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PFeventJon of ocular iDflamma o8 by m 'rine prednisolone and cyc)ooxygenase and Hpox'ygenaseinbibitors

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ABSTRACT Lens protein-induced ocular inflammation in rabbits was used to stuåy the action' mechanism of some an inflammatory agents.]ndomethaejna cyclooxy nase inhibiœr markedly reduced PGE_1 and PGF1« in the is and cUiary body at 2 h but PGE_2 on1y at 4 h. REV 5901 a' lipoxygenas in-hibitor only signMca\t}y reduced PGE2 levels in the ciliary body at 4 h. PGFm levels were not affected by REV 5901. When indomethacin: and REV 5901 were combined both PGE_1 and PGf1« were suppressed at 2 and 4 h in both iris and cili&fY body. Neither matrj.ne nor predn ìsolone produecd significant effects on the levels of PGE_1 and POF_{1c1} owever prednisolone exhibited the greatt reduction in chemotaxis of leukocytes fa110wed by REV 5901. Indomethacin on the contra produced a significant increase chemotaxis of leuk ytes. Matrine produced a decrease in leukocyte unts but was not statistically sign ficant. These rcsults indicate that indomethac ÏD is effective in- the early phase of inflammation to reduce PG's production whereas prednisolone and REV 5901 were more effec ve in the late phase of inflammation. Combined use of REV 5901 and indomethacin could become a drug of thoice for the treatment of ocu ar inflammation without inducing corticosteroidal side effects.

KEY WORDS eye; inflammation; matrine; prednisolone; non−steroidal anti-inflammatory agents; üpoxygenase inhibito 'prostaglandins E; prostagland ins F

The two major classes of anti-inflammatory agents .used after ocular surgery are steroidal and non-steroidal anti-ittflammato ry agents. Neither of t:hese two classes is ideal. The major ocular side-effect wÀich l mits the uses of steroidal agents is the induction of aD' iDcrease in in.tra-ocular- pressureω-Non-steroidal anti-in ßammatory drugs SAID) on the o er hand have e potentiai of WOJ!c B.mg the inflammation especially during the late phase of ' the inflammatioD. Tbis is due to the fact that a11 present clinicaUy av.ai ble NSAIP are prim y cyclooxygenase inhibitors. Blocking the cyclooxygenase arm of the arachidomc acid (AA) cascade potentiates the productioD of lipoxygenase metaboli s which are ultjlnately the leukotrienes (LT). The LT are responsible for the late phase of tnflammation and the chemotaxis of leukocytes(2-4}. In a previous study(S) a new synthetic lipoxygenase inhibitor R.EV 5901 was demonstrated to be effective in reducing the late phase of inflammation and using REV 5901 with indom èthacin was demonstrated to be as e.ffective as prednisolone in reducing inflammation. However when RE5901 is used alone m treatment of lens protein-induced ocular inflam.mation" tbere was an mcrease in the early phase of inflammation. This observation was attFibuted \o an' increase in the production of prostaglattdins caused by tl1e inhib tion of the lipoxygenase arIU of the AA cascade.

Another new agent which sh øwed promhse tObe am eRective ocu ar aD -inflammatory drug is matrine(6). Matrineis a natural substance iso ated from the plant *Sophora SUbp1ostrata*. The mechanism of action of matrine is unknown' but matrine has been demonstrated to possess analgesic antipyretic and anti-inflammatory pFopertles[6-8]

In this study attempts have been made to deter nme the mechanism of action of matrille

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and conarm the mechanism of action of indomethacin REV 5901 and prednisolone-This was done by determining the concentra on of PGEpPGFM'and LTB4in Ele iris ciliary body and the rdinsby mdioimmunoaωy. Theeffmencotonsf theωse dU OD the aωCCωumu tiωOB øfleukoωCYteωs in tbe antωerior chamber of the eye during inflammatiωon was 81so studied.

MATERIALS AND METHODS

Materials Indomethacin was purchased from Sigma (St Louis MO).REV5901wts obtained from Revlon Health Care Group (Tuckahoe NY). Prednisolone used was a 1% ophthalmic solution (1010 Econopred) purchased from Alcon (Fort Worth TX). Matrine was isolated from the root of *Sophora subprostrata* by a previously reported method(8). Both indomethacin and REV 5901 were dissolved in polyethylene glycol (M_r 200) and then diluted with aqueous solution to the Enal concentration of 1%.h4atTine was dissolved in normal saline to final concentrations of 1% and 2%. All drugs were applied topicany in aliquots of 50µl to one eye of the rabbit 1 h prior to intraeameral admi stration of lens proteins. In the other eye of the rabbit the solvent was applied as a control.

