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Angiostasis and Vascular Regression in Chronic Granulomatous Inflammation Induced by Diclofenac in Combination with Hyaluronan in Mice

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Abstract

Angiostasis and vascular regression in chronic granulomatous inflammation was assessed in mice induced with diclofenac in combination with hyaluronan.

The local injection of 0·1 mL HYAL EX-0001 (0·18% diclofenac in 2·5% hyaluronan) reduced granulomatous development after six days treatment from 150·4 ± 13·8 (0·18 saline) to 117·1 ± 17·8 mg (dry weight, n = 10), but not significantly when compared with 0·1 mL 2·5% hyaluronan alone or diclofenac in 0·1 mL saline. Diclofenac administered in saline had no significant effect when compared with saline control. The vascular density, expressed as carmine content per mg dry weight tissue, in those animals treated with HYAL EX-0001 was also significantly reduced to 5·27 ± 0·55 μg mg^{-1} ($P < 0·1$, n = 10) when compared with saline control (7·99 ± 1·0), hyaluronan alone (7·20 ± 1·0), and diclofenac in saline. (7·36 ± 1·28). A similar profile of activity was seen on topical application except that all treatments did not affect granulomatous tissue development.

On therapeutic dosing of mice daily with HYAL EX-0001 from day 7 after induction of the granulomatous tissue, the granulomatous tissue development was dramatically reduced from 111·67 ± 4·40 mg (n = 14 on day 7) to 60·23 ± 7·22 ($P < 0·001$, n = 8 on day 14) and 54·98 ± 7·88 ($P < 0·001$, n = 8 on day 21). HYAL EX-0001 after 14 days of application significantly reduced granulomatous tissue mass when compared with the hyaluronan-dosed control on day 21 (89·58 ± 7·49, $P = 0·01$, n = 8). The granulomatous tissue lost weight on the application of hyaluronan for 14 days by 19·8% ($P < 0·01$).

The vascular density of the tissues was 15·05 ± 0·63 μg mg^{-1}, which HYAL EX-0001 significantly reduced to 11·96 ± 1·14 ($P < 0·05$) after 7 days application and 11·25 ± 1·21 μg mg^{-1} ($P < 0·02$) after 14 days application. The latter was significantly inhibited when compared with the day 21 hyaluronan control (14·02 ± 1·39 μg mg^{-1}, $P < 0·05$). The day 7 vascular density was not significantly reduced by the topical application of hyaluronan from day 7 to 21.

The results suggest that hyaluronan is acting as a novel and effective drug delivery system, and may explain the therapeutic effectiveness of HYAL CT-1101 on basal cell carcinoma and actinic keratosis.

New blood vessel formation is essential for the development of tumours and chronic inflammatory granulomatous tissue such as pannus in rheumatoid arthritis. The restriction of angiogenesis in such disease states (Folkman 1972; Colville-Nash & Seed 1993) is a focus for the development of agents which would restrict tissue growth.

Case reports and clinical trials have shown that the combination of hyaluronan and the non-steroidal anti-inflammatory drug diclofenac (HYAL CT-1101) induces remission of basal cell carcinoma (Harper 1993). The 80% response rate with either remission or fragmentation into smaller nests appeared to follow a classic regression of the carcinoma, which is consistent with a reduction in vascularity.

Diclofenac is a potent non-steroidal anti-inflammatory drug (NSAID) and as such inhibits prostaglandin synthesis. Prostaglandin E_2 (PGE_2) is reported to be angiogenic in the rabbit cornea (Ben Ezra 1978) and in the chick chorio-allantoic membrane (CAM) assay (Form et al 1982). PGE_2 also stimulates endothelial cell tube formation in-vitro (Murota et al 1990). Stable prostacyclin analogues are also effective in inducing angiogenesis in the CAM assay (Oktsu et al 1988), whilst prostaglandins are involved in basic fibroblast growth factor-induced angiogenesis in the CAM assay (Spisni et al 1992).

