IN VITRO MODULATION OF ANDROGEN AND ESTROGEN RECEPTORS IN HUMAN PROSTATE CELLS BY ESSENTIAL FATTY ACIDS

BY

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"Clearly there is need for a stronger evidence base, and more creative thinking on the part of the health-care profession, to help engage men of all ages in caring for their own health. Most importantly, it is essential that men themselves take an active part in the process of researching and developing their own care. The accomplishments of the women's health movements in recent years should provide ample stimulus and inspiration."

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May you all be richly blessed with everlasting peace and joy!

To my Creator, Saviour and Encourager, I acknowledge Thee, I thank Thee and I praise Thee!

ABBREVIATIONS

A_{260}	-	Absorbance at 260 nm
A_{280}	-	Absorbance at 280 nm
ALA	-	α-Linolenic acid
AR	-	Androgen receptor
ARE	-	Androgen response element
bp	-	base pair
BP	-	Binding protein
BPH	-	Benign prostatic hyperplasia
°C	-	degrees Celsius
Ca^{2+}	-	Calcium cationе
CAG	-	Cytosine Adenine Guanine
cDNA	-	complementary deoxyribonucleic acid
Ci	-	Curie
COX	-	Cyclooxygenase
CSPD	-	Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2' -(5' chloro)-tricyclo[3.3.1.1^{3,7}]decan}-4-yl) phenyl phosphate
CsTFA	-	Cesium trifluoroacetate
DAB	-	3,3'-diaminobenzidine tetrahydrochloride
DHA	-	Docosahexaenoic acid
DTT	-	Dithiotreitol
ddATP	-	Dideoxyadenine triphosphate
ddNTP	-	Dideoxynucleotide triphosphate
ddUTP	-	Dideoxyuridine triphosphate
DEPC	-	Diethyl pyrocarbonate
DES	-	Diethylstilbestrone
DESdP	-	Diethylstilbestrone diphosphate
DHT	-	Dihydrotestosterone

DIG	-	Digoxigenin
DNA	-	Deoxyribonucleic acid
dpm	-	disintegrations per minute
E		Estrogen
E_2	-	Estradiol
EMEM	-	Eagle's minimum essential medium
EDTA	-	Ethylenediamine tetraacetic acid disodium salt
EFA	-	Essential fatty acid
EFAs	-	Essential fatty acids
EGF	-	Epidermal growth factor-urogastrone
EPA	-	Eicosapentaenoic acid
ER	-	Estrogen receptor
ERα	-	Estrogen receptor alpha
ERβ	-	Estrogen receptor beta
EpRE	-	electrophile/antioxidant response element
et al.	-	and others
EVS	-	Essensiële vetsure
FAs	-	Fatty acids
FAS	-	Fatty acid synthase
FCS	-	Fetal calf serum
FGF-2	-	Fibroblast growth factor 2
FSH	-	Follicle-stimulating hormone
G3PDH	-	Glyceraldehyde-3-phosphodehydrogenase
GAPDH	-	Glyceraldehyde-3-phosphodehydrogenase
GH	-	Growth hormone
GLA	-	γ-Linolenic acid
[^{3}H]-E_2	-	Radiolabelled estradiol
[^{3}H]-T	-	Radiolabelled testosterone
HRE	-	Hormone response element
HRP	-	Horse radish peroxidase
hsp	-	heat shock protein

IGEPAL	-	(Octylphenoxy)polyethoxyethanol
IGF-I	-	Insulin-like growth factor I
IgG	-	Immunoglobulin Gamma
kDa	-	kilo Dalton
KGF	-	Keratinocyte growth factor
LH	-	Luteinizing hormone
LH-RH	-	Luteinizing hormone-releasing hormone
LA	-	Linoleic acid
M	-	Molarity
MDA	-	Malondialdehyde
ml	-	millilitre
mmol	-	millimoles
MOPS	-	3-[N-Morpholino]propanesulphonic acid
mRNA	-	Messenger ribonucleic acid
MUFAs	-	Monounsaturated fatty acids
NLS	-	Nuclear localization signal
nM	-	nano Molar
NO	-	Nitric oxide
NGS	-	Normal goat serum diluted 1 in 5 with TBS
NSAID	-	Nonsteroidal anti-inflammatory drugs
OA	-	Oleic acid
PAGE	-	Polyacrylamide gel electrophoresis
par	-	pararagraph
PBS	-	Phosphate buffered saline
PEP	-	Phosphoenolpyrovate
PIN	-	Prostatic intraepithelial neoplasia
PKC	-	Protein kinase C
pmol	-	picomoles
PMS	-	Premenstrual syndrome
PMSF	-	Phenyl-methyl-sulphonyl fluoride
PR	-	Phenol red

PSA	-	Prostate specific antigen
PUFAs	-	Poly unsaturated fatty acids
RIPA	-	Radioimmuno precipitation assay
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
RSHBG	-	SHBG receptor
SA	-	South Africa
SDS	-	Sodium dodecyl sulphate
SFAs	-	Saturated fatty acids
SHBG	-	Steroid hormone binding globulin
SH	-	Steroid hormone
SHR	-	Steroid hormone receptor
SH-SHR	-	Steroid hormone – steroid hormone receptor complex
SS	-	Saline solution
SSC	-	Saline sodium citrate
T	-	Testosterone
TAF	-	Transcription activation factor
TBS	-	Tris buffered saline
TBS-T	-	Tris buffered saline Tween 20
TE	-	Tris-EDTA
TEBG	-	Testosterone estradiol binding globulin
TeBG	-	Testosterone binding globulin
TGF-α	-	Transforming growth factor alpha
Tris	-	Tris(hydroxymethyl)-amino-methane
Tween 20	-	Polyoxyethylenesorbitan
UK	-	United Kingdom
uPA	-	Urokinase type plasminogen activator
USA	-	United States of America
μl	-	microlitre
VEGF	-	Vascular endothelial growth factor

