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Influence of Mitomycin C on Endothelial Monolayer Regeneration In Vitro

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Abstract This study examines the effect of Mitomycin C, a fungal toxin which inhibits DNA synthesis, on the regeneration of partially denuded large vessel endothelium in vitro. Monolayers of bovine pulmonary artery endothelial cells were treated with Mitomycin C prior to or immediately following partial denudation and were incubated in the continuing presence of Mitomycin C; the effects of this treatment on monolayer repair, cell proliferation, and other aspects of endothelial phenotype were monitored. Cell proliferation, DNA, RNA, and protein synthesis were all reduced in a dose dependent manner in treated cultures. Incubation with Mitomycin C for 48 h or longer resulted in reduced cell spreading, and rounding up and loss of cells from both intact and partially denuded cultures. Effects were less severe with lower doses and shorter incubation times. However, significant reductions in monolayer regeneration occurred within 8 h of incubation, sufficiently early to suggest that Mitomycin C may affect aspects of the regeneration process independent of cell proliferation. Polarization/spreading of cells at the denudation edge was monitored by fluorescence staining for golgi with C_5-DMB-ceramide, and for centrioles with antibodies to tubulin. Centrioles and golgi rapidly reoriented to a location at the putative leading edge of control cultures. Mitomycin C treatment had no effect on centriole reorientation, but caused a significant delay in golgi localization. These results suggest that Mitomycin C inhibits endothelial monolayer regeneration by mechanisms independent of cell proliferation and DNA synthesis, perhaps by interfering with cell spreading or translocation at the wound edge.

Key words: endothelium, cell proliferation, cell migration, centrosome

Repair of intimal damage to large blood vessels may require both vascular endothelial cell migration and proliferation [Schwartz et al., 1979; Gordon and Staley, 1990; Gotlieb, 1990; Rosen et al., 1990]. This process of intimal repair can be studied in vitro with partially denuded confluent monolayers of cultured large vessel endothelial cells. The relative roles of cell spreading/translocation and cell proliferation in monolayer regeneration can then readily be examined under controlled conditions with this model [Coomber and Gotlieb, 1990; Coomber, 1991a].

Previous work has established that the mechanisms of monolayer regeneration in vitro and in vivo is somewhat dependent upon the size of the denudation. Very small injuries, involving loss of a few endothelial cells, rapidly repair via spreading of adjacent cells, with or without additional cell migration [Wong and Gotlieb, 1984; Reidy and Schwartz, 1981; Ramsay et al., 1982]. Larger denudations require the coordinated action of cell spreading, cell migration, and cell proliferation to regenerate the endothelium [Jackman et al., 1988; Coomber and Gotlieb, 1990; Muthukrishnan et al., 1991]. Numerous studies have examined the effects of various treatments on this repair process in vitro, notably cytoskeletal disrupting agents [Selden and Schwartz, 1979; Coomber and Gotlieb, 1990; Gotlieb, 1990; Wong and Gotlieb, 1990] and growth factors and cytokines [Heimark et al., 1986; Coomber, 1991a]. This present study was devised to use Mitomycin C, a Streptomyces toxin known to inhibit DNA synthesis, to determine whether previously reported bFGF enhanced monolayer repair [Coomber, 1991a] can occur in the absence of cell proliferation. Unexpectedly, however, evidence was obtained that Mitomycin C suppresses monolayer regeneration by mechanism(s) in addition to that of cell proliferation [Coomber, 1991b]. This report describes experiments which examine possible

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mechanisms of Mitomycin C induced reductions in endothelial monolayer regeneration.

METHODS

Cell Culture

Cells used for these studies are bovine pulmonary artery endothelium (BPAEC, CCL 209), obtained from American Type Culture Collection. Cells were grown in complete media which consisted of EMEM + 10% FBS + 6 μg/ml gentamycin sulfate (GIBCO BRL, Burlington, Ont.). When confluent, cultures were passaged with trypsin/EDTA (GIBCO BRL) and plated into multiwell plates or 35 mm petri dishes containing sterile glass coverslips, as appropriate. Cells for study were used between passages 19 and 27. All cultures were incubated at 38°C, 5% CO_2 in a humid atmosphere and fed complete media unless otherwise indicated. Cell morphology was monitored with inverted phase contrast microscopy. Mitomycin C (Boehringer Mannheim, Montreal) was dissolved in sterile water at a concentration of 1 mg/ml and stored in aliquots at −20°C until used. Complete media supplemented with 5, 10, or 20 μg/ml of Mitomycin C was used for these studies. Some cultures were pretreated with Mitomycin C for 24 h prior to experimental manipulation, and in other studies, Mitomycin C incubation was delayed for 4 h after denudation.