Tumours (Bennett et al 1977; Goodwin et al 1980), tumour cells (Owen et al 1980), as well as inflammatory tissue (Vane 1976) synthesize prostaglandins in appreciable quantities. The inhibition of prostaglandin synthesis by diclofenac has been shown to result in a reduction in tumour growth and vascularization in rat hepatoma (Petersen 1983, 1984). Indomethacin is also reported to reduce both tumour development and vascularization (Mira et al 1988; Sundbeck et al 1981) whilst ibuprofen has been shown to inhibit the growth of implanted C6 astrocytoma (Farrell et al 1988). Angiogenesis associated with corneal wounds is inhibited by flurbiprofen, indomethacin and ketorolac (Deutsch & Hughes 1979; Cooper et al 1980; Mahoney & Waterbury 1985; Haynes et al 1989).

Hyaluronan is used not only to aid the delivery of drugs, but also to target delivery to pathological sites. Topical hyaluronan enhances the ocular delivery of dexamethasone (Grecomoro et al 1992), gentamycin (Moreira et al 1991) and tobramycin (Gandolfi et al 1992). 5-Fluorouracil

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uptake by rat mammary carcinoma and subcutaneous Fischer bladder carcinoma is enhanced by hyaluronan (Klein et al 1994) as well as cyclosporin A (Falk 1994). This can be related to the presence of hyaladherins such as CD44 and the receptor for hyaluronan-mediated motility, termed RHAMM (Knudson & Knudson 1993), as well as the recent identification of inter-cellular adhesion molecule-1 (ICAM-1) as an extremely high affinity hyaladherin binding protein (McCourt et al 1994), during neoplastic and inflammatory disease (Henrich & Hawkes 1989; Cronstein & Weissman 1993). The expression and up-regulation of these receptors would cause specific binding of hyaluronan and drug targeting. The identification of ICAM-1 as a high-affinity hyaladherin has specific relevance to targeting during inflammation, being up-regulated on endothelial cells as well as recruited inflammatory cells.

Relating the effectiveness of topical HYAL CT-1101 on basal cell carcinoma (Harper 1993), the NSAID's effects on angiogenesis, and the targeting properties of hyaluronan, we considered whether angiostasis and the induction of vascular regression in inflammation could be effected by this formulation.

Using a model of murine subcutaneous chronic granulomatous inflammation which lends itself to the topical and local administration of agents, we have assessed whether diclofenac formulated with hyaluronan influences angiogenesis in murine chronic granulomatous tissue, and whether this inclusion of hyaluronan improves efficacy. We have previously found that angiogenesis in granulomatous tissue is significantly reduced by indomethacin and ibuprofen (Colville-Nash et al 1992), as do the angiostatic steroids (Colville-Nash et al 1995). However, pharmacologically-induced regression of the neovasculature in granulomatous tissue has never before been demonstrated. We have assessed whether diclofenac with hyaluronan is effective in inhibiting angiogenesis, and more importantly, whether regression of the neovasculature can be induced.

Materials and Methods

Induction of inflammation

Air pouches were induced in 25–30 g female Tuck original mice by the subcutaneous injection of 3 mL sterile air under halothane anaesthesia. Twenty-four hours later, chronic granulomatous inflammation was induced by the injection of 0·5 mL Freund's complete adjuvant with 0·1% croton oil (Kimura et al 1986; Appleton et al 1993).

Assessment of vascularity

The mice were dosed for six days and the vascular content assessed by the formation of a vascular cast as described by Kimura et al (1986) and modified by us (Colville-Nash & Seed 1993; Colville-Nash et al 1995). One millilitre 25% carmine red in 10% gelatin was injected at 40°C into mice which had been maintained at 37°C for 10 min, this latter step leading to improved peripheral perfusion and reproducibility of results (Orlandi et al 1988). The carcasses were chilled and the granulomatous air pouch linings dissected. These were dried at 56°C, weighed, and papain-digested (Farndale et al 1986). The dye can then be dissolved by the addition of 1 mL 0·05 M NaOH, and the samples were then centrifuged at 2500 *g* for 20 min. After filtration, the absorbances were read at 490 nm using a multi-well plate reader (Biotek). The results were expressed either as μg dye content per sample or as the vascular index as μg dye (mg dry weight)$^{-1}$.

