Countermeasures for Radiation Induced Skin Injury

Combined Injury Workshop
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Center for Biophysical Assessment and Risk Management Following Irradiation

Disclosures

Companies supporting conferences: Amgen, Varian, Electa, BrainLAB (all unrestricted educational grants)

Collaborations or Advisory positions: BD inc, Raybiotech, Litron, Varian, BrainLAB, Amgen

No paid speaking engagements for industry

Grants: NCI, NIAID, DOD, ACS, BrainLAB, RTOG Foundation, Hope Foundation (SWOG), Wilmot Foundation, Breast Cancer Coalition

Significant Interest: patents related to extracranial SRS and drugs that are radiation protectors, Eva Pharmaceuticals LLC, Rtek Inc.

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Molecular Mechanisms of Cutaneous Radiation Damage

Mitigation and Therapy of Radiation Cutaneous Syndromes

- Curcumin
- Celebrex
- Pentoxifylline
- EsA

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CYTOKINE HOMEOSTATIC FEEDBACK IS ODDLY CONTROLED LEADING TO IMBALANCE

INFLAMMATORY FACTORS IN SKIN AND OTHER SOFT TISSUES

TGFs
TNF
IL-1,2,3,6,8,11,12
MIP
MCP
FGF1,2,7,10 (KGFs)
Cox-2

Cell Types

- Fibroblasts
- Macrophage
- T-Cells
- Epithelial cells
- Endothelium

Same cytokines present in different doses or at different times can be deleterious or beneficial

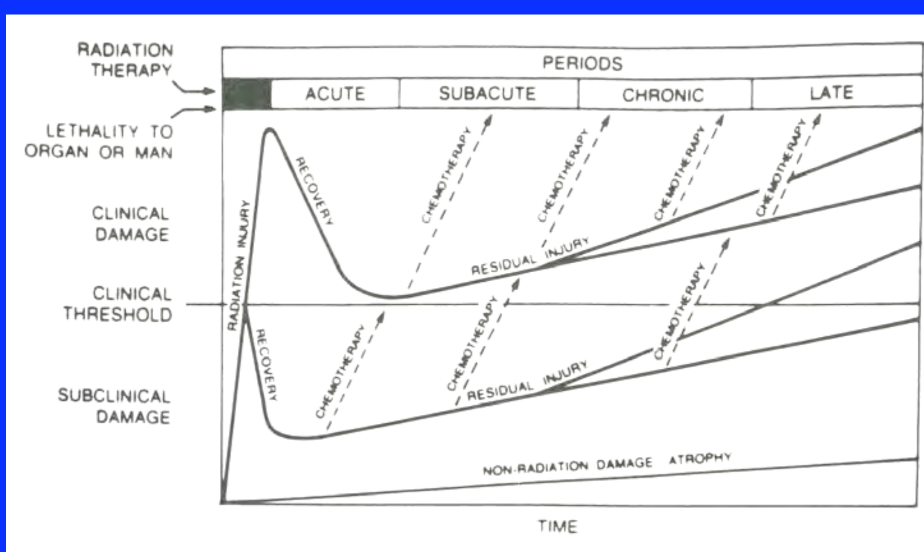
- Keratinocytes make IL-1
- Macrophage chemotaxis & activation
- Local Macrophage make MCP-1 & TGF ß
- Fibroblasts proliferate and make more MCP-1 and TGFß
- Positive feedback with Keratinocytes to make more IL-1
- Need CARS to stop cycle

IL-1

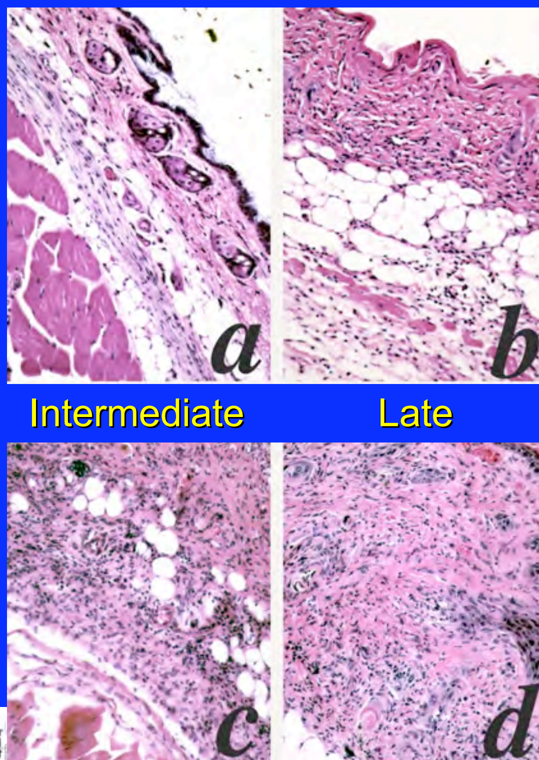
Positive Feedback
Self Maintaining
Over-expression

TGFß1

Inflammation with <u>fibroblast</u> proliferation and <u>mononuclear</u> infiltrate



Rubin Empirical Model



Pattern of progression from the papillary dermis, though the dermis and then to the superficial muscle and then in a process that resembles invasion penetration and replacement of the muscle and skin is not consistent with a purely random cell inactivation that is expected by classical biology

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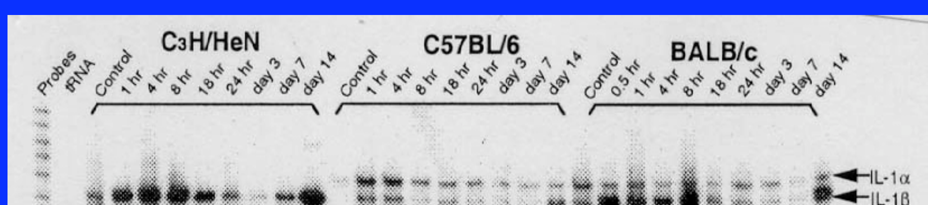
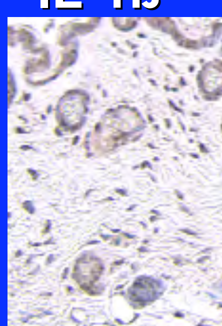


IL-1ß

C57 /WT



Control

- **There are strain specific differences in the expression and timing of IL-1 alpha and IL-1ß in response to radiation**