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Film developer and fixer solutions

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ABSTRACT

Cancer of the prostate is one of the leading causes of cancer related deaths in black and white men. An important role in the development of prostate cancer is played by androgens and androgen ablation is therefore currently used in cancer treatment. In the past, estrogens were widely used in treatment of prostate cancer, but there are indications that estrogens could also be involved in carcinogenesis. In contrast with saturated fats, some essential fatty acids (EFAs) are associated with a decrease in prostate cancer incidence. Lately, much research has been done on the modulation of the binding of steroid hormones to their receptors by essential fatty acids (EFAs), which could interfere with the steroid hormone's message. Therefore, the aim of this study was to determine in whole DU-145 human prostate cells the effect of EFAs and their metabolites on the binding and affinity of the estrogen receptor (ER) and androgen receptor (AR) to estradiol (E_2) and testosterone (T), respectively. Binding studies were carried out with radioactive T and E_2 with and without an excess of cold steroid. Data was analyzed according to the Scatchard method. Western and northern blotting were applied. The results showed that the EFAs under investigation inhibited the AR's capacity (B_{max}), in contrast to the ER's capacity, which was stimulated. However, the dissociation constants (K_d) of the AR and ER complexes in the presence of the fatty acids (FAs) were as follows. Except for eicosapentaenoic acid (EPA) which decreased the AR dissociation constant and EPA and alpha-linolenic acid (ALA) which increased the ER dissociation constant, the remaining FAs had no significant effect on the K_d values of both the AR and ER complexes. The decrease in the AR K_d and B_{max} values implied that EPA caused less T to bind to AR, but more tightly. In contrast, the increase in the ER B_{max} and K_d values implied that both EPA and ALA caused more E_2 to bind to ER, but more loosely. The overall inhibition of T binding to AR and stimulation of E_2 binding to ER by n-3 EFA may be beneficial during androgen withdrawal with or without estrogen therapy. Molecular studies showed that EFAs had no effect on the expression of the steroid receptors and further studies are necessary to

investigate the effect of EFAs on steroid receptor expression. According to these preliminary results it is concluded that men should benefit from a diet rich in certain essential polyunsaturated fatty acids although its function remains to be clarified.

KEY WORDS: Estrogen receptor α, Estrogen receptor β, Androgen receptors, Prostatic cancer, DU-145 cells, Essential fatty acids, Scatchard analysis, Northern blotting, Immunocytochemistry, Western blotting.

OPSOMMING

Prostaatkanker is een van die belangrikste oorsake van kankerverwante sterftes onder swart en wit mans. Androgene speel 'n belangrike rol in die ontwikkeling van prostaatkanker en derhalwe word androgeen onttrekking in die behandeling van kanker behandeling gebruik. In die verlede was estrogene algemeen gebruik in die behandeling van prostaatkanker, maar daar is aanduidings dat estrogene ook betrokke kan wees in karsinogenese. Onlangs is heelwat navorsing gedoen op die modulering van die binding van steroïed reseptore aan hulle reseptore deur essensiële vetsure (EVS), wat met die steroïed hormoon se boodskap kan inmeng. Daarom was die doel van hierdie studie om die effek van EVS en hulle metaboliete op die bindingskapasiteit en die affiniteit van die estrogeen reseptor (ER) en die androgeen reseptor (AR) aan estradiol (E_2) en testosteroon (T) in heel DU-145 menslike prostaat selle onderskeidelik te bepaal. Scatchard binding studies, asook noordelike en westelike klad is uitgevoer. Die resultate het getoon dat die EVS wat ondersoek was die AR se kapasiteit (B_{max}) geïnhibeer het, in teenstelling met die ER se kapasiteit wat gestimuleer was. Die dissosiasie konstantes (K_d) van die AR en ER komplekse in die teenwoordigheid van EVS was as volg. Behalwe vir eicosapentaenoaat (EPA) wat die AR dissosiasie konstante verminder het en EPA en alpha-linolenaat (ALA) wat die ER dissosiasie konstante vermeerder het, het die oorblywende vetsure geen betekenisvolle effek op die K_d waardes van beide die AR en ER komplekse gehad nie. The afname in die AR K_d en B_{max} waardes beteken dat EPA veroorsaak het dat minder T aan die AR gebind het, maar stywer. In teenstelling daarmee, het EPA en ALA veroorsaak dat meer E_2 aan ER gebind het, maar losser. Die algehele inhibering van T binding aan ER deur EPA en stimulering van E_2 binding aan ER mag voordelig wees tydens androgeen onttrekking met of sonder estrogeen behandeling. Hoewel geen effek op die uitdrukking van AR en ER verkry was nie, word verdere studies benodig om te bepaal of EVS die uitdrukking van steroïed reseptore sou affekteer. Volgens hierdie voorlopige resultate kan afgelei word dat mans bevoordeel sou

word deur 'n diëet ryk in sekere essensiële poli-onversadigde vetsure, hoewel die funksies nog opgeklaar moet word.

SLEUTELWOORDE: Estrogeen reseptor α, Estrogeen reseptor β, Androgeen reseptore, Prostaat kanker, DU-145 selle, Essensiële vetsure, Scatchard analise, Noordelike klad, Immunositochemie, Westelike klad.