Letter

Presence of a 15-Ketoprostaglandin Δ^{13}-Reductase in Porcine Cornea

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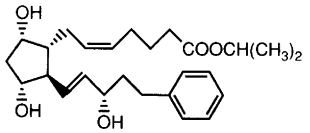
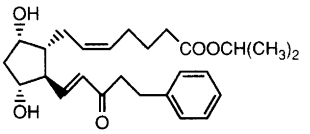
Primary prostaglandins (PGs) regulate a variety of physiological and biochemical processes in the body.[1,2] They are known to occur in various eye tissues and $PGF_{2\alpha}$ has been shown to possess hypotensive properties when applied topically to the eye.[3,4] $PGF_{2\alpha}$ is rapidly metabolised to 15-keto-$PGF_{2\alpha}$ by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and subsequently to 15-keto-13,14-dihydro-$PGF_{2\alpha}$ by 15-ketoprostaglandin Δ^{13}-reductase (Δ^{13}-reductase).[5,6] Both these enzymes are widely distributed e.g. in lung, kidney, liver and spleen, and, interestingly, low 15-PGDH and high Δ^{13}-reductase activities are seen in the porcine brain.[7] Very little is known about the existence of 15-PGDH and Δ^{13}-reductase in the cornea. However, it has been claimed that eye tissues lack inactivating enzymes for prostaglandins.[8] Since topically applied drugs penetrate the cornea we have investigated the corneal metabolism of phenyl-substituted prostaglandin esters which have been developed as drug candidates for treatment of glaucoma.[9]

Experimental

17-Phenyl-18,19,20-trinor-$PGF_{2\alpha}$ 1-isopropyl ester (PhDH100A) and 15-keto-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ 1-isopropyl ester (PhXA12) analogues of $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ (Fig. 1) were synthesized as described elsewhere[10] and tritium labelled at the C-9β position at Kabi Pharmacia, Uppsala, Sweden.[§] Briefly, ^{3}H-PhDH100A was prepared by reducing 9-keto-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ 1-isopropyl ester (synthesized at Kabi Pharmacia) with NaB^3H_4 in the presence of cerium chloride in tetrahydrofuran and methanol at room temperature. The epic mixture of ^{3}H-9α and ^{3}H-9β compounds obtained was separated by reversed-phase HPLC to give the pure ^{3}H-9β-PhDH100A isomer. ^{3}H-PhXA12 was prepared by treating ^{3}H-PhDH100A with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in dioxane at room temperature. The product was purified by column chromatography (silica gel; ethyl acetate–ether 1:1). Porcine eyes were obtained from a local slaughter house and dissected on ice within an hour. The tritium-labelled test substances (PhDH100A, spec. act. 555 GBq mmol^{-1} and PhXA12, spec. act. 94 GBq mmol^{-1}) were studied in an isolated cornea incubation chamber consisting of two compartments separated by an excised porcine cornea. The transfer of the drugs across cornea occurred by passive diffusion. This *in vitro* chamber has also been used to study permeability and metabolism of drugs.[11] The compartment on the epithelial and that on the endothelial sides of the cornea had a volume of 1 and 6 ml, respectively. The compartments were filled with glutathione bicarbonate Ringer's (GBR) solution (pH 7.5) and saturated with 95 % O_2 and 5 % CO_2 to maintain constant pH and the temperature was kept at 34–35 °C. GBR solution on the epithelial side of the compartment was substituted with [^{3}H]PhDH100A (37 KBq per 10 or 20 μM) and [^{3}H]PhXA12 (37 KBq per 15 or 30 μM) in GBR solution. After 240 min of incubation at 34–35 °C all samples from both compartments were withdrawn and stored at −20 °C. Later the thawed samples were acidified to pH 3.5 with formic acid (1 M). PhDH100A, PhXA12 and their metabolites were extracted with ethyl acetate (1:1) and separated by reversed-phase HPLC with on-line radioactivity detection. A gradient solvent system with acetonitrile and water containing 0.1 % acetic acid was used. Major peaks were subjected to GC–MS identification after *tert*-butyldimethylsilyl derivatization (Hewlett Packard 5890 GC with silica column HP-5, Finnigan MAT 90).



PhDH 100A



PhXA12

Fig. 1. Chemical structures of PhDH100A and PhXA12.

[§] All compounds were identified and characterized by GC–MS and NMR spectrometry. Purity of the radiolabelled compounds was checked by reversed-phase HPLC with on-line radioactivity detection.