Monolayer Regeneration

Monolayer regeneration was assayed as previously described [Coomber, 1991a]. Briefly, a portion of the confluent monolayer of cells grown on glass coverslips was scraped away with a sterile Teflon spatula, fed fresh media with or without Mitomycin C, and incubated. Subsequent monolayer regeneration was quantified by monitoring, with inverted phase contrast microscopy, the advance of the monolayer relative to the original denudation edge. This study compares monolayer regeneration in control cultures to cultures treated for various times with 10 μg/ml Mitomycin C.

Fluorescence Staining

Fluorescence staining was used to visualize microtubules and golgi in regenerating cultures after various incubation times in the presence or absence of Mitomycin C. Cultures were fixed with 3% paraformaldehyde in PBS, then permeabilized by incubation with 0.1% Triton X-100 in PBS for 4 min. Microtubules were stained by incubating with a mouse monoclonal antibody to tubulin [Leslie et al., 1984], followed by fluorescein-conjugated goat anti-mouse IgG (Sigma). Microtubule organizing center (MTOC) location was scored relative to the location of denudation edge and the nucleus in cells at the leading edge of the regenerating monolayer. Golgi was visualized in treated monolayers by a modification of Pagano's method [Pagano et al., 1989]. Briefly, treated cultures are rinsed with serum free medium, then incubated with serum free medium containing 2.5 μg/ml C_5-DMB-ceramide (Molecular Probes, Eugene, OR) plus 0.4 mg/ml fatty acid free bovine serum albumin (faf BSA) (Boehringer Mannheim) at 38°C for 5 min. Cultures were further rinsed with several changes of serum free medium containing faf BSA, then fixed in 3% paraformaldehyde, rinsed in PBS, and mounted with aqueous media. Golgi orientation relative to the denudation and nucleus in cells at the leading edge of the regenerating monolayer was scored after various incubation times for control and Mitomycin C treated monolayers. All manipulations were performed at room temperature unless otherwise noted. Coverslips were examined and photographed with a Zeiss Standard microscope equipped for epifluorescence.

Metabolic Labeling

The effects of Mitomycin treatment on DNA, RNA, and protein synthesis in these endothelial cells was monitored by measuring incorporation of [^{3}H]-thymidine, uridine, and proline, respectively. Endothelial cells were seeded into 96 well flat bottom plates and grown in complete media until confluent. Cultures were then treated with fresh media containing Mitomycin C and 2 μCi/ml of the appropriate isotopes, and incubated for an additional 24 h. Media was then replaced with trypsin/EDTA, and plates were incubated with shaking to detach the cells. Cells were harvested onto glass fibre filter mats using LKB cell harvester, and filter mats were counted in a 1205 Betaplate flatbed scintillation counter (LKB, Wallac). DNA synthesis in regenerating monolayers of endothelial cells was quantified using a cell proliferation immunohistochemistry kit (Amersham, Oakville, Ont.). Cultures were fed at the time of denudation with complete media containing BrdU and FdU (± Mitomycin C), and incubated for 24 h. Cultures were fixed in acid ethanol, then incubated with a

primary antibody to BrdU followed by a secondary antibody conjugated with HRP, and coloured reaction product was produced with DAB incubation (kit components). DNA synthesis was quantified by determining relative numbers of cells with labelled nuclei at the edge of control and treated monolayers.

Statistical Analysis

All experiments were performed at least in triplicate, and values were pooled to calculate mean and standard error of mean. ANOVA was used to examine differences within and between groups, and Student's *t*-test was used to compare means when *F* values were significant. A 95% confidence interval was used for all tests.

