1-

**PHARMACEUTICAL CARE OF PATIENTS WITH HEPATOCELLULAR CARCINOMA: COULD GAGS BE USEFUL AS A DIAGNOSTIC AND PROGNOSTIC TUMOR MARKER?**

Despite the very high incidence of hepatocellular carcinoma (HCC) in Egypt, surgery is considered as the only curative treatment option. However, most patients are diagnosed at advanced stages thereby surgery will be of little value. The aim of the current study was to provide a pharmaceutical care for patients with HCC who were treated with the investigational drug mistletoe as well as to assess whether glycosaminoglycans (GAGs) could be useful as an effective screening tumor marker for early diagnosis and to investigate the significance of total GAGs serum concentrations in patients with HCC. Blood samples were collected from three groups: the first group (n=50) with HCC, the second with cirrhosis (n=15) and the third was a control (n=15). Liver and kidney functions laboratory tests and total GAGs concentrations were measured. Only 23% of the patients achieved objective responses to the investigational drug but monitoring of the supportive treatments as well as drug side effects and toxicities were strongly recommended. Patients with HCC showed a significant increase in serum GAGs as compared with the control group (p<0.01). However, there were no significant differences between cirrhotic group HCC group or with control group. A significant positive correlation between serum level of GAGs and tumor size (r = 0.39, p<0.005) was observed, however there are no correlations between serum concentration of GAGs with other patients or with tumor characteristics. No significant relationship was found between serum level of GAGs and overall survival. We could conclude that pharmaceutical care is very important in patients with HCC to improve their quality of life and serum level of GAGs may have a role in HCC, and their serum level may be related to a high tumor burden.

2-

**THE ROLE OF SOME BIOCHEMICAL MARKERS IN PREDICTING BONE METASTASES IN BREAST CANCER PATIENTS**

The present study aims to assess serum levels of BALP, CA 15-3, TGF-pi and PTHrP in female patients located into stages II and IV of breast cancer in order to evaluate the role of these substances in the development and pathogenesis of bone metastases. This study was performed on 66 female patients with breast cancer, 42 patients with stage II and 24 patients with stage IV breast cancer (12 with bone metastases and 12 with non bone metastases). 12 healthy women was used as control group. Liver function tests, kidney function tests and bone metabolism markers (serum calcium, phosphorous and bone specific alkaline phosphatase levels) were determined. Urinary calcium/creatinine & phosphorous/creatinine ratios and the incidence of proteinuria were calculated. Serum CA 15-3, TGF-pl and PTHrP levels were estimated by enzyme linked immunosorbent assay (ELISA). Breast cancer patients with non bone metastases exhibited an increase in serum ALT and AST activities and globulin concentration and a decrease in serum total protein, albumin and A/G ratio in comparison with other breast cancer patients. Serum levels of creatinine & uric acid and incidence of proteinuria were increased in breast cancer patients, while serum phosphorous level was decreased in breast cancer when compared with control group.
Urinary calcium/creatinine ratio was elevated significantly (p<0.05) in stage IV bone metastases patients when compared with stage II. Bone metastases patients were characterized by highly (p<0.001) elevated serum total alkaline phosphatase and BALP levels than non bone metastases patients. CA 15-3 level was highly increased in stage IV patients either with bone or non bone metastases when compared with either stage II or control groups. Serum TGF-pM and PTHrP levels increased significantly (p<0.05) in breast cancer patients as compared to that value in control subjects. Only PTHrP showed a significant increase in serum of stage IV patients as compared with the stage II patients and it was useful to differentiate between bone metastases and non bone metastases patients. Total alkaline phosphatase and PTHrP levels showed significant increase in hypercalcemic patients compared with normocalcemic patients in stage IV with bone metastases, but serum level of TGF-fjl had no significant change in predicting bone metastases or incidence of hypercalcemia. Total alkaline phosphatase, BALP and PTHrP are good predictors for bone metastases in breast cancer patients. Urinary calcium/creatinine ratio was very useful marker in differentiation between early and advanced stages of breast cancer. Also, it is a promising and sensitive marker for differentiation between hypercalcemic and normocalcemic patients whatever the stage of the disease.

3-

Some Glycoproteins, Glycoprotein Derivatives and Their Degrading Enzymes in Egyptian Patients with Cirrhosis and Hepatocellular Carcinoma

