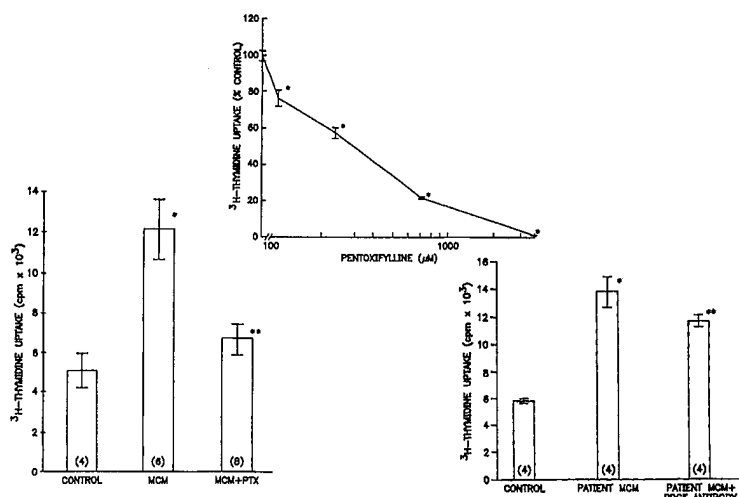




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(54) Title: NEW USES FOR COMPOUNDS WHICH REDUCE *c-jun* GENE EXPRESSION

(57) Abstract

In accordance with the present invention, it has been discovered that monocyte conditioned medium (MCM) obtained from patients with liver disease stimulates the proliferation of fibroblasts. Platelet derived growth factor (PDGF) has also been found to stimulate fibroproliferation of fibroblasts, and to be at least partially responsible for the fibroproliferative effect of the MCM. Further in accordance with the present invention, the effect of MCM and PDGF on the expression of *c-fos* and *c-jun* has been investigated, because *c-fos* and *c-jun* form AP-1 complexes which can stimulate genes involved in proliferation. It has recently been reported that pentoxifylline inhibits platelet derived growth factor-stimulated proliferation. Studies were conducted to determine whether pentoxifylline altered the expression of *c-fos* and *c-jun*. While PDGF was found to induce the expression of both *c-fos* and *c-jun*, pentoxifylline was found to effectively reduce the effect of PDGF-induced *c-jun* gene expression, without altering *c-fos* gene expression. These results suggest that pentoxifylline inhibits PDGF-stimulated proliferation by decreasing *c-jun* expression. These results further suggest a variety of diseases and/or conditions which may also be successfully treated with compounds, such as pentoxifylline, which reduce the transcription of *c-jun* gene.

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New Uses for Compounds which Reduce *c-jun* Gene ExpressionFIELD OF THE INVENTION

The present invention relates to novel uses for compounds which reduce *c-jun* gene expression. In a particular aspect, the present invention relates to methods
5 for the treatment of diseases and/or conditions characterized by elevated levels of such factors as platelet derived growth factor (PDGF), inflammatory cytokine(s), and the like.

BACKGROUND OF THE INVENTION

10 Collagenous colitis was first described by Lindstrom as chronic watery diarrhea (Pathol. Eur. 11(1):87-89 (1976)). Collagenous colitis is characterized by collagen deposition, likely resulting from an imbalance between collagen production by mucosal fibroblasts and
15 collagen degradation. Very little is understood, however, regarding the mechanism by which collagenous colitis results in secretory diarrhea.

The incidence of collagenous colitis is similar to primary biliary cirrhosis. This disease has an annual
20 incidence of 1.8 per 100,000 and a prevalence of 15.7 per 100,000, which is similar to primary biliary cirrhosis (12.8 per 100,000) and lower than ulcerative colitis (234 per 100,000), Crohn's disease (146 per 100,000) or celiac disease (5 per 100,000). In patients with chronic
25 diarrhea, about 0.3 to 5% have collagenous colitis.

In efforts to characterize the patients under study, sera and monocyte conditioned media (MCM) from patients with collagenous colitis have been assessed for their ability to stimulate fibroproliferation. Cytokine

antibodies were used to characterize the fibroproliferative component of patient samples.

Previous studies have suggested that MCM samples obtained from patients with liver disease are capable of stimulating proliferation of fibroblasts (see Peterson and Isbrucker, in *Hepatol.* 15(2):191-197 (1992)). It has also been established that several genes involved in proliferation possess AP-1 binding sites, and thus would be expected to be susceptible to regulation by the immediate early genes *c-fos* and *c-jun* (see, for example, Schafer et al., in *Biochem. Biophys. Res. Commun.* 221:111-116 (1996) and Bamberger et al., in *Proc. Natl. Acad. Sci. USA* 93:6169-6174 (1996)).

In order to determine whether these immediate early genes are upregulated by MCM obtained from patients with liver disease, the effect of MCM from patients with liver disease was investigated. MCM exerts many effects due to PDGF (see, for example, Peterson and Isbrucker in *Hepatol.* 15(2):191-197 (1992) and Peterson and Tanton in *Can. J. Gastroenterol.* 10:S76 (1996)). Indeed, it has been established that PDGF itself stimulates proliferation of fibroblasts (see Peterson, T.C. in *Hepatol.* 17(3):486-493 (1993) and Peterson et al., in *Immunopharmacol.* 28:259-270 (1994)). Thus, the question of whether PDGF upregulates the expression of *c-fos* and *c-jun* was addressed.

Prior studies have indicated that pentoxifylline inhibits PDGF and MCM stimulated proliferation (see, for example, Peterson, T.C. in *Hepatol.* 17(3):486-493 (1993), Peterson et al., in *Immunopharmacol.* 28:259-270 (1994) and Peterson and Neumeister in *Immunopharmacol.* 31:183-193 (1996)). The mechanism for this effect of pentoxifylline remains unclear, but does not appear to involve competing

for the PDGF receptor or adenosine receptor activation (see Peterson, T.C. in Biochem. Pharmacol. 52:597-602 (1996)). One possible mechanism for the inhibitory effect of pentoxifylline on PDGF-stimulated proliferation is inhibition of PDGF post-receptor signalling. Thus, in accordance with the present invention, it was undertaken to determine if compounds such as pentoxifylline modified the effect of PDGF on immediate early gene expression.

Accordingly, there is a need in the art to achieve a better understanding of the mechanism by which compounds such as pentoxifylline are effective to treat diseases and conditions such as collagenous colitis. Based upon a more complete understanding of the mechanism by which such agents are effective for the treatment of collagenous colitis, it may be possible to apply this understanding to the development of new methods of treatment for a variety of indications which are in no way related to collagenous colitis as well as identifying additional compounds which could be employed to treat fibroproliferative diseases and conditions.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, it has been discovered that monocyte conditioned medium (MCM) obtained from patients with liver disease stimulates the proliferation of fibroblasts. Platelet derived growth factor (PDGF) has also been found to stimulate fibroproliferation of fibroblasts, and to be at least partially responsible for the fibroproliferative effect of the MCM.

Further in accordance with the present invention, the effect of MCM and PDGF on the expression of *c-fos* and *c-jun* has been investigated, because *c-fos* and *c-jun* form

AP-1 complexes which can stimulate genes involved in proliferation.

In accordance with the present invention, it has been discovered that compounds which reduce *c-jun* gene expression inhibit platelet derived growth factor-stimulated proliferation. Studies have been conducted to determine whether such compounds altered the expression of *c-fos* and *c-jun*. While PDGF was found to induce the expression of both *c-fos* and *c-jun*, pentoxifylline was found to effectively reduce the effect of PDGF-induced *c-jun* gene expression, without altering *c-fos* gene expression. These results suggest that compounds such as pentoxifylline inhibit PDGF stimulated proliferation by decreasing *c-jun* expression. These results further suggest a variety of diseases and/or conditions which may also be successfully treated with such compounds.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A illustrates the effect on fibroproliferation of MCM obtained from patients with liver disease when incubated either with 240 μ M pentoxifylline, or without added pentoxifylline.

Figure 1B illustrates the effect on fibroproliferation of MCM obtained from patients with liver disease when incubated with increasing amounts of pentoxifylline.

Figure 1C illustrates the effect on fibroproliferation of MCM obtained from patients with liver disease, with and without preincubation with PDGF antibody.

For each of Figures 1A, 1B and 1C, fibroproliferation was assessed as tritiated thymidine uptake by fibroblasts in counts per minute (cpm). MCM was prepared from blood obtained from liver disease patients (n=6) as described in Example 1. Pentoxifylline (PTX) was assessed over a range of concentrations, as indicated in Figure 1B. PDGF antibody (50 µg/ml) was preincubated with MCM for 1 hr prior to testing on fibroblasts. Results are expressed as mean $\pm$ S.E. Each treatment was done on wells in quadruplicate and experiments were repeated. *(p<0.05) compared to fibroblasts incubated with control medium. **(p<0.05) compared to fibroblasts stimulated with MCM in the absence of pentoxifylline. *** (p<0.05) compared to fibroblasts stimulated with MCM in the absence of PDGF antibody.

Figure 2 presents the results of phosphorimaging analysis illustrating the effect of MCM obtained from patients with liver disease, with and without incubation with pentoxifylline (3.5 mM, for 30 min), on the expression of *c-fos* and *c-jun*. The figure combines the results obtained from three independent experiments. *=p<0.05 compared to control.

Figure 3 presents the results of phosphorimaging analysis illustrating the effect of PDGF, with and without pentoxifylline, on the expression of *c-fos* and *c-jun*. The figure combines the results of three independent experiments. *=significantly different (p<0.05) compared to control or PTX alone. +=significantly different (p<0.05) compared to PDGF.

Figure 4 illustrates the effect of pentoxifylline on PDGF stimulated proliferation. Fibroproliferation was assessed as tritiated thymidine uptake by fibroblasts in

counts per minute (cpm). Pentoxifylline (PTX) was assessed at 3.5 mM (1mg/ml), PDGF was tested at 8ng/ml. Results were expressed as mean $\pm$ S.E. Each treatment was done on wells in quadruplicate and experiments were repeated.
5 *(p<0.05) compared to fibroblasts without PDGF. +(p<0.05) compared to fibroblasts stimulated with PDGF in the absence of pentoxifylline.

Figure 5 presents a graph illustrating the effect of neural graft on brain collagen concentrations in rats.

10 Figure 6 presents a graph illustrating the reduction of PDGF-stimulated proliferation of dermal fibroblasts by antisense *c-jun* oligonucleotide.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there
15 are provided methods for the treatment of a subject afflicted with a disease and/or condition characterized by one or more of the following:

 elevated platelet derived growth factor (PDGF)
 levels (e.g., elevated levels of PDGF-AA,
20 PDGF-BB, PDGF-AB),
 elevation of Jun kinase,
 elevation of c-Jun,
 activation of NF-kappaB or NF-kappaB p65,
 neutrophil infiltration, or
25 elevated levels of inflammatory cytokine(s)
 (e.g., elevated levels of tumor necrosis
 factor (TNF), interleukin-1 (IL-1),
 interleukin-4 (IL-4), interleukin-12
 (IL-12), insulin growth factor-1 (IGF-1),
30 insulin growth factor-2 (IGF-2), TGF-alpha,
 TGF-beta, epidermal growth factor (EGF),
 nerve growth factor (NGF), acidic fibroblast

growth factor (aFGF), basic fibroblast growth factor (bFGF), and the like), provided, however, that said disease or condition is not collagenous colitis, said method comprising administering
5 to said subject an amount of a compound which reduces *c-jun* gene expression effective to ameliorate the symptoms of said disease and/or condition.