Lens protein Lens protein was prepared and protein concentration was deterlnined according to procedures reported previously(2). 'IEe quantity of lens protein in the lens protein preparatiωused for is study was 29.67 mg-mr1.

Rabbit prepara íoß New Zealand albìno rabbits of ei sex weigbing 2.5-3.5 kg w used for thIS study.The rabbits werR anes tized by intramuscular jin eωωOn'00 keta m hydrochloride 25mg-kg-land xyhnne5mg-kg-1.The anesthesia was maintained by injection of LPfqmJ"n hydrochlodqf12.25mg-kiland xylazine 2.5.mg-kg every hOUT through012t the experiment.After the rabbits w m t - the d we ;::::;

apPEed tome eye md the other eye received 50µ1 of the solvent. One hour after the appHcation of the drugs 25µ1of lens protein weTe injected intracamerany with k 30 gauge needidextreme care was taken to avoid contact with tne iris. Rabbits were killed by an overdose of sodium pentpbarbital.

RadioimmunoωBSY 'Fhe radioimmunoassay (R11A) was perføflned by: using the kits DUTchased hom Advanced Magnetics (Cambridge MA).kits were used to determine the concentration of PG;E2' PGP 2g' and 1TB4 in the iris ciliary body and ret a

For the RIA one group of rabbits w killed at 2 and another at 4 h artCr intracameral injection of the lens protein. The wet weights of ciliary body and rea weTe immediately obtamed. The tissues were homogenized in 1 ml of nor a1 sa1ine acid to pH 3-4. Two ml' of ethy1 acetate were added to the homogenate and vortexed for 30 s. The homogenate was then centrifuged at 1000 x *g* for 20 min. The eth yl acetate layer of the homogenate was removed and evaporated under nitrogen. The tissue samples were resuspended in buffer solution provided in the RIA kits. The prostaglandin RIA was performed in samples resuspended in 0.5 ml of buffer solu.tion and the leukotriene RIA in samp]es resuspended in 0..3 ml of buffer. The RIA was performed according to instructions provided in the kits. The RIA saInples were centrifuged at 1000 x *g* for 20 min after the addition of charcoal (provided in. the RIA kit). 'Fhe supernatant was decanted and 5 ml of scintiilation cocktail solution. (Uriiversal cocktait ICN Biochemical Irvine CA) was added.The sam ples were counted in aliquid scin llatioD Counter (LS 5000 CE BeckmaD FullertOD CA) for 10 min. The results were calculatedas per instrωtìons. The c?ncenation of prostaglandin and leukptnm wm expressed as pg (mgoftis - SUlet •

aLeukocyte count RabbIts killed at4h alter injecti øn of lens ptotein were used in

seedon of-the study. Thezaqueoua humor was obtained from the eye by puncturing the cornea with a 23 gauge needle and 3-ml syr - inge. After insertion of the needle into the an.. terior chamber" even mixing of the aqueous humor was ensured by sU ctioning and FD - leasing several times with the syringe øefore drawing the sample. The aqueous humor was en placed in an improved Neubauer cham - ber and the leukocytes were counted.

RESVLTS

The results ofthe RIA are shown in Tab 1 and 2. Indomethacin significantly reduced the concentration of PGE_2 and PGF:za. in the iris and ciliary body at 2 h (early ph:ase). Indomethacin however produced a signifi - cant inα'ease in chemotaxis of leukocytes (Tab 3). REV 5901 only produced a significant de - crease in PGE_2 leveis in the ciliary body at 4 h σab 1). t also caused a significant reduction in leukocyte counts (Tab 3). When indomethac ǹ and REV 5901 were combinedt .there w.s • :signif. lC8l. \l decrease of $PG'B_2$ and PGP'74 at 2 and 4 h in both the iris and the ciliary body (Tab 1 and 2).

Neither matrine nor prednisÐlone pro- duced a statistical1y significant change li the levels of. PGE_2 or PGF2« .(Tab 1 and; '

'Fne prostaglandin level in the retina without drug treatments is lower than those m the iris and ciliary body in al1 cases (Tab 1 and 2). The levels of prostaglandin in the retina were nøt significantly different in treated and. control 'eyes foany of the drugs tested except a borderline reduction of PGE_2 by indomethacin plus REV 5901 (Tab 1 nd 2). These results indicate that the inflammation induced by intracameral injection of lens pro - tein does not affect the retina and that the top - ical application of drugs to the eye does not af - fect the retina either.