RESULTS

Mitomycin C significantly reduces cell proliferation as revealed by cell counts and [^{3}H] thymidine incorporation in BPAEC (Table I). Incubation of intact or partially denuded monolayers of endothelial cells with doses of 20 μg/ml or higher for 24 h, or with lower doses for longer periods of time, results in detachment of cells and monolayer disruption (Fig. 1). If partially denuded monolayers were treated with 10 μg/ml Mitomycin C at the time of denudation, significant reduction in monolayer regeneration is noted

TABLE I. Effect of Mitomycin C on BPAEC Proliferation†

	% of control cell number	% of control [^{3}H] thymidine incorporation
Control	100 (2.23)	100 (2.22)
5 μg/ml Mitomycin C	72 (2.84)*	52 (4.49)***
10 μg/ml Mitomycin C	73 (2.03)*	25 (2.12)****
20 μg/ml Mitomycin C	58 (2.80)**	14 (1.20)*****

†For cell counts, BPAEC were incubated with various doses of Mitomycin C for 24 h and then trypsinized. Duplicate counts of cell suspensions were made with a hemocytometer and phase contrast microscope. For thymidine incorporation, BPAEC were incubated with various doses of Mitomycin C and 2 μCi/ml [^{3}H] thymidine for 24 hours, followed by cell harvesting and scintillation counting. Values shown are mean (standard error). Thymidine incorporation has been corrected for cell number.
*Significantly different from Control cell number ($P < 0.05$).
**Significantly different from other values ($P < 0.05$).
***Significantly different from control thymidine ($P < 0.05$).
****Significantly different from control and 5 μg/ml values ($P < 0.05$).
*****Significantly different from other thymidine values ($P < 0.05$).

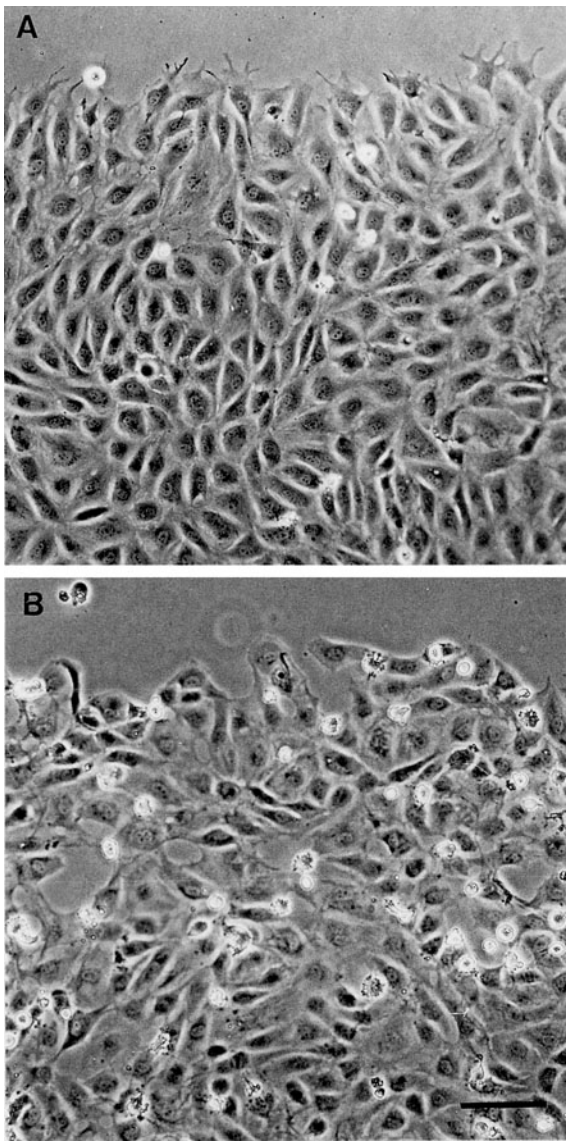

Fig. 1. Phase contrast micrographs of BPAEC 8 h after denudation. **A:** Control. **B:** Pretreated for 24 h with 10 μg/ml Mitomycin C. Direction of monolayer repair is towards the top of the figure. Pretreated cultures show reduced cell spreading, rounding up, and loss of cells from the monolayer. This becomes more pronounced with increasing incubation times. Cultures treated with the same concentration of Mitomycin C at time of denudation show little morphological alteration from control cultures. Scale bar = 100 μm.

within 8 h of incubation, without obvious evidence of cell loss (Fig. 2). The relative reduction in monolayer regeneration increases with increased incubation (Fig. 2) and is not ameliorated by delaying Mitomycin C treatment for 4 h (data not shown). Thus, this reduction in monolayer regeneration is likely due to sustained, rather than transient, effects of Mitomycin C on the regenerative process. Preincubation of mono-

