinical investigations are attempting to elucidate the doxorubicin induced cardiotoxcity drugs are used to scavenge the toxicity or to determine the possible mechanisms of this cardiac toxicity. Role of autonomic nervous system in this regard has extensively been investigated. We have tried to find the physiological interactions of DOX with muscarinic receptors in presence of Atropine (Antagonist) and evaluated the effects for up and down regulation of muscarinic receptors. The results showed synergistic activity of DOX with atropine and hence DOX is said to be a muscarinic, antagonist.
Keywords: Doxorubicin, Muscarinic receptor antagonist.

INTRODUCTION

The use of Doxorubicin is limited due to its dose related cardiotoxicity1 where as Chemotherapy2'3 plays a significant role in the management of the 60% of cancer patients who are not curable by regional modalities (i.e. surgery and radiation4). Improvement in the use of chemotherapy are the result of a number of factors, including newtechniques, drugs & the combinations of the two.

Drugs are now routinely used earlier in the course of the patients management often in conjunction with radiation or surgery to treat malignancy when

it is most curable and when the patient is best able to tolerate treatment. Doxorubicin is effective for the treatment of soft tissue, osteogenic and other sarcomas, Hodgkin's disease, non-Hodgkin lymphomas.

Doxorubicin is produced by the fungus streptococcus peucetius var. Caesius since its introduction for clinical use in the early 1970s, more than two million patients have received doxorubicin[5]. Several hundred analogues of doxorubicin have been obtained by partial or total synthesis and tested in experimental tumor models in an attempt to improve the biological activity of the parent compounds[6'7'8']. Doxorubicin has shown a wide range of activity in human solid tumours is the most effective single agent against soft tissue sarcomas in adults, although it is rarely curative in advanced disease[9]. Doxorubicin is probably the best available agent[10]. Doxorubicin is not absorbed orally due to hydrolysis in the GIT and so it must be administered by parental i.v. infusion, the drug undergoes metabolism and the predominant metabolites are adriamycinol respectively[11'12]. The enzyme responsible is a cytoplasm aldo-ketoreductase which is found in all tissues but kidneys have the highest activity[13]. Most of the information on this microsomal metabolism has been given by Bachur from studies of the drug metabolits present in the urine of patients under treatment with doxorubicin. The drug is rapidly distributed from the plasma following i.v. injection and is accumulated to a high degree in all tissues except the CNS[14'15]

Elimination via the urinary route is low; less than 6% of the administered dose of Doxorubicin is excreted in the urine over 5 days[16].

Role in Cancer Chemotherapeutic:

Since the first clinical trial of doxorubicin little more than a decade ago. It has gained rapid acceptance as major therapeutic agents in the treatment of cancer.

Robinson and Giri[17]observed the chronic effects of administration of Doxorubicin on myocardial a-adrenergic receptors histamine, cyclic nucleotides, calcium, norepinephrine, calmodulin and guanylate cyclase activity and plasma catecholamines in rats and concluded that the depression of CAMP indicates damage to the membrane bound enzyme, adenylate cyclase, and that the membrane interaction of DOX appeared to be an integral part of biochemical mechanism of toxicity.

MATERIAL & METHODS

Langendorff's Heart Preparation was used. The heart was obtained from male healthy rabbits, which were acquired in-groups and acclimatized to housing conditions one week before the experiment. Solution was freshly prepared for each set of experiment. Langendorff Perfusion Apparatus (C-18-1940) was provided by Harvard apparatus Ltd. The Langendorff apparatus and the recording instrument were calibrated before experimentation. The Kreb's Ringer Buffered Physiological solution was freshly prepared for every set of experiment. It was equilibrated with 0_2 for at least 30 minutes and pH was adjusted 7.4 each time.

For extraction of heart, the rabbit was heparinized intra peritoneally and dissected at mid chest incision. The organ was mounted on isolated heart assembly and temperature of perfusion fluid was kept at 37˚C. After 15-20 minutes of stabilization the activity of heart was observed with drugs which were administered through a 1 c.c. syringe which was connected to the rubber tube near the aorta. Drugs were prepared in distill water. Each drug was repeated 5 times & separate readings were taken.

STATISTICAL ANALYSIS

Percentage change was calculated from control and plotted on graph paper. The dose is plotted on X-axis and the percentage change along Y-axis was

applied to compare the two drugs.

RESULTS

Evaluation of atropine effect on rabbit (perfused) heart;

Table-1 shows the percentage changes in amplitude and rate after the administration of 10" ^{6}gm and 10"^{5}gm of atropine from normal. The mean value at dose 10"6gm of atropine the amplitude of contraction was 1.04±5%(5) and rate was 3.6±5%(5) from normal, while at dose 10-5 gm of atropine the amplitude was found to be 2.1±0.7%(5) and rate was increased to 3.7±1.05%(5) from normal.

Table-l. Evaluation of atropine effect on rabbit heart (perfused).

Dose (gm)	%age change	
	Amplitude (n=5)	+Rate(n=5)
Zero	00.00	00,00
10'6	1.04±0.5*	3.610.51
io-5	2.U0.7	3.U1.05

*Mean±SEM.

Evaluation of atropine effect on rabbit (perfused) heart.