Administration of diclofenac with hyaluronan

HYAL EX-0001 was prepared by the dissolution of 0·18% diclofenac in 2·5% hyaluronan. Topical applications of HYAL EX-0001 (0·1 mL, 6 mg kg^{-1} diclofenac), diclofenac in aqueous cream BP (0·1 mL, 6 mg kg^{-1}), 0·1 mL aqueous cream alone, or 0·1 mL 2·5% hyaluronan alone were made daily to the surface of the depilated air pouch, rubbing in a circular motion twenty times clockwise, then anti-clockwise.

Administration into the pouch was carried out by the injection into the air-pouch of 0·1 mL HYAL EX-0001, hyaluronan alone, diclofenac in sterile saline, or saline alone.

Drugs were administered daily from 1 h before induction of the air pouch to termination at day 6 for the assessment of the effect on the development of angiogenesis. Therapeutic dosing was carried out by topical application to the existing neovasculature at day 7 daily, to termination at day 21.

Statistics

Results are expressed as mean ± s.e.m., and comparisons carried out using the Kruskall-Wallace non-parametric multicomparison test.

Materials

Hyaluronan (500–800 kDa) was supplied by the Hyal Pharmaceutical Company, Toronto, Canada. Diclofenac was from Sigma, UK, and aqueous cream BP was from Evans Medical, Horsham, UK.

Results

Local injection of HYAL EX-0001

The local injection of 0·1 mL HYAL EX-0001 (6 mg kg^{-1} diclofenac in 2·5% hyaluronan) reduced granulomatous development after six days treatment to 117·1 ± 17·8 mg (dry weight, $P < 0·05$, n = 10) when compared with 0·1 mL saline alone (150·4 ± 13·8 mg, n = 10), but not significantly when compared with 0·1 mL 2·5% hyaluronan alone (129·0 ± 5·85 mg, n = 10) or diclofenac in 0·1 mL saline (133·7 ± 7·75 mg, n = 10). Diclofenac administered in saline had no significant effect when compared with saline control.

The vascular volume, expressed as carmine content, in those animals treated with HYAL EX-0001 was also significantly reduced to 0·562 ± 0·059 mg (n = 10) when compared with saline control (1·143 ± 0·151 mg, n = 10, $P < 0·002$), hyaluronan alone (0·917 ± 0·12 mg, n = 10, $P < 0·05$), and diclofenac in saline (0·928 ± 0·128 mg, n = 10, $P < 0·05$). Diclofenac administered in saline was not significantly different from saline control. The derived vascularity index (VI) of the granulomatous tissues after six days treatment with HYAL EX-0001 was reduced by 26·8% ($P < 0·05$), 34·1% ($P < 0·05$), and 28·5% (not significant) when compared with hyaluronan, saline alone, and diclofenac in saline, respectively (Table 1).

Table 1. The reduction in the vascularity of the murine chronic granulomatous air pouch by HYAL EX-0001. Diclofenac (6 mg kg^{-1}) was injected into the air pouch administered in a volume of 0·1 mL sterile saline or 2·5% hyaluronan. On the sixth day after induction of inflammation the vascular index of the tissue was expressed as μg carmine dye per mg granulomatous tissue dry weight.

Treatment	Vascular index ($\mu g\ mg^{-1}$)
Saline	7·98 ± 1·02
Saline + diclofenac	7·36 ± 1·28
Hyaluronan	7·20 ± 0·96
HYAL EX-0001	5·27 ± 0·55*+

Results are expressed as mean ± s.e.m. (n = 8, *P < 0·05 HYAL EX-0001 vs saline; +P < 0·05 HYAL EX-0001 vs hyaluronan; HYAL EX-0001 vs diclofenac in saline was not significantly different).

Neither hyaluronan nor diclofenac in saline had any other significant effect.

Topical application of HYAL EX-0001

A similar profile of activity was seen on topical application except that HYAL EX-0001, as well as the other treatments, did not affect granulomatous tissue development significantly (HYAL EX-0001: 154·6 ± 13·8 mg; control cream: 179·6 ± 21·26 mg; hyaluronan: 176·2 ± 5·61 mg; diclofenac in aqueous cream: 166·3 ± 11·58 mg, n = 8).