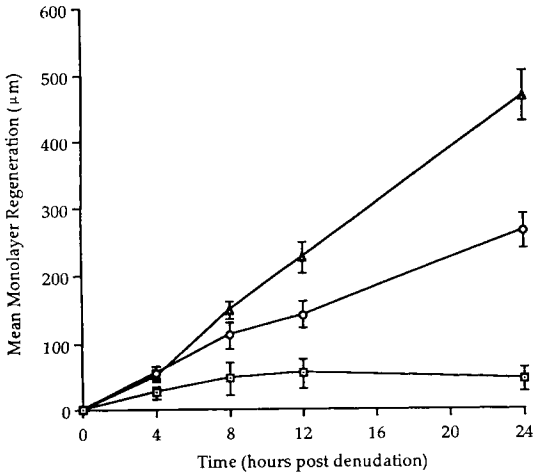

Fig. 2. Monolayer regeneration during the first 24 h after denudation. Values represent means with standard error bars: Control (△), 10 μg/ml Mitomycin C at time of denudation (○), 10 μg/ml Mitomycin for 24 h prior to denudation (□). Significant reductions in Mitomycin C treated monolayers occur after 4 h for preincubated cultures and after 8 h for cultures treated at the time of denudation ($P < 0.05$).

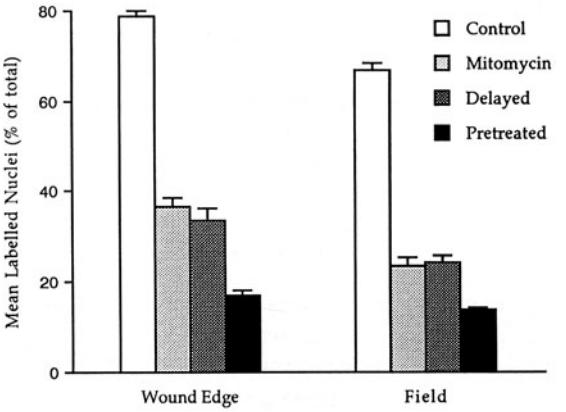

Fig. 3. Relative numbers of labelled nuclei in cells at the wound edge and within the first 8 rows (field) of regenerating monolayers incubated with BrdU at the time of denudation and subsequently immunostained. Bars represent standard errors. Cultures treated with 10 μg/ml Mitomycin C at time of denudation, or 4 h after denudation, had labelling indices significantly lower than control and significantly higher than pretreated cultures, but not significantly different from each other ($P < 0.05$).

layers for 24 h with 10 μg/ml Mitomycin C leads to rapid and persistent reduction in monolayer regeneration, due to loss of cells from the coverslip (Fig. 2).

Concomitant with reduced monolayer repair as seen in phase contrast microscopy, there is a reduction in DNA synthesis as shown by immunostaining for incorporated BrdU, in Mitomycin C (10 μg/ml) treated monolayers undergoing regeneration. This significant decrease is location and time dependent. It is enhanced by preincubating monolayers for 24 h prior to denudation and occurs both at the edge of the regenerating monolayer as well as within the first several rows of cells (Fig. 3). If treatment with Mitomycin C is delayed until 4 h after denudation, there is no significant improvement in relative numbers of labelled cells as compared to cultures incubated with Mitomycin C at time of denudation (Fig. 3).

In addition to cell proliferation, spreading and translocation of cells at the edge of a denuded monolayer of endothelium are important components of the regeneration process. It has been proposed that centrosome reorientation is a prerequisite for endothelial cell migration from the denuded edge [Gotlieb, 1990]. MTOC orientation in cells at the denudation edge was examined in cultures stained for tubulin and incubated for various times with 10 μg/ml Mitomycin C. There were no significant alterations in the relative numbers of denudation edge cells with MTOC oriented towards the putative direction of cell migration (Figs. 4A, 5). Staining of similarly treated cultures with fluorochrome labelled ceramide resulted in significant reductions in relative numbers of edge cells with golgi oriented in the direction of monolayer regeneration (Figs. 4B, 6). These reductions were detectable within 2 h of incubation and were sustained for at least 8 h. In contrast to results obtained for monolayer regeneration and BrdU incorporation, delay of Mitomycin C treatment for 4 h had no significant effect on golgi orientation, suggesting that this aspect of the repair process is only vulnerable to Mitomycin C induced effects shortly after denudation.

These studies also examined the effect of Mitomycin C on other synthetic processes which may be involved in modulating the regeneration response. Protein synthesis, as measured by incorporation of [^{3}H] proline, was significantly reduced in BPAEC treated with 10 μg/ml Mitomycin C or higher (Table II). Incorporation of [^{3}H] uridine, as an indication of RNA production, was dramatically reduced in a dose dependent manner (Table II). These results suggest that Mitomycin C may also be influencing monolayer regeneration by disrupting translation and/or transcription of essential gene products.