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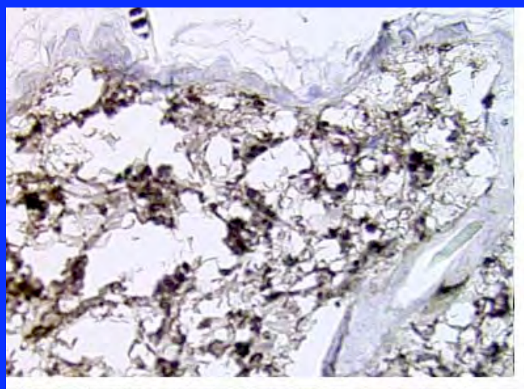
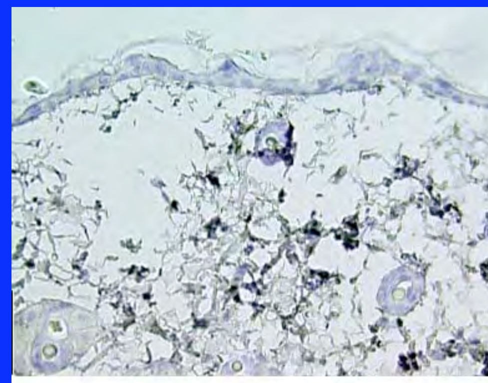
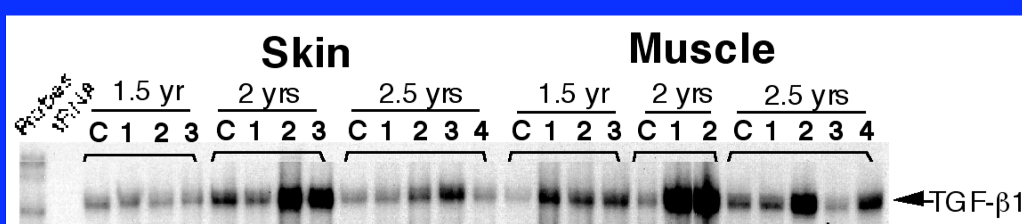
Immunohistochemical Staining (ED1) of Cutaneous Macrophage at 20 Days Post-Radiation



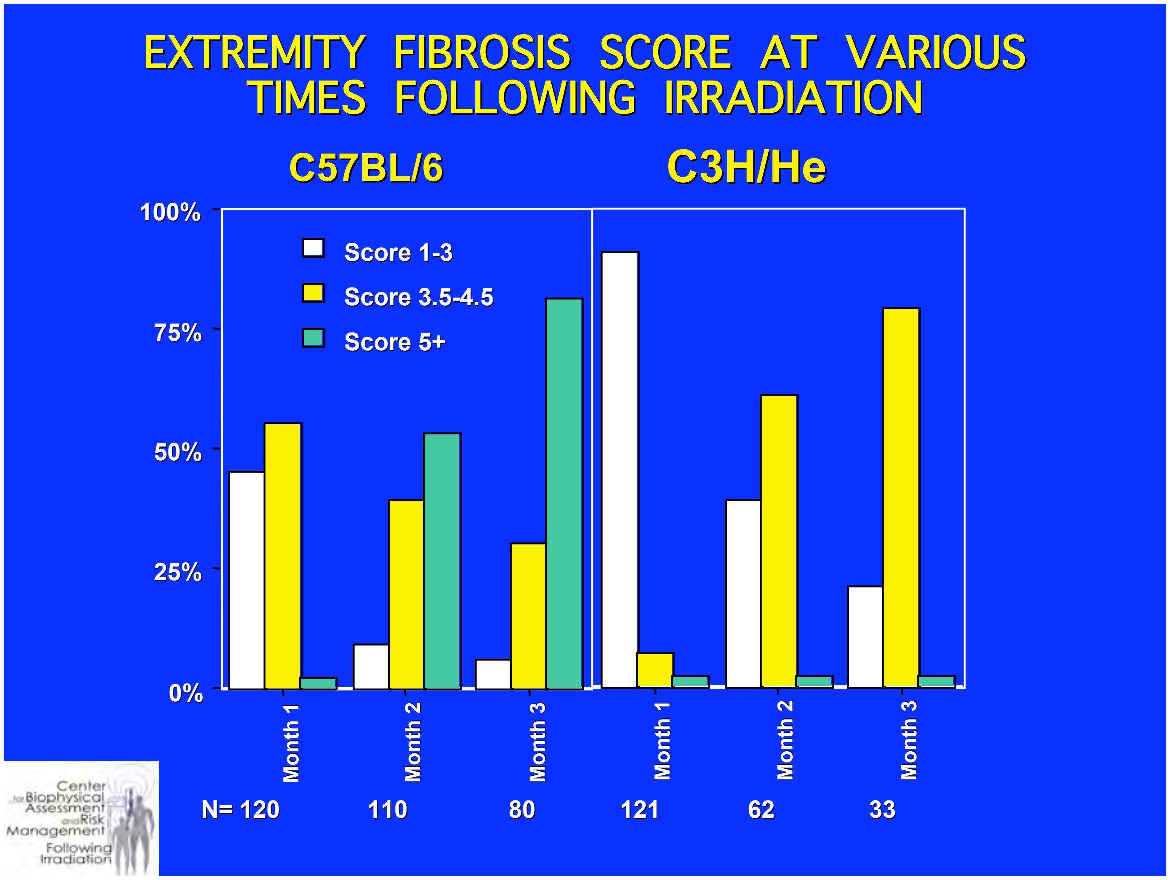


Celebrex reduces IL-1 and macrophage chemotaxis to tissue

mRNA
Detected by RNase Protection Assay



Mice followed for 1.5 to 2.5 years after 20 Gy irradiation had elevation of TGFß1 commensurate with their level of fibrovascular changes


