Prostaglandin activity in sustained inflammation of human skin before and after aspirin

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Summary

1. Pharmacologically active mediators of inflammation were obtained from suction bullae raised on normal and inflamed human abdominal skin. These contained a clear inflamatory exudate, which was analysed for known mediators of inflammation.

2. The exudates were examined for smooth muscle-contracting activity by a superfusion cascade bioassay, for prostaglandin F2α by radioimmunoassay and by Lipidex 5000 gel-partition chromatography for other prostaglandins and related compounds.

3. Tetrahydrofurfuryl nicotinate (Trafuril) was applied topically before and after systemic administration of aspirin. Trafuril alone caused a sustained inflammatory response within minutes of application, which was reduced by prior administration of aspirin (a known prostaglandin synthetase inhibitor).

4. Exudate from inflamed skin showed increased prostaglandin activity compared with exudate from contralateral non-inflamed skin. However, aspirin prevented this increase in prostaglandin activity. Analysis by thin-layer and gas-liquid chromatography further suggested that Trafuril-induced inflammation was mediated by certain prostaglandins and related compounds.

5. No evidence was obtained to suggest any change in histamine or bradykinin after Trafuril. We suggest that the response caused by Trafuril is mediated by increased synthesis of prostaglandins. Aspirin, by blocking prostaglandin synthesis, prevents or reduces the erythema.

Key words: aspirin, human skin, inflammation, prostaglandin, tetrahydrofurfuryl nicotinate.

Abbreviation: PG, prostaglandin.

Introduction

Tetrahydrofurfuryl nicotinate (Trafuril, Ciba) is a compound employed therapeutically for the relief of muscular pain and rheumatism. Topical application of Trafuril cream to human skin causes a sustained inflammatory response after a latent period of 10–15 min. The response to Trafuril does not appear to be associated with any of the classical mediators of skin inflammation and its mode of action is little understood.

Studies in perfused human skin failed to show involvement of histamine, bradykinin, 5-hydroxytryptamine or acetylcholine in Trafuril-induced erythema (Winkelmann, Wilhelmj & Homer, 1965; Sondergaard & Greaves, 1970). The apparent absence of pharmacological mediators has led to the suggestion that Trafuril acts directly on skin vasculature. However, unlike most forms of human skin inflammation the response to Trafuril is inhibited by aspirin (Truelove & Duthie, 1959; Winkelmann & Wilhelmj, 1963), which is known to block prostaglandin synthesis at therapeutic doses (Vane, 1971; Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971).

We have therefore studied prostaglandin activity during Trafuril-induced erythema before and after systemic aspirin administration.
Methods

Subjects
Ten normal healthy volunteer subjects and 21 patients with localized skin disorders not involving the abdomen were studied. The mean age of the subjects, 18 of whom were males, was 44.8 years (SEM 3.0, range 17–76). The study was approved by the Institute’s Ethical Committee and each subject gave informed consent to the study beforehand.

Trafuril inflammation

Inflammation was produced by application of 1 ml of Trafuril cream to 120 cm² of clinically normal abdominal skin. The abdomen was chosen because of its greater sensitivity to Trafuril (Winkelmann & Wilhelmj, 1963). Each subject remained in a supine position throughout the procedure.

Inflammatory exudate

Soon after the appearance of an erythema Perspex cups (Kiiistala, 1968; Black, Greaves, Hensby & Plummer, 1976) were inverted on the surface of the skin. Suction bullae, containing exudate, were produced by application of 200 mmHg vacuum. Exudates were always obtained within 6 h of Trafuril application and stored at −20°C until analysed. Light- and electron-microscopy showed that suction bullae arose as a result of a split of the dermo-epidermal junction between the plasma membrane of the basal cells and the basal lamina. Control samples were obtained from the contralateral non-inflamed side of each subject. In 11 of the subjects 3.6 g of aspirin was subsequently given, 600 mg in six divided doses over a 24 h period, before a second application of Trafuril.