The glycosylation of proteins is changed in tumor cells during the development of hepatocellular carcinoma (HCC). Such alterations in the structure of glycoproteins and other glycoconjugates on the surfaces of cancer cells are important in the phenomenon of metastasis. Therefore, the diagnostic accuracy of some glycoproteins (α-fetoprotein and vitronectin), some of their degrading enzymes (hyaluronidase and α2-glucuronidase) and some of their degradation products (hyaluronic acid, sialic acid and glucuronic acid) was assessed as biochemical markers of HCC in 90 HCC patients, 45 cirrhotic patients in addition to 45 healthy subjects as a control group. In the present study, circulating level of vitronectin was decreased significantly in both cirrhosis and HCC, without significant difference observed between cirrhotic and HCC patients. Serum hyaluronic acid, sialic acid and glucuronic acid were significantly increased in patients with cirrhosis and HCC when compared to control group, also a significant increase was observed in serum level of these acids in HCC group compared to cirrhotic group. Serum α2-glucuronidase was increased significantly in HCC group in comparison with control group, while serum hyaluronidase activity was decreased significantly in HCC group when compared to control group. In conclusion, serum vitronectin level reflects the degree of hepatic dysfunction in cirrhosis and HCC, but it is not useful as a tumour marker in screening for HCC patients, since it is not able to differentiate HCC patients from cirrhotic patients. Serum levels of hyaluronic acid, sialic acid and glucuronic acid could be used in diagnosis of HCC. In addition, hyaluronic acid could be used to indicate the severity of liver damage. Further studies are necessary to elucidate the biological and clinicopathological significance of estimating lysosomal enzymes in patients with HCC.

4-
Alteration of growth factors and neuronal death in diabetic retinopathy: what we have learned so far

PURPOSE: Diabetic retinopathy (DR) is a leading cause of blindness in American adults. Over the years, DR has been perceived as a vascular disease characterized by vascular permeability, macular edema, and neovascularization that can lead to blindness. Relatively new research on neurodegeneration is expanding our views of the pathogenesis of DR. Evidence has begun to point to the fact that even before vascular complications begin to manifest, neuronal cell death and dysfunction have already begun. Based on the literature and our own studies, we address whether neuronal death is associated with loss of neurotrophic support due to less production of a given growth factor or due to impairment of its signaling events regardless of the level of the growth factor itself.

METHODS: In this article we aimed to review the literature that looks at the neuronal side of DR and whether retinal neurons are adversely affected due to the lack of neurotrophic levels or activity. In particular, we examine the research looking at insulin, insulin-like growth factor, vascular endothelial growth factor, pigment epithelium-derived growth factor, brain-derived neurotrophic factor, and nerve growth factor.

RESULTS: Research shows that insulin has neurotrophic properties and that the loss of its pro-survival pathways may have a role in diabetic retinopathy. There is also evidence to suggest that exogenously administered insulin may have a role in the treatment of DR. Insulin-like growth factor has been shown to have a role in retinal neurogenesis and there is early evidence that it may also have neuroprotective effects. While there is evidence of neuroprotective effects of vascular endothelial growth factor, paradoxically, there is also an increased amount of apoptotic activity in retinal neurons despite an increased level of VEGF in the diabetic eye. Further research is necessary to elucidate the exact mechanisms involved. Pigment epithelium derived growth factor has retinal neuroprotective effects and shows evidence that it may be an avenue for future therapeutic use in DR. Brain-derived growth factor has been shown to have neuroprotective effects in the retina and there is also some evidence in diabetic rats that it may have some therapeutic potential in treating DR. Nerve growth factor has also been shown to have neuroprotective effects and research has begun to elucidate some of the pathways and mechanisms through which these effects occur.

CONCLUSIONS: Research has shown that there is some degree of neuronal death involved in DR. It is also evident that there are many growth factors involved in this process. Some of these growth factors have shown some potential as future therapeutic targets in DR. These findings should encourage further investigation into the mechanism of these growth factors, their potential for therapy, and the possibility of a new horizon in the clinical care of DR.

Diabetes-induced peroxynitrite impairs the balance of pro-nerve growth factor and nerve growth factor, and causes neurovascular injury

AIMS/HYPOTHESIS: Diabetic retinopathy, the leading cause of blindness in working-
age Americans, is characterised by reduced neurotrophic support and increased proinflammatory cytokines, resulting in neurotoxicity and vascular permeability. We sought to elucidate how oxidative stress impairs homeostasis of nerve growth factor (NGF) and its precursor, proform of NGF (proNGF), to cause neurovascular dysfunction in the eye of diabetic patients.

METHODS: Levels of NGF and proNGF were examined in samples from human patients, from retinal Müller glial cell line culture cells and from streptozotocin-induced diabetic animals treated with and without atorvastatin (10 mg/kg daily, per os) or 5,10,15,20-tetrakis (4-sulfonatophenyl)porphyrinato iron (III) chloride (FeTPPs) (15 mg/kg daily, i.p.) for 4 weeks. Neuronal death and vascular permeability were assessed by TUNEL and extravasation of BSA-fluorescein.

RESULTS: Diabetes-induced peroxynitrite formation impaired production and activity of matrix metalloproteinase-7 (MMP-7), which cleaves proNGF extracellularly, leading to accumulation of proNGF and reducing NGF in samples from diabetic retinopathy patients and experimental models. Treatment of diabetic animals with atorvastatin exerted similar protective effects that blocked peroxynitrite using FeTPPs, restoring activity of MMP-7 and hence the balance between proNGF and NGF. These effects were associated with preservation of blood-retinal barrier integrity, preventing neuronal cell death and blocking activation of RhoA and p38 mitogen-activated protein kinase (p38MAPK) in experimental and human samples.