EXTREMITY FIBROSIS SCORE AT VARIOUS TIMES FOLLOWING IRRADIATION
C57BL/6
C3H/He
100%
75%
50%
25%
0%
Score 1-3
Score 3.5-4.5
Score 5+
Month 1
Month 2
Month 3
Month 1
Month 2
Month 3
N= 120
110
80
121
62
33
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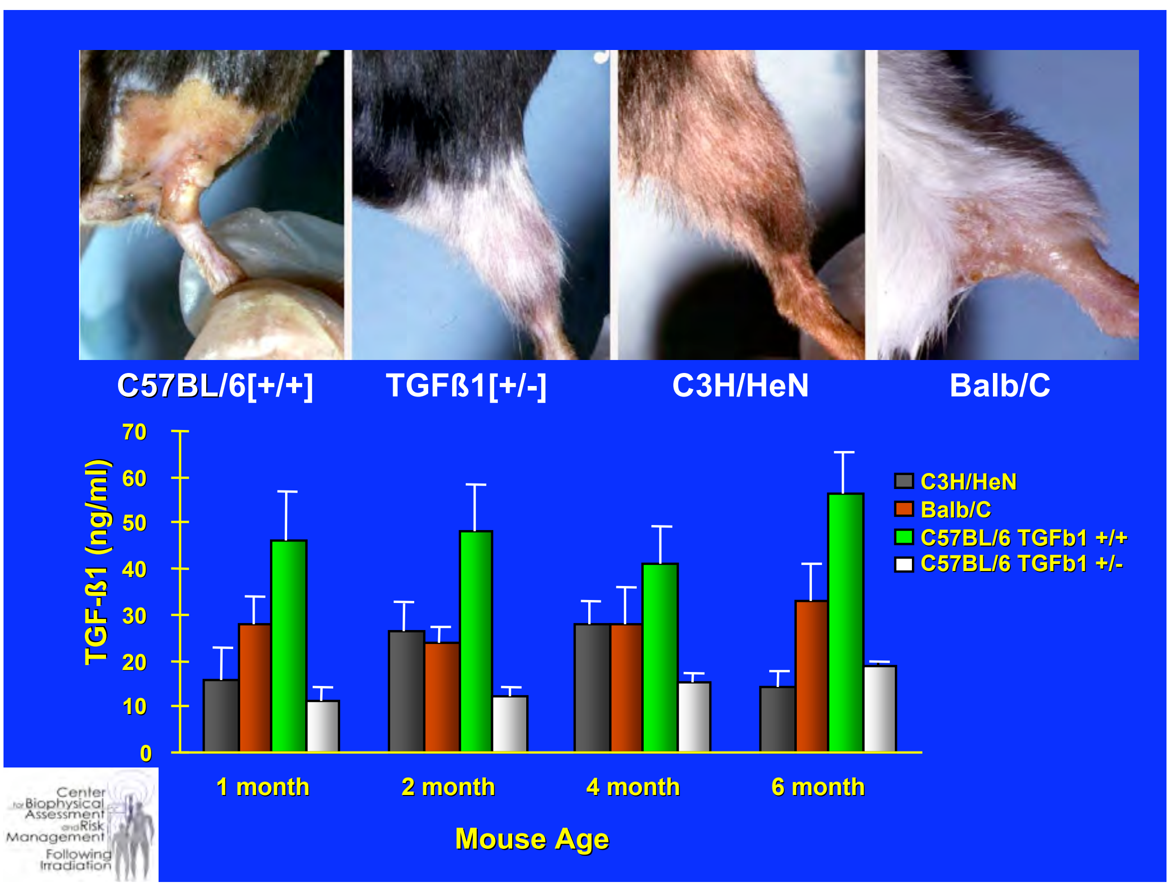
C57BL/6[+/+]
TGFß1[+/-]
C3H/HeN
Balb/C
TGF-ß1 (ng/ml)
70
60
50
40
30
20
10
0
C3H/HeN
Balb/C
C57BL/6 TGFb1 +/+
C57BL/6 TGFb1 +/-
1 month
2 month
4 month
6 month
Mouse Age
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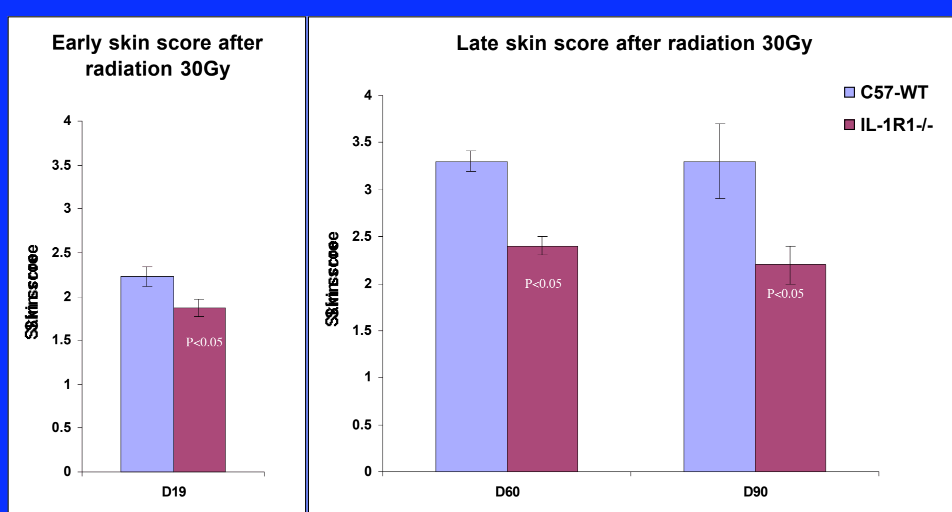
Whole Body Irradiation $LD_{50/30}$ Dose for Various Mouse Strains

Strain	Fibrosis Sensitivity	$LD_{50/30}$
C3H/HeN	low	7.4 ± 0.2 Gy
Balb/C	intermediate	7.0 ± 0.1 Gy
C57BL/6	high	8.7 ± 0.1 Gy
TGFβ1[+/+]	high	8.9 ± 0.5 Gy
TGFβ1[+/-]	low	9.4 ± 0.4 Gy

Altered expression of TGFβ1, whether intrinsic or genetically defined by the knockout model, is predictive of susceptibility to late fibrovascular effects. Intrinsic radiation sensitivity as measured by $LD_{50/30}$ or cell survival curves was not helpful in distinguishing differential sensitivity to fibrovascular complications.

Radiation induced early and late skin toxicity in C57 wild type (WT) and C57-IL-1R1-/- mice

30 Gy single fraction



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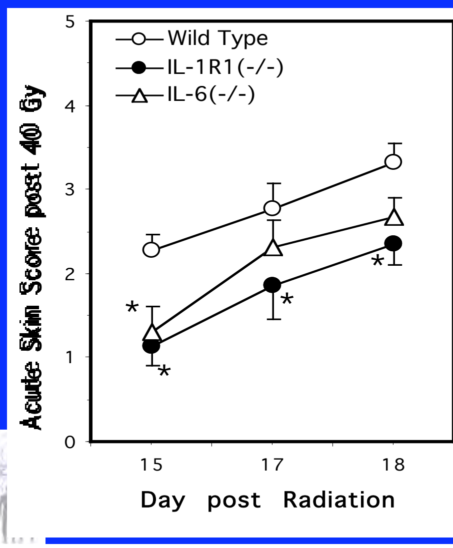
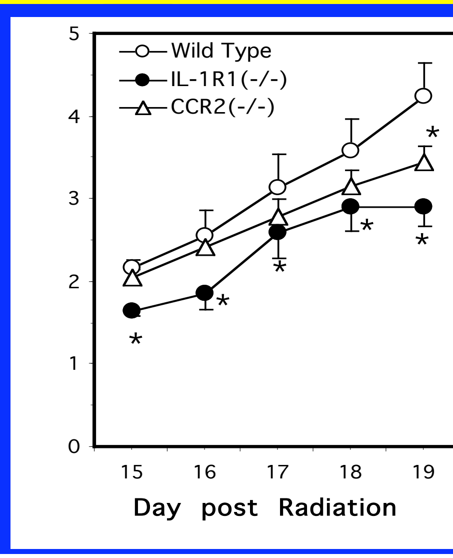
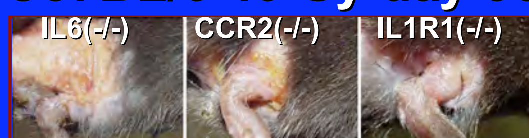
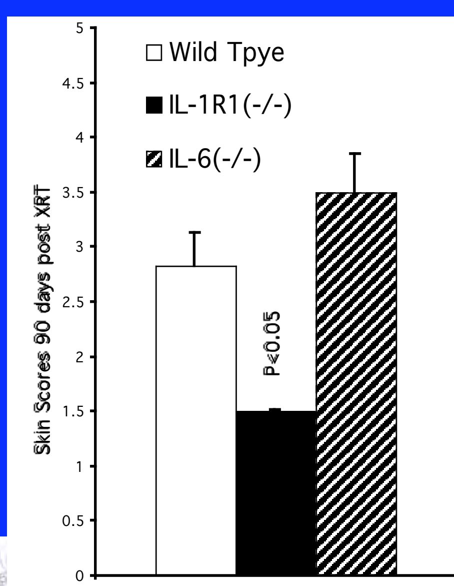
C57Bl/6 40 Gy day 19