As employed herein, reference to "elevated platelet derived growth factor (PDGF) levels" embraces
10 species such as PDGF-AA, PDGF-BB, PDGF-AB, and the like, which are present at levels which are significantly higher than levels which are considered to be normal.

As employed herein, the phrase "elevated levels of inflammatory cytokine(s)" embraces such cytokines as
15 tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-4 (IL-4), interleukin-12 (IL-12), insulin growth factor-1 (IGF-1), insulin growth factor-2 (IGF-2), TGF-alpha, TGF-beta, epidermal growth factor (EGF), nerve growth factor (NGF), acidic fibroblast growth factor
20 (aFGF), basic fibroblast growth factor (bFGF), and the like, wherein said cytokines are present at levels which are significantly higher than levels which are considered to be normal.

As readily recognized by those of skill in the
25 art, a wide variety of diseases and/or conditions are characterized as described herein, i.e., by one or more of the following:

 elevated PDGF levels,
 elevation of Jun kinase,
30 elevation of c-Jun,
 activation of NF-kappaB or NF-kappaB p65,
 neutrophil infiltration, or
 elevated levels of inflammatory cytokine(s).

A variety of disease states and/or conditions are contemplated for treatment according to the invention, excluding collagenous colitis. Examples of disease states and/or conditions contemplated include interstitial lung disease, human fibrotic lung disease (e.g., idiopathic pulmonary fibrosis (IPF), adult respiratory distress syndrome (ARDS), tumor stroma in lung disease, systemic sclerosis, Hermansky-Pudlak syndrome (HPS), coal worker's pneumoconiosis (CWP), chronic pulmonary hypertension, AIDS associated pulmonary hypertension, and the like), human kidney disease (e.g., nephrotic syndrome, Alport's syndrome, HIV-associated nephropathy, polycystic kidney disease, Fabry's disease, diabetic nephropathy, and the like), glomerular nephritis, nephritis associated with systemic lupus, peritoneal fibrosis, liver fibrosis, myocardial fibrosis, pulmonary fibrosis, Grave's ophthalmopathy, drug induced ergotism, cardiovascular disease, cancer (including desmoid tumor), Alzheimer's disease, scarring, scleroderma, glioblastoma in Li-Fraumeni syndrome, sporadic glioblastoma, myeloid leukemia, acute myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, gynecological cancer (e.g., ovarian cancer, Lynch syndrome, and the like), Kaposi's sarcoma, Hansen's disease, inflammatory bowel disease (including stricture formation in Crohn's disease and microscopic colitis), and the like.

As used herein, the phrase "compound which reduces *c-jun* gene expression" refers to any compound which inhibits the transcription and/or translation of *c-jun*. Examples of compounds which reduce *c-jun* gene expression include pentoxifylline or functional derivatives/metabolites thereof, antisense *c-jun* oligonucleotide (including the oligonucleotide set forth in SEQ ID NO:3), antibodies specific to the *c-jun* gene or

protein (preferably monoclonal antibodies), paclitaxel, and the like. In a preferred embodiment of the present invention, the compound will reduce the expression of *c-jun* but will not affect the expression of other signalling
5 proteins including *c-fos*, NF kappaB, Sp1, and the like.

As used herein, the phrase "pentoxifylline or functional derivatives/metabolites thereof" refers to the compound 1-(5-oxohexyl)-3,7-dimethylxanthine (pentoxifylline), and oxidation-, reduction-, substitution-
10 and/or rearrangement-products thereof, such as, for example, metabolite-1 through metabolite-7 as described by Luke and Rocci in J. Chromatogr. 374(1):191-195 (1986) (e.g., 1-(5-hydroxyhexyl)- 3,7-dimethyl-xanthine (metabolite-1)), as well as synthetic variants thereof
15 (e.g., propentofylline).

The active components described for use herein can be delivered in a suitable vehicle, thereby rendering such compositions amenable to oral delivery, transdermal delivery, subcutaneous delivery (e.g., intravenous
20 delivery, intramuscular delivery, intraarterial delivery, intraperitoneal delivery, and the like), topical delivery, inhalation delivery, osmotic pump, and the like.

Depending on the mode of delivery employed, the above-described compositions can be delivered in a variety
25 of pharmaceutically acceptable forms. For example, the above-described compositions can be delivered in the form of a solid, solution, emulsion, dispersion, micelle, liposome, and the like.

Pharmaceutical compositions contemplated for use
30 in the practice of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the like, wherein the resulting

composition contains one or more of the active compounds contemplated for use herein, as active ingredients thereof, in admixture with an organic or inorganic carrier or excipient suitable for enteral or parenteral applications.

5 The active ingredients may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used include glucose,

10 lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or

15 liquid form. In addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used. The active compounds contemplated for use herein are included in the pharmaceutical composition in an amount sufficient to produce the desired effect upon the target

20 process, condition or disease.

Pharmaceutical compositions containing the active ingredients contemplated herein may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or

25 granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions. In addition, such compositions may contain one or more agents selected

30 from a sweetening agent (such as sucrose, lactose, or saccharin), flavoring agents (such as peppermint, oil of wintergreen or cherry), coloring agents and preserving agents, and the like, in order to provide pharmaceutically elegant and palatable preparations. Tablets containing the

35 active ingredients in admixture with non-toxic

pharmaceutically acceptable excipients may also be manufactured by known methods. The excipients used may be, for example, (1) inert diluents such as calcium carbonate, lactose, calcium phosphate, sodium phosphate, and the like; 5 (2) granulating and disintegrating agents such as corn starch, potato starch, alginic acid, and the like; (3) binding agents such as gum tragacanth, corn starch, gelatin, acacia, and the like; and (4) lubricating agents such as magnesium stearate, stearic acid, talc, and the 10 like. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract, thereby providing sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl 15 distearate may be employed. They may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

In some cases, formulations for oral use may be 20 in the form of hard gelatin capsules wherein the active ingredients are mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate, kaolin, or the like. They may also be in the form of soft gelatin capsules wherein the active ingredients are mixed with 25 water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

The pharmaceutical compositions may be in the form of a sterile injectable suspension. This suspension may be formulated according to known methods using suitable 30 dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Sterile, fixed oils are

conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids (including oleic acid), naturally occurring vegetable oils
5 like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or synthetic fatty vehicles like ethyl oleate or the like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

Compositions contemplated for use in the practice
10 of the present invention may also be administered in the form of suppositories for rectal administration of the active ingredients. These compositions may be prepared by mixing the active ingredients with a suitable non-irritating excipient, such as cocoa butter, synthetic
15 glyceride esters of polyethylene glycols (which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the active ingredients), and the like.

In addition, sustained release systems, including
20 semi-permeable polymer matrices in the form of shaped articles (e.g., films or microcapsules) can also be used for the administration of the active compound employed herein. Sustained release pentoxifylline compositions also include liposomally entrapped pentoxifylline.

25 Since individual subjects may present a wide variation in severity of symptoms and each active ingredient has its unique therapeutic characteristics, it is up to the practitioner to determine a subject's response to treatment and vary the dosages accordingly. Effective
30 pentoxifylline levels for a given patient can readily be achieved by monitoring the patient using commonly available techniques, such as, for example, by subjecting serum obtained from the patient to high performance liquid

chromatography (HPLC). The practitioner can then determine whether increased or decreased dosages of pentoxifylline are indicated, based on the level detected in the patient's serum.

5 Typical daily doses of the active component, in general, lie within the range of from about 10 μ g up to about 100 mg per kg body weight, and, preferably within the range of from about 50 μ g up to about 50 mg per kg body weight and can be administered up to four times daily. The
10 daily dose lies within the range of from about 1 μ g to about 100 mg per kg body weight, and, preferably, within the range of from 10 μ g to 10 mg per kg body weight. Where one will operate within the above ranges will vary based on a variety of considerations, such as, for example, the age
15 of the patient, the size of the patient, what, if any, other medications the patient may be taking (especially steroids), and the like.

 As readily recognized by those of skill in the art, the active compounds contemplated for use herein can
20 be administered as part of a slow release formulation, as a single bolus for rapid administration, as part of a depot formulation, as part of a nutritional supplement, and the like.

 In another aspect of the present invention, a
25 combination treating agent can be employed for the treatment of diseases and/or conditions characterized by elevated levels of such factors as platelet derived growth factor (PDGF), inflammatory cytokine(s), and the like, wherein inhibitor(s) of cytochrome P-450 are combined with
30 a "compound which reduces *c-jun* gene expression," as defined herein.

In another aspect of the present invention, a combination treating agent can be employed for the treatment of diseases and/or conditions characterized by elevated levels of such factors as platelet derived growth factor (PDGF), inflammatory cytokine(s), and the like, wherein kinase inhibitors are combined with a "compound which reduces *c-jun* gene expression," as described herein. Those of skill in the art can readily identify a variety of kinase inhibitors suitable for this purpose. Presently preferred kinase inhibitors include cyclic AMP-dependent kinase inhibitors, JNK inhibitors, and the like. Examples of kinase inhibitors include H7, staurosporine, genistein, SP-203580, PD98059, and the like.

In accordance with the present invention, the effect of MCM and PDGF on the expression of *c-fos* and *c-jun* has been investigated. MCM samples obtained from patients with liver disease have been found to stimulate fibroproliferation and collagen synthesis. The ability of these MCM samples to stimulate proliferation of fibroblasts is reduced (though not totally blocked) by preincubation of MCM with PDGF antibody. These results indicate that MCM obtained from patients with liver disease upregulates *c-fos* and *c-jun* gene expression in dermal fibroblasts. MCM samples from such patients significantly stimulate *c-fos* and *c-jun* mRNA compared to basal levels. These results indicate that the stimulation of fibroproliferation which occurs with MCM samples from patients with liver disease likely involves an upregulation in *c-fos* and *c-jun* gene expression. The results also indicate that the stimulation of *c-fos* and *c-jun* mRNA by MCM samples from such patients is not altered by 30 minute incubation with pentoxifylline. These results indicate that the effect of invention compounds, such as pentoxifylline, on fibroproliferation stimulated by MCM samples from a liver disease patients is

likely not due to an effect on *c-fos* or *c-jun* gene expression.