CONCLUSIONS/INTERPRETATION: Oxidative stress plays an unrecognised role in causing accumulation of proNGF, which can activate a common pathway, RhoA/p38MAPK, to mediate neurovascular injury. Oral statin therapy shows promise for treatment of diabetic retinopathy.

Epicatechin blocks pro-nerve growth factor (proNGF)-mediated retinal neurodegeneration via inhibition of p75 neurotrophin receptor proNGF expression in a rat model of diabetes

AIMS/HYPOTHESIS: Accumulation of pro-nerve growth factor (NGF), the pro form of NGF, has been detected in neurodegenerative diseases. However, the role of proNGF in the diabetic retina and the molecular mechanisms by which proNGF causes retinal neurodegeneration remain unknown. The aim of this study was to elucidate the role of proNGF in neuroglial activation and to examine the neuroprotective effects of epicatechin, a selective inhibitor of tyrosine nitration, in an experimental rat model of diabetes.

METHODS: Expression of proNGF and its receptors was examined in retinas from streptozotocin-induced diabetic rats, and in retinal Müller and retinal ganglion cells (RGCs). RGC death was assessed by TUNEL and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays in diabetic retinas and cell culture. Nitrotyrosine was determined using Slot-blot. Activation of the tyrosine kinase A (TrkA) receptor and p38 mitogen-activated protein kinase (p38MAPK) was assessed by western blot.
RESULTS: Diabetes-induced peroxynitrite impaired phosphorylation of TrkB-Y490 via tyrosine nitration, activated glial cells and increased expression of proNGF and its receptor, p75 neurotrophin receptor (p75(NTR)), in vivo and in Müller cells. These effects were associated with activation of p38MAPK, cleaved poly-(ADP-ribose) polymerase and RGC death. Treatment of diabetic animals with epicatechin (100 mg kg(-1) day(-1), orally) blocked these effects and restored neuronal survival. Co-cultures of RGCs with conditioned medium of activated Müller cells significantly reduced RGC viability (44%). Silencing expression of p75(NTR) by use of small interfering RNA protected against high glucose- and proNGF-induced apoptosis in RGC cultures.

CONCLUSIONS/INTERPRETATION: Diabetes-induced peroxynitrite stimulates p75(NTR) and proNGF expression in Müller cells. It also impairs TrkB receptor phosphorylation and activates the p75(NTR) apoptotic pathway in RGCs, leading to neuronal cell death. These effects were blocked by epicatechin, a safe dietary supplement, suggesting its potential therapeutic use in diabetic patients.

Impact of Lifestyle and Oxidative Stress on the Management of Patients with Type II Diabetes Mellitus

Purpose: This study was conducted to investigate the effect of different lifestyle factors (e.g., diet, smoking, caffeine consumption & physical activities) and oxidative stress on the management of patients with T2DM; aiming to give those patients the best plan for behavioral and lifestyle conduct to decrease the morbidity and severity of their illness.

Methods: Patient history was taken for 102 (44 females and 58 males) patients who suffered from type 2 diabetes mellitus (T2DM). Those patients were interviewed for completion of a standardized questionnaire regarding personal medical history, current treatments and lifestyle behaviors especially diet, caffeine consumption, smoking habits as well as their physical activities. Blood samples were taken and used for measurement of the study parameters e.g., glucose and HbA1c as well as to detect any drug-drug interactions. Lipid profile, hydrogen peroxide, malondialdehyde (MDA) level, catalase and glutathione peroxidase (GPx) activities were also estimated.

Results: Only 70 patients (30 females and 40 males) aged between 36 and 70 years (mean±SE = 52.940.75±1 years) continued our research study. No patient was initially responded to dietary modification alone. All patients required pharmacological treatment to control their blood glucose level. No severe adverse effects nor significant drug-drug interactions were found in the treated patients. Smoker patients showed a significant increase in serum glucose, HbA1c, oxidative stress markers as hydrogen peroxide, MDA and antioxidant activity like catalase and GPx than non-smoker patients (p<0.05).

Conclusion: Patients with T2DM should be educated about the vital role of lifestyle in the management of this illness. From our results, it was concluded that, restricted diet could improve patient’s glycemic control, lipid profile and antioxidant activity. Smoking increases both oxidative stress and antioxidant enzymes activities. Also, caffeine consumption leads to improvement in lipid profile however, it has neither effect in the glycemic control, oxidative stress nor antioxidant activity. Increased physical activity could improve the glycemic control, lipid profile as well as antioxidant activity.
Impact of diabetes and metabolic syndrome on patients with non alcoholic stetohepatitis-NASH

Objectives: This current study was undertaken to characterize the association between hepatic steatosis and the lipid profile in normal and over weight patients with fatty liver with or without diabetes. In addition, we tried to assess whether there are relationships between oxidative stress and antioxidant system in patients with non alcoholic stetohepatitis (NASH) and its relation to over weight and diabetes. Moreover, we investigated the relationship between lipid profile, oxidative stress and antioxidants in patients with metabolic syndrome in presence and absence of NASH. This was done in order to investigate the role of these factors on the treatment regimen and pharmaceutical care of these patients.