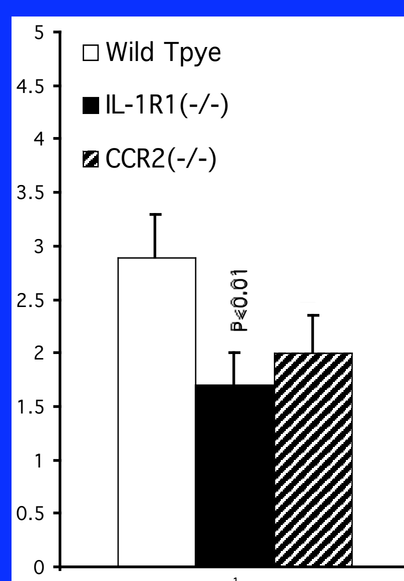




C57BL/6 40 Gy day 90







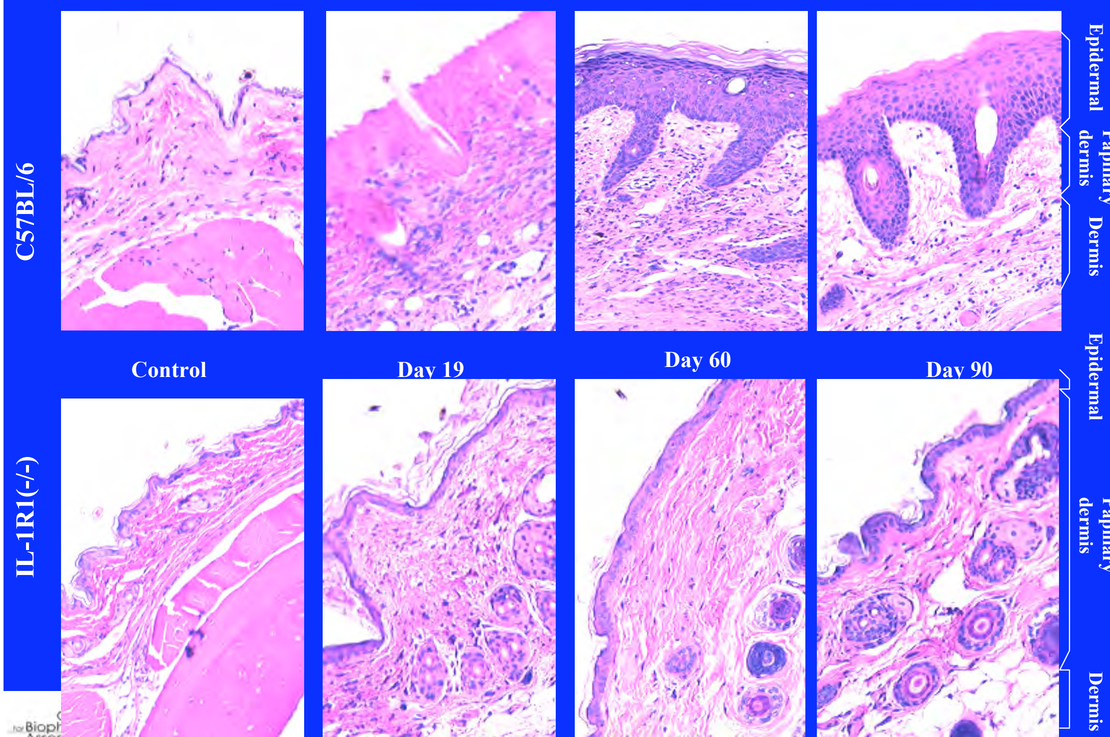
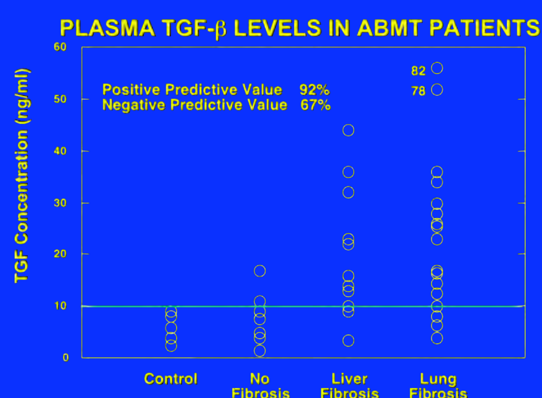
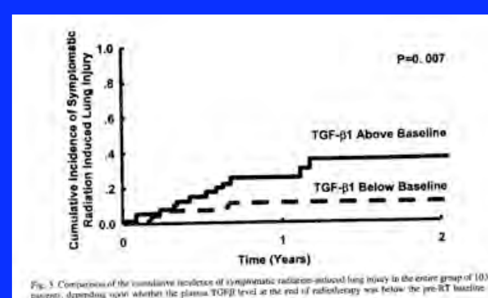
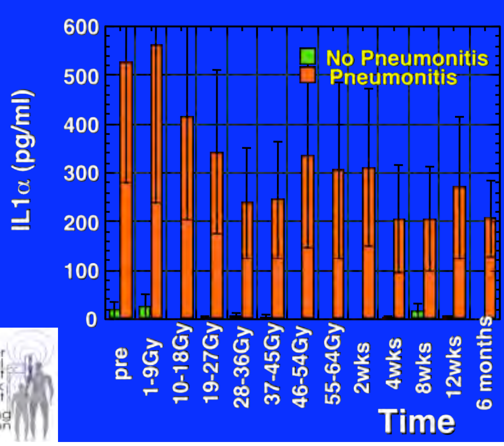


Fig. 1. Liu et al.