The results of the leukocyte counts are sbown in Tab 3. Prednisolone exhibited the greatest percent reduction in chemotaxis of leukocytes. Matrine produced a decrease in

Tab 1. Effects *01* anti-inflammatory ageQts 00 PGE_1 productioD ioduced by le08 proteins at early pbase and late pbase 01inllammatlo . • $P > 0.05$ *p*0" 05 control.

	n	Control eyes Iris		Cillary body		Retina		Treated eyes Iris		Ciliary body		Retina	
Early phase <at 2 h)													
Prednisolone	4	95.9	25.1	35.7	10	10.0	10	111.2	34.1•	59.1	12.2-	10.0	1.7
Indomethacin	6	183.1	42.0	93.5	17.0	26.7	19.0	22.5	14.0..	11.0	2.0..	10.3	2.0.
REV 5901	8	183.8	34.3	157.3	35.5	39.0	14.7	123.0	46.0	120.0	45.1.	46.7	18.0•
Indomethacin +REV 5901	4	94.4	5.8	72.6	11.0	9.2	2.6	.4	5.4".	19.3	8.1..	10.7	3.9•
Matrine	6	164.4	37.7	118.7	42.8	24.9	7.8	88.7	29.1	55.3	5.8•	27.5	10.6*
Late pbase <at 4 b)													
Prednisolone	4	122.	148.3	61.3	17.1	31.5	12.2	61.5	19.6*	114.0	45.5.	22.9	8.9
lndometbacin	4	158.3	22.0	129.1	41.0	16.9	2.6	27.5	12.	22.0	8.S–.	33.3	27.8•
REV 5901	6	185.5	46.8	90.5	22.8	13.3	2.4	1r2.6	43.3	51.6	12.γ111	26.8	18.1
Indomethacin +REV 5901	14	171.0	44.5	84.5	20.0	12.2	3.5	22.0	7.8..	20..6	6.γ·	8.5	2.0
Matrine	6	123.1	16.1	115.9	1 8.1	31.0	10.3	105.6	14.9	69.7	16.1•	45.1	20.3•

EarlY...ω2'h)											
D $()l<>nc	4	194	4.8	7.1	l.l	7.6	1.1	13.4	4.8•	*1S.2*	5.3.
IlltJÖ1C ac 1	6	*S02*	16.0	S2:t	r6.3	12.6	2.1	3.0	2.5.	12.	3.7
ØV5901	6	26.6	4.3	19.7	4.4	16.5	2.4	19.5	4.2 •	17.1	t:-
fndometbac il +REY S901	4	33.2	4.8	20.9	1 . 9	4.7	0.7	'2.. ':/	0.9..	5.2	1 . 3-
Ma e	6	38.3	7.0	*38.S*	12.1	29.6	10.8	2 1	1-1.	20.1	63'
La e (.t4)											
Predniso lone	20.0	9τ		11.8	3.4	16.3	5.0	*13.8*	*2.2.*	20 1 :t7.8-	12.8	1O'
Indomethacln	*50.3*	204		56.1	33.'0	7.8	1.4	*3.1*	*I* '	6.1 1.	17.2	2.9.
qv 90t	39.8	13.4		29.1	1.4	11.6	4.9	*2$.6*	*5.3•*	22.3 3.9-	'20.1	6.7.
Indømetha -fREV *S901*	30.9	7.9		20.8	5.2	6.2	0.6	*2.4*	—	24 0.3..	4.5	0.6*
Matrino	26.0	*5.S*		22.1	3.7	22'.5	10.r	*207*	*3.7.*	r5.5::t 3 .5.	*26.S*	1:1348*'

Yb 3. Eft tI ofi andτ ßamm.atof ageQ koeyte dt @ lDduced bJ. leD8 p tew t late eot fl.m tio 1. B0.05 V$ωB 1.

Pcugs{1%)	n(	Control ceE-g		Treatoo (lk.mm		αWlgê >
Prednisolone	4	90.0	20.4	10.0	4.1 •	-89
Indomethacin	6	158 0	'41.0	298:0	:t70;0	+88
REV 5901	6	395.0	90.5	140.0	30.5'.	6S
Matride (2%)	4	252.5	*1.5*	1325		−48

kukocyte countsakhough not statistically sig - hificant.

Attempts to deterrnine the levels of lêukotri ee centrati øn i ra1)b t 0eul8. J' tis - mewere ndoned after sever ttempts due pextmely low ieve18 0 l@u ωs es: all eaωsurements in iris cilia J Y bQdy and retma were 1 an6pg-mg-t even afker8h after injection of the lens fotm Attemptswmalso m to measure e concentration of leukotri ne ill he aqueou ß humor after ij jectiQn of lens prote 'fbe 1esults were not sigI1ifica{11 dif Ier from tbose obtained frøm oculat tissues.

