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The depletion of substance P by diclofenac in the mouse

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Abstract

Diclofenac in hyaluronan is analgesic and angiostatic. The depletion of substance P may be a common mechanism. Mice received diclofenac, diclofenac in hyaluronan, or saline i.v. for 5 days and snout substance P assessed: saline 2.80 ± 0.23; 0.5 mg/kg diclofenac 2.03 ± 0.20 ($P < 0.05$); diclofenac in hyaluronan 1.88 ± 0.21 ($P < 0.02$); capsaicin 1.45 ± 0.26 fmol/mg tissue ($P < 0.005$). Substance P recovered by 5 days (diclofenac in hyaluronan, capsaicin) and 24 h (diclofenac). Diclofenac may deplete substance P in analgesia, and hyaluronan prolong the depletion.

Keywords: Diclofenac; Substance P; Analgesia

Diclofenac is a non-steroidal antiinflammatory drug (NSAID), and an effective analgesic attributable to peripheral prostaglandin synthesis inhibition. However, other mechanisms may be involved (Brune et al., 1991). These may include central and peripheral nitric oxide synthase inhibition, central prostaglandin suppression, and pain receptor down-regulation (Björkman, 1995). Small primary sensory nociceptive neurones contain substance P and capsaicin-induced substance P depletion is analgesic (Holzer, 1991). Diclofenac also reduces synovial fluid substance P in rheumatoid arthritis (Sacardote et al., 1995). We reasoned that the analgesic actions of diclofenac may possess commonality in the involvement of substance P with other actions such as the inhibition of inflammation and angiogenesis (Moore et al., 1996). We have tested for diclofenac-induced substance P depletion and whether drug delivery in hyaluronan would result in an improvement.

Murine snout substance P was quantified by a modification of the technique of Malcangio and Bowery (1993). Snouts were weighed and placed into 1 ml of glacial acetic acid and boiled prior to supernatant desalting and extraction on Sep-Pak C_{18} reverse silica gel cartridges (Waters, USA). Substance P was assayed by scintillation proximity radio-immunoassay (Amersham, UK). Mice (female Tuck strain TO, 25 g) were dosed i.v. daily for 5 days prior to assay with diclofenac sodium and/or 0.3% hyaluronan (molecular weight 500 000, Hyal Pharmaceutical, Canada) in 0.1 ml for drug delivery. Results were compared by Tukey-Kramer multi-comparison.

Control snouts contained 2.80 ± 0.23 fmol/mg tissue ($n = 8$) after 5 days i.v. administration of 0.1 ml saline. Diclofenac (0.5 mg/kg/day) in saline reduced this to 2.03 ± 0.20 fmol/mg (-28%; $P < 0.05$, $n = 7$); diclofenac in hyaluronan depleted substance P similarly to 1.88 ± 0.21 fmol/mg (-33%; $P < 0.02$, $n = 7$). Capsaicin (50 mg kg single subcutaneous dose) reduced substance P to 1.45 ± 0.26 fmol/mg (-48%; $P < 0.005$, $n = 8$) 7 days after administration. 0.1 ml 0.3% hyaluronan elicited 2.35 ± 0.25 fmol/mg (not significant, $n = 8$).

We tested the rate of recovery from depletion. Snouts from mice treated with capsaicin remained depleted by 61% 7 days post-administration (Fig. 1) and recovered over the following 6 days to 32%. Diclofenac (5 days, 0.5 mg/kg/day, i.v.) reduced substance P by 48%, returning to control within 3 days after cessation of therapy. Depletion by diclofenac in hyaluronan was similar to diclofenac alone, but repletion was retarded significantly, only nearing control levels by day 6. In a separate experiment, recovery from diclofenac-induced depletion was tested 24 h post-administration and was found to be complete (saline: 2.53 ± 0.31; diclofenac: 2.61 ± 0.27 fmol/mg, $n = 8$).

Subsequent study of the dose relationship revealed it was inverse between 0.03 and 1.0 mg/kg diclofenac. Dosed at 0.03, 0.1, 0.3 and 1.0 mg/kg, diclofenac gave substance P levels of 1.55 ± 0.09 ($P < 0.05$, $n = 8$), 1.52 ± 0.20 ($P < 0.05$, $n = 7$), 1.89 ± 0.22 and 1.94 ± 0.20,

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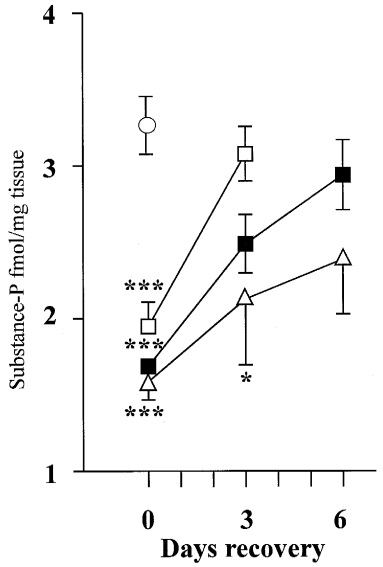

Fig. 1. Depletion and recovery of murine snout substance P after the i.v. administration of 0.1 ml saline (open circle), 0.5 mg/kg diclofenac (open square); diclofenac in 0.3% hyaluronan (closed square) for 5 days, or from 7 days after capsaicin s.c. (open triangle).

compared to saline control 2.24 ± 0.12 fmol/mg ($n = 14$), indicating that a more potent effect could be achieved with lower doses.

Diclofenac depleted peripheral substance P, and formulation in hyaluronan prolonged this effect, hyaluronan itself being inactive. Comparable improvements with diclofenac formulated in hyaluronan are seen with osteoarthritic pain and angiostasis and these effects could be attributed in part to substance P depletion (Moore et al., 1996). Although the analgesic mechanisms of NSAIDs are unknown, prostaglandins induce hyperalgesia by sensitising afferent nociceptors to inflammatory mediators and are involved in central processing of nociceptive signals (reviewed Appleton, 1997; Björkman, 1995). Cyclooxygenase-2 is present in the central nervous system, and upregulated on neuronal stimulation. Intrathecal NSAIDs block substance P or *N*-methyl-D-aspartate tail flick responses, and intrathecal arachidonic acid potentiates the formalin response. Diclofenac may also deplete substance P from the central projections of these neurones, ketotifen reduces substance P within spinal cord and hypothalamus (Dubordieu and Dray, 1989).

Through these interactions diclofenac may deplete peripheral substance P. Drug delivery with hyaluronan extends the period of depletion which may allow less frequent dosing and reduced risk. Whether these phenomena contribute to the analgesic activity of diclofenac remains to be investigated. NSAID analgesia is thus complex and possibly involves pathways in addition to prostaglandin synthesis inhibition.

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