Anscher et al: Baseline TGFß predicts for chemotherapy and radiation induced pneumonitis



Chen et al: Baseline IL-1a and IL-6 predicts for radiation induced pneumonitis

MECHANISMS, PREVENTION, and MITIGATION of RADIATION DERMATITIS

- A preventable and reversible component of cutaneous damage is mediated by a fast acting, dynamic feedback system controlling inflammation
- The system has many control points that can often be re-regulated by chemical or genetic normalization
- Many of the critical control factors are known
- Optimal benefit is likely to be achieved when multiple interventions are combined and sequenced

Molecular Mechanisms of Cutaneous Radiation Damage

Mitigation and Therapy of Radiation Cutaneous Syndromes

- Curcumin
- Celebrex
- Pentoxifylline
- EsA

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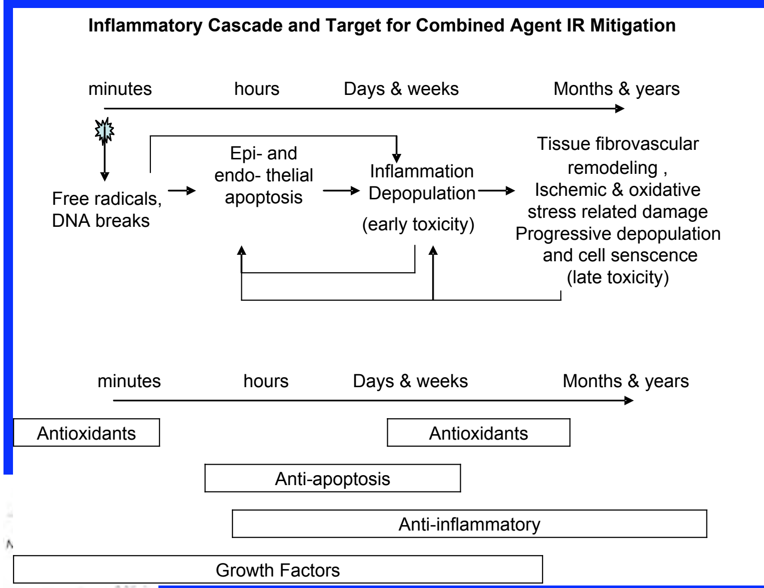
Global Plan



<u>Agents</u>
Pentoxifylline
Curcumin
EsA
Celebrex

<u>Pentoxifylline</u>

Inhibits cAMP phosphodiesterase and thereby increases cAMP and ATP in red blood cells.

It also improves flow by inhibiting ICAM expressing and thus reduces leukocyte adherence to endothelial cells.

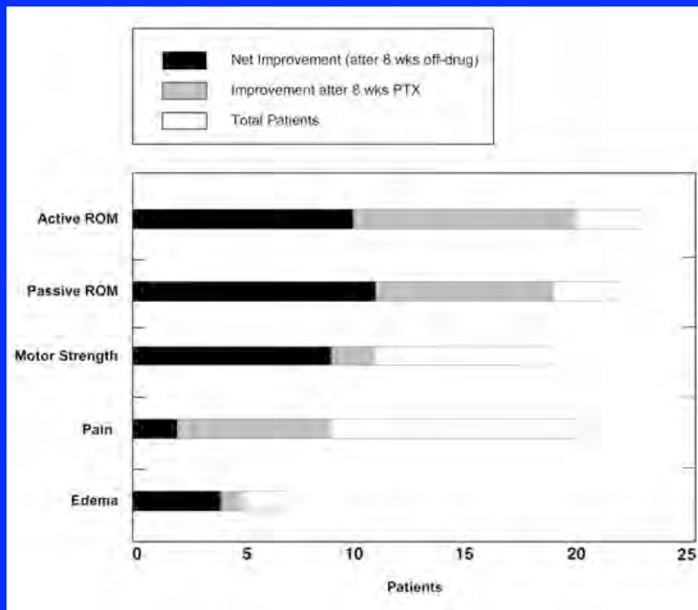
It increases prostacyclin production and thus inhibits platelet aggregation.

Pentoxifylline inhibits IL-1ß and PDGF induced fibroblast proliferation in-vitro.

It reduces TNF expression

<u>Pentoxifylline doesn't help if there isn't hypoxia and aberrant blood flow</u>

Predilection of Acute and Late Toxicity



Patients with late fibrovascular complications of radiation have greatly elevated bFGF levels

Pentoxifylline reduced the bFGF and reduced complications.

None of the toxicity scores were from CTC, RTOG or EORTC.

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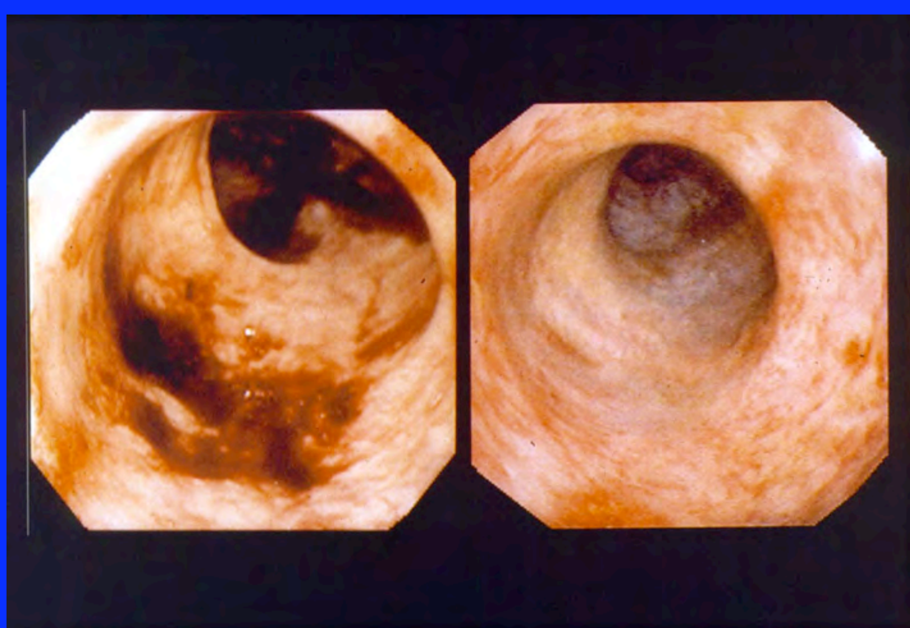

Healing of chronic rectal ulcer years after prostate radiation

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COX-2 INHIBITORS

Several Cox-2 inhibitors have been shown to have benefit for early and sometimes later radiation reactions

Because of a small risk of increased thromboembolic events Cox-2 inhibitors are less widely used

Cox-2 is produced indirectly through cellular interactions often initiated by IL-1 and MCP-1 with numerous cell types

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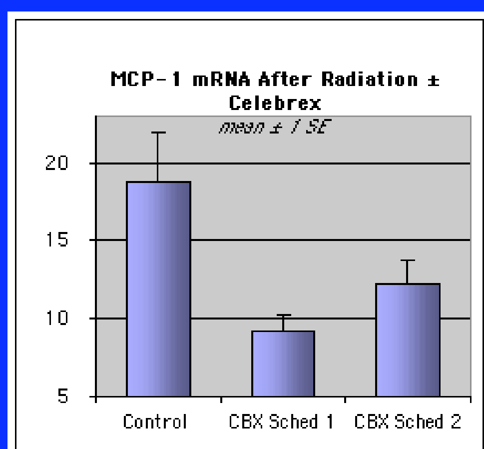
Effects of Celebrex on normal soft tissues in irradiated C57BL/6 mice