Carmine dye content was significantly reduced from 2·40 ± 0·345 mg (n = 8) for the control cream, 1·845 ± 0·213 mg (n = 7) for hyaluronan alone, and 1·873 ± 0·236 mg (n = 8) for diclofenac in aqueous cream, to 1·036 ± 0·243 mg (n = 8, P = 0·01, P < 0·05 and P < 0·05, respectively) by HYAL EX-0001. This was translated into a similar and significant reduction in VI with HYAL EX-0001 by 52·6 (P = 0·02), 41·6 (P < 0·05), and 45·9% (P < 0·05) for control cream, hyaluronan alone and diclofenac in control cream, respectively (Table 2).

The induction of vascular regression by HYAL EX-0001

Therapeutic dosing of mice daily was carried out from day 7 after induction of the granulomatous tissue with HYAL EX-0001 or with hyaluronan alone as control.

The granulomatous tissue development was dramatically reduced from 111·67 ± 4·40 mg (n = 14) on day 7 to 60·23 ± 7·22 (P < 0·001, n = 8) on day 14 and 54·98 ± 7·88 (P < 0·001, n = 8) on day 21 by topical HYAL EX-0001. HYAL EX-0001 after 14 days of application significantly reduced granulomatous tissue mass when compared with the hyaluronan-dosed control on day 21 (89·58 ± 7·49, P = 0·01, n = 8). The granulomatous tissue lost weight on the application of hyaluronan for 14 days by 19·8% (P < 0·01).

Table 2. The reduction in the vascularity of the murine chronic granulomatous air pouch by topical HYAL EX-0001. Diclofenac (6 mg kg^{-1}) was administered in a volume of 0.1 mL sterile aqueous cream or 2·5% hyaluronan.

Treatment	Vascular index ($\mu g\ mg^{-1}$)
Aqueous cream	13·89 ± 2·01
Aqueous cream + diclofenac	12·16 ± 1·58
Hyaluronan	10·31 ± 1·11**+
HYAL EX-0001	6·58 ± 1·23

Results are expressed as mean ± s.e.m. (n = 8, **P = 0·02 HYAL EX-0001 vs aqueous cream; +P < 0·05 HYAL EX-0001 vs hyaluronan; HYAL EX-0001 vs diclofenac in aqueous cream was significantly different at P < 0·05).

The effects on the vascular volume were found to be similar. The vascular bed held 1·738 ± 0·657 mg carmine at day 7, which HYAL EX-0001 significantly reduced to 0·696 ± 0·081 mg (P < 0·001) after 7 days application and 0·666 ± 0·140 mg (P < 0·0002) after 14 days application. The latter was significantly inhibited when compared with the day 21 hyaluronan control (1·187 ± 0·150 mg, P < 0·05). The day 21 vascular volume was reduced (P < 0·01) by the topical application of hyaluronan from day 7 to 21.

However, the VI value of 7-day tissues was reduced only by topical HYAL EX-0001 treatment (Fig. 1) for 7 (20·5%, P < 0·05) and 14 days (25·5%, P = 0·02). Hyaluronan alone applied for 14 days had no effect on the vascular density, the inclusion of diclofenac significantly reducing it by 19·8% (P < 0·05). Animal body weights did not differ significantly between groups.

Discussion

The murine chronic granulomatous air pouch was developed for its profound angiogenic component (Kimura et al 1986). This has been utilized to assess the influence of angiostatic and angiogenic therapy (Colville-Nash & Seed 1993; Colville-Nash et al. 1995). Cortisone in combination with heparin retards the development of the vasculature in this model, whilst heparin given orally enhances it. The anti-inflammatory drugs indomethacin and ibuprofen significantly reduce the vascular development of the granulomatous tissue vasculature, dramatically reducing dye content and the derived VI (Colville-Nash et al 1992). Interestingly, both of these NSAIDs increase the granuloma dry weight, an effect probably due to the immunomodulatory effects of PGE_2 synthesis inhibition in chronic inflammation.