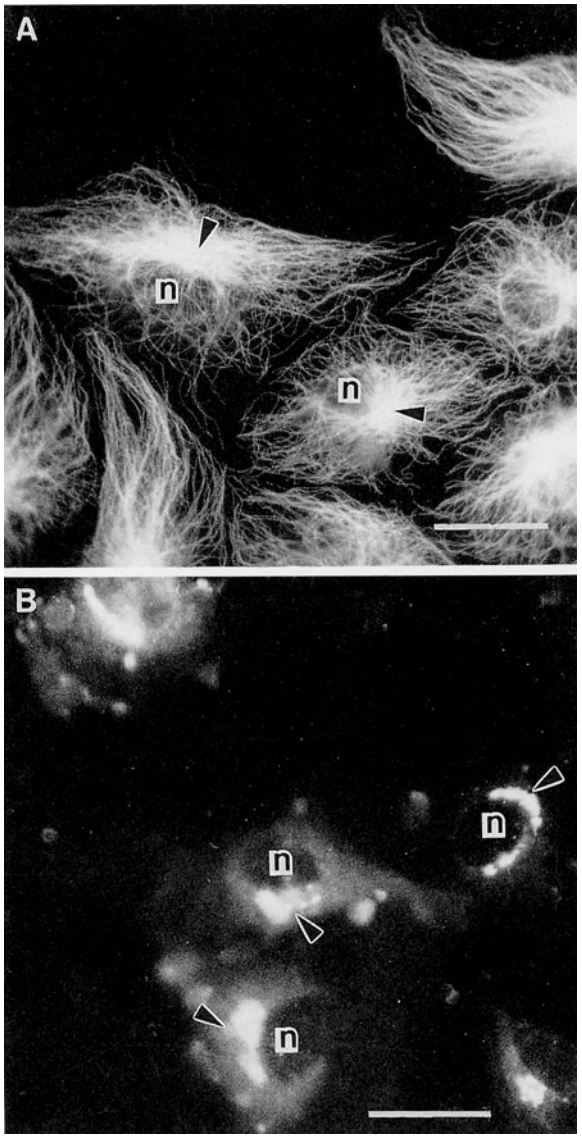

Fig. 4. **A:** Micrograph showing denudation edge cells stained for microtubules via immunofluorescence in a control culture incubated for 8 h. Denuded portion of coverslip is towards the top of the figure. Arrowheads indicate location of centriole relative to nucleus (n). Scale bar = 25 μm. **B:** Micrograph showing denudation edge cells stained for golgi in a Mitomycin C treated culture incubated for 8 h, then labelled with C_5-DMB-ceramide. The golgi can be clearly seen as the brightly staining structure (arrowhead) clustered around the nucleus (n). Denuded portion of coverslip is towards the top of the figure. Scale bar = 25 μm.

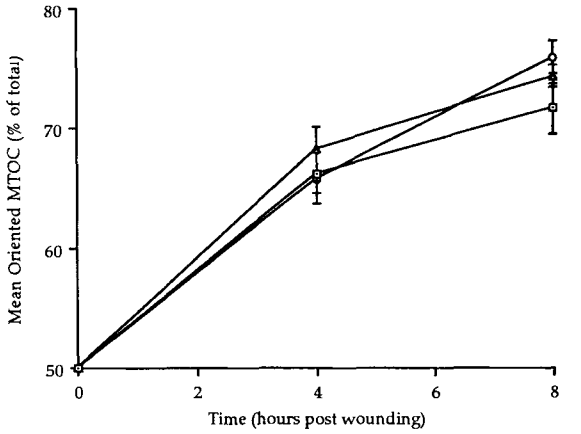

Fig. 5. Quantification of relative numbers of edge cells with centrioles reoriented towards denudation. Bars represent standard error. There are no significant differences in centriole orientation between Control cultures (△) and cultures treated with 10 μg/ml Mitomycin C at time of denudation (○), or cultures pretreated with 10 μg/ml Mitomycin C for 24 h prior to denudation (⊡) ($P < 0.05$).