Laboratory analysis

The diluted exudates (1:5) were examined for smooth muscle-contracting activity by the superfusion cascade method of Vane (1964). Histamine was detected from that part of the contractile response of the guinea-pig ileum which was inhibited by mepyramine. Relaxation of the rat duodenum indicated the presence of kinin-like activity. Prostaglandin-like activity was recognized by contractions of the rat stomach strip and rat colon in the presence of antagonists to other known inflammatory mediators (Gilmore, Vane & Wyllie, 1968).

The exudates were also examined for prostaglandin activity by a double antibody radioimmunoassay for PGF₂α. The antisera showed minimal cross-reactivity to the major prostaglandin classes except the D and F series. The F₃ series showed total cross-reactivity, whereas that to PGD₁ and PGD₂ was approximately 3%. The assay was capable of detecting as little as 3 pg of authentic PGF₂α.

In order to identify a wider spectrum of prostaglandins and related compounds the exudates were pooled and diluted three times with distilled water. The pH was adjusted to 4.0 with hydrochloric acid (0.1 mol/l) and the resulting solution extracted three times with an equal volume of redistilled ethyl acetate. The ethyl acetate fractions were combined and taken to dryness under reduced pressure at 40–45°C. The residues were then treated with methanolic ethereal ¹⁴C-labelled diazomethane to produce ¹⁴C-labelled methyl esters. These were then separated by gel-partition chromatography on a Lipidex 5000 column with heptane/chloroform solvent (4:1, v/v) (Brash & Jones, 1974; Hensby, 1975). The position of the radioactive peaks was compared with the positions of authentic ³H-labelled and unlabelled prostaglandin methyl esters, which were detected by liquid-scintillation counting and gas–liquid chromatography respectively.

The presence of certain prostaglandins was further examined by thin-layer chromatography on silica gel G plates (Anderman) with the F.V1 solvent system of Anderson (1969: ethyl acetate/acetone/acetic acid, 180:20:1, by vol.). The inclusion of silver nitrate (30 g/l) in the silica gel allowed the separation of prostaglandins of the same series but differing degree of unsaturation. Authentic standards were detected by spraying the plates with saturated ethanolic solution of phosphomolybdic acid, followed by heating at 120°C for 5–10 min. Before this 1 cm zones were scraped from the plate and eluted with methanol and an aliquot was assayed for radioactivity by liquid-scintillation counting. PGE₂ and PGF₂α were further identified by gas–liquid chromatography as the methyl ester n-butyl oxime trimethylsilyl ether and methyl ester trimethylsilyl ether respectively. These were separated on a glass column packed with 3% OV-1 on Chromosorb W. The carrier gas
(nitrogen) flow rate was 35–40 ml/min and the oven temperature 260°C.

Exudate protein concentrations were estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

Student’s t-test was used for all statistical analyses.

Results

Trafuril

Inflammatory response. Topical application of Trafuril caused an inflammatory response in all subjects studied. The erythema appeared in 10–15 min and usually persisted for up to 24 h. The protein concentration in exudate from normal skin was 24.0 g/l (SEM 2.4, n = 10). This increased to 37.5 g/l (SEM 3.0, n = 11) in Trafuril-inflamed skin, which was significantly greater (P < 0.005) than the normal concentration. The number of platelets in exudates from normal and Trafuril-treated skin of six subjects was not significantly different at 2.5 x 10^10/1 and 2.6 x 10^10/1 (SEM 0.8 and 0.6) respectively (Table 1).

Histamine and bradykinin. Histamine was detected in only one of 11 exudates from normal skin. After Trafuril the concentration in this same subject remained unchanged, whereas one of the other subjects now showed some activity. Similarly, Trafuril failed to produce any changes in kinin-like activity in the exudates (Table 1).

Prostaglandins. Prostaglandin-like activity was detected by bioassay in the samples from normal skin. The mean concentration, measured as PGE2 equivalents, was 54.5 fmol/l (SEM 17.3, n = 12). After Trafuril this increased to 129.6 fmol/l (SEM 19-6, n = 17, P < 0.01) (Table 1).

Similar results were obtained with the radioimmunoassay for PGF2a (Table 1).