The fibroproliferation stimulated by the liver disease patient's MCM was partially attenuated by PDGF antibody, indicating that PDGF is one of the components of the MCM involved in the stimulation of proliferation. It has previously been established that PDGF stimulates proliferation of skin fibroblasts (see Peterson, T.C. in *Hepatology*. 17(3):486-493 (1993) and Peterson et al., in *Immunopharmacology*. 28:259-270 (1994)) and myofibroblasts (see Isbrucker and Peterson in *Hepatology*. 22:4 (1995)). Pentoxifylline has been shown to block PDGF-stimulated fibroproliferation in a dose related manner (see Peterson et al., in *Immunopharmacology*. 28:259-270 (1994)). Pentoxifylline inhibits proliferation with a similar IC_{50} to trapidil (see Peterson et al., supra), a drug reported to act at the PDGF receptor (see Kuratsu and Ushio in *Neurosurgery*. 73:436-40 (1990) but pentoxifylline does not compete for the PDGF receptor (see Slys and Peterson, supra). An interference in the post-receptor signalling of PDGF by pentoxifylline is a reasonable mechanism to investigate because pentoxifylline can increase intracellular cAMP (see Meskini et al., in *Biochemical Pharmacology*. 47:781-788 (1994) and Bessler et al., in *J. Leukocyte Biology*. 40:747-754 (1986)) and compounds that elevate cAMP have been reported to inhibit PDGF postreceptor signalling (see Wu et al., in *Science* 262:1065-9 (1993)).

It has previously been reported that the MCM obtained from patients with liver disease contains IL-1 and activated oxygen intermediates (see Peterson in *Biochemical Pharmacology*. 43(5):1166 (1992)) in addition to PDGF. It is likely that these and other factors contribute to the proliferative effect of MCM because PDGF antibody only

partially reduces the fibroproliferative activity of the MCM. A recent report (see Lo et al., in J. Biol. Chem. 271:15703-7 (1996)) suggests that these other factors can stimulate immediate early gene expression, thus suggesting
5 that the increased expression of these genes induced by MCM likely represents the combined effect of several factors. The lack of effect of pentoxifylline on MCM induced *c-jun* is likely due to the complex nature of the MCM and the variable effect of pentoxifylline on the individual
10 components of the MCM. Since it has previously been established that the effect of PDGF on proliferation is blocked by pentoxifylline, the effect of PDGF on immediate early gene expression, and its potential modification by pentoxifylline was investigated.

15 The results of these investigations indicate that PDGF stimulates *c-fos* expression and that the induction is not reduced by pentoxifylline. These results are in agreement with several reports suggesting that PDGF will stimulate *c-fos* gene expression (see, for example, Marra et al., in FEBS Lett. 376:141-5 (1995), Buchdunger et al., in
20 Cancer Res. 56:100-4 (1996), Rosenwald et al., in Cell Prolif. 28:631-44 (1996) and Terano et al., in Lipids S301-4 (1996)). Thus, the stimulation of *c-fos* mRNA by PDGF is maintained when cells are exposed to pentoxifylline. In
25 contrast, however, PDGF stimulated fibroproliferation is abolished when the cells are exposed to pentoxifylline. These results indicate that the action of pentoxifylline on PDGF stimulated fibroproliferative activity is not via an effect on PDGF receptor signalling, through *c-fos*, or
30 through *c-fos* itself.

PDGF postreceptor signalling was then studied to understand the action of PDGF on proliferation of fibroblasts. Though the fact that PDGF upregulates *c-fos*

expression was known and assumed to play a major role in the mitogenic effects of PDGF, the upregulation of *c-jun* expression by PDGF and its potential role in proliferation has not been previously described. The results obtained
5 herein indicate that PDGF increases *c-jun* expression 7-fold over basal expression.

The PDGF receptor is a tyrosine kinase receptor which upon activation transmits signals to mitogen activated protein kinases (MAP kinases). MAP kinases are
10 a family of extracellular signal regulated kinases whose enzyme activity is increased in response to most mitogens, such as those acting on the receptor protein tyrosine kinases (see, for example, Krespo et al., in J. Biol. Chem. 269:21103-21109 (1994) and Palech, S.L., in Curr. Biol.
15 3:513-516 (1993)). This signalling from the PDGF receptor includes a cascade of protein kinases. The MAP kinases are phosphorylated, thereby increasing their enzymatic activity (see McCormick, F. in Nature 363:15-16 (1993) and Schlesinger, J. in Trends Biochem. Sci. 18:273-275 (1993)).
20 In turn the MAP kinases phosphorylate and regulate the activity of key enzymes and nuclear proteins (Davis, R.J. J. Biol. Chem. 268:14553-14556 (1993)) which are responsible for increased expression of the *c-fos* gene.

Jun kinase controls the activity of *c-jun*, which
25 then complexes with *c-fos* to make the AP-1 transcription factor. Homodimers of the jun protein product or heterodimeric complexes of *jun-fos* form the AP-1 nuclear transcription factor which controls the expression of genes possessing this regulatory element, including genes
30 involved in proliferation (see, for example, Schafer et al., in Biochem. Biophys. Res. Commun. 221:111-6 (1996) and Bamberger et al., in Proc. Natl. Acad. Sci. USA 93:6169-74 (1996)).

The role of these two nuclear proteins was assessed because the activity of AP-1 is regulated at the level of *jun* and *fos* gene transcription (see Herschman, H. in Ann. Rev. of Biochem. 60:281-319 (1991) and Hunter and
5 Karin in Cell 70:375-387 (1992)). The results obtained indicate that both *c-fos* and *c-jun* are upregulated by PDGF.

It is well accepted that PDGF activates MAP kinase (see Coso et al., in J. Biol. Chem. 270:5620-5624 (1995)). However, consistent with the results obtained
10 herein, a recent paper suggests that PDGF may also activate Jun kinase (see Bogoyevitch, et al., in J. Biol. Chem. 270(50):29710-17 (1995)). The mechanism for this effect is not known, but PDGF may activate both pathways via an effect on Ras. Recent results suggest that Ras activation
15 results in phosphorylation of Jun kinase and MAP kinase (see Xie and Herschman in J. Biol. Chem. 270:27622-28 (1995)). The results reported herein clearly show that PDGF rapidly and reproducibly increases the gene expression of both *c-fos* and *c-jun*.

20 To study the mechanism of action of pentoxifylline in the inhibition of PDGF-stimulated proliferation, the effect on PDGF postreceptor signalling of compounds which inhibit *c-jun* gene expression, including pentoxifylline, was determined. Ultimately an inhibitory
25 effect on the pathway that leads from PDGF to *c-fos* or from PDGF to *c-jun* (or both) would block proliferation. The results presented herein indicate that PDGF upregulates both *c-fos* and *c-jun* expression in dermal fibroblasts and that compounds which reduce *c-jun* gene expression, e.g.,
30 pentoxifylline, preferentially inhibit the upregulation of *c-jun*. A recent report suggests that compounds that elevate cAMP may act upstream from MAP kinase at the Ras-Raf interaction (see Burgering et al., in EMBO Journal

12:4211-29 (1993)). Such an interaction would be expected to alter *c-fos* expression. In contrast, the results presented herein would suggest that pentoxifylline does not affect the Ras-Raf interaction, but instead interferes with an alternate PDGF signalling pathway which signals from Ras through Jun kinase, or a pathway which crosstalks between MAP kinase and Jun kinase. Accordingly, pentoxifylline would only alter the expression of *c-jun* and not affect the expression of *c-fos*.

10 The results presented herein provide evidence for the induction of *c-jun* expression by PDGF and provides a mechanism for the inhibitory effect of pentoxifylline on PDGF stimulated proliferation by altering PDGF post-receptor signalling. An understanding of this mechanism of action could prove to be of great importance, since an elevation in PDGF has been implicated in a wide variety of diseases and/or conditions, including interstitial lung diseases (see, for example, Uebelhoer et al., in Chest. 107:701-5 (1995) and Shaw et al., in Am. Rev. Respir. Dis. 143:167-73 (1991)), glomerular nephritis (see, for example, Gesualdo et al., in Lab Invest. 65:160-7 (1991)), nephritis associated with systemic lupus (see, for example, Nakamura et al., in Clin. Immunol. Immunopathol. 63:173-81 (1992)), liver fibrosis (see, for example, Peterson and Isbrucker in Hepatol. 15(2):191-197 (1992)), Graves' ophthalmopathy (see, for example, Imai et al., in Acta Endocrinol. Copenh. 126:541-52 (1992)), drug induced ergotism (see, for example, Pietrogrande et al., in Angiology 46:633-6 (1995)), cardiovascular disease (see, for example, Cimminiello et al., in Angiology 45:289-93 (1994) and Pesonen, E. in Eur. Heart J. 15 Suppl C:57-61 (1994)), cancer (see, for example, Ross et al., in Cancer Epidemiol. Biomarkers Prev. 4:485-9 (1995)), and the like.

In accordance with another embodiment of the present invention, there are provided in vitro assays to determine whether compounds which reduce *c-jun* gene expression are likely to be effective for treatment of a
5 subject afflicted with a disease and/or condition characterized by one or more of the following:

elevated PDGF levels,
elevation of Jun kinase,
elevation of c-Jun,
10 activation of NF-kappaB or NF-kappaB p65,
neutrophil infiltration, or
elevated levels of inflammatory cytokine(s),
said method comprising determining the uptake by test cells in suitable media therefor, of a labeled building block
15 indicative of cell proliferation, wherein said uptake is determined in the presence of serum obtained from said subject, and in the presence and absence of a compound which reduces *c-jun* gene expression,

wherein test cells suitable for use herein are
20 selected from the group consisting of fibroblasts, glial cells, smooth muscle cells and transformed cells obtained from the particular organ of interest, and

wherein reduction of uptake by test cells of labeled building block in the presence of said compound,
25 relative to the uptake by test cells of labeled building block in the absence of said compound, is predictive of the efficacy of said compound for the treatment of diseases or conditions characterized as set forth above.

As employed herein, the phrase "labeled building
30 block" refers to any compound which is taken up by DNA during active DNA synthesis, wherein such compound can be readily detected by any suitable means. Such labeled materials can be rendered detectable by a variety of means, e.g., as a result of radioactive labeling, by colorimetric

detection, and the like. Examples of suitable labeled building blocks contemplated for use herein include tritiated thymidine, bromouridine, and the like.

Suitable test cells contemplated for use herein
5 include fibroblasts, glial cells, smooth muscle cells or transformed cells derived from the organ which is the disease target, e.g., liver myofibroblasts are useful to test sera samples from liver fibrosis patients, pulmonary fibroblasts or smooth muscle cells (mesenchymal cells) are
10 useful to test sera samples from pulmonary fibrosis patients, glial cells are useful to test sera samples from Alzheimer's patients, intestinal fibroblasts and intestinal smooth muscle cells are useful to test sera samples from patients with inflammatory bowel disease, and the like.
15 Other test cells contemplated for use herein include cultured human skin fibroblasts, vascular smooth muscle cells, mesangial cells (kidney), hematopoietic cells (leukemias), Kaposi sarcoma-derived cells (Kaposi sarcoma), epithelial cells, and the like.

20 As readily recognized by those of skill in the art, suitable media appropriate for use with a test cell will depend on the particular test cell being employed. Those of skill in the art can readily determine appropriate media to employ once the test cell of choice has been
25 identified.