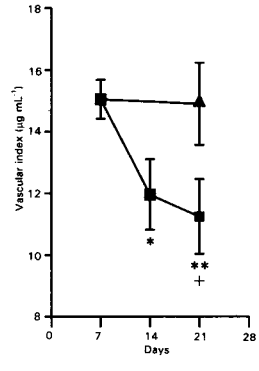

FIG. 1. The regression of the granulomatous tissue neovasculature induced by the daily topical application of HYAL EX-0001 to established 7-day.old murine chronic granulomatous air pouches. The vascularity was assessed after a further 7 and 14 days. The vascularity index is expressed as μg carmine red per mg dry granulomatous tissue. Points represent mean ± s.e.m. of 0·1 mL hyaluronan applied alone (▲) and HYAL EX-0001 (■) (n = 15, *P < 0·05, **P < 0·02 compared with 7 day control; +P < 0·05 compared with hyaluronan control at day 21).

The local injection and topical application of HYAL EX-0001 did not exacerbate the development of the granulomatous tissue as reported for orally active NSAIDs; however HYAL EX-0001 did significantly reduce granulomatous inflammation when injected into the lesion, and reduced the granulomatous tissue vascular content when given via both routes. The vascular index was reduced by HYAL EX-0001 when administered via both routes, topical application appearing to be more effective, with a significant inhibition of 53% as compared to a non-significant inhibition of 34% by local injection. Hyaluronan alone had no significant effect on any of the parameters, neither did diclofenac given in placebo cream. This suggests that the co-administration with hyaluronan has a permissive effect on the action of diclofenac on the development of new blood vessels when given by these two routes, and confirms the delivery and targeting described earlier.

We have previously established that the effects of the chronic administration of NSAIDs are not due to acute vasodilator prostaglandin synthesis inhibition since, under the controlled conditions used, the method used in this study is insensitive to acute pharmacological alterations in vascular tone and plasma extravasation, such as that found with bolus indomethacin (Colville-Nash & Seed 1993; Colville-Nash et al 1995). This indicates that the action of chronic HYAL EX-0001 therapy on the carmine dye content is a reflection of an alteration in the vascular volume of the tissue, and when expressed as a function of granulomatous tissue mass (the vascular index), reflecting an inhibition of vascular development.

It is important to note, however, that the dosage regimens used are prophylactic. Chronic inflammatory and neoplastic disease present as existing lesions. The induction of regression of the neovasculature, as well as angiostasis, is an important goal for the therapy of existing inflammatory and neoplastic lesions. HYAL EX-0001 applied topically to the 7-day chronic granulomatous air pouch resulted in a significant 25% regression of the neovasculature over 14 days. It also provided a significant inhibition when compared with controls given hyaluronan alone. The natural history of the granulomatous tissue is a 30% reduction in mass over this period (Colville-Nash & Seed 1993), which is also seen with carmine content. Thus the significant reduction in tissue mass and vascular volume seen with the hyaluronan control is not an effect of its application, neither is it due to an effect on angiogenesis since the vascular index remained unaltered. Combination with diclofenac significantly accelerated granulomatous tissue regression, led by a reduction in vascular density and regression of the neovasculature. This demonstrates for the first time that regression of the neovasculature in granulomatous inflammation can be achieved, and that HYAL EX-0001 is effective in inducing it.

The consequences of angiostatic therapy is a reduction in granulomatous tissue development (Dunn & Galinet 1991; Colville-Nash et al 1993), accompanied by a reduced vascular index, inflammatory cell recruitment, extracellular matrix deposition, tissue destruction, and a reduction in the resultant granulomatous tissue mass. It is interesting to note that HYAL EX-0001 therapy is more effective in inhibiting granulomatous tissue development when given therapeutically rather that prophylactically. The populations of inflammatory cells in the chronic phase are different from those found in the acute phase, involving macrophages and fibroblasts, and the expression and localization of growth factors and cytokines is altered (Appleton et al 1993). A selective action on these processes would explain the therapeutic efficacy of HYAL EX-0001.