Results and discussion

The results show that 8.4 ± 1.0 % ($\bar{x}$ ± SEM, n = 4) PhDH100A was hydrolysed to its free acid and 4.3 ± 0.9 % of PhDH100A was further metabolised on the epithelial side of the cornea. The corresponding figures on the endothelial side were 99.9 ± 0.1 and 2.0 ± 1.2 % (Fig. 2, left-hand side). In contrast, 20.7 ± 3.4 % (n = 6) of PhXA12 was hydrolysed to its free acid and 9.5 ± 2.3 % PhXA12 was further metabolised to 15-keto-13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ (out of the total metabolism 12.2 ± 2.1 %) on the epithelial side. On the endothelial side PhXA12 was found to be completely hydrolysed to its free acid and most surprisingly 86.9 ± 2.2 % of the free acid was metabolised to 15-keto-13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ (out of the total metabolism 95.7 ± 1.3 %) by the reduction of the 13,14-double bond (Fig. 2, right-hand side).

Thus high esterase activity was present in the porcine cornea as has previously been shown by Bito and Baroody in the rabbit.[12] The almost quantitative reduction of the 13,14-double bond of PhXA12 indicates the presence of Δ^{13}-reductase in porcine cornea which, to our knowledge, has not been reported to date. The low 15-PGDH activity seen in this study should be treated with caution since PhDH100A was shown to be a relatively poor substrate for 15-PGDH compared with $PGF_{2\alpha}$.[13] However, we have previously shown that the cornea, along with other ocular tissues, possesses a low 15-PGDH activity.[13] The low 15-PGDH and a high Δ^{13}-reductase activities in the cornea resemble the activities of these enzymes in the porcine brain[7] although there is no direct embryological relationship between the brain and the cornea.

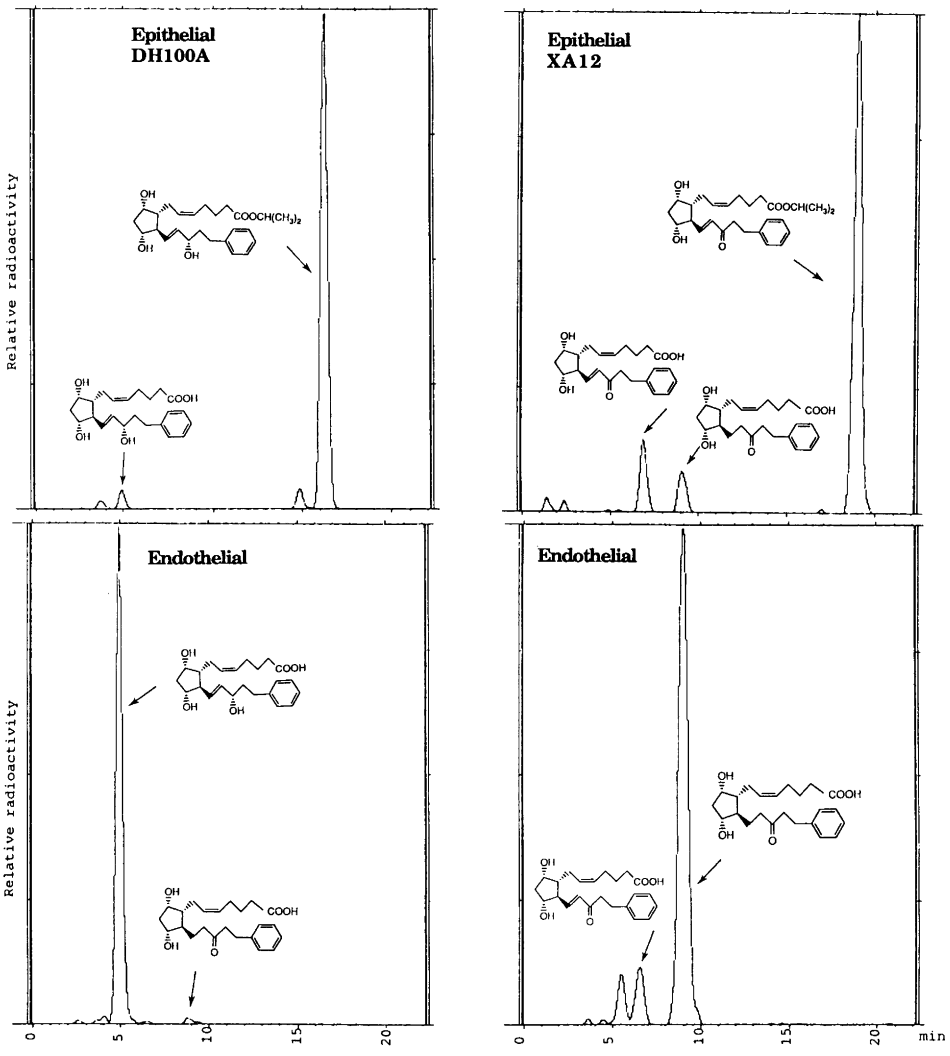


Fig. 2. Reversed-phase HPLC chromatograms with radioactivity detection of the bioconversion products after 240 min of incubation of ^{3}H-PhDH100A (10 μM, left-hand side) and ^{3}H-PhXA12 (30 μM, right-hand side) from the epithelial side (upper panel) and endothelial side (lower panel) of porcine cornea. Identification of the compounds was based on retention times and confirmed by GC–MS.

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