In accordance with yet another embodiment of the present invention, there are provided kits which are useful for assays to determine whether a compound which reduces *c-jun* gene expression is likely to be effective for
30 treatment of diseases and/or conditions characterized by one or more of the following:

- elevated PDGF levels,
- elevation of Jun kinase,

elevation of c-Jun,
activation of NF-kappaB or NF-kappaB p65,
neutrophil infiltration, or
elevated levels of inflammatory cytokine(s),

5 said kit comprising:

10 cultured cells selected from the group consisting
of fibroblasts, glial cells, smooth muscle
cells and transformed cells obtained from
the particular organ of interest, in
suitable media therefor,
a compound which reduces c-jun gene expression,
and
labeled building block indicative of cell
proliferation.

15 Cultured cells contemplated for use herein
include human skin fibroblasts, intestinal smooth muscle
cells, liver myofibroblasts, pulmonary fibroblasts,
mesenchymal cells, glial cells, intestinal fibroblasts,
intestinal smooth muscle cells, vascular smooth muscle
20 cells, mesangial cells, hematopoietic cells, Kaposi
sarcoma-derived cells, epithelial cells, and the like.

In accordance with a still further embodiment of
the present invention, there are provided methods to
quantify the collagen content of a tissue sample, said
25 method comprising:

optionally rolling said tissue sample
longitudinally,
fixing said sample, and
optionally embedding said sample in paraffin,
30 sectioning said sample,
deparaffinizing a section of said sample, if
paraffined,

staining said sample with Sirius red and Fast green,

removing said dyes from the stained sections, and determining the collagen concentration of said sample. Thus, collagen concentrations in a variety of tissues (e.g., colonic tissue, heart tissue, brain tissue, lung tissue, and the like) can be determined by removing the desired tissue for analysis from the host and treating the tissue as described herein. The sample can readily be fixed in a variety of methods and fixing agents known to those of skill in the art. Examples of fixing agents include formalin, glutaraldehyde, and the like. The specific dyes employed allow one to distinguish collagenous protein from non-collagenous protein since Sirius red stains collagenous protein while Fast green stains non-collagenous protein. These dyes can be removed from the tissue sections using suitable solvent, e.g., sodium hydroxide methanol solution, and the collagen concentration determined with reference to the maximal absorbance of Sirius red and Fast green.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1

Details of blood test

Fibroproliferative activity of sera (500 μ l) and/or monocyte conditioned medium (MCM) samples (500 μ l) is assessed by tritiated thymidine uptake using cultured human skin fibroblasts and/or intestinal smooth muscle cells (ATCC). Pentoxifylline (Sigma Chemical Company, St. Louis, MO.) is tested at 240 μ M. MCM is prepared from peripheral blood (10 ml) as has previously been described (see Peterson in Hepatol. 15(2):191-197 (1992)).

To assess fibroproliferative activity, medium is removed by aspiration from flasks containing confluent intestinal smooth muscle cells or monolayers of human fibroblasts (at passage 5 to 12). The cells are briefly
5 rinsed with 10 - 20 ml of sterile saline to remove any remaining medium. Fibroblasts are removed from the flasks by the addition of 5 ml trypsin-EDTA (Gibco, Ontario, Canada) for 30 seconds with gentle rocking at room temperature, followed by pouring off of most of the trypsin
10 solution and incubation at 37°C for 3 - 5 minutes.

Cells are resuspended in 10 ml Dulbecco's (DBE, Gibco, Canada), antibiotic/antimycotic (Gibco, Canada) and 0.5% CPSR-2 (Sigma Chemical Co., St. Louis, Mo.). CPSR-2 is a serum replacement (control processed serum
15 replacement-2) which has low mitogenic activity. Two hundred microliter aliquots of cell suspension (8×10^3 cells) are added to 0.32 cm² flat bottom wells of 96 well microtiter plates and incubated for 24 hours at 37°C in 5% CO₂ in air. The media are removed from each well by
20 aspiration, replaced by 200 μ l of DBE supplemented with factors incubated as above for a further 22 hours.

PDGF A/B, (R&D Systems Inc. Minneapolis, MN) 8ng/ml is the positive control and is used to stimulate proliferation of fibroblasts in the presence or absence of
25 pentoxifylline (Sigma Chemical Co., St. Louis, MO.).

Methyl-³H-thymidine 0.5 μ Ci (Amersham, Ontario, Canada) is added to each well and the incubation continued for an additional 2 hours. To harvest the fibroblasts, the medium is removed by aspiration and replaced by 100 μ l of
30 trypsin-EDTA for a few minutes at 37°C. This trypsinization is verified as sufficient to detach all fibroblasts. The loose fibroblasts are then aspirated onto glass fiber filters using a Brandel Cell Harvester (Xymotech

Biosystems, Ontario, Canada), washed 8-10 times with phosphate buffered saline and the radioactivity is determined by liquid scintillation. All samples are tested in quadruplicate. The ^3H -thymidine uptake assay has been
5 verified as a good measure of proliferation of fibroblasts, in response to stimuli or inhibitors, by manual cell counts and MTT assay.

The results produce a patient disease index (PDI) and a drug sensitivity index (DSI).

10

Example 2

Fibroproliferation

The fibroproliferative activity of PDGF was assessed by modification of the tritiated thymidine incorporation method of Dohlman et al. in Immunol. 52:577-
15 584 (1984) using normal human skin fibroblasts as reported by Peterson (see Hepatol. 15(2):191-197 (1992)). Briefly, cells were resuspended in Dulbecco's (DBE, Gibco, Canada), antibiotic/antimycotic (Aa, Gibco, Canada) and 0.5% CPSR-2 (Sigma Chemical Co., St. Louis, Mo.). CPSR-2 is a serum
20 replacement (control processed serum replacement-2) which has low mitogenic activity. Two hundred microliter aliquots of cell suspension (8×10^3 cells) are added to 0.32 cm^2 flat bottom wells of 96 well microtiter plates and incubated for 24 hours at 37°C in 5% CO_2 in air. The media
25 are replaced by 200 μl of DBE supplemented with factors incubated as above for a further 22 hours. PDGF B/B, (R&D Systems Inc. CA) 8ng/ml is used to stimulate proliferation of fibroblasts in the presence or absence of pentoxifylline (Sigma Chemical Co., St. Louis, MO.).

30

Monocyte conditioned medium (MCM) is prepared from monocytes obtained from patients with liver disease, as previously described by Peterson in Hepatol. 15(2):191-

197 (1992)). Samples are assessed for their ability to stimulate fibroproliferation and also assessed for the effect of pentoxifylline on that proliferative effect. Methyl-³H-thymidine 0.5 μ Ci (Amersham Canada) is added to each well, incubated for an additional 2 hours and then the cells are harvested by aspiration onto glass fiber filters using a Brandel Cell Harvester (Xymotech Biosystems, Ontario, Canada) and the radioactivity is determined by liquid scintillation. All samples are tested in quadruplicate. The ³H-thymidine uptake assay has been validated by manual cell counts and MTT assay as a measure of fibroblast number following treatment with either proliferative or antiproliferative agents (see, for example, Peterson et al., in Immunopharmacol. 28:259-270 (1994) and Denizat & Lang, in Immunol. Methods 89:271-277 (1986)).

Example 3

Assessment of immediate early gene expression

The effect of MCM obtained from patients with liver disease on *c-fos* and *c-jun* expression and the effect of pentoxifylline on this gene expression is assessed. To do this, MCM is prepared from blood samples obtained from 6 patients with liver disease as previously described (see Peterson and Isbrucker in Hepatol. 15(2):191-197 (1992)). Fibroblasts are then incubated with MCM for 30 min with and without addition of pentoxifylline prior to assessment of *c-fos* and *c-jun* mRNA.

Measurement of *c-fos* and *c-jun* mRNA is done by Northern analysis using specific oligomers. mRNA was isolated from dermal fibroblasts using Ribosept kits (VWR, Canada). RNA is subjected to electrophoresis overnight in a denaturing 1% agarose gel, transferred onto a Nytrans⁺

nylon membrane (ICN Biomedicals Inc., Montreal, Canada) and cross-linked with an ultraviolet crosslinker (Stratagene UV Stratalinker 2400). Specific probes (45-mer) for human *c-fos* (SEQ ID NO:1) and human *c-jun* (SEQ ID NO:2) as follows are obtained from Genosys:

SEQ ID NO:1:

5'-CAGCGGGAGGATGACGCCTCGTAGTCTGCGTTGAAGCCCGAGACC-3'

SEQ ID NO:2:

5'-GCATGAGTTGGCACCCACTGTTAACGTGGTTCATGACTTTCTGTT-3'

10 Probes are labelled with ^{32}P using 3'-endlabelling kit (Amersham, Canada). Blots are subsequently stripped and reprobbed with ^{32}P -labelled β -actin as internal control. Initial experiments allow one to optimize conditions and show that *c-fos* mRNA is increased in dermal fibroblasts
15 treated with PDGF for 30 min compared to controls.

The effect of pentoxifylline on PDGF stimulated *c-fos* and *c-jun* gene expression is also assessed by Northern analysis using mRNA prepared from dermal fibroblasts which had been treated with PDGF (8ng/ml) for
20 30 min (in the presence and absence of pentoxifylline) for 30 min and 2 hrs.

Statistical analysis. An unpaired Students t test is used to compare two variables, and an analysis of variance (ANOVA) and Student-Neuman-Keuls test is used when
25 more than 2 variables were compared (see Zar, J.H. in Biostatistical methods. Prentice-Hall (Englewood Cliffs, N.J., 1974)).

Results. Monocyte conditioned medium (MCM) samples obtained from six patients with liver disease are

seen to stimulate proliferation of fibroblasts 2.5-fold over basal proliferation (see Figure 1A). Incubation of MCM samples with pentoxifylline significantly reduced the effect of MCM on proliferation in an concentration related manner (see Figure 1B). Preincubation of MCM with PDGF antibody (50 μ g/ml) significantly reduced the fibroproliferative effect. This indicates that one of the components of the MCM which stimulates proliferation is PDGF (see Figure 1C).

10 The hypothesis that the stimulation of fibroproliferation which occurs with MCM samples from a liver disease patient involves an upregulation in *c-fos* gene expression due to the presence of PDGF was then tested. A Northern blot of such samples shows the effect
15 of MCM obtained from a liver disease patient on *c-fos* and *c-jun* gene expression in human fibroblasts and clearly indicates that MCM obtained from patients with liver disease increases the production of *c-fos* and *c-jun* mRNA in fibroblasts. Phosphorimaging analysis showed that MCM
20 increased *c-fos* and *c-jun* mRNA 6-fold and 2-fold, respectively.

To test the hypothesis that the upregulation in *c-fos* and *c-jun* gene expression by MCM samples obtained from a liver disease patient is downregulated by pentoxifylline, mRNA was also assessed from cells treated
25 with MCM in the presence of pentoxifylline. The results indicate that the upregulation in *c-fos* and *c-jun* which occurs due to exposure of fibroblasts to MCM obtained from patients with liver disease is not inhibited by 30 min of exposure of MCM to pentoxifylline. The results also show
30 that pentoxifylline did not alter *c-fos* and *c-jun* mRNA levels. It is of interest to note that this same

concentration of pentoxifylline was sufficient to abolish MCM stimulated fibroproliferation by 95% (see Figure 1B).