These investigations have shown that the combination of hyaluronan and diclofenac (HYAL EX-0001), given either topically or directly into the lesion, results in reduced vascular development during granulomatous inflammation. Topical application to the existing neovasculature results in vascular regression and in accelerated granulomatous tissue resolution. The results suggest that hyaluronan is acting as a novel and effective drug delivery system, and may explain the therapeutic effectiveness of HYAL CT-1102 on basal cell carcinoma and actinic keratosis.

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References

Appleton, I., Tomlinson, A., Colville-Nash, P. R., Willoughby, D. A. (1993) Temporal and spatial immuno-localisation of cytokines in murine chronic granulomatous tissue. Lab. Invest. 69: 405–414

Ben Ezra, D. (1978) Mediators of immunological reactions function as inducers of neovascularisation. Metab. Ophthalmol. 21: 339–341

Bennett, A., Charlier, E. M., McDonald, A. M., Simpson, J. S., Stamford, I. F., Zebro, T. (1977) Prostaglandins and breast cancer. Lancet 2: 624–626

Colville-Nash, P. R., Seed, M. P. (1993) The current state of angiostatic therapy, with special reference to rheumatoid arthritis. Curr. Opin. Invest. Drugs 2: 763–813

Colville-Nash, P. R., Seed, M. P., Willoughby, D. A. (1992) Anti-rheumatic drugs and the development of the vasculature in murine chronic granulomatous air pouches. Br. J. Pharmacol. 107: 421P

Colville-Nash, P. R., El-Ghazaly, M., Willoughby, D. A. (1993) The use of angiostatic steroids to inhibit cartilage destruction in an in vivo model of granuloma-mediated cartilage degradation. Agents Actions 38: 127–134

Colville-Nash, P. R., Alam, C. A. S., Brown, J. R., Appleton I., Seed, M. P., Willoughby, D. A. (1995) The pharmacological modulation of angiogenesis in chronic granulomatous inflammation. J. Pharmacol. Exp. Ther. In press

Cooper, C. A., Bergamini, M. V. W., Leopold, I. H. (1980) Use of flurbiprofen to inhibit corneal neovascularisation. Arch. Ophthalmol. 98: 1102–1105

Cronstein, B. N., Weissman, G. (1993) The adhesion molecules of inflammation. Arthritis Rheum. 36: 147–157

Deutsch, T. A., Hughes, F. H. (1979) Suppressive effects of indomethacin on thermally induced neovascularisation of rabbit corneas. Am. J. Ophthalmol 87: 536–540

Dunn, C. J., Galinet, L. A. (1991) Angiostatic cortexone-heparin combination treatment suppresses chronic granulomatous inflammation in mice. Drug Dev. Res. 23: 241–248

Falk, R. (1994) Effect of hyaluronic acid on the penetration and targeting of drugs. Roy. Soc. Med. Round Table Ser. 33: 2–10

Farndale, R. W., Buttle, D. J., Barrett, A. J. (1986) An improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim. Biophys. Acta 883: 173–177

Farrell, C. L., Megyesi, J., Del-Maestro, R. F. (1988) Effect of ibuprofen on tumour growth in the C6 spheroid implantation glioma model. J. Neurosurg. 68: 925–930

Folkman, J. (1972) Anti-angiogenesis: new concept for therapy of solid tumours. Ann. Surg. 175: 409–416

Form, D. M., Sidky, Y. A., Kubai, L., Auerbach, R. (1982) Prostaglandin E_2 induced angiogenesis. Prostaglandins Leukotrienes Related Lipids 2: 685–689

Gandolfi, S. A., Massari, A., Orsoni, J. G. (1992) Low-molecular weight sodium hyaluronate in the treatment of bacterial corneal ulcers. Graefe's Arch. Clin. Exp. Ophthalmol. 230: 20–23

Goodwin, J. S., Husby, G., Williams, R. C. (1980) Prostaglandin E and cancer growth. Cancer Immunol. Immunother. 8: 3–7

Grecomoro, G., Piccione, F., Letizia, G. (1992) Therapeutic synergism between hyaluronic acid and dexamethasone in the intra-articular treatment of osteoarthritis of the knee: a preliminary open study. Curr. Med. Res. Opinion 13: 49–55