Methods: This research study was carried out on 110 patients. These patients were selected from the outpatient clinics of Internal Medicine Unit in Mansoura University, in the period between October 2006 and February 2008. Patients were classified into three groups. Group I consists of patients with NASH and type II diabetes mellitus (T2DM). Group II consists of patients with NASH only. Group III consists of patients with metabolic syndrome (MS) without NASH. Oxidative stress was assessed by measuring H2O2 and malondialdehyde (MDA) (as marker of lipid peroxidation) levels and antioxidant status by measuring blood catalase and glutathione peroxidase (GPx) activities.

Results: We found that patients with both NASH and T2DM showed a significant increase in H2O2 and MDA levels as compared with patients with NASH only or the control subjects. Patients with NASH only showed a significant increase in antioxidant enzymes (catalase and GPx) as compared with patients with both NASH and T2DM or the control group. In addition, patients with both MS and NASH showed a significant increase in H2O2 and MDA levels as compared with patients with MS alone or the control group. Furthermore, patients with MS alone showed a significant decrease in catalase and GPx activities as compared with patients with both MS and NASH or the control subjects.

Conclusion: Clinical observations indicated that oxidative stress plays an important mechanism in obesity associated with metabolic syndrome in the development of diabetes and in NASH. Patients with NASH and T2DM or those with MS and NASH developed a redox imbalance, which is characterized by lower than normal antioxidant potential, as catalase and GPx and an enhanced free-radical activity, as lipid peroxidation and H2O2 activity. This view has stimulated the quest for interaction between diabetes, metabolic syndrome and over weight and their effect on the drug interaction of those diseases.

Cannabinoid 1 receptor activation contributes to vascular inflammation and cell death in a mouse model of diabetic retinopathy and a human retinal cell line

AIMS/HYPOTHESIS:

Recent studies have demonstrated that cannabinoid-1 (CB(1)) receptor blockade ameliorated inflammation, endothelial and/or cardiac dysfunction, and cell death in models of nephropathy, atherosclerosis and cardiomyopathy. However the role of CB(1)
receptor signalling in diabetic retinopathy remains unexplored. Using genetic deletion or pharmacological inhibition of the CB(1) receptor with SR141716 (rimonabant) in a rodent model of diabetic retinopathy or in human primary retinal endothelial cells (HREC) exposed to high glucose, we explored the role of CB(1) receptors in the pathogenesis of diabetic retinopathy.

METHODS:

Diabetes was induced using streptozotocin in C57BL/6J Cb(1) (also known as Cnr1)(+/+) and Cb(1)(-/-) mice aged 8 to 12 weeks. Samples from mice retina or HREC were used to determine: (1) apoptosis; (2) activity of nuclear factor kappa B, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), poly (ADP-ribose) polymerase and caspase-3; (3) content of 3-nitrotyrosine and reactive oxygen species; and (4) activation of p38/Jun N-terminal kinase/mitogen-activated protein kinase (MAPK).

RESULTS:

Deletion of CB(1) receptor or treatment of diabetic mice with CB(1) receptor antagonist SR141716 prevented retinal cell death. Treatment of diabetic mice or HREC cells exposed to high glucose with SR141716 attenuated the oxidative and nitrative stress, and reduced levels of nuclear factor κB, ICAM-1 and VCAM-1. In addition, SR141716 attenuated the diabetes- or high glucose-induced pro-apoptotic activation of MAPK and retinal vascular cell death.

CONCLUSIONS/INTERPRETATION:

Activation of CB(1) receptors may play an important role in the pathogenesis of diabetic retinopathy by facilitating MAPK activation, oxidative stress and inflammatory signalling. Conversely, CB(1) receptor inhibition may be beneficial in the treatment of this devastating complication of diabetes.

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Anti-inflammatory Effect of Simvastatin-Aspirin Combination

The aim of this study is to investigate the anti-inflammatory effects of simvastatin alone and in combination with aspirin, the most widely used analgesic, antipyretic and anti-inflammatory agent, and to evaluate the effect of using them in combination which may produce synergistic effect and lower the dose required for each agent. 72 Male Wistar albino rats suffering from air pouch granuloma were used. They were divided into 12 groups each comprising 6 rats, they received either simvastatin (20 mg/kg/day) or aspirin (25 mg/kg/day) or combined therapy (simvastatin & aspirin) for either 3 or 6 executive days. The control groups received the solvents only for the same periods. Biochemical markers of inflammation as serum tumor necrosis factor-\(\alpha\), interleukin-6 and interleukin-4 were measured by an
Elisa method. Antioxidant activity was calorimetrically assessed by measuring serum nitric oxide concentration. Results indicated that treatment with simvastatin alone had no significant difference from treatment with aspirin alone which give solid ground for the predicted anti-inflammatory effects of simvastatin. Furthermore, our study demonstrated that treatment with the combined therapy reduced the extent of inflammation as compared to treatment with simvastatin alone or aspirin alone indicating that aspirinsimvastatin combination represents a synergistic combination concerning the immune response to the inflammatory challenge. The combination treatment with agents that inhibit different aspects of the signal transduction pathways will be transformational and have better efficacy with fewer side effects.