MCM contains PDGF but also contains other factors including IL-1 and activated oxygen intermediates which can stimulate proliferation and could potentially stimulate *c-fos* and/or *c-jun* and which individually may or may not be inhibited by pentoxifylline. On the other hand it has been established that PDGF stimulates proliferation and that this effect is blocked by pentoxifylline. Further study, therefore, focussed on PDGF itself. Thus, tests were conducted to determine whether the effect of PDGF on proliferation involves upregulation of immediate early genes and whether the actions of pentoxifylline are the result of changes in immediate early gene expression.

C-fos and *c-jun* gene expression in skin fibroblasts stimulated to proliferate with PDGF in the presence and absence of pentoxifylline was then assessed, compared to controls (cells stimulated with basal medium alone). Thus, the effect of PDGF, with and without pentoxifylline, on the expression of *c-fos* and *c-jun* can be visualized by Northern blot, wherein the following are compared:

- mRNA isolated from fibroblasts incubated in basal medium;
- mRNA isolated from fibroblast samples stimulated with PDGF (8ng/ml);
- mRNA from skin fibroblasts stimulated with PDGF plus pentoxifylline (3.5 mM);
- mRNA levels in skin fibroblasts stimulated with pentoxifylline alone.

The blots are probed with the 45-mer for *c-fos* described above (SEQ ID NO:1), the 45-mer for *c-jun* described above (SEQ ID NO:2), and oligomer for β -actin.

Thus, incubation of PDGF (30 min) increases *c-fos* and *c-jun* expression in skin fibroblasts. Phosphorimaging analysis shows that PDGF causes increased levels of *c-fos* and *c-jun* mRNA 2-fold and 7-fold, respectively.

5 Pentoxifylline itself did not alter *c-fos* or *c-jun* mRNA levels in control cells and did not reduce the level of *c-fos* mRNA in cells treated with PDGF. However, pentoxifylline markedly reduced *c-jun* mRNA levels in cells treated with PDGF. Phosphorimaging analysis also shows

10 that pentoxifylline decreases *c-jun* mRNA (53%) in cells treated with PDGF compared to cells treated with PDGF alone.

When the same blot is probed with a β -actin probe, equal loading in all four lanes of the blot is

15 verified. Phosphorimaging analysis of results obtained from 3 independent experiments indicates that PDGF significantly increases the expression of *c-jun* and *c-fos*, and that pentoxifylline significantly reduces the expression of *c-jun*, but does not alter *c-fos* expression

20 induced by PDGF.

Cells treated in the same manner were also assessed for fibroproliferative activity. The results (see Figure 2) verify that this concentration of PDGF (8ng/ml) stimulates proliferation and that this concentration of

25 pentoxifylline (3.5mM) blocks proliferation of fibroblasts.

Example 4

Assessment of the Effect of Antisense *c-jun* Oligonucleotide on PDGF-stimulated Proliferation of Fibroblasts

30 To verify the involvement of *c-jun* in the stimulation of proliferation by PDGF, experiments were

conducted using antisense *c-jun* oligonucleotides (OGNs) according to the method of Yoshida et al. (Molec Cell Biol 17(7):4014-4023 (1997)). An antisense phosphothioate analog of the oligonucleotides to the 5' end of *c-jun* was used. The OGN was obtained from Genesys:

SEQ ID NO:3

5' -GCAGTCATAGAACAGTCCGTCACCTTCACGT-3'

Incubation of human microvascular endothelial cells with 20 μ M of this antisense *c-jun* oligonucleotide specifically decreased cellular levels of c-Jun as assessed on SDS polyacrylamide gel using a specific c-Jun antibody.

To assess the role of *c-jun* in the stimulation of proliferation, dermal fibroblast cells which had been grown in Dulbecco's containing 10% CPSR-1 were transferred to Dulbecco's containing 10% CPSR-2, 24 hours prior to assay. The cells were then trypsinized and plated onto 96 well plates at a density of 40,000 cells per ml in a total volume of 200 μ l. 24 h later the Dulbecco's medium was removed and cells were incubated with antisense *c-jun* OGN in 150 mM NaCl. The antisense *c-jun* OGN was administered in 20 μ l volume in concentrations ranging from 2nM to 20 μ M. Following this, 80 μ l of Dulbecco's medium supplemented with 0.5% CPSR-2 was added to each well. The plates were then incubated for 3 hours at 37°, following which PDGF was added to the wells in DBE plus 0.5% CPSR-2 (final concentration PDGF 8 ng/ml) to stimulate proliferation. Incubation was continued for 24 hours, during the last two hours of which tritiated thymidine was

added to each well. At 24 hours, cells were harvested as described above for the fibroproliferation assay. Trypan blue exclusion tests were done to test for potential toxicity. Incubation of cells with antisense *c-jun* OGN (up
5 to 20 μ M) did not affect viability as assessed by trypan blue exclusion.

Treatment of dermal fibroblasts with antisense *c-jun* OGN significantly reduced PDGF-stimulated proliferation by 85% (Figure 6). This almost total block
10 of PDGF-stimulated proliferation by antisense *c-jun* OGN, indicates that *c-jun* plays a critical role in PDGF-stimulated proliferation. In these experiments, pentoxifylline (0.066 mg/ml) reduced PDGF-stimulated proliferation by 40%. In human fibroblasts that were
15 treated with *c-jun* antisense plus pentoxifylline, PDGF-stimulated proliferation was reduced by approximately 88% suggesting that antisense *c-jun* ODN and pentoxifylline were less than additive but this may reflect the major knockout of the *c-jun* by antisense *c-jun* OGN using the
20 20 μ M concentration, thus masking any further effects due to addition of pentoxifylline. These results indicate that treatment of cells with antisense *c-jun* OGN (2 μ M) also blocked PDGF-stimulated proliferation and that antisense *c-jun* oligonucleotides (2 μ M and 20 μ M) inhibited basal
25 proliferation. Incubation of cells with a lower concentration of antisense oligonucleotide (1 μ M) inhibited PDGF-stimulated proliferation (35%) while not affecting basal proliferation.

Example 5Effect of pentoxifylline on C-kinase pathway

To investigate the possibility that the effect of pentoxifylline on proliferation was due to direct action on the C-kinase pathway, a series of experiments were designed using C-kinase inhibitor H7 (1-(5-isoquinolinylsulfonyl)-2-methyl-piperazine, Sigma Chemical Co., St. Louis, MO). To investigate the role of the C-kinase pathway in PDGF-stimulated proliferation, fibroblasts were pre-incubated with H7 in increasing concentrations (at concentrations ranging from 0.1 μ M to 50 μ M) for 30 minutes prior to stimulation with PDGF. H7 (10 μ M and 50 μ M) was toxic to fibroblasts while H7 (0.1 μ M and 1 μ M) was not toxic to fibroblasts as determined by trypan blue exclusion.

Assessment of tritiated thymidine uptake showed that H7 at 1 μ M and 0.1 μ M did not significantly affect PDGF-stimulated proliferation. These results suggest that the C-kinase pathway does not play a critical role in PDGF-stimulated proliferation and would argue against a C-kinase pathway as a target for pentoxifylline. To further substantiate this effect, experiments were done where fibroblasts were incubated with pentoxifylline (0.066 mg/ml), where fibroblasts were incubated with the combination of pentoxifylline (0.066 mg/ml) and H7 (1 μ M) and where fibroblasts were incubated with H7 at 1 μ M alone. As suggested previously, H7 (1 μ M) did not inhibit PDGF-stimulated fibroproliferation. Pentoxifylline (0.066 mg/ml) alone decreased PDGF-stimulated proliferation (37%). The combination of pentoxifylline (0.066 mg/ml) and

H7 (1 M) resulted in a more dramatic effect on PDGF-stimulated proliferation i.e., H7 plus pentoxifylline reduced PDGF-stimulated fibroproliferation by 54%. This potentiation of the effect of pentoxifylline further suggests that pentoxifylline and H7 do not work on the same pathway since the two drugs were supra-additive. Such a synergistic effect could be explained by a simultaneous block at sequential steps or an effect where one drug inhibits an enzyme that destroys the second drug or an effect where one drug promotes entry of a second drug through the cell membrane.

The combination of pentoxifylline and H7 suggests that pentoxifylline and H7 do not work at the same point in the PDGF-stimulated proliferation pathway. Also because H7 is a cyclic AMP-dependent kinase inhibitor, these results further suggest that the activity of pentoxifylline on PDGF-stimulated proliferation is likely not due to its effect on phosphodiesterase and therefore is not dependent on an increase in cyclic AMP. These results are consistent with previous studies which suggest that the effect of PTX on PDGF driven proliferation was cAMP-independent. These results further demonstrate the synergistic effect of combining such compounds (i.e., pentoxifylline and H7) compared to the effects of each compound (pentoxifylline and H7 alone) on PDGF-stimulated proliferation.

Example 6 Collagen Assay

Collagen concentration in colonic, brain or heart tissue is determined using the method of Lopez de Leon and

Rojkind (see J. Histochem. and Cytochem. 33(8):737-743 (1985)). Colons are dissected from rats and rolled longitudinally, fixed in formalin or glutaraldehyde, and embedded in paraffin for sectioning. Brains or hearts are
5 dissected from rats and fixed in formalin or glutaraldehyde. Heart tissue is further embedded in paraffin for sectioning. Sections of colon, brain or heart (8mm) are deparaffinized, if paraffined for sectioning, and stained with Sirius red and Fast green. Sirius red stains
10 collagenous protein while Fast green stains non-collagenous protein. The dyes are then removed from these sections using sodium hydroxide methanol solution and the collagen concentration determined with reference to the maximal absorbance of Sirius red and Fast green.

15 While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

SEQ ID NO:1:

5' -CAGCGGGAGGATGACGCCTCGTAGTCTGCGTTGAAGCCCGAGACC-3'

SEQ ID NO:2:

5' -GCATGAGTTGGCACCCACTGTTAACGTGGTTCATGACTTTCTGTT-3'

SEQ ID NO:3

5' -GCAGTCATAGAACAGTCCGTCACTTCACGT-3'

That which is claimed is:

1. A method for the treatment of a subject afflicted with a disease or condition characterized by one or more of the following:

elevated platelet derived growth factor (PDGF)

5 levels,

elevation of Jun kinase,

elevation of c-Jun,

activation of NF-kappaB or NF-kappaB p65,

neutrophil infiltration, or

10 elevated levels of inflammatory cytokine(s),

provided, however, that said disease or condition is not collagenous colitis, said method comprising administering to said subject an amount of a compound which reduces c-jun gene expression effective to ameliorate the symptoms of said disease.

2. A method according to claim 1 wherein said disease or condition is interstitial lung disease, human fibrotic lung disease, human kidney disease, glomerular nephritis, nephritis associated with systemic lupus, 5 peritoneal fibrosis, liver fibrosis, myocardial fibrosis, pulmonary fibrosis, Grave's ophthalmopathy, drug induced ergotism, cardiovascular disease, cancer, Alzheimer's disease, scarring, scleroderma, glioblastoma in Li-Fraumeni syndrome, sporadic glioblastoma, myeloid leukemia, acute 10 myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, gynecological cancer, Kaposi's sarcoma, Hansen's disease or inflammatory bowel disease not including collagenous colitis.

