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THE EFFECTS OF DIGOXIN-SPECIFIC ANTISERA TO OUABAIN TOXICITY

Kevser EROL (*)

Mehmet GÜNGÖR(**)

SUMMARY :

The rabbits were immunized with digoxin-Bovine Serum Albümin (Dig-BSA) conjugates. Sera from all immunized animals contained antibodies to digoxin. Five rabbits given 150 µg/kg ouabain exhibited toxic symptoms of this glycoside and died within 60 minutes. 1.5 ml/kg immune serum and 150 µg/kg ouabain were administered to nine rabbits. Three of them showed disturbances of cardiac rhythm, reversed in 15 to 60 minutes and lived. The remaining six animals showed towic symptoms and died between 10 minutes and 3 hours. It was found that antidigoxin antibodies decreased the toxic affects of ouabain, but did not reverse.

UVABAİN TOKSİSİTESİNE KARŞI DİGOKSİNE ÖZGÜ ANTİSERUMUN ETKİLERİ

ÖZET :

Digoksin-Bovine Serum Albumin (Dig-BSA) konjugatıyla immünize edilen tavşanlardan alınan serumlarda digoksine karşı antikorlar bulunmaktaydı. 150 µg/kg uvabain verilen beş tavşan bu glikozidin toksik semptomlarını gösterdi ve 60 dakika içinde öldü. Dokuz tavşana 1.5 ml/kg immün serum ve 150 µg/kg uvabain verildi. Bunlardan üç tanesi kardiyak ritm bozuklukları gösterdi, bu bozukluklar 15-60 dakika arasında normale döndü ve hayvanlar yaşamaya devam etti. Geri kalan altı hayvan toksik belirtiler gösterdi ve 10 dakika ile 3 saat arasında öldü. Antidigoksin antikorların uvabainin toksik etkilerini azalttığı, ancak tamamen ortadan kaldıramadığı görülmüştü.

INTRODUCTION :

The rapidly acting cardioactive glycoside ouabain has been widely used in both clinical and physiological investigations. It is much less lipidsoluble and rarely bound to plasma albumin than other glycosides. It was reported that there was a cross-reactivity among related steroid compounds when tested against steroid-specific antisera. The antidigoxin antibodies were found to bind digoxin

(*) Anadolu University, Department of Pharmacology, Faculty of Medicine, Eskişehir, Turkey.
(**) İstanbul University, Department of Pharmacology, Faculty of Medicine, İstanbul, Turkey.

one-tenth as well as digitoxin (1). The ovine digoxin-specific antibodies in animal experiments and treatment of some patients with digoxin poisoning displayed a modest degree of cross-reactivity with digitoxin in vitro (2,3,4,5).

The present study was carried out in rabbits to investigate whether antidigoxin antibodies were effective for protecting the toxic effects of ouabain.

METHODS :

Production of Antibodies

Digoxin was oxidized with periodate and bound to bovine serum albumin (BSA) (1). Rabbits were immunized (by the injection of Dig-BSA) 1 mg/ml in complete Freund's adjuvant mixture. After an initial series of three weekly 0.4 ml injections (0.1 ml into each of four individual toe pads), subsequent 0.4 ml boster injections were given at 2 week intervals.

The presence of antibodies to the protein antigens used in the present study was determined by the bis-diazotized benzidine hemagglutination method,using human group "A,Rh^+" erythrocytes coated with Dig-BSA, as previously described (2,6). Sera were obtained by decapitation from rabbits which had been immunized. Thimerosal (in a final concentration of 1: 10.000) was added as a preservative and the sera were stored at 4°C until used.

Animal Experiments

The rabbits were anesthetized with Dial-urethane (Ciba), and connected to a polygraph for recording the electrocardiogram (Lead II) and main arterial pressure. A single toxic dose of ouabain (150 µg/kg, i.v) was given to the control group (5 animals). 1.5 ml/kg immune serum and then toxic dose of ouabain were administered intravenously to the experimental group (9 animals). The beginning of the development of ventricular irregularities and the occurence of death were recorded.

RESULTS :

The toxic dose of ouabain caused abnormalities of cardiac rhythm and disturbances of AV conduction. Five animals in the control group exhibited ventricular extrasystole, ventricular tachycardia and died of ventricular fibrilation. These animals died between 7 and 60 minutes (Figure: I). The sinus rhythm did not return to normal until the time of death. On the nine animals of the experimental group given digoxin-specific antisera and toxic dose of ouabain, three did not exhibit ventricular tachycardia, and disturbances of cardiac rhythm reversed between 15 and 60 minutes. The normal sinus rhythm recovered later on. These three animals did not die during the examination period and were sacrificed. Arrhythmia were seen in the six remaining animals, two of which exhibited ventricular tachycardia continuing from 2 to 3 minutes. The normal cardiac rhythm was not observed at anytime in any of six rabbits. These six animals died between 10 minutes and 3 hours.

DISCUSSION :

It was demonstrated that digoxin-specific antibodies cross-reacted with digitoxin (1,7,8), deslanatoside (1) and ouabain (1,7) and that antidigitoxin antibodies cross-reacted with digoxin (8). But it was found that antidigoxin antidigoxin antibodies reacted 20 to 30 times more effectively with digoxin than digitoxin (1,7). The affinity of antidigoxin antibody for digitoxin was found 10-fold lower as determined by hapten displacement studies (1). It was shown that purified Fab fragments of antidigoxin antibodies reversed severe digitoxin intoxication in man (9,10).