**Type 2 Diabetes Mellitus-Induced Hyperglycemia in Patients with NAFLD and Normal LFTs: Relationship to Lipid Profile, Oxidative Stress and Pro-inflammatory Cytokines**

Type 2 diabetes mellitus is associated with dyslipdemia, insulin resistance and non alcoholic fatty liver disease. The purpose of the current study was to assess whether type 2 diabetes mellitus- induced hyperglycemia has an effect on the lipid profile and release of oxidative stress markers and inflammatory mediators in patients with non alcoholic fatty liver disease and normal liver function tests which may in turn lead to enhancing the pathogenicity of this liver disease. For this purpose, one hundred and five outpatients, matched in age and weight, were classified into two groups: the first group consists of patients with non alcoholic fatty liver disease and the second group consists of patients with non alcoholic fatty liver disease in conjunction with hyperglycemia due to the presence of type 2 diabetes mellitus. In all patients, lipid profile, oxidative stress, and inflammatory mediators were assessed by measuring serum concentrations of triglycerides, low density lipoprotein, hydrogen peroxide, malondialdehyde, tumor necrosis factor-alpha and interleukin-6, respectively. In the studied population, it was found that the presence of type 2 diabetes mellitus-induced hyperglycemia significantly impaired lipid profile, and significantly enhanced the formation of hydrogen peroxide and malondialdehyde as well as significantly increased the release of tumor necrosis factor-alpha and interleukin-6 in the second group of patients. In addition, plasma glucose level showed significant positive correlation with hydrogen peroxide, malondialdehyde, tumor necrosis factor-alpha and interleukin-6. From the previous results, it was concluded that the presence of type 2 diabetes mellitus-induced hyperglycemia results in significant increase in lipid profile, oxidative stress markers and inflammatory mediators in patients with non alcoholic fatty liver disease and normal liver function tests. For this reason, further research studies may be essential to evaluate the benefit of adding suitable antioxidant and anti-inflammatory drugs to the treatment regimen for this group of patients. In addition, regular monitoring of blood glucose levels and liver function tests should be advised to this category of patients to reduce liver fat deposition and avoid the development of non alcoholic steatohepatitis, cirrhosis or liver cancer and their related complications.
Reactive Oxygen Species Mediate Nonalcoholic Fatty Liver Injury Through TNF-\(\alpha\)±: Relation to Body Weight

Nonalcoholic fatty liver disease (NAFLD) becomes widespread in developed countries. It is associated with obesity. Therefore, the study was directed to investigate the effect of obesity on the production of reactive oxygen species (ROS) and antioxidants in NAFLD. Moreover, the influence of produced ROS on tumor necrosis factor (TNF)-\(\alpha\)± production and fatty liver pathogenicity was also examined. Seventy Six outpatients from outpatient clinics of Internal Medicine department at Specialized Medical Hospital at Mansoura University with NAFLD were classified into three groups: obese (18 patients), overweight (24 patients) and normal weight (34 patients). The results were compared with the results of the control group including 25 normal subjects. Oxidative stress was assessed by measuring the activities of leukocyte hydrogen peroxide (H2O2) and superoxide anion (O2-). Antioxidant status was assessed by measuring the activities blood catalase and glutathione peroxidase (GPx). In addition, we assessed tissue damage by measuring serum malondialdehyde (MDA) and the inflammatory mediator TNF-\(\alpha\)±. We found that, obese patients showed significant increase in serum TG, H2O2 and O2- levels as compared with normal weight patients. In addition, obese fatty liver patients showed a significant decrease in glutathione peroxidase activity only as compared with the normal weight patients. Moreover, obese patients showed a significant increase in serum MDA and TNF-\(\alpha\)± as compared with normal weight patients. In conclusion, oxidative stress or redox imbalance that results from hyperlipidemia may be an important mechanism in obesity induced fatty liver complications by formation of MDA, which in turn increases the inflammatory mediator TNF-\(\alpha\)±.

Thioredoxin interacting protein is a novel mediator of retinal inflammation and neurotoxicity

BACKGROUND AND PURPOSE Up-regulation of thioredoxin interacting protein (TXNIP), an endogenous inhibitor of thioredoxin (Trx), compromises cellular antioxidant and anti-apoptotic defences and stimulates pro-inflammatory cytokines expression, implying a role for TXNIP in apoptosis. Here we have examined the causal role of TXNIP expression in mediating retinal neurotoxicity and assessed the neuroprotective actions of verapamil, a calcium channel blocker and an inhibitor of TXNIP expression.