Gel-partition chromatography of the extracted exudates, after conversion into [14C]methyl esters, produced six main peaks of radioactivity (Fig. 1). When compared with authentic prostaglandin methyl esters it can be seen that the first peak, which was the largest, was at the same position as oleic acid and arachidonic acid. The second peak ran in the same position as PGA and 13,14-dihydro-15-keto-PGE2. It is probable that C20 hydroxy fatty acids will also run in this position. Peak 3 corresponded to 13,14-dihydro-15-keto-PGF2a and 15-keto-PGE2. The fourth peak resembled PGD2 and 15-keto-PGF2a, and peak 5 ran with PGE1 and PGE2. The final peak ran with PGF2a.

The presence of compounds which resembled arachidonic acid, PGD2, 15-keto-PGF2a, PGE2

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<td>Bioassay</td>
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<td>Kinin-like</td>
<td>134±4.7</td>
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<td>(fmol/l)</td>
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<tr>
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<td>PG-like*</td>
<td>545±17.3</td>
<td>1296±19.6</td>
<td>709±31.8</td>
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<td></td>
<td>(fmol/l)</td>
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<td>(n=17, P&lt;0.01)</td>
<td>(n=5, P&lt;0.6)</td>
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<td>Radioimmunoassay</td>
<td>PGF2a*</td>
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<td>78.6±24.8</td>
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<td></td>
<td>(fmol/l)</td>
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<td>Others</td>
<td>Protein</td>
<td>24±0.2</td>
<td>37.5±3.0</td>
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<td>(n=11, P&lt;0.005)</td>
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<td>10^10 x No. of platelets</td>
<td>2.5±0.8</td>
<td>2.6±0.6</td>
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* Assayed in term of PGE2 equivalents.
and PGF\textsubscript{2\alpha} was also indicated by thin-layer chromatography in the presence of silver nitrate (30 g/l). Gas-liquid chromatography was used to further identify PGE\textsubscript{2} and PGF\textsubscript{2\alpha}. PGE\textsubscript{2} could not be demonstrated in our extracts by either thin-layer or gas-liquid chromatography. No activity was detected which had the same chromatographic properties as authentic PGF\textsubscript{1\alpha} on any of the systems tested.

The elution pattern from the Lipidex 5000 column remained essentially unchanged with exudates from normal and Trafuril-inflamed skin (Fig. 1). However, the amount of radioactivity eluted in each peak was different for each experimental group. The activity in all six peaks increased after Trafuril. The largest increases were in the peaks which were in the same position as PGE\textsubscript{2}, prostaglandin metabolites and possibly C\textsubscript{20} hydroxy fatty acids.

**Aspirin**

Prior administration of aspirin reduced or abolished the Trafuril-induced erythema, but appeared to have no effect on the concentrations of histamine, kinin-like activity or protein in the exudates (Table 1). However, aspirin blocked the increase in prostaglandin-like activity (as determined by bioassay and immunoassayable PGF\textsubscript{2\alpha}) in Trafuril-inflamed skin (Table 1). This reduction of prostaglandin activity by aspirin was confirmed by gel-partition chromatography. The activities in the fractions at the same position as peaks 4, 5 and 6 were no different from that seen in exudate from normal skin (Fig. 2). The activity in peak 3 was less than that seen after Trafuril. However, after aspirin the activity in peaks 1 and 2, resembling arachidonic acid and possibly C\textsubscript{20} hydroxy fatty acids, increased.

**FIG. 1.** (a) Elution pattern of \textsuperscript{3}H-labelled (---) and unlabelled (- - - -) authentic prostaglandin methyl esters from a Lipidex 5000 gel-partition column with chloroform/heptane solvent (1:4, v/v). The radioactivity was detected as disintegrations/s (d.p.s.) by liquid-scintillation counting and mean peak heights by gas-liquid chromatography respectively. OA, oleic acid; AA, arachidonic acid; 15-KH-E\textsubscript{2}, 13,14-dihydro-15-keto-PGE\textsubscript{2}; 15-KH-F\textsubscript{2\alpha}, 13,14-dihydro-15-keto-PGF\textsubscript{2\alpha}; 15-K-E\textsubscript{2}, 15-keto-PGE\textsubscript{2}; 15-K-F\textsubscript{2\alpha}, 15-keto-PGF\textsubscript{2\alpha}. (b) Elution pattern of \textsuperscript{14}C-labelled methyl esters of extracted exudates from normal (●) and Trafuril-treated skin before (▲) and after (■) systemic administration of aspirin and correction for 1 ml of exudate from the same Lipidex 5000 column.
Prostaglandins and aspirin in skin erythema