3. A method according to claim 1 wherein said compound which reduces *c-jun* gene expression is delivered orally, transdermally, intravenously, intramuscularly, topically, by inhalation or rectally.

4. A method according to claim 1 wherein said compound which reduces *c-jun* gene expression is delivered orally.

5. A method according to claim 1 wherein an effective amount of said compound which reduces *c-jun* gene expression comprises in the range of about 200 mg - 1 g per dose, administered to the subject at least two times a day.

6. A method according to claim 5 wherein said compound which reduces *c-jun* gene expression is administered to said subject at least four times a day.

7. A method according to claim 1 wherein said compound which reduces *c-jun* gene expression is administered to the subject by sustained release.

8. A method according to claim 1, wherein said compound which reduces *c-jun* gene expression is pentoxifylline or functional derivatives/metabolites thereof.

9. A method according to claim 1 wherein said pentoxifylline or functional derivatives/metabolites thereof are selected from the group consisting of 1-(5-oxohexyl)-3,7-dimethylxanthine (pentoxifylline),

- 5 1-(5-hydroxyhexyl)-3,7-dimethylxanthine (metabolite-1), and propentofylline.

10. A method according to claim 1, wherein said compound which reduces *c-jun* gene expression is an antisense *c-jun* oligonucleotide.

11. A method according to claim 1 wherein said compound which reduces *c-jun* gene expression is administered in combination with an inhibitor of cytochrome P-450.

12. A method according to claim 1, wherein said compound which reduces *c-jun* gene expression is administered in combination with a kinase inhibitor.

13. A method according to claim 12, wherein said compound is a cyclic AMP-dependent kinase inhibitor.

14. A method according to claim 1 further comprising monitoring the level of a compound which reduces *c-jun* gene expression in serum from said patient, and adjusting the dosage of said compound based on the level detected in the patient's serum.

15. An in vitro assay to determine whether a compound which reduces *c-jun* gene expression is likely to be effective for treatment of a subject afflicted with a disease and/or condition characterized by one or more of
5 the following:

elevated PDGF levels,

elevation of Jun kinase,
elevation of c-Jun,
activation of NF-kappaB or NF-kappaB p65,
10 neutrophil infiltration, or
elevated levels of inflammatory cytokine(s),
said method comprising determining the uptake by test cells
in suitable media therefor, of a labeled building block
indicative of cell proliferation, wherein said uptake is
15 determined in the presence of serum obtained from said
subject, and in the presence and absence of said compound,
wherein test cells are selected from the group
consisting of fibroblasts, glial cells, smooth muscle cells
and transformed cells obtained from the particular organ of
20 interest, and
wherein reduction of uptake by test cells of
labeled building block in the presence of said compound,
relative to the uptake by test cells of labeled building
block in the absence of said compound, is predictive of the
25 efficacy of said compound for the treatment of diseases or
conditions characterized as set forth above.

16. An assay according to claim 15 wherein said
labelled building block is tritiated thymidine.

17. An assay according to claim 15 wherein said
test cell is selected from liver myofibroblasts, pulmonary
fibroblasts, smooth muscle cells, mesenchymal cells, glial
cells, intestinal fibroblasts, intestinal smooth muscle
5 cells, cultured human skin fibroblasts, vascular smooth
muscle cells, mesangial cells, hematopoietic cells, Kaposi
sarcoma-derived cells or epithelial cells.

18. An assay according to claim 15 wherein said test cells comprise a monolayer of cultured human skin fibroblasts.

19. An assay according to claim 15 wherein said test cells comprise confluent intestinal smooth muscle cells.

20. A kit useful for assays to determine whether pentoxifylline or functional derivatives/metabolites thereof are likely to be effective for treatment of diseases and/or conditions characterized by one or more of
5 the following:

 elevated PDGF levels,
 elevation of Jun kinase,
 elevation of c-Jun,
 activation of NF-kappaB or NF-kappaB p65,
10 neutrophil infiltration, or
 elevated levels of inflammatory cytokine(s),

said kit comprising:

 cultured cells selected from fibroblasts, glial
 cells, smooth muscle cells and transformed
15 cells obtained from the particular organ of
 interest in suitable assay medium,
 a compound which reduces *c-jun* gene expression,
 and
 labeled building block indicative of cell
 proliferation.

21. A kit according to claim 20 wherein said cultured cells are selected from human skin fibroblasts, intestinal smooth muscle cells, liver myofibroblasts, pulmonary fibroblasts, mesenchymal cells, glial cells, 5 intestinal fibroblasts, intestinal smooth muscle cells, vascular smooth muscle cells, mesangial cells, hematopoietic cells, Kaposi sarcoma-derived cells or epithelial cells.

22. A kit according to claim 20 wherein said labeled building block is tritiated thymidine or bromouridine.

23. A kit according to claim 20 further comprising PDGF-AA, PDGF-BB, PDGF-AB, TNF, IL-1, IL-4, IL-12, IGF-1, IGF-2, TGF-alpha, TGF-beta, EGF, NGF, aFGF or bFGF.

24. A method to quantify the collagen content of a tissue sample, said method comprising:

optionally rolling said tissue sample longitudinally,

5 fixing said sample, and

optionally embedding said sample in paraffin, sectioning said sample,

deparaffinizing a section of said sample, if paraffined,

10 staining said sample with Sirius red and Fast green,

removing said dyes from the stained sections, and

determining the collagen concentration with reference to the maximal absorbance of Sirius red and Fast green.