EXPERIMENTAL APPROACH Retinal neurotoxicity was induced by intravitreal injection of NMDA in Sprague-Dawley rats, which received verapamil (10\(\mu\)g/ml·kg(-1), p.o.) or vehicle. Neurotoxicity was examined by terminal dUTP nick-end labelling assay and ganglion cell count. Expression of TXNIP, apoptosis signal-regulating kinase 1 (ASK-1), NF-\(\kappa\)B, p38 MAPK, JNK, cleaved poly-ADP-ribose polymerase (PARP), caspase-3, nitrotyrosine and 4-hydroxy-nonenal were examined by Western and slot-blot analysis. Release of TNF-\(\alpha\)± and IL-1\(\beta\) was examined by elisa. KEY RESULTS NMDA injection enhanced TXNIP expression, decreased Trx activity, causing increased
oxidative stress, glial activation and release of TNF-\(\alpha\) and IL-1\(\beta\). Enhanced TXNIP expression disrupted Trx/ASK-1 inhibitory complex leading to release of ASK-1 and activation of the pro-apoptotic p38 MAPK/JNK pathway, as indicated by cleaved PARP and caspase-3 expression. Treatment with verapamil blocked these effects.

CONCLUSION AND IMPLICATIONS Elevated TXNIP expression contributed to retinal neurotoxicity by three different mechanisms, inducing release of inflammatory mediators such as TNF-\(\alpha\) and IL-1\(\beta\), altering antioxidant status and disrupting the Trx-ASK-1 inhibitory complex leading to activation of the p38 MAPK/JNK apoptotic pathway. Targeting TXNIP expression is a potential therapeutic target for retinal neurodegenerative disease.

14-

Circulating adiponectin: a potential prognostic marker for hepatocellular carcinoma

Objective
We planned this study to investigate the relation between serum adiponectin level and hepatocellular carcinoma (HCC): risk, features and prognosis.

Methods
The study included 100 patients with HCC and 40 healthy control subjects. Adiponectin levels were determined by an enzyme-linked immunosorbent assay kit.

Results
In the subset of patients with compensated cirrhosis, the mean serum adiponectin level was significantly lower in HCC cases compared to healthy controls (88.6 versus 115 ng/mL; \(P = 0.012\)). In addition, serum adiponectin levels correlated negatively with tumor size (\(P = 0.004\)) and were significantly lower in patients with vascular invasion and distant metastases (\(P = 0.03\) and \(P = 0.02\) respectively). Furthermore, the median overall survival was significantly higher in the high adiponectin group than the low adiponectin group (median 12.5 versus 9.5 months; log rank = 4.6, \(P = 0.03\)).

Conclusion
Decreased circulating adiponectin level may play a role in the development of HCC and is a potential poor prognostic marker. These data should be validated in further prospective studies. Also the mechanisms by which adiponectin affect the course of HCC need to be clarified.

15-

Retinal microglial activation and inflammation induced by amadori-glycated albumin in a rat model of diabetes

OBJECTIVE:
During diabetes, retinal microglial cells are activated to release inflammatory cytokines that initiate neuronal loss and blood-retinal barrier breakdown seen in diabetic retinopathy (DR). The mechanism by which diabetes activates microglia to release those inflammatory mediators is unclear and was therefore elucidated.

RESEARCH DESIGN AND METHODS:
Microglia activation was characterized in streptozocin-injected rats and in isolated microglial cells using immunofluorescence, enzyme-linked immunosorbent assay, RT-PCR, and Western blot analyses.
RESULTS:
In 8-week diabetic retina, phospho-extracellular signal-related kinase (ERK) and P38 mitogen-activated protein kinases were localized in microglia, but not in Mueller cells or astrocytes. At the same time, Amadori-glycated albumin (AGA)-like epitopes were featured in the regions of microglia distribution, implicating a pathogenic effect on microglial activation. To test this, diabetic rats were treated intravitreally with A717, a specific AGA-neutralizing antibody, or murine IgG. Relative to nondiabetic rats, diabetic rats (IgG-treated) manifested 3.9- and 7.9-fold increases in Iba-1 and tumor necrosis factor (TNF)-\( \alpha \) mRNAs, respectively. Treatment of diabetic rats with A717 significantly attenuated overexpression of these mRNAs. Intravitreal injection of AGA per se in normal rats resulted in increases of Iba-1 expression and TNF-\( \alpha \) release. Guided by these results, a cultured retinal microglia model was developed to study microglial response after AGA treatment and the mechanistic basis behind this response. The results showed that formation of reactive oxygen species and subsequent activation of ERK and P38, but not Jun NH2-terminal kinase, are molecular events underpinning retinal microglial TNF-\( \alpha \) release during AGA treatment.

CONCLUSIONS:
These results provide new insights in understanding the pathogenesis of early DR, showing that the accumulated AGA within the diabetic retina elicits the microglial activation and secretion of TNF-\( \alpha \). Thus, intervention trials with agents that neutralize AGA effects may emerge as a new therapeutic approach to modulate early pathologic pathways long before the occurrence of vision loss among patients with diabetes.

16-

Fish oil improves lipid metabolism and ameliorates inflammation in patients with metabolic syndrome: Impact of nonalcoholic fatty liver disease

Context: Nonalcoholic fatty liver disease (NAFLD) is increasingly prevalent in Egypt, in parallel with increasing obesity. NAFLD can lead to liver inflammation, fibrosis and cirrhosis. NAFLD appears tightly linked with metabolic syndrome (MetS).