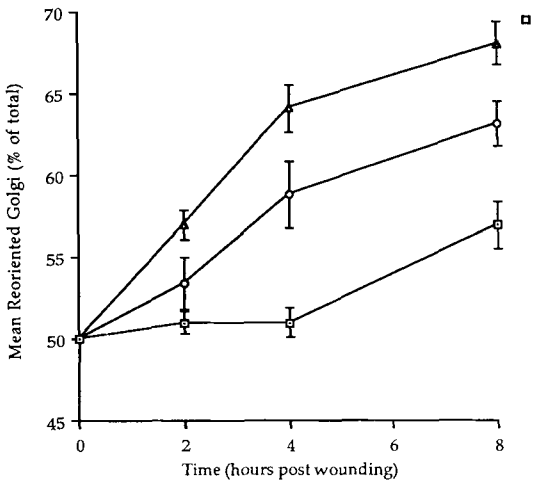

Fig. 6. Quantification of relative numbers of edge cells with golgi reoriented towards denudation. Bars represent standard error. Control cultures (△) have significantly higher proportions of cells with oriented golgi than cultures treated with 10 μg/ml Mitomycin C at time of denudation (○) and than cultures with 24 h prior incubation (⊡) ($P < 0.05$). Golgi orientation in cultures initially incubated for 4 h with control media, followed by media containing 10 μg/ml Mitomycin C, was not significantly different from control cultures at 8 h post denudation (□) ($P < 0.05$).

DISCUSSION

Continuous incubation of BPAEC with Mitomycin C reduces monolayer regeneration by reducing cell proliferation and by affecting other aspects of the repair/regeneration response. While endothelial cell proliferation is an essential component for repair of large denudations *in* vitro and in vivo, it has been shown that monolayer regeneration does occur initially when cell proliferation has been prevented [Sholley et al., 1977; Klein-Soyer et al., 1990]. This limited monolayer repair is due to cell spreading and

TABLE II. Effect of Mitomycin C on Protein and RNA Synthesis in BPAEC†

	% of control [^{3}H] proline incorporation	% of control [^{3}H] uridine incorporation
Control	100 (2.18)	100 (2.18)
5 μg/ml Mitomycin C	97 (2.59)	77 (2.59)**
10 μg/ml Mitomycin C	67 (1.99)*	35 (1.90)***
20 μg/ml Mitomycin C	68 (2.67)*	28 (2.56)***

†BPAEC were incubated with various doses of Mitomycin C for 24 h in the presence of 2 μCi/ml [^{3}H] proline or uridine, followed by cell harvesting and scintillation counting. Values shown are mean (standard error). Proline and uridine incorporation have been corrected for cell number.
*Significantly different from control proline ($P < 0.05$).
**Significantly different from control uridine ($P < 0.05$).
***Significantly different from control and 5 μg/ml values ($P < 0.05$).

migration, processes which seem able to occur independent of cell proliferation in denuded monolayers [Coomber and Gotlieb, 1990].

Previous studies have found that transient treatment of intact endothelial monolayers with Mitomycin C prior to denudation did not disrupt the repair process [Schleef and Birdwell, 1982; Defilippi et al., 1991]. Long-term (several days) incubation with Mitomycin C results in less than 10% reduction in endothelial monolayer repair [Bell and Madri, 1989]. This is in contrast to the results of the present study, which found that Mitomycin C treatment leads to significant reductions in monolayer repair within 8 h of denudation. This discrepancy is likely due to the length of time cells are exposed to Mitomycin C and differences in endothelium used (cell line vs. primary isolates).

Mitomycin C is a potent inhibitor of DNA synthesis and is often used to selectively block cell proliferation in vitro (Antonelli-Orlidge et al., 1989; Pepper et al., 1989). Inhibition of DNA synthesis is due to Mitomycin C induced covalent crosslinking between complementary strands of DNA [Iyer and Szybalski, 1963; Tomaz et al., 1986]. This present study found significant reductions in [^{3}H] thymidine incorporation, cell number, and BrdU labelling index in Mitomycin C treated endothelial cells, in agreement with others [Schleef and Birdwell, 1982; Klein-Soyer et al., 1990; Defilippi et al., 1991]. However, cell proliferation and DNA synthesis do not begin until at least 16 h after denudation of cultured endothelial monolayers [Schwartz et al., 1979]. Therefore, Mitomycin C induced delays in monolayer regeneration, which are significant 8 h after denudation in this study, must be due to factors other than Mitomycin C effects upon endothelial DNA synthesis.

