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THROMBOXANE A_2 GENERATION BY THROMBOCYTES OF WHITE CARNEAU PIGEONS

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INTRODUCTION

ATHEROSCLEROSIS MAY BE INITIATED BY FACTORS - SUCH AS HYPERLIPIDEMIA, CERTAIN METABOLITES, INFECTIOUS AGENTS OR IMMUNOLOGICAL INJURY - THAT ALTER THE ENDOTHELIAL CELL LINING OF VASCULAR TIESSUES, EXPOSING THE SUBENDOTHELIAL COLLAGEN LAYER (ROSS ET AL, 1976). BLOOD PLATELETS CAN ADHERE, THEN AGGREGATE AND RELEASE CELLULAR CONSTITUENTS (ROSS AND GLOMSET, 1976). THIS MAY BE FOLLOWED BY SMOOTH MUSCLE CELL PROLIFERATION AND THE SYNTHESIS AND DEPOSITION OF PROTEOGLYCANS, LIPIDS AND OTHER MOLECULES (HARKER ET AL, 1976). THESE EVENTS ULTIMATELY LEAD TO THE FORMATION OF A PRIMARY LESION. THE PRIMARY LESION FORMATION MAY BE PREVENTED BY INHIBITION OF PLATELET CONSUMPTION (HARKER ET AL, 1976).

INTERACTION BETWEEN THE DAMAGED VASCULAR WALL AND PLATELETS RESULTS IN THE RELEASE OF THROMBOXANE A_2 (TxA_2) FROM THE PLATELETS (GRYGLEWSKI ET AL, 1976). ARACHIDONIC ACID IS A PRECURSOR TO TXA_2 AND OTHER METABOLITES IN PLATELETS (FIGURE 1). A ROLE OF TXA_2 IN ATHEROSCLEROSIS HAS BEEN SUGGESTED USING A RABBIT MODEL (GRYGLEWSKI ET AL, 1978, DEMBINSKA-KIEC ET AL, 1979 AND GRYGLEWSKI, 1980).

THIS STUDY USES THE RANDOM BRED WHITE CARNEAU PIGEON (RBWC) AND WHITE CARNEAU II PIGEON (WC-II). THE RBWC AND WC-II ARE GENETICALLY DIFFERENT IN THEIR SUSCEPTIBILITY TO ATHEROSCLEROSIS; WITH THE WC-II EXHIBITING GREATER SUSCEPTIBILITY. STUDIES (INCLUDING PRITCHARD ET AL, 1964, SUBBIAH ET AL, 1976 AND LEWIS ET AL, 1977) INDICATE THAT THE WHITE CARNEAU PIGEON DEVELOPS SPONTANEOUS ATHEROSCLEROTIC LESIONS LIKE THE HUMAN.

SINCE THE RBWC AND WC-II DIFFER IN THEIR SUSCEPTIBILITY TO ATHEROSCLEROSIS IT IS REASONABLE TO CONDUCT A COMPARATIVE STUDY WITH RESPECT TO TxA_2 IN THE PIGEONS.