1JSSION

DISC

Z is srugy i nïurrt ed previo\1sfuJ<1. ing that inàomξ adhibited PG øduc tiØtl effectiv èlyω. O Ihe othet ha EO REV 5ØØ} inÐibè 1ewukoωcytes at 1ate pMase df m arnJnJ uωdn{ω whe:reass incjrømt aωci enli ânced —ab 33. Co b ìned use o îindomethacin a .d RJ1V S 1 mMbi d "G productiQ 1 ewn b ttet ω e use ofpredniSolone alqne.

1\1 ough matrine was shø -J to inh it QC1!1lar inflam a od it d ìd Q bitfG oductiail (Tab 1 and 2) o1\' 1 oc;tç e J1emotws 8i tftcantly (Tab . These rt - sults indicate at either matrine is 8 weBk allh-inflammatøry agent Oli it T'e'p r BIS-m entirely new class of aa FiZEamm ti agøt Which Suppress innammation w hout eCg

aracltidonic cascade.

It is interesting to note that lens protein ct èd ùl acamerally induced PG syntheses main.}y . ' the anterior part of. e eyes (iris and diary body but not in Ehe back of the eves (re a). F'ur er leukotriene pzoducedm is lens protein-induced inflam'm'atioD' modei was to ø litt le to be detected witb RIA. There fore lcukocytc chemotaxis has to bc used to Jepresent the late phase of in. ammation.

REFERENCES

1 Polansky IR Weinreb RN. Antiinflammatofy agents steroids as antiinflammatory agents. fu: Seam M ed.pharmacoJogy of the eye-Berh: Springer 1985 : 459-538. (Bom GVR Fatab A Herken ß Welch AP eds. *Handbook of experimeut41 pharmacology;* vol 69)

2 Miyanø K Chiou GCY. Pharmacological prevention of ocular inflammation induced by lens proteins. *Ophthalmic Res* 1984; 16 : 2S6-63

3 Chiou LV Chiou QCY. Ocular anti-inflammatory action „01 ÜPOXYIOD880 tory action \ rabbit. *J Ocular Phørmøcol 1985:* 1 : 383–9 43hattacherjee Z EZmmond B; Bckins i(.. BfYeeta of Upoxygcnase inhibitor in the leukocyte accumulation in the rabbit eye - In: SamuelsOD B. 'Paole R. eds Leulco *trienes and other llpøxyge7IIUe products.* NY:' Raven Press, 1982 : 32S-3Ð

S' Chang MS. 'Chiou GGY. Prevcntion of 1mB protein induced ocular inflammation with cyclooxygenase and Upoxygenase inhibitors. *J Ocular Pharmacol"* 1989; 5 : 363-70

6Cbuang CY Xiao JG chiou Gey-Ocular anti-inflammatory actions of matrine. *J OcuJar Pharmacol* 1987; 3: 129-34

Che CH Chuang CY Chen CF. Study of th è aotipyretic IIctiVity of matrine: å lupin alkaloid isolated from *Sophora subprostrata. Planta Med* 1986; 52 : 343

8 Cho CH Chuang CY. Study of the anti - inflammatory action of matrine: an alkaloid isolated frorn *Sophora subprostrata. IRCS Med Sci* 1986; 14 : 441-2

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Positive inotropic and toxic action of direct lytic factor 00 isolated workg guinea pig hcarts[1]

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ABSTRACT The positive inotropic and 'the toxic ef - fects of direct lytic factor (DLF) on tbe otated work - mg guinea pig hearts were studied. As compared Widll baseline values, DLF 1-10μg. mr-! increased aortic flow up to 138% cardiac outpt 116% left ventricular pressure volume work 136% left ventricular pressure 114% dP / d mu 130% V_{max} 8% and mean aortic systolic pressure 114% but ron y flow was decreased by 16% on an average =6)-However head mte remained constanb myocardial o gen consumption anq efficiency wel'e cllaaged tittle. TÀe cardiolonic effect of DLF was al - so ebserved by recording the isometric contractions of the isoia d guinea pig papillary uscles and by de termining the left ventricular pressure and dP / dt_{mall} in anesthe ed dogs. Neither sponta - aeously beating rate of rig11t .. iUin nor the excitabili - ty ofleft atrium ';*110* was affected by DLF. The re- sults show that ÐLF is one of. the cardiotonic agents . witbout chronotropic effect and its coronary vasooonstnctioll effect plays an ùnportant part in heart faUure.

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KEY WORDS oob 11 venoms; direct lytic