An intriguing discovery is the influence of Mitomycin C on protein and RNA synthesis in these endothelial cells. Actinomycin D treatment at a dose which does not completely suppress [^{3}H] uridine incorporation will prevent corneal endothelial monolayer regeneration [Gordon and Staley, 1990]. It is possible that the reduced [^{3}H] uridine incorporation seen reported here may indicate insufficient production of gene products required for BPAEC repair. Although this study did not examine Mitomycin C induced reductions in specific RNA species, it is likely that significant reductions in gene transcription have occurred in Mitomycin C treated corneal cells. The effect of Mitomycin C on induction of early response genes in cells other than endothelium has also been examined. Prolonged treatment of cultured cells with Mitomycin C can induce the "U V response," which is a characteristic induction of specific genes, likely involving the transcription promoter AP-1 complex. Mitomycin C treatment has been shown to induce message for *c-jun,* collagenase, *c-fos,* and other genes in HeLa cells [Stein et al., 1989; Angel and Karin, 1991], but its effects on mRNA levels for these products have not been reported in endothelial cells. Therefore Mitomycin C induced DNA alterations may prevent transcription of gene products essential for normal monolayer regeneration. Alternatively, Mitomycin C treatment may enhance the production of early response genes (as is seen in non-endothelial cells treated with Mitomycin C) which may then influence transcription of gene products inhibitory for monolayer repair. An overall reduction in mRNA production does not preclude the possibility that Mitomycin C is able to selectively enhance transcription of certain early response genes which may suppress or reduce monolayer regeneration. The possibility that such Mitomycin C induced gene products are likely mediated via the AP-1 promoter complex is currently under investigation in this laboratory.

The influence of Mitomycin C upon other aspects of endothelial cell function in vitro has not been well studied. Pepper et al. [1989] examined gap junction coupling between cells at the edge of a denuded monolayer and found no effect

of Mitomycin C on this process in small vessel endothelial cells. It is therefore unlikely that disrupted communication between cells at the wound edge is responsible for the reduction in monolayer regeneration seen in the present study, although further work is required to confirm this.

Another novel effect found in this study is the influence of Mitomycin C on aspects of cell polarization/spreading at the denudation edge. The golgi apparatus is involved in packaging and modification of membrane and secretory proteins and is intimately associated with the MTOC/centrosome. Secretory vesicles are transported to the cell surface along the microtubule array emanating from the centrosome [Kreis, 1990]. These vesicles then fuse with the plasma membrane to form the leading edge of migrating cells [Singer and Kupfer, 1986]. In most migrating cells, the reorientation of centrosome and golgi is rapid and coordinated [Singer and Kupfer, 1986] and is thought to be an indication of and requirement for directed cell migration [Albrecht-Buehler and Bushnell, 1979]. Recent evidence suggests that this preferential centrosome location may be more a consequence of lamellipodia protrusion and cell spreading than an indication of cell polarization and directional migration [Euteneuer and Schliwa, 1992]. The current study is the first to report uncoupling of centrosome and golgi reorientation in Mitomycin C treated regenerating endothelial monolayers. Although the mechanism for this uncoupling is unclear, it is possible that delayed golgi orientation may lead to subsequent reductions in cell spreading or cell translocation in these Mitomycin C treated endothelial cells.

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REFERENCES

Albrecht-Buehler G, Bushnell A (1979): The orientation of centrioles in migrating 3T3 cells. Exp Cell Res 120:111–118.

Angel P, Karin M (1991): The role of Jun, Fos and AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072:129–157.

Antonelli-Orlidge A, Saunders KB, Smith SR, D'Amore PA (1989): An activated form of transforming growth factor β is produced by cocultures of endothelial cells and pericytes. Proc Natl Acad Sci USA 86:4544–4548.

Bell L, Madri JA (1989): Effect of platelet factors on migration of cultured bovine aortic endothelial and smooth muscle cells. Circ Res 65:1057–1065.

Coomber BL (1991a): Cytoskeleton in TGF-β- and bFGF-modulated endothelial monolayer repair. Exp Cell Res 194:42–47.

Coomber BL (1991b): Mitomycin C inhibits endothelial monolayer regeneration in vitro. J Cell Biol 115:366a.

Coomber BL, Gotlieb AI (1990): In vitro endothelial wound repair: Interaction of cell migration and proliferation. Arteriosclerosis 10:215–222.

Defilippi P, Truffa G, Stefanuto G, Altruda F, Silengo L, Tarone G (1991): Tumor necrosis factor α and interferon γ modulate the expression of the vitronectin receptor (integrin β_3) in human endothelial cells. J Biol Chem 266:7638–7645.

Euteneuer U, Schliwa M (1992): Mechanisms of centrosome positioning during the wound response in BSC-1 cells. J Cell Biol 116:1157–1166.