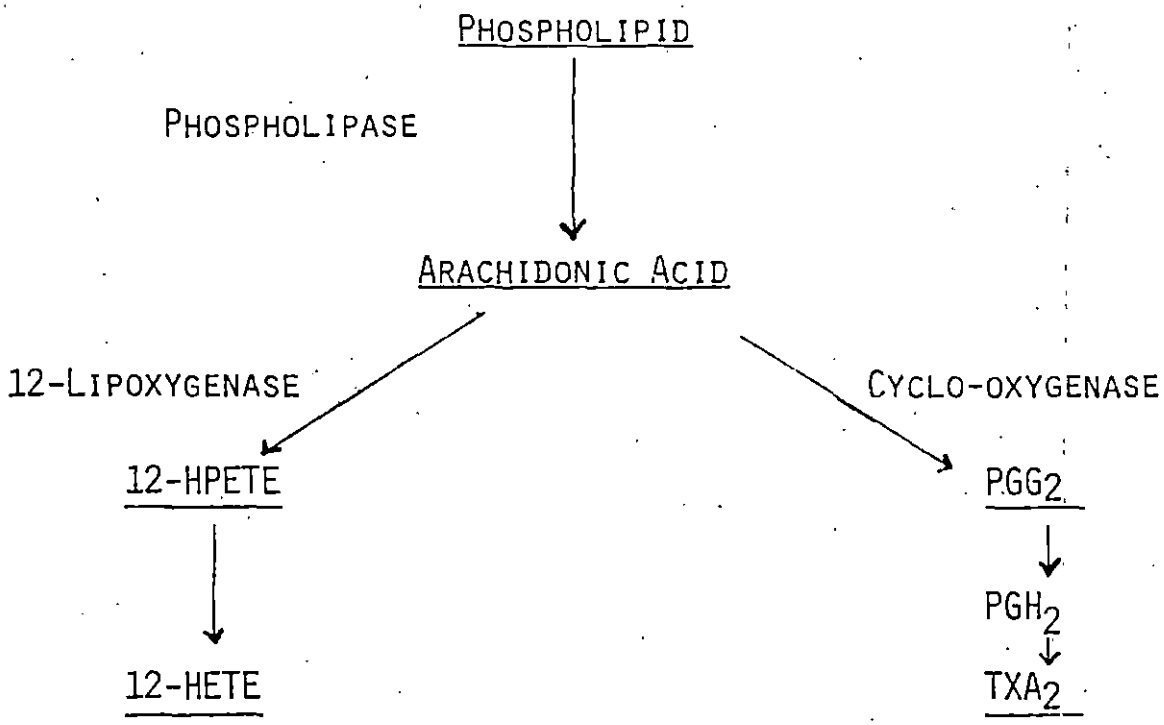

FIGURE I. ARACHIDONIC ACID METABOLISM BY TWO DIFFERENT ENZYMATIC PATHWAYS IN PLATELETS (GRANSTRÖM ET AL, 1982).

METHODS

RADIOIMMUNOASSAY (RIA) WHICH MEASURED THROMBOXANE B_2 (TXB_2), A STABLE METABOLITE OF TXA_2, WAS USED TO DETERMINE TXA_2 LEVELS IN THE PLASMA OF 15 EACH RBWC AND WC-II. RIA WAS ALSO USED TO MEASURE TXB_2 FORMATION BY RBWC AND WC-II THROMBOCYTES INCUBATED WITH AND WITHOUT ARACHIDONIC ACID.

BLOOD SAMPLES AND THROMBOCYTE ENRICHED PLASMA (TEP) PELLETS WERE PREPARED AS DESCRIBED BY TAYLOR AND LEWIS (1981).

PLASMA TXB_2 DETERMINATIONS

1). PLASMA + INDOMETHACIN (1 UG/ML PLASMA)

2). ACIDIFIED TO pH 3.0 AND EXTRACTED IN ETHYL ACETATE

3). ORGANIC LAYER TRANSFERED TO FLASK OF ROTO-EVAPORATOR AND EVAPORATED UNDER REDUCED PRESSURE

4). RESIDUE REDISPERSED IN RIA BUFFER

5). RIA PERFORMED

NOTE: THROMBOCYTE COUNTS OF BLOOD WERE DETERMINED FOR RBWC AND WC-II

TXB_2 FORMATION BY TEP PELLETS INCUBATED IN PRESENCE AND ABSENCE OF ARACHIDONIC ACID

1). TEP + PUCKS SOLUTION

2). PLATELET COUNTS WERE PERFORMED SO TXB_2 LEVELS COULD BE EXPRESSED AS PG TXB_2 PER 10^8 CELLS

3). Arachidonic acid = 2.5 ug/ml Pucks.

4). Incubation = 3 minutes at 41°C

5). Incubation terminated by acidification to pH = 3.0

6). Sample extraction of aliquots was performed as described for plasma determinations

7). RIA performed

RIA was performed according to the specifications of the producer (New England Nuclear) of the RIA materials. A Packard Model 300C Scintillation Counter was used and DPM were calculated to determine the pg yield of TXB_2.

RESULTS AND DISCUSSION

THROMBOCYTE COUNTS OF THE PIGEON BLOOD WERE PERFORMED SINCE THE TXB_2 DETERMINATIONS IN PLASMA MIGHT BE INFLUENCED BY DIFFERENCES IN THROMBOCYTE LEVELS OF THE RBWC AND THE WC-II. THESE LEVELS ARE INDICATED IN TABLE 1, AND USING ANOVA IT WAS DETERMINED THE MEANS WERE NOT SIGNIFICANTLY DIFFERENT. SO THE LEVELS OF TXB_2 IN PLASMA WERE NOT INFLUENCED BY DIFFERENCES IN THE NUMBER OF THROMBOCYTES.