A

Normal Trafuril Aspirin and Trafuril

FIG. 2. Radioactivity in each of the six peaks (○, peak 1; ▲, peak 2; ■, peak 3; ○, peak 4; ▲, peak 5; □, peak 6) eluted from a Lipidex 5000 gel-partition column with chloroform/heptane solvent (4:1, v/v) after extraction of exudates from Trafuril-treated skin before and after systemic administration of aspirin relative to that found in exudate from normal skin.

above that seen in the Trafuril-treated skin, which had not been pretreated with aspirin.

Discussion

Our results suggest that Trafuril stimulates synthesis of compounds with the same chromatographic properties as arachidonic acid, various prostaglandins and their metabolites. This probably reflects an initial increase in arachidonic acid, which then acts as a substrate for prostaglandin synthetase, so increasing synthesis of prostaglandins of the 2 series. It is interesting that Trafuril appears to increase the synthesis of both PGE₂ and PGF₂α, since the latter is thought to be anti-inflammatory (Willinghoughy, 1968). However, when other prostaglandin-like compounds, such as PGD₂, are taken into consideration the net effect may be pro-inflammatory. The elevated amounts of prostaglandins and related compounds up to 6 h after Trafuril application suggests that these compounds may mediate the inflammatory response induced by Trafuril. In addition, our unpublished studies at 24 h after Trafuril application, when erythema was absent or minimal, showed that the concentration of these prostaglandin-like compounds was the same as in exudate from normal skin, again suggesting an intimate involvement of prostaglandins in the response to Trafuril.

A most significant finding in this study was that aspirin, a non-steroidal anti-inflammatory drug, prevented both the appearance of erythema and increase of compounds resembling the major prostaglandins (PGD₂, PGE₂ and PGF₂α). In 1971 Vane proposed that the anti-inflammatory action of aspirin arises from inhibition of prostaglandin biosynthesis. His view has subsequently been supported by reports that aspirin can inhibit prostaglandin synthesis in human platelets (Smith & Willis, 1971) and semen (Collier & Flower, 1971; Horton, Jones & Marr, 1973), reduce urinary excretion of prostaglandins (Hamberg, 1972) and cause accumulation of arachidonic acid in human skin after blockage of prostaglandin synthetase (Ziboh, 1975). Our demonstration of inhibition of both increased prostaglandin activity and inflammation in man thus provides new confirmation of Vane's hypothesis. Our results further suggest that aspirin inhibits an enzyme-mediated step in the synthesis of prostaglandin, since after aspirin arachidonic acid appears to accumulate at the expense of the prostaglandins.

The origin of the prostaglandins found in skin exudate is unknown, but at least three sources are possible. Goldyne, Jordan & Winkelmann (1975) described the production of prostaglandins by isolated human epidermal cells but this has not been investigated in the present study. The second possible origin is from platelets, but this seems unlikely as equal numbers of platelets were found in the exudate from normal and Trafuril-inflamed skin, and this only represents 10% of the platelet concentration of normal blood. It is also possible that the prostaglandins originate from leucocytes (Higgs & Youlten, 1972), which are present in small numbers in skin inflamed by Trafuril. However, further investigation is required to determine the origin of the increased prostaglandin activity that we have observed.

This study, like others (Winkelmann et al., 1965; Sondergaard & Greaves, 1970), failed to show that histamine and bradykinin are involved in the response to Trafuril. It is not known if there is a relation between prostaglandins and other mediators, such as histamine and kinins.
Acknowledgments

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References