SKIN SCORES (Mean ± 1 SE)	Early (14 d) 60 Gy	LATE (90 d) 30 Gy	LATE (90 d) 60 Gy
Control	3.3 ± 0.3	3.0 ± 0.3	4.5 ± 0.2
Celebrex t = 4h+	1.4 ± 0.1*	1.8 ± 0.1*	2.3 ± 0.5*
Celebrex t = 7d+	2.8 ± 0.3*	2.2 ± 0.1*	4.2 ± 0.4

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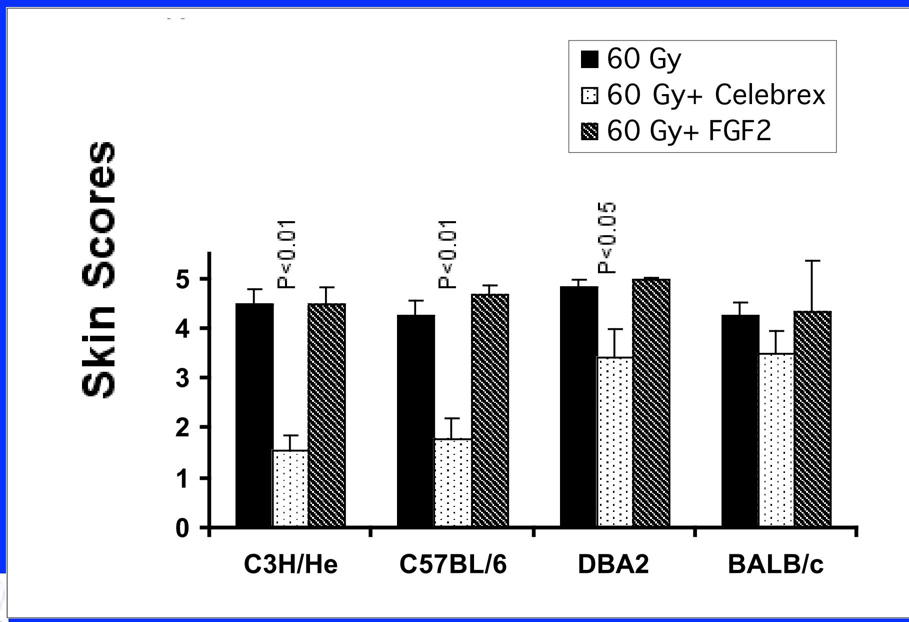
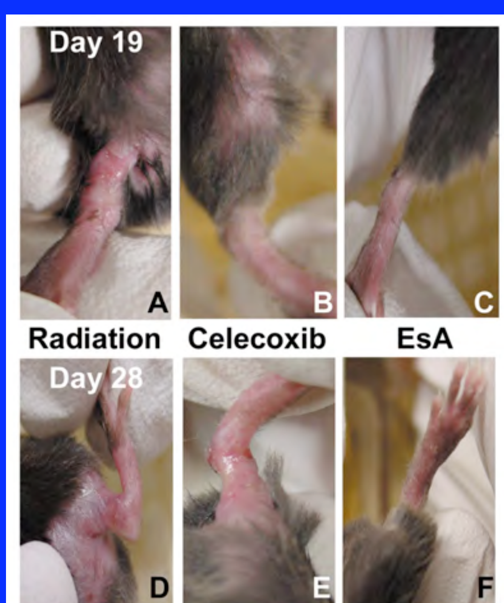
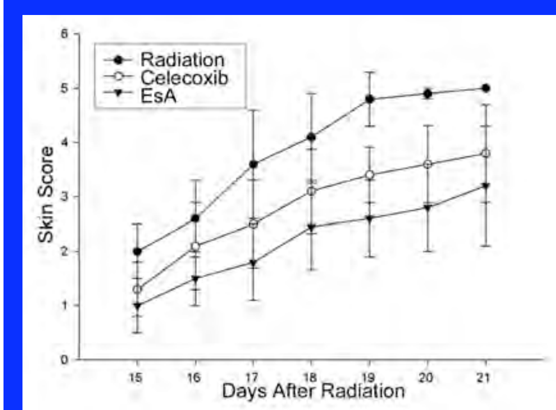
Effects of Celebrex on soft tissue damage in three mouse strains







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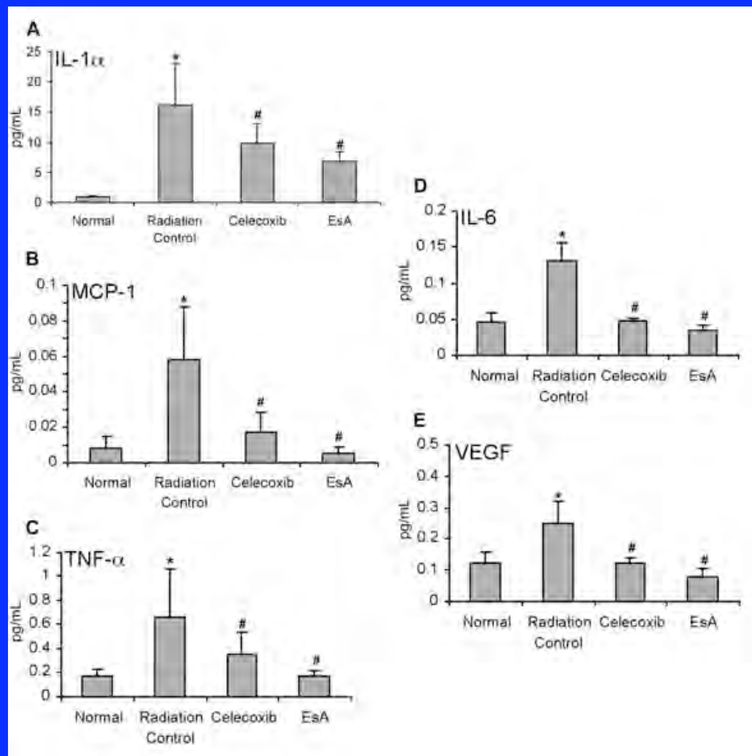
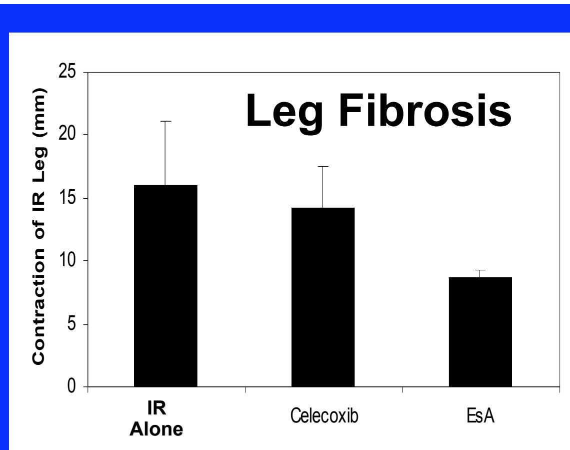
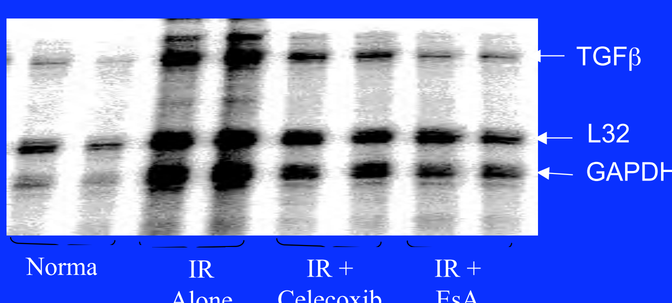
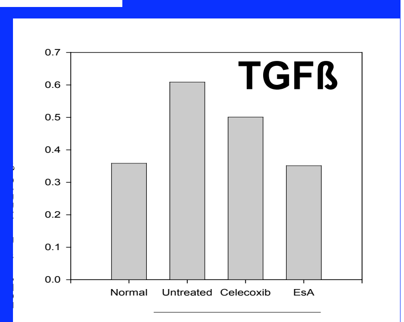