Harper, D. (1993) A review of current clinical trials with HYAL EX-0001, HYAL AT-2101 and HYAL AV-2201. Roy. Soc. Med. Round Table Ser. 33: 60–63

Haynes, W. L., Proia, A. D., Klintworth, G. K. (1989) Effect of inhibitors of arachidonic acid metabolism on corneal neovascularisation in the rat. Invest. Ophthalmol. Vis. Sci. 30: 1588–1593

Henrich, C. J., Hawkes, S. P. (1989) Molecular weight dependence of hyaluronic acid produced during oncogenic transformation. Cancer Biochem. Biophys. 10: 257–267

Kimura, M., Amemiya, K., Yamada, T., Suzuki, J. (1986) Quantitative method for measuring adjuvant-induced granuloma angiogenesis in insulin treated diabetic mice. J. Pharmacobiodyn. 9: 442–446

Klein, E. S., He, W., Shmizu, S., Asculai, S., Falk, R. E. (1994) Hyaluronic acid enhances tritiated fluorouracil uptake in experimental cancer. Roy. Soc. Med. Round Table Ser. 33: 11–15

Knudson, C. B., Knudson, W. (1993) Hyaluronan-binding proteins in development, tissue homeostasis, and disease. FASEB J. 7: 1233–1241

Mahoney, J. M., Waterbury, L. D. (1985) Drug effects on the neovascularisation response to silver nitrate cauterization of the rat cornea. Curr. Eye Res. 4: 531–535

McCourt, P., El, B., Forsberg, N., Gustafson, S. (1994) Intercellular adhesion molecule-1 is a cell surface receptor for hyaluronan. J. Biol. Chem. 269: 30081–30084

Mira, L., Hunter, N., Furuta, Y., Nishiguchi, I., Runkel, S. (1988) Antitumour effects of indomethacin alone and in combination with radiotherapy: role of inhibition of tumour angiogenesis. Int. J. Radiat. Biol. 60: 65–70

Moreira, C. A., Moreira, A. T., Armstrong, D. K., Jellife, R. W., Woodford, C. C., Liggett, P. E., Trousedale, M. D. (1991) In vitro and in vivo studies with sodium hyaluronate as a carrier for intra-ocular gentamicin. Acta Ophthalmol. 69: 50–56

Murota, S., Kanayasu, T., Nakano-Ilyashi, J., Morita, I. (1990) Involvement of eicosanoids in angiogenesis. Adv. Prostaglandin Thromboxane Leukotriene Res. 21: 622–626

Oktsu, A., Fujii, K., Kurozumi, S. (1988) Induction of angiogenic response by chemically stable prostacyclin analogues. Prostaglandins Leukotrienes Essential Fatty Acids 33: 35–39

Orlandi, C., Dunn, C. J., Cutshaw, L. G. (1988) Evaluation of angiogenesis in chronic inflammation by laser Doppler flowmetry. Clin. Sci. 74: 119–121

Owen, K., Gomolka, D., Droller, M. J. (1980) Production of prostaglandin-E_2 by tumour cells in vitro. Cancer Res. 40: 3167–3171

Petersen, H. I. (1983) Effects of prostaglandin synthesis inhibitors on tumour growth and vascularisation. Invasion Metastasis 3: 151–159

Petersen, H. I. (1984) Effects of prostaglandin synthesis inhibition on tumour growth and vascularisation—experimental studies in the rat. In: Courtice, F. C., Garlick, D. G., Perry, M. A. (eds) Progress in Microcirculation Research II. Committee in Postgraduate Medical Education, Sydney, The University of New South Wales, pp 45–47

Spisni, E., Manica, F., Tomasi, V. (1992) Involvement of prostanoids in the regulation of angiogenesis by polypeptide growth factors. Prostaglandins Leukot. Essent. Fatty Acids 47: 111–115

Sundbeck, A., Karlsson, L., Lilja, J., Petersen, H. I. (1981) Inhibition of tumour vascularisation by tranexamic acid. Experimental studies on possible mechanisms. Anticancer Res. 1: 299–304

Vane, J. R. (1976) Prostaglandins as mediators of inflammation. Adv. Prostaglandin Thromboxane Leukotriene Res. 2: 791–801