WC-II PIGEONS, MORE SUSCEPTIBLE TO ATHEROSCLEROSIS THAN RBWC, HAD SIGNIFICANTLY HIGHER (TABLE 2) PLASMA TXB_2 LEVELS THAN THE RBWC. WHILE THE PLASMA LEVELS REPORTED MAY NOT REFLECT EXACT ENDOGENOUS BIOLOGICAL LEVELS BECAUSE OF THE ARTIFACTUAL CONTRIBUTION INITIATED BY THE COLLECTION OF THE BLOOD SAMPLE THE RBWC AND WC-II WERE TREATED THE SAME.

IT SHOULD ALSO BE NOTED THAT ALBUMIN MAY COVALENTLY BIND TXA_2 AND THEREFORE ALL TXA_2 IS NOT CONVERTED TO TXB_2 (FITZPATRICK ET AL, 1977 AND MACLOUF ET AL, 1980).

THE LEVELS OF TXB_2 GENERATED BY TEP PELLET CELLS INCUBATED IN THE PRESENCE AND ABSENCE OF ARACHIDONIC ACID ARE EXPRESSED IN TABLE 3. THE TXB_2 LEVEL WITHOUT ARACHIDONIC ACID STIMULATION WAS SIGNIFICANTLY HIGHER ($F = 7.52$, $P = 0.05$) IN THE WC-II THAN THE RBWC. THE TXB_2 GENERATION WITH ARACHIDONIC ACID STIMULATION WAS ALSO GREATER IN THE WC-II COMPARED TO THE RBWC.

IN ALL PHASES OF THIS STUDY THE PIGEON GENETICALLY MORE SUSCEPTIBLE TO ATHEROSCLEROSIS EXHIBITED GREATER TXB_2 LEVELS THAN THE PIGEON GENETICALLY LESS SUSCEPTIBLE TO ATHEROSCLEROSIS.

TABLE 1. THROMBOCYTE COUNTS IN RBWC AND WC-II

PIGEON	THROMBOCYTES PER MM^3 BLOOD
RBWC	$\bar{X}$ = 522,000
WC-II	$\bar{X}$ = 524,000

TABLE 2. TXB_2 LEVELS IN PLASMA

PIGEON	PG TXB_2 PER ML PLASMA
RBWC	$\bar{X}$ = 17.7
WC-II	$\bar{X}$ = 156.0

TABLE 3. TXB_2 GENERATION BY TEP PELLET CELLS INCUBATED IN THE PRESENCE AND ABSENCE OF ARACHIDONIC ACID

PIGEON	INCUBATION WITH ARACHIDONIC ACID PG TXB_2 PER 10^8 CELLS	INCUBATION WITHOUT ARACHIDONIC ACID PG TXB_2 PER 10^8 CELLS
RBWC	$\bar{X}$ = 14.3	$\bar{X}$ = 5.3
WC-II	$\bar{X}$ = 66.2	$\bar{X}$ = 19.5

GRYGLEWSKI (1980) GIVES SEVEN LINES OF EVIDENCE TO SUPPORT THE HYPOTHESIS THAT TXA_2 IS IMPORTANT IN THE ETIOLOGY OF ATHEROSCLEROSIS.

IT IS ALSO TEMPTING TO SPECULATE THAT CONTINUOUS GENERATION OF TXA_2 BY PLATELETS ADHERING TO DAMAGED WALLS OF CORONARY ARTERIES RESULTS IN THEIR LOCALIZED VASOCONSTRICTION. AT THE SAME TIME THE TXA_2 MAY INDUCE CIRCULATING PLATELETS TO AGGREGATE AND FORM A MURAL THROMBUS AT THIS SITE SINCE ARACHIDONATE - AGGREGATED PLATELETS OF SOME PATIENTS SUFFERING FROM MYOCARDIAL INFARCTION GENERATE MORE TXA_2 THAN PLATELETS OF HEALTH SUBJECTS, (GRYGLEWSKI, 1980).

THUS TXA_2 MAY HAVE ROLES IN ATHEROGENESIS AND CORONARY SPASM AND THE RESULTS OF OUR STUDY INDICATE THERE ARE GENETICAL IMPLICATIONS IN THE ABILITY OF PLATELETS TO GENERATE TXA_2,

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