EFFECTS OF DICLOFENAC AND L-745,337/ NS-398/ DFU, SELECTIVE CYCLOOXYGENASE-2- INHIBITORS, ON PROSTAGLANDIN- AND LEUKOTRIENE RELEASE IN BURSA SUBACROMIALIS TISSUE

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Introduction:
It is generally known, that prostaglandins (PG) and leukotrienes (LT) are strong mediators of inflammation. Local concentrations of PG and LT are found to be elevated in inflamed tissues. PG are synthesized by the two cyclooxygenase (cox) iso-enyzmes cox-1 and -2-. While cox-1 is constitutively expressed in almost any tissue and PG, generated via the cox-1 pathway play an essential role in physiological homoeostasis, little or no cox-2 is found in resting cells or tissues.
In contrast to cox-1, cox-2 gene expression is dramatically induced by cytokines or mitogens in inflammatory tissues and -cells. Likewise, PG synthesized via the cox-2 pathway support inflammation and contribute to clinical symptoms like oedema, redness, swelling and pain (Vane, Botting 1995).

Material and methods:
We compared the effects of both, classical, non-steroidal antiinflammatory drugs (NSAID),like diclofenac, and selective cox-2-inhibitors, like L-745,337, NS-398 and DFU, on release of PGE_2, 6-keto-$PGF1_\alpha$ and LTC_4 in inflamed bursa subacromialis tissue. Tissue was obtained from patients, suffering from impingement syndrome and taken by open surgery (n= 43).
At the time of operation, patients were at least 2 weeks without NSAID therapy and least 4 weeks without glucocorticoid therapy. Bursal specimens, 150-200 mg wet weight (w.w.) were incubated in modified tyrode solution at presence of graded concentrations of diclofenac, L-745,337, NS-398 and DFU (0,1-1000 µM).Each single specimen was analyzed histologically. Levels of PGE_2, 6-keto-PGF1α and LTC_4 were measured by means of radioimmunoassay and formation of LTC_4 was stimulated using Calcium Ionophore A 23187 (5 mg/ 1 ml DMSO). Statistical analyses were performed using student´s test for matched pair data.

Results:
Inflamed bursal tissue basic release (n=16) was 7,46 ± 1,7 ng PGE_2/ g w.w., 20,24 ± 3,08 ng 6-keto-PGF1α/ g w.w. and 33,1 ± 7,8 ng LTC_4/ g w.w..
Administration of 10 µM diclofenac decreased the release of PGE_2 to 5,5 ± 1,1 ng/ g w.w., of 6-keto-$PGF1_\alpha$ to 11,4 ± 2,32 ng/ g w.w. and increased release of LTC_4 to 37,08 ± 8,5 ng/ g w.w. respectively.
This equals 15 ± 9,07 % ($p< 0,05$), 42,23 ± 6,6 % ($p< 0,001$) inhibition and 24,88 ± 11,42 % stimulation respectively.
Administration of 0,1/ 1/ 10/ 100/ 1000 µM diclofenac (n=7) caused the following inhibition of release:
PGE_2: 9,47 ± 29,69 %/ 50,07 ± 20,21 %/ 66,73 ± 5,78 %/ 46,29 ± 23,61 %/ 72,94 ± 11,4 %. IC_{50} value is 2,63 * 10^{-6} M.
6-keto-$PGF1_\alpha$: 23,09 ± 25,39 %/ 43 ± 21,281 % ($p< 0,05$)/ 54,52 ± 16,6 % ($p< 0,05$)/ 67,45 ± 13,35 % ($p< 0,05$)/ 53,38 ± 34,02 % . IC_{50} value is 5,09 * 10^{-6} M.
Administration of 10/ 100/ 1000 µM L-745,337 (n=7) caused the following inhibition of release:
PGE_2: 15,47 ± 18,46 %/ 51,9 ± 8,86 % ($p< 0,025$)/ 69,7 ± 4,49 % ($p< 0,005$). IC_{50} value is 1,7 * 10^{-4} M.
6-keto-$PGF1_\alpha$: -4,54 ± 20,24 %/ 67,45 ± 13,35 % ($p< 0,01$)/ 54,36 ± 24,03 % ($p< 0,05$). IC_{50} value is 2,36 * 10^{-4} M.
LTC_4: -16,22 ± 20,15 %/ 16,39 ± 11,85 %/ 32,32 ± 14,75 %.
Administration of 10/ 100/ 1000 µM NS-398 (n=7) caused the following inhibition of release:
PGE_2: -69,78 ± 61,64 %/ 26,74 ± 15,3 %/ 84,36 ± 6,89 % ($p< 0,025$).
IC_{50} value is 2,95 *.10^{-4} M.
6-keto-$PGF1_\alpha$: 13,33 ± 17,92 %/ 57 ± 6,77 % ($p< 0,01$)/ 65,88 ± 7,57 % ($p< 0,025$). IC_{50} value is 1,49 * 10^{-4} M.
LTC_4: -42,09 ± 24,71 %/ -7,1 ± 20,23 %/ -21,46 ± 20,38 %.
Administration of 1/ 10/ 100 µM DFU (n=3) caused the following inhibition of release:
PGE_2: -67,83 ± 89,5 %/ -27,46 ± 28,3 %/ 23,74 ± 26,66 %.
6-keto-$PGF1_\alpha$: -36,15 ± 31,07 %/ -34,87 ± 45,78 %/ 2,84 ± 58,59 %.

Discussion:
Concentrations of all agents in the range of µM inhibit PG formation in bursal tissue. However, L-745,337, NS-398 and DFU inhibit PG formation in bursal tissue only at high concentrations (≥100 µM) that also inhibit cox-1 activity (Panara et al. 1995). At concentrations of L-745,337, NS-398 and DFU, that are selective for inhibition of cox-2 (< 10 µM) no significant inhibition on PG release can be detected. The data strongly suggest that the bulk of PG, generated in bursal tissue, is derived from the cox-1 pathway and that in therapy of the impingement syndrome, administration of cox-2 selective NSAID might be of little value.
Both, in acute and chronic cases of the impingement syndrome, 6-keto-$PGF1_\alpha$, which is exclusively synthesized by endothelial cells, is released in high levels. A possible explanation could be the sizeable angiogenesis within granulating and chronically granulating bursal tissue respectively, revealed in the corresponding histologies.
Furthermore, levels of PG and LT seem to correspond directly to the extent of damage in the subacromial space and within the rotator cuff. Apparently, bursal tissue also performs mechanical tasks, especially if the rotator cuff is ruptured.

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