1/8

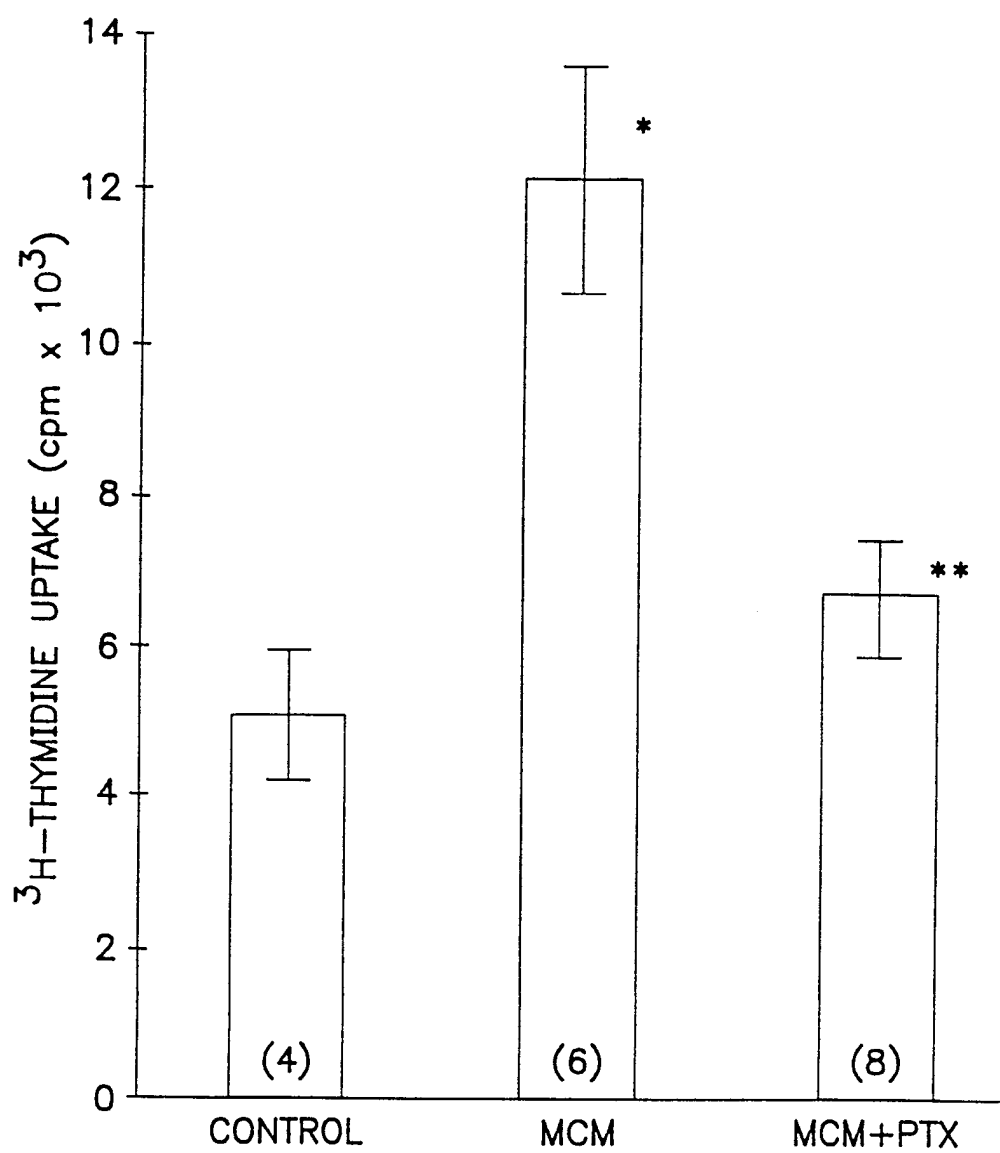


FIG. 1A

2/8

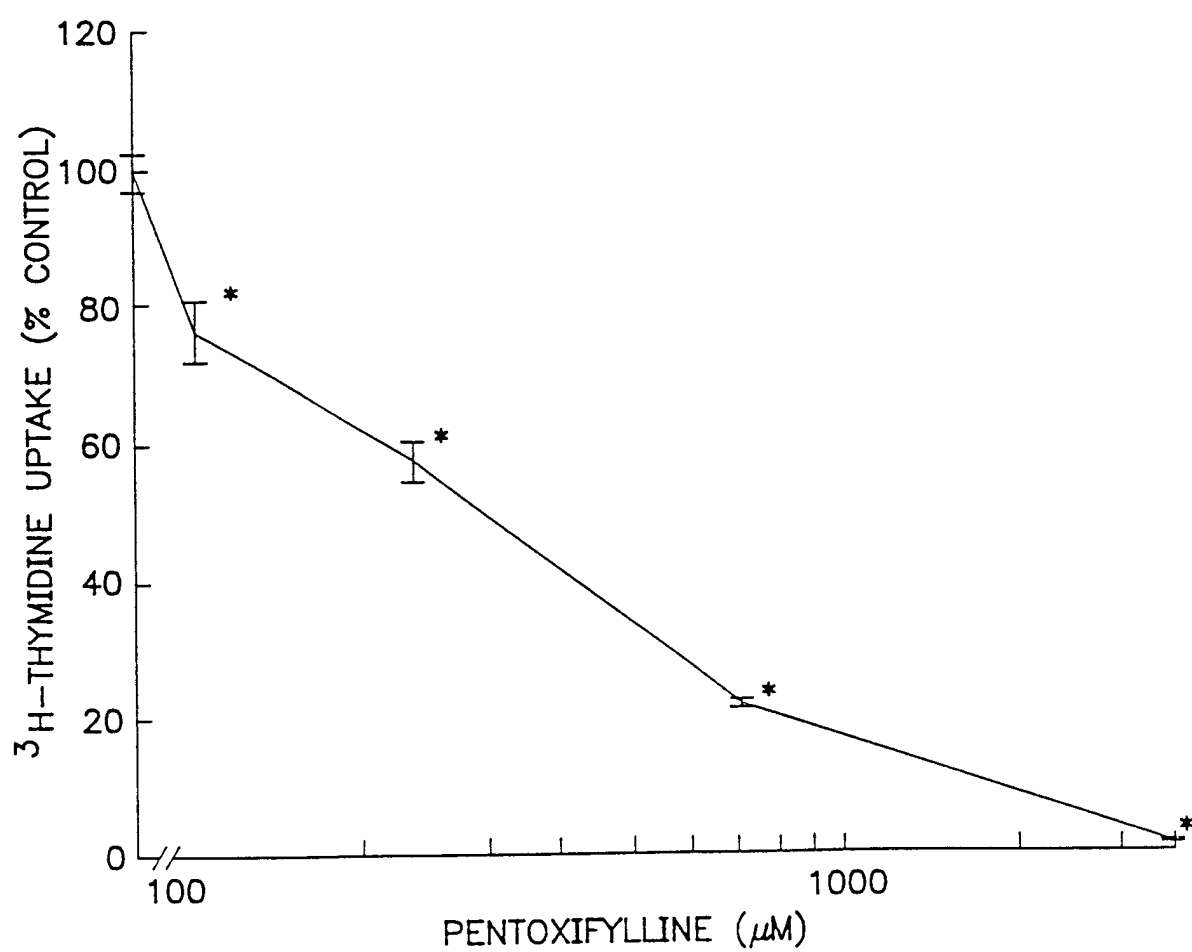


FIG. 1B

3/8

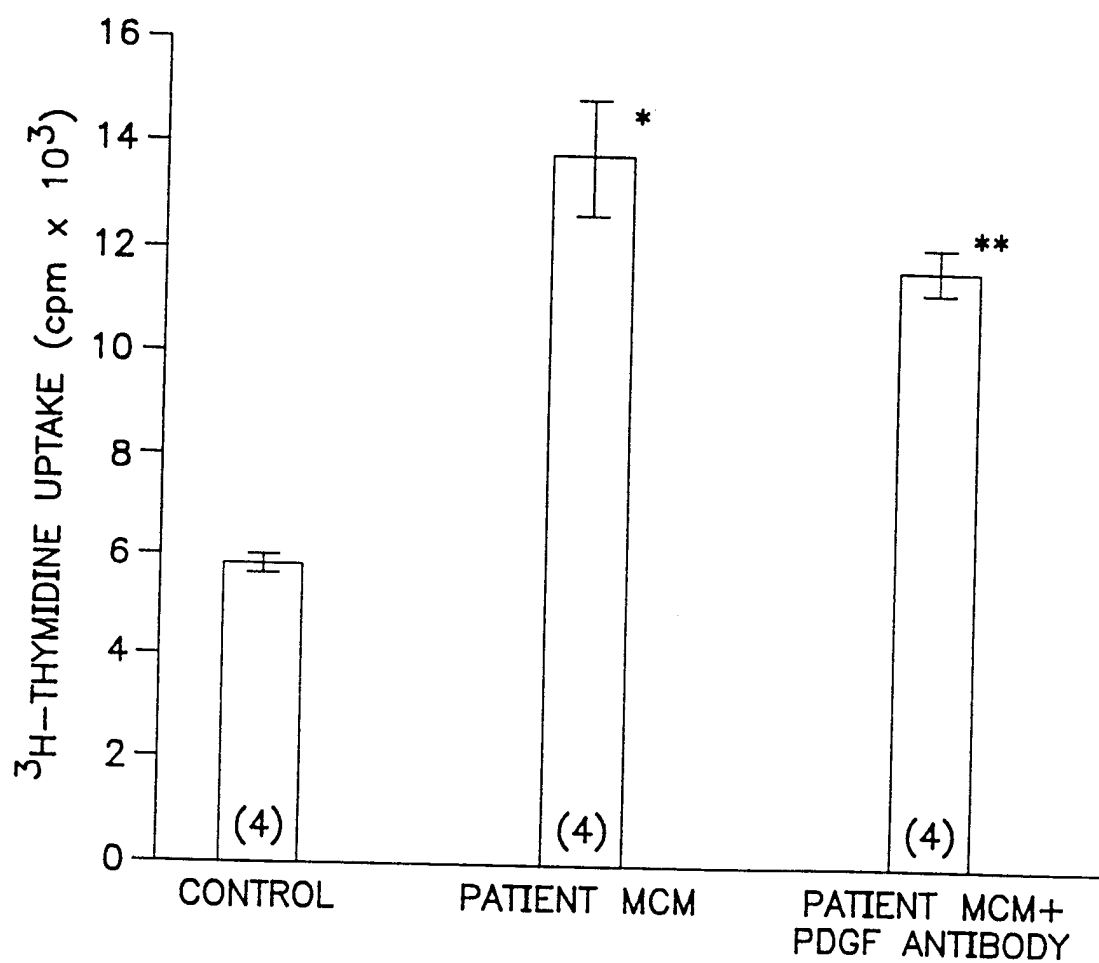


FIG. 1C

4/8

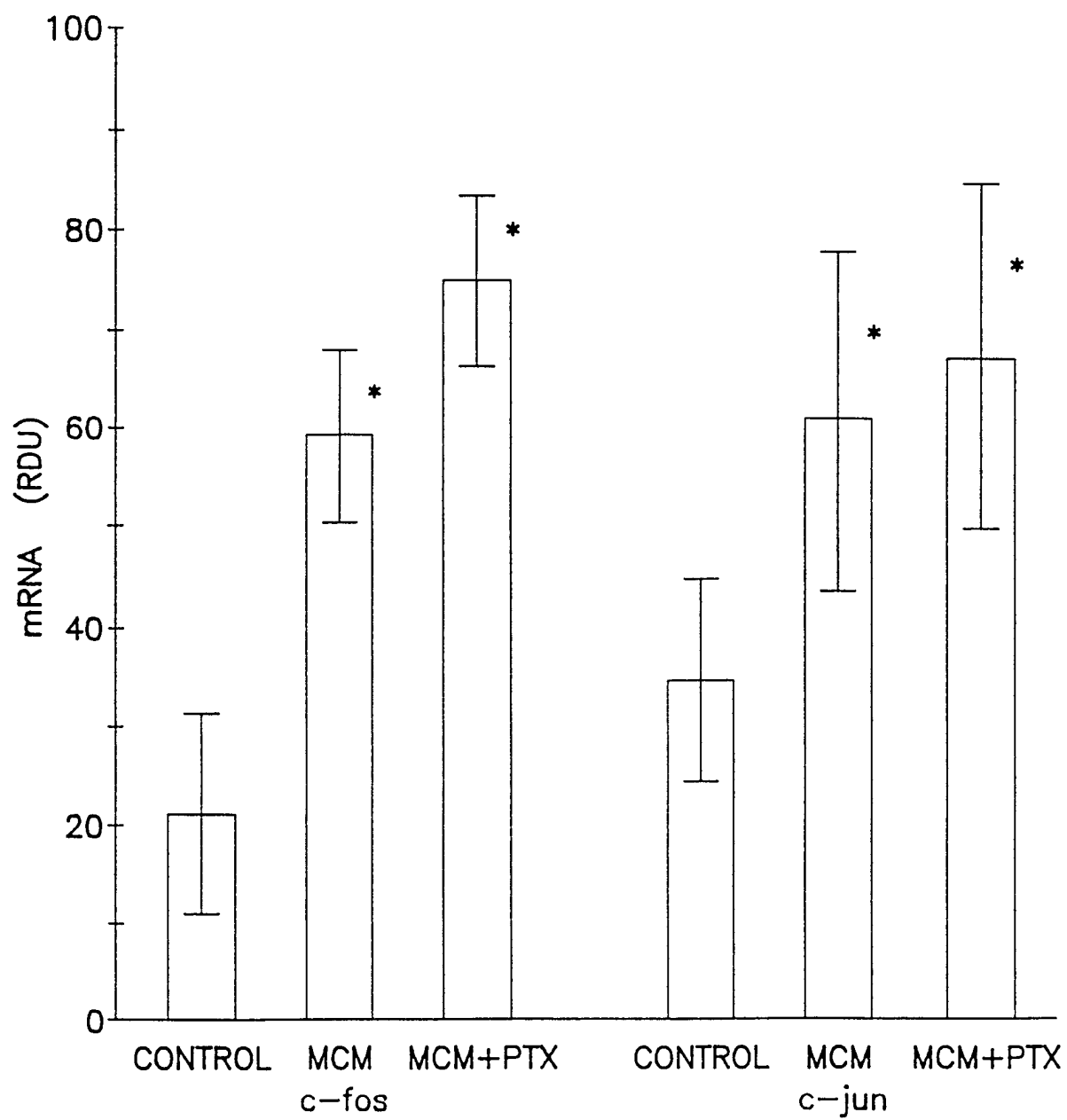


FIG. 2