Objective: Examine the impact of dietary fish oil on human patients with MetS and NAFLD.

Materials and methods: One hundred and forty patients were enrolled in the current study and classified into two groups: patients with both MetS and NAFLD and patients with MetS alone. Sixty-four patients were treated with daily supplementation of 2\( \% \) of fish oil for 6 months. Markers of hyperlipidemia and oxidative stress, hydrogen peroxide (H2O2) and malondialdehyde (MDA), as well as proinflammatory cytokines, tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) and interleukin-6 (IL-6), were analyzed.

Results: Patients without fish oil exhibited significant increases in triglycerides (TGs), low-density lipoprotein (LDL), H2O2 and MDA that were associated with significantly elevated TNF-\( \alpha \) and IL-6 compared to controls. Furthermore, patients with both NAFLD and MetS showed significant increase in H2O2, MDA, TNF-\( \alpha \) and IL-6 levels compared with MetS group (p\( \% \)
Discussion and conclusion: Patients with NAFLD had bad lipid profile through a mechanism that involved developed redox imbalance, characterized by boosted free-radical activity and lipid peroxidation enhancing the release of proinflammatory cytokines leading to increased MetS risk and liver damage. However, daily treatment of patients with fish oil for 6 months improved lipid profile and blocked the oxidative stress and cytokines release.

Relevance of Serum Levels of Interleukin-6 and Syndecan-1 in Patients with Hepatocellular Carcinoma

Syndecan-1 is a trans-membrane heparan sulfate proteoglycan that localizes in epithelial cells and has been shown to be present in normal hepatocytes. It is thought to be involved in processes such as cell growth, differentiation and adhesion. However, the clinical data regarding syndecan-1 in patients with hepatocellular carcinoma (HCC) are scarce and controversial. Therefore, we need to evaluate the effects of HCC on the serum levels of syndecan-1. Thus, 40 patients with HCC and 31 patients with liver cirrhosis were physically examined. Blood samples were taken for measurements of routine markers (sGPT, sGOT, bilirubin, albumin, and α-fetoprotein), as well as serum levels of interleukin (IL)-6 and syndecan-1. Patients with liver cirrhosis showed significant increase in serum IL-6 as compared with HCC patients and the control subjects. Serum level of syndecan-1 was significantly increased in HCC patients as compared with the cirrhotic and control groups. In addition, significant positive correlations between syndecan-1 and serum levels of ALT, AST in HCC patients were found. Moreover, syndecan-1 increased significantly with increasing stage of Barcelona-Clinic Liver Cancer Group diagnostic and treatment strategy. In conclusion, the development of HCC is accompanied by a significant elevation in serum syndecan-1 levels. The increase in serum syndecan-1 may be linked with progression of HCC.

Reno-protective effect of NECA in diabetic nephropathy: implication of IL-18 and ICAM-1

Diabetic nephropathy (DN) remains the most common cause of end-stage renal disease. Although, adenosine acts as a local modulator with a cytoprotective function, extracellular adenosine usually disappears quickly due to a rapid uptake into adjacent cells. Therefore, we investigated the effect of 5′-(N-ethylcarboxamido)-adenosine (NECA), a stable, nonselective adenosine receptor agonist, on diabetes-induced increases in inflammatory cytokines and adhesion molecules. The enhancement of adenosine receptor action by NECA was examined in the renal tissues of rats with streptozotocin-induced diabetes. Daily i.p. injections of NECA at 0.3 mg/kg/day were given to rats, over a two-week period, six weeks after the induction of diabetes. Morphological changes were assessed in kidney sections. Oxidative stress was examined by measuring tissue malondialdehyde. Gene expression of interleukin (IL)-18, tumor necrosis factor (TNF)-α, and intercellular adhesion molecule (ICAM)-1 was measured by real-time PCR. Activation of cellular, proapoptotic pathways was demonstrated by measuring the activation of c-Jun NH(2)-terminal kinases (JNK)-mitogen-activated-protein kinase (MAPK). We found that diabetes-induced malondialdehyde formation activated the
production of IL-18, TNF-\( \alpha \)± and ICAM-1, which, in turn, activated pro-apoptotic pathways in diabetic rats. Treatment with NECA protected diabetic rats by exerting hypoglycemic and antioxidant effects as well as reducing gene expression of proinflammatory cytokines. These effects were associated with deactivation of JNK-MAPK. In addition, diabetic rats treated with NECA showed mild glomerular effects and vacuolation of tubular epithelium. We can conclude that activation of adenosine receptors is a potential therapeutic target in DN. NECA acts via multiple mechanisms including: reducing diabetes-induced oxidative stress, inhibiting gene expression of IL-18, TNF-\( \alpha \)± and ICAM-1, and blocking activation of the JNK-MAPK pathway.

Electroporation-mediated gene delivery of cleavage-resistant pro-nerve growth factor causes retinal neuro- and vascular degeneration

PURPOSE:

Neurotrophins, including nerve growth factor (NGF), are secreted by glia as a pro-form (proNGF) that is normally cleaved into the mature ligand. Increases of proNGF has been well documented in retinal neurodegenerative diseases. Since systemic overexpression of proNGF exhibits embryonic lethality, we aimed to establish a model that specifically and stably overexpresses a cleavage-resistant mutant of proNGF (proNGF123) plasmid in the retina using electroporation.