A
IL-1α
pg/mL
Normal
Radiation Control
Celecoxib
EsA
B
MCP-1
pg/mL
Normal
Radiation Control
Celecoxib
EsA
C
TNF-α
pg/mL
Normal
Radiation Control
Celecoxib
EsA
D
IL-6
pg/mL
Normal
Radiation Control
Celecoxib
EsA
E
VEGF
pg/mL
Normal
Radiation Control
Celecoxib
EsA


Leg Fibrosis
Contraction of IR Leg (mm)
IR Alone
Celecoxib
EsA

90 days

*


TGFβ
L32
GAPDH
Norma
IR Alone
IR + Celecoxib
IR + EsA


TGFß
Normal
Untreated
Celecoxib
EsA
30 Gy Irradiation

Curcumin & COX2

Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-κB activation

Chun KS etal Carcinogenesis. 2003 Sep;24(9):1515-24.

Radioprotective effects of Curcumin

Radioprotective action of curcumin extracted from Curcuma longa LINN: inhibitory effect on formation of urinary 8-hydroxy-2'-deoxyguanosine, tumorigenesis, but not mortality, induced by γ-ray irradiation
Inano H, Onoda M. Int J Radiat Oncol Biol Phys. 2002;53:735-43.

Prevention of radiation-induced mammary tumors
Inano H, Onoda M. Int J Radiat Oncol Biol Phys. 2002;52:212-23

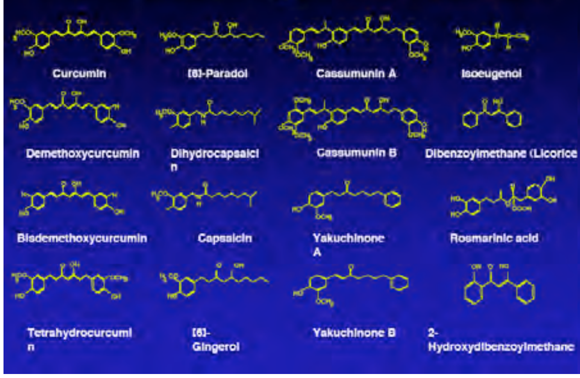
Potent preventive action of curcumin on radiation-induced initiation of mammary tumorigenesis in rats
Inano H, Onoda M, Inafuku N, Kubota M, Kamada Y, Osawa T, Kobayashi H, Wakabayashi K. Carcinogenesis. 2000;21:1835-41.

Chemoprevention by curcumin during the promotion stage of tumorigenesis of mammary gland in rats irradiated with gamma-rays.
Inano H, Onoda M, Inafuku N, Kubota M, Kamada Y, Osawa T, Kobayashi H, Wakabayashi K. Carcinogenesis. 1999;20:1011-8.

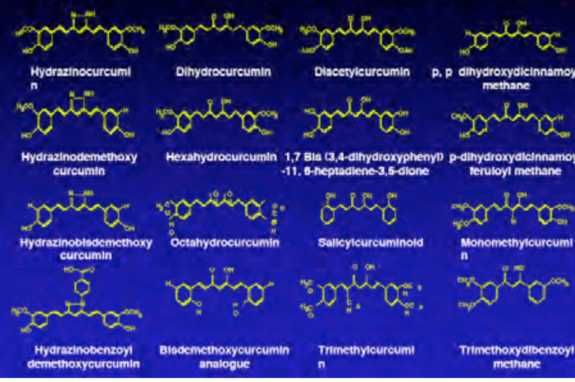
Protective effect of curcumin, ellagic acid and bixin on radiation induced genotoxicity.
Thresiamma KC, George J, Kuttan R. J Exp Clin Cancer Res. 1998;17:431-4.

Slides from Aggarwal

Natural analogs of curcumin



Synthetic analogs of curcumin

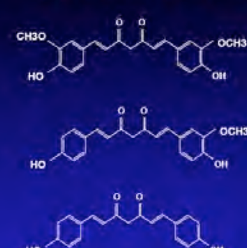


NF-κB and sunburn

A role for NF-κB-dependent gene transactivation in sunburn

Abeyama K, et al.
Journal of Clinical Investigation
2000;105:1751-9.

Structure of curcumin



Curcumin I (77%)

Curcumin II
Demethoxycurcumin (17%)

Curcumin III
Bis-Demethoxycurcumin (3%; less active)

Curcumin & Wound-healing

Dermal wound healing processes with curcumin incorporated collagen films.
Gopinath D. etal Biomaterials. 2004 May;25(10):1911-7.

Protective effects of curcumin against oxidative damage on skin cells in vitro: its implication for wound healing.
Phan TT etal J Trauma. 2001 Nov;51(5):927-31.

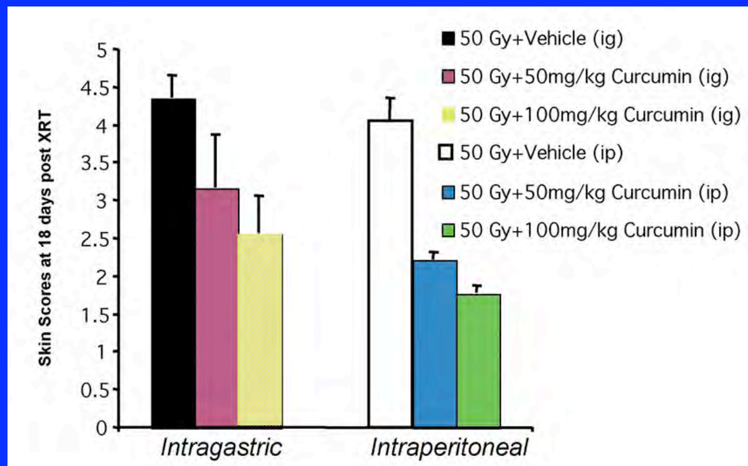
Enhancement of wound healing by curcumin in animals.
Sidhu GS etal , Wound Repair Regen. 1998 Mar-Apr;6(2):167-77.