Table II shows the percentage change in amplitude and rate after treating the heart with a fixed effective dose of doxorubicin and then administration of the two consecutive doses of doxorubicin. The percentage change in amplitude at dose 10~6gm of atropine with DOX was 2.3±0.85%(5) and rate was 3.0±0.7% (5), whereas at a higher dose of 105 gm of atropine in DOX treated heart the amplitude was 3.5±0.72%(5) and rate was increased to 4.8±0.5%(5).

Comparison of percentage change in amplitude alone and after treating with a fixed dose of doxorubicin on rabbit (perfused) heart;

Table 111 shows the amplitude at 10"6gm of atropine in DOX treated heart was 2.3±0.85%(5) which is insignificant (P>0.05) as compared to percentage change in amplitude 1.04±0.5%(5) produced independently, while at a higher dose 10"5gm of atropine with DOX the amplitude was 3.5±0.72%(5) which is non-significant (P>0.05) as compared to the amplitude 2.1±0.7%(5) observed with atropine independently.

Table-It. Evaluation of atropine effect in doxorubicin treated rabbit (perfused) heart

Dose (gm)	%age change	
	Amplitude (n=5)	+Rate(n=5)
Zero	00.00	00.00
10'6	2.3±0.85*	-3.0±0.7
10'5	3.5±0.72	-2.8±0.5

*Mean±SEM. *No. of beats/min.

Table-Ill. Comparison of effect of atropine independently and with a fixed dose of Doxorubicin(amplitude)

Dose (gm)	%age change		P value
	Atropine (n=5)	Dose +atropine (n=5)	
Zero	00.00	00.00	
10'6	1.04±0.5*	2.3±0.85	NS
io-5	2.U0.7	3.5±0.72	NS

*Mean±S£M. n= No. of observations.

Comparison of the effect of atropine independently and with a fixed dose of DOX on rate of rabbit (perfused) heart:

Table IV shows that at dose 10"6 gm of atropine with DOX the percentage change was 3.0±0.7%(5) from normal which is non-significant (P>0.05) as compared to rate 3.6±0.5%(5) with atropine independently, whereas at dose 105 gm of atropine with DOX the rate was 4.8±0.5%(5) is statistically significant (P<0.05) as compared to the rate 3.7±1.0%(5) increased by atropine without DOX.

Table-IV. Comparison of effect of atropine independently and with a fixed dose of Ooxorubicin (rate)*

Dose (gm)	%age change		P value
	Atropine (n=5)	Dose +atropine (n=5)	
Zero	00.00	00.00	
1C"6	3.6±0.5	-3.0±0.7	<0.01
ID'5	2.1±0.7	-2.810.5	<0.01

Note: Amplitude without drug is treated as zero. * Beats/minute

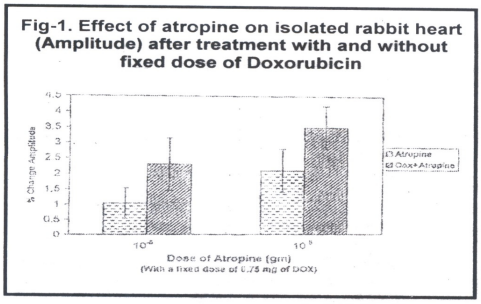

Fig-1. Effect of atropine on isolated rabbit heart (Amplitude) after treatment with and without fixed dose of Doxorubicin

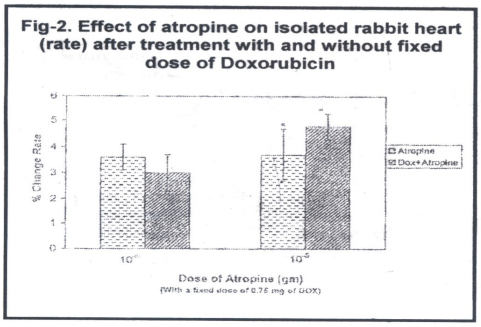

Fig-2. Effect of atropine on isolated rabbit heart (rate) after treatment with and without fixed dose of Doxorubicin

DISCUSSION

Reviews published by Blinks[18] and black[19] evaluated the mechanism of action of cardio-selective and cardiotoxic drugs which has been a topic of great stress since long. The techniques have successfully employed for cardiotoxic tendency of doxorubicin[20] an anthracycline antibiotic, which has shown an absolute response in malignant tumors.

This study was designed to find interaction of DOX on Muscarinic receptor in the presence of Atropine (a muscarinic antagonist).

i If inotoprism is considered as a response indicator it enhances the effect of atropine statistically significant (P<0.05) for all trial doses, this proportional relationship strengthen the probability.

ii If chronotropism isconsidered as response criteria then DOX looks to counteracts the response of atropine. Although the difference between different observations is statistically insignificant (P>0.05) this proportional relationship supports the hypothesis.

These observations reveal that DOX possess antimuscarinic activity in rabbit (perfused heart). Our results clearly show that DOX acts on Muscarinic receptors in a particular distribution. Its role is perhaps vague on other receptors while prominent on muscarinic receptors which is also supported by the study of Hagane[20] and his colleagues[21'22] who reported transient positive[23] inotropic and negative chronotropic effects in guineapigs. Temma[24] et al reported similar observations which reveal that doxorubicin possess a direct antimuscarnic action in guinea pig heart. These results suggests that DOX may act as competitive antagonist at muscarnic receptors in rabbit (perfused heart) the possibility therefore the

muscarinic antagonistic action of DOX contributes to the development of acute cardiotoxicity. This interpretation does not exclude other cardiotoxic actions operating at membrane level or at intercellular sites[25].

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