METHODS:

Male Sprague-Dawley rats were injected intravitreally with pGFP or pGFP-proNGF123 plasmids, then electroporated with various settings for optimization. Retinal cell death and ganglion cell count were assessed by TUNEL and immunostaining with anti-Brn3. Expression of proNGF, NGF, and their receptors was examined by western blot. Retinal vascular permeability was assessed by extravasation of bovine serum albumin-fluorescein. Development of acellular capillaries was assessed by periodic acid-Schiff and hematoxylin staining.

RESULTS:

Successful pGFP-proNGF123 gene delivery and expression of proNGF was demonstrated by western blot and extensive proNGF immunostaining in retina sections. Overexpression of proNGF reduced NGF expression while inducing the expression of neurotrophin receptors, including p75(NTR) and tyrosine receptor kinase A, but not sortilin. Overexpression of proNGF resulted in ~50% reduction in ganglion cell count and fivefold increase in TUNEL-positive cells in rat retina. In addition, overexpression of proNGF induced breakdown of the blood-retina barrier evident by twofold increase in extravasation of bovine serum albumin-fluorescein after 1 week and induced the development of acellular capillaries after 4 weeks.

CONCLUSIONS:

Electroporation can successfully incorporate and express biologically active cleavage-
resistant proNGF locally in rat retinas. Overexpression of cleavage-resistant proNGF can be a useful tool to investigate specific molecular mechanisms by which proNGF causes neurodegeneration and vascular injury in the retina.

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**Diabetes and Overexpression of proNGF Cause Retinal Neurodegeneration via Activation of RhoA Pathway**

Our previous studies showed positive correlation between accumulation of proNGF, activation of RhoA and neuronal death in diabetic models. Here, we examined the neuroprotective effects of selective inhibition of RhoA kinase in the diabetic rat retina and in a model that stably overexpressed the cleavage-resistance proNGF plasmid in the retina. Male Sprague-Dawley rats were rendered diabetic using streptozotosin or stably express cleavage-resistant proNGF plasmid. The neuroprotective effects of the intravitreal injection of RhoA kinase inhibitor Y27632 were examined in vivo. Effects of proNGF were examined in freshly isolated primary retinal ganglion cell (RGC) cultures and RGC-5 cell line. Retinal neurodegeneration was assessed by counting TUNEL-positive and Brn-3a positive retinal ganglion cells. Expression of proNGF, p75(NTR), cleaved-PARP, caspase-3 and p38MAPK/JNK were examined by Western-blot. Activation of RhoA was assessed by pull-down assay and G-LISA. Diabetes and overexpression of proNGF resulted in retinal neurodegeneration as indicated by 9- and 6-fold increase in TUNEL-positive cells, respectively. In vitro, proNGF induced 5-fold cell death in RGC-5 cell line, and it induced >10-fold cell death in primary RGC cultures. These effects were associated with significant upregulation of p75(NTR) and activation of RhoA. While proNGF induced TNF-\(\alpha\) expression in vivo, it selectively activated RhoA in primary RGC cultures and RGC-5 cell line. Inhibiting RhoA kinase with Y27632 significantly reduced diabetes- and proNGF-induced activation of proapoptotic p38MAPK/JNK, expression of cleaved-PARP and caspase-3 and prevented retinal neurodegeneration in vivo and in vitro. Taken together, these results provide compelling evidence for a causal role of proNGF in diabetes-induced retinal neurodegeneration through enhancing p75(NTR) expression and direct activation of RhoA and p38MAPK/JNK apoptotic pathways.

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**Contribution of TNF-\(\alpha\) to the development of retinal neurodegenerative disorders.**

During the late 1970s, tumor necrosis factor alpha (TNF-\(\alpha\)) was initially recognized as an endotoxin-induced substance that was mainly produced by macrophages, and able to cause the lysis of certain tumor cells. Subsequent research demonstrated that TNF-\(\alpha\) mediates a broad range of cellular activities, including proliferation, survival, differentiation and apoptosis. It is also considered to be essential for the induction and maintenance of the inflammatory immune responses. Meanwhile, visual impairment imposes a substantial disease burden on society. It is associated with both significant economic impact and reduction in quality of life. Visual impairment raises serious social challenges for both patients and their families, interfering with day-to-day life, and can limit employment possibilities. Many of the most common, irreversible blinding pathologies involve neuronal loss from the retina, which is the light-sensing tissue of the eye. The retina, being part of the central nervous system, is unable to regenerate neurons lost to disease. Therefore, in the current review we will discuss the association between
increased expression of TNF-$\alpha$ with neurodegenerative disorders, downstream cellular signaling mechanisms following interaction of TNF-$\alpha$ with its receptors, and the role of TNF-$\alpha$ as a possible target in the treatment of retinal neurodegenerative disorders.