Gordon SR, Staley CA (1990): Role of the cytoskeleton during injury-induced cell migration in corneal endothelium. Cell Motil Cytoskel 16:47–57.

Gotlieb AI (1990): The endothelial cytoskeleton: Organization in normal and regenerating endothelium. Toxicol Pathol 18:603–617.

Heimark RL, Twardzik DR, Schwartz SM (1986): Inhibition of endothelial regeneration by type-beta transforming growth factor from platelets. Science 233:1078–1080.

Iyer VN, Szybalski W (1963): A molecular mechanism of mitomycin action: Linking of complementary DNA strains. Proc Natl Acad Sci USA 50:355–362.

Jackman RW, Anderson SK, Sheridan JD (1988): The aortic intima in organ culture. Response to culture conditions and partial endothelial denudation. Am J Pathol 133:241–251.

Klein-Soyer C, Beretz A, Cazenave J-P, Driot F, Maffrand J-P (1990): Behavior of confluent endothelial cells after irradiation. Modulation of wound repair by heparin and acidic fibroblast growth factor. Biol Cell 68:231–238.

Kreis TE (1990): Role of microtubules in the organization of the golgi apparatus. Cell Motil Cytoskel 15:67–70.

Leslie RJ, Saxton WM, Mitchison TJ, Neighbors B, Salmon ED, McIntosh JR (1984): Assembly properties of fluorescein-labelled tubulin in vitro before and after fluorescence bleaching. J Cell Biol 99:2146–2156.

Muthukrishnan L, Warder E, McNeil PL (1991): Basic fibroblast growth factor is efficiently released from a cytolsolic storage site through plasma membrane disruptions of endothelial cells. J Cell Physiol 148:1–16.

Pagano RE, Sepanski MA, Martin OC (1989): Molecular trapping of a fluorescent ceramide analogue at the golgi apparatus of fixed cells: Interaction with endogenous lipids provides a trans-golgi marker for both light and electron microscopy. J Cell Biol 109:2067–2079.

Pepper MS, Spray DC, Chanson M, Montesano R, Orci L, Meda P (1989): Junctional communication is induced in migrating capillary endothelial cells. *J Cell Biol 109:3027–*3038.

Ramsay MM, Walker LN, Bowyer DE (1982): Narrow superficial injury to rabbit aortic endothelium. Atherosclerosis 43:233–243.

Reidy MA, Schwartz SM (1981): Endothelial regeneration. III: Time course of intimal changes after small defined injury to rat aortic endothelium. Lab Invest 44:301–308.

Rosen EM, Moromsky L, Setter E, Vinter DW, Goldberg ID (1990): Quantitation of cytokine-stimulated migration of endothelium and epithelium by a new assay using microcarrier beads. Exp Cell Res 186:22–31.

Schleef RR, Birdwell CR (1982): The effect of fibrin on endothelial cell migration in vitro. Tissue Cell 14:629–636.

Schwartz SM, Selden SC III, Bowman P (1979): Growth control in aortic endothelium at wound edges. In Sato G, Ross R (eds): "Hormones and Cell Culture," Volume 6 Book B. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp 593–610.

Selden SC III, Schwartz SM (1979): Cytochalasin B inhibition of endothelial proliferation at wound edges in vitro. J Cell Biol 81:348–354.

Sholley MM, Gimbrone MA Jr, Cotran RS (1977): Cellular migration and replication in endothelial regeneration. A study using irradiated endothelial cultures. Lab Invest 36:18–25.

Singer SJ, Kupfer A (1986): The directed migration of eukaryotic cells. Annu Rev Cell Biol 2:337–365.

Stein B, Rhamsdorf HJ, Steffen A, Litfin M, Herrlich P (1989): UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-fos, and metallothionein. Mol Cell Biol 9:5169–5181.

Tomaz M, Chowdary D, Lipman R, Shimotakahara S, Veiro D, Walker V, Verdine GL (1986): Reaction of DNA with chemically or enzymatically activated mitomycin C: Isolation and structure of the major covalent adduct. Proc Natl Acad Sci USA 83:6702–6706.

Wong MKK, Gotlieb AI (1984): In vitro reendothelialization of a single-cell wound. Role of microfilament bundles in rapid lamellipodia-mediated wound closure. Lab Invest 51:75–81.

Wong MKK, Gotlieb AI (1990): Endothelial monolayer integrity. Perturbation of F-actin filaments and the dense peripheral band-vinculin network. Arteriosclerosis 10:76–84.