5/8

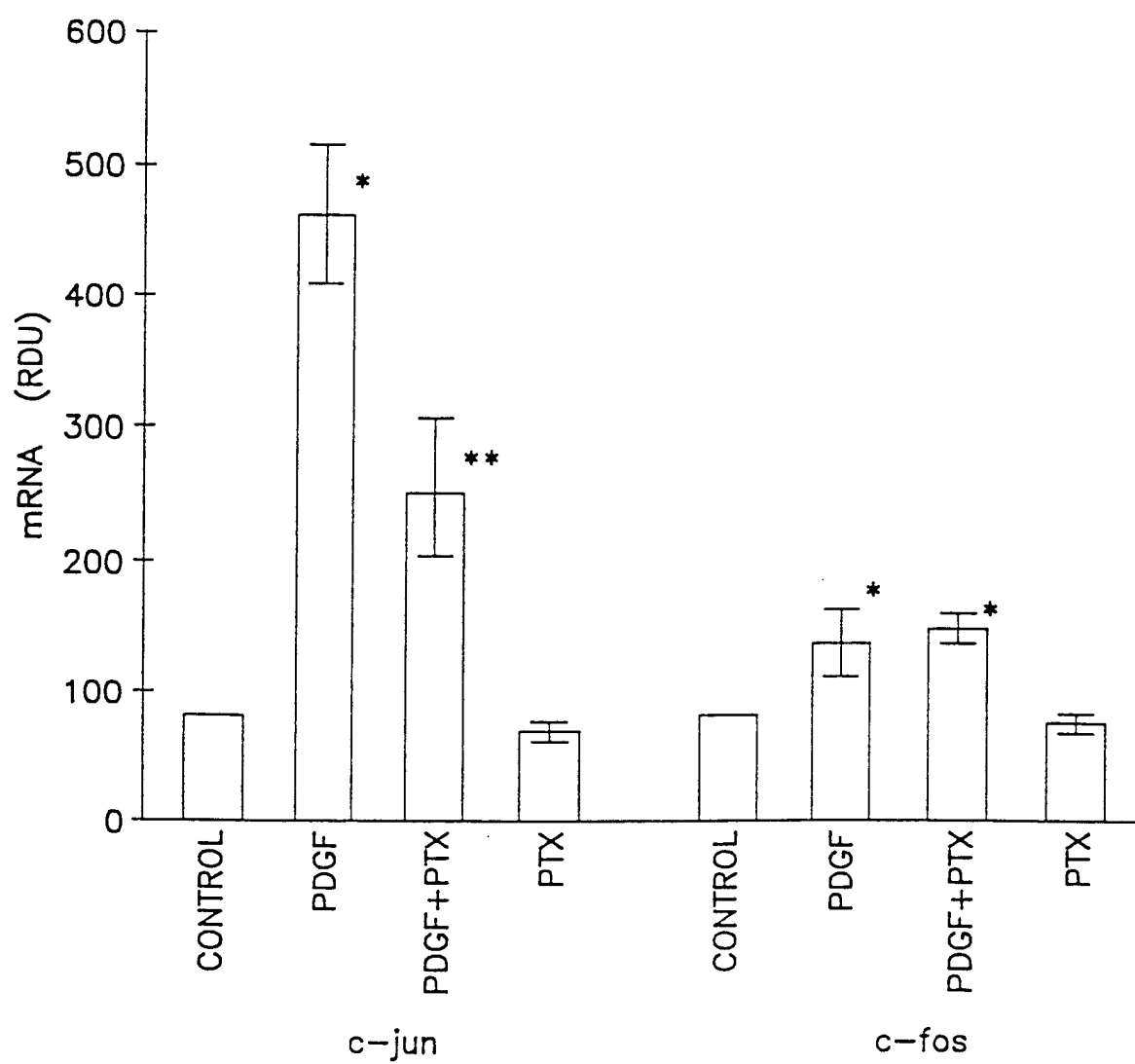


FIG. 3

6/8

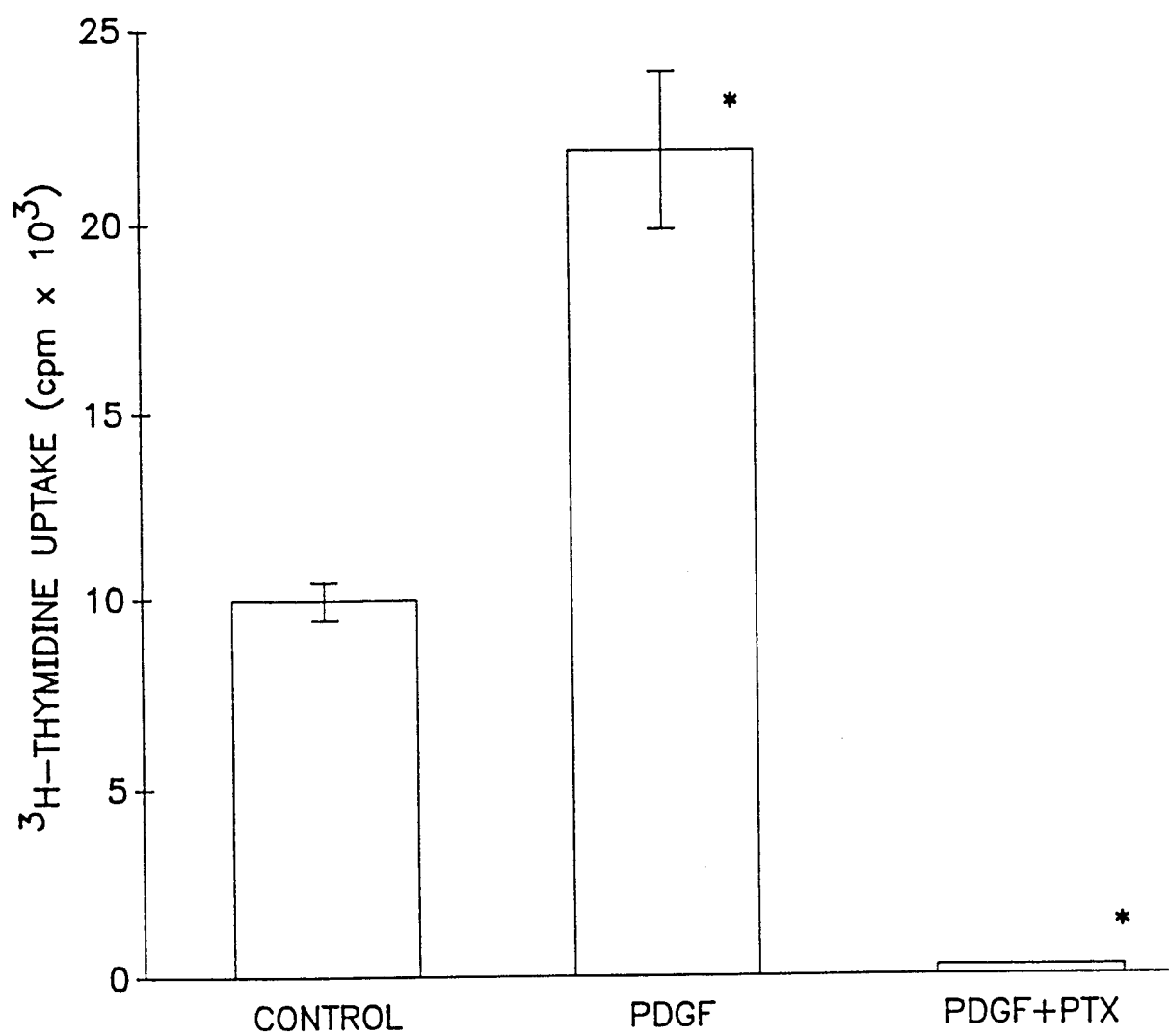


FIG. 4

7/8

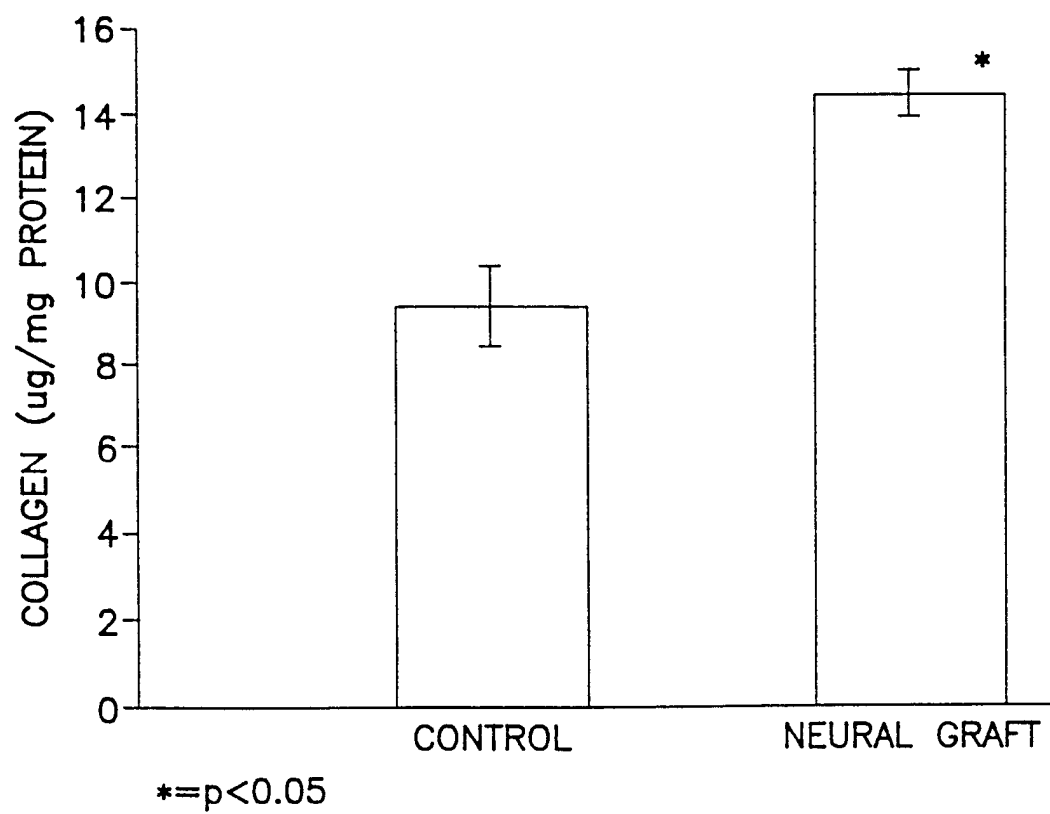


FIG. 5

8/8

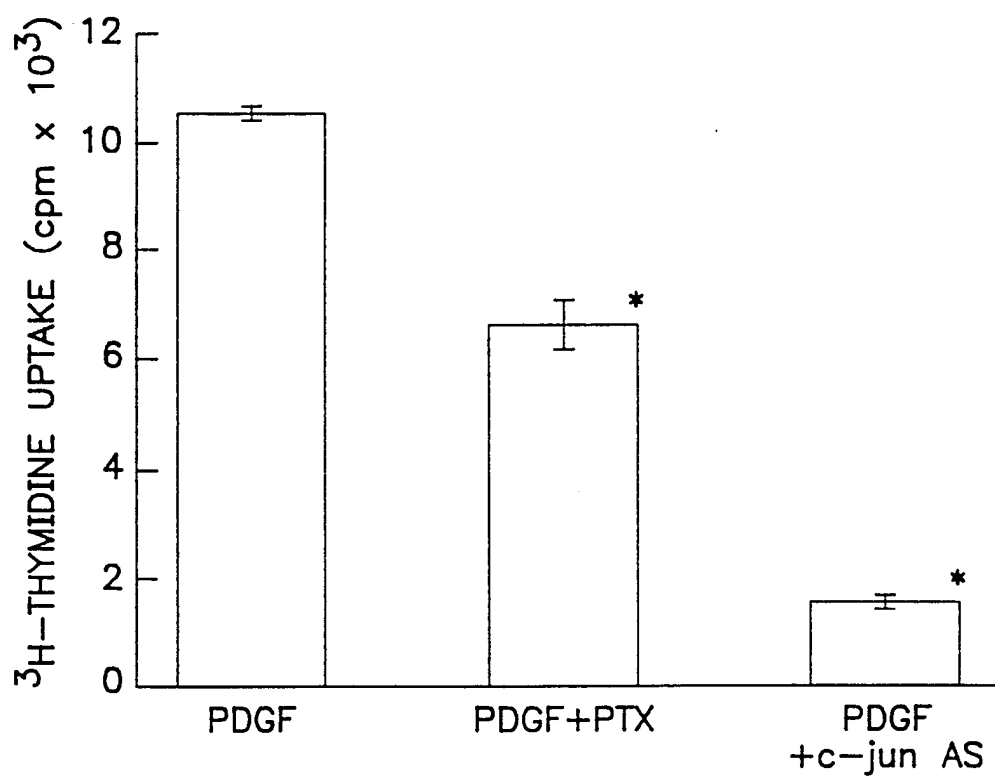


FIG. 6