The results of the present study indicated that in vivo antidigoxin antibodies had the ability to decrease the toxicity of ouabain, but these antibodies did not completely reverse all the toxic effects of

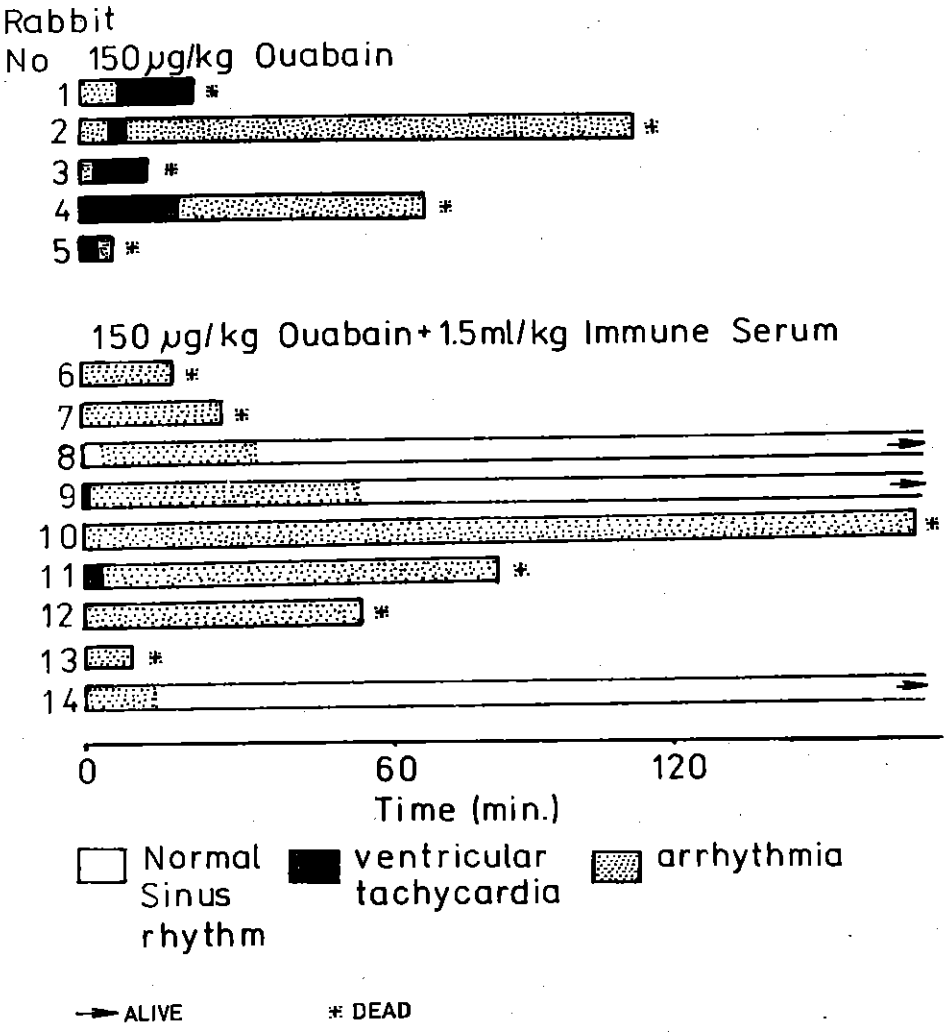

Figure 1: The course of ouabain-induced arrhythmias and the effect of digoxin-specific antibodies on ouabain toxicity.

ouabain, in contrast to what had been observed in the case of digoxin. The steroid-specific antibodies have generally high affinity and specificty for related steroid compounds. Digoxin exhibits minor strictural differences from digitoxin. Digitoxin lacks the hydroxyl group at the carbon-12 position in the steroid ring of aglycone, while ouabain differs from digoxin in both the lacton ring and sugar radical (Figure: 2). It was indicated that the major determinants of binding specificity resided in the steroid portjon of molecule (1). Therefore the affinity of antidigoxin antibody decreases against ouabain.

In the present investigation the same amount of antidigoxin antibodies reversed completely the toxicity of digoxin, while it only decreased the toxicity of ouabain and did not completely reverse

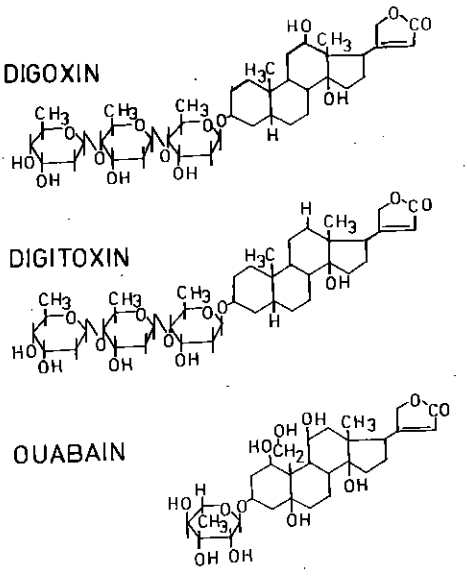

Figure 2: The structural formulas of digoxin, digitoxin and ouabain.

it. The different steroidal structure of digoxin and ouabain apparently decreases the affinity of digoxin-specific antibodies to ouabain.

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