Inhibitory effect of curcumin on PMA-induced increase in ODC mRNA in mouse epidermis.
Lu YP...Conney AH, Carcinogenesis. 1993 Feb;14(2):293-7.

Inhibitory effect of dietary curcumin on skin carcinogenesis in mice.
Limtrakul P., Cancer Lett. 1997 Jun 24;116(2):197-203.

Turmeric and curcumin as topical agents in cancer therapy.
Kuttan R., Tumori. 1987 Feb 28;73(1):29-31.

Slides courtesy of Aggarwal



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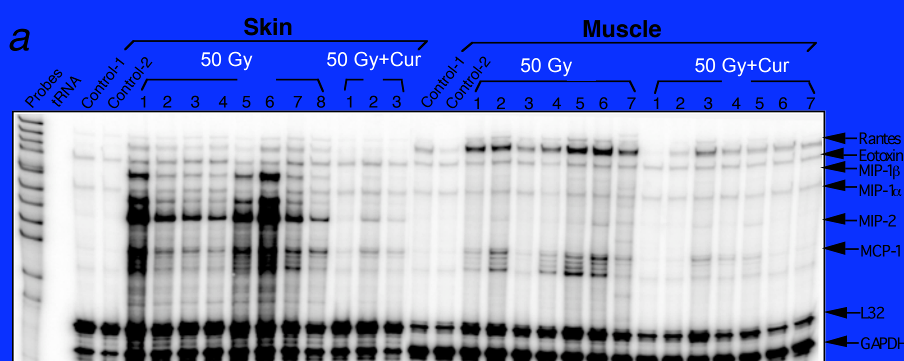
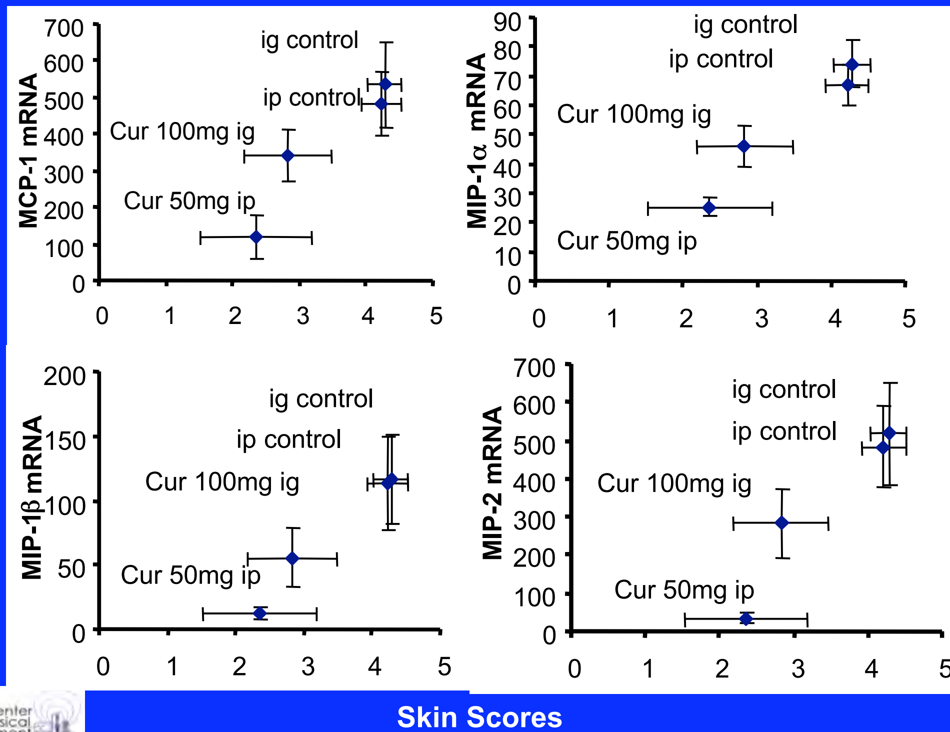


Table 1 Effects of curcumin on chemokine mRNA expression in irradiated skin and muscle tissues

		RANTES	Eotoxin	MIP-1β	MIP-1α	MIP-2	MCP-1
Skin	50 Gy	19 ± 11	51 ± 15	117 ± 94	71 ± 20	505 ± 323	514 ± 272
	50 Gy + cur 50 mg/kg i.p.	8 ± 6*	28 ± 5**	14 ± 9**	26 ± 5**	40 ± 23**	124 ± 101**
	50 Gy + cur 100 mg/kg i.g.	13 ± 11	45 ± 16	57 ± 51	47 ± 15*	288 ± 203	348 ± 159
Muscle	50 Gy	212 ± 89	59 ± 21	24 ± 12	45 ± 16	27 ± 17	185 ± 125
	50 Gy + cur 50 mg/kg i.p.	54 ± 57**	41 ± 11*	13 ± 5**	34 ± 8*	15 ± 8*	64 ± 28**
	50 Gy + cur 100 mg/kg i.g.	105 ± 26**	55 ± 15	17 ± 4*	46 ± 13	19 ± 1	78 ± 32**

* : $p < 0.05$, **: $p < 0.01$, compared with 50Gy radiation. Mean±SD.
Mice in 50 Gy group represent the combination of I.g and I.p. vehicle controls



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Key people in lab since 2005

Center for Biophysical Assessment and Risk Management Following Irradiation

Richard Hill
(Project 5)

Paul Okunieff
(Principal Investigator, Project 1 & Core A)

Jack Finkelstein
(Project 2 & Core B)

Sally Thurston
(Core D)

Harold Swartz
(Project 3)

Yuhchyau Chen
(Project 4)

Jacky Williams
(Core C)

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October 21st 2005
First CBARMFI Retreat

FUTURE PLANS

• Pentoxifylline: This agent is available off patent and will not be needed urgently after an event. It is therefore not needed for the stockpile. Physicians can and do already offer it with success. We need only better understand it to make it of public health value.

• Celebrex: This agent is available and was most effective when given for several weeks around the time of the exposure. Clinical trials in cancer patients are being proposed at RTOG, SWOG, and CURED to determine utility and schedule in humans. At best studies will open in about 2 years.

• Curcumin: CCOP studies are being held up for need of an IND at the FDA for the past 2 years. The plan is to use it in breast cancer patients.

• EsA: The phase I STTR is complete. The agent has been licensed. The Phase II STTR will be submitted later this week.

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