状となる．いたみ，しびれ，びりびりなど異常感覚としての患者が訴えることから，これらの症状を陽性症状という．侵される線維の種類によってその症状は異なってくる．糖尿病発症早期で，神経線維の脱落が目立たない状態でも，高血糖から神経細胞は膜興奮の変化から刺激に対する過敏反応を示す．すなわち，糖尿病での感覚・触覚過敏で代表される症状となる[17]．一方，長期経過により神経変性・脱落が進行し，再生障害が起こると自発痛のような自発性のインパルスが発生する．すなわち，慢性期にはじっくりと出現する自発痛，異常感覚が主体となり，治療にも抵抗性の場合が多い．理論的には陽性症状は神経線維が残存している限り出現することになり，神経障害の進展，すなわち病期の進展を反映していない．これに対し，病期の進展を反映する病理学的特徴は神経線維の進行性脱落であり，感覚低下(感覚鈍麻)の症候として反映される．患者は症状として訴えないことから陰性症状と呼ばれている[18]．感覚低下が明らかな場合には，患者には壊疽の危険性を熟知させ，徹底的な指導の必要性があることになる．すなわち，感覚低下の部位の拡大や，その程度を調べることが患者のケアの指標となるばかりでなく，直接病理学的な病期の進展を示すことになる．

おわりに

糖尿病性神経障害，とくに多発神経障害(ポリニューロパチー)の病理，および病変の成り立ちに限って述べた．高血糖の持続により，末梢神経の機能的異常から形態学的異常がもたらされる．末梢神経の病変としては進行性の神経線維の脱落に代表される．そこでは，神経組織自体での代謝異常とともに，細小血管障害が大きく関与している．初期には可逆的状態だが，しだいに不可逆的状態に陥る．神経組織は可塑性の高い組織でもあり，初期の不可逆的病変は可逆的状態にある組織により代償的に機能は補完されている．しかしながら，さらに進展すると代償不能となり，病変は全体として広く修復されないことになる．現在のところ，point of no returnはいつであるのか不明であり，可能な限り早期から病変を進行させない努力が大切となる．最近の研究では自覚症状の発現は病変の進展を直接には反映せず，進行性の神経機能の悪化こそが客観的な指標となることが示されている[19]．したがって，症状はなくとも積極的に，常に患者の他覚的所見に注意して，管理・治療にあたることが不可欠である．

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ラット末梢神経の虚血・再灌流傷害に対するFK506（タクロリムス）の効果*

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目　的

FK506（タクロリムス）は、1984年藤沢薬品探索研究所により茨城県筑波山近郊の土壌から分離され、現在では肝・腎・骨髄移植後の拒絶反応を抑制する免疫抑制剤として日・米・欧州など16カ国で承認されている[1),2)]。FK506はさらにアトピー性皮膚炎、慢性関節リウマチなどにも用いられ、また中枢・末梢神経組織において神経細胞保護作用が報告され、FK506が単なる免疫抑制剤に留まらない可能性を示唆している。特にラット末梢神経の急性虚血性ニューロパチーでは、少量のFK506投与により軸索変性がほぼ完璧に抑制され[3)]、脳梗塞モデルでは再灌流後の急性炎症反応を抑制するとされる[4)]。本研究ではFK506のラット末梢神経虚血・再灌流傷害時の急性炎症反応および神経線維保護作用に対する効果について電気生理学的・組織学的に検討した。

方　法

手術方法：

虚血・再灌流の惹起方法については既に報告したのと同じ手技を用いた[5)]。雄ウィスターラット（体重250–300g）にネンブタール麻酔下、腹部大動脈・右総腸骨動脈・右大腿動脈など右後肢を支配する動脈へのクリッピングにて右後肢に高度の虚血を起こし、4時間の虚血後にクリップを遠位部から順次外し再灌流をおこした。

FK506投与：

虚血開始直後にFK506 5.0mg/kg s.c.を投与、術後24時間にはFK506 1.0mg/kg s.c.を2回、その後は1.0mg/kg s.c.を1日1回投与した。コントロールラットには同量生食を同様に投与した。ラット数は、FK投与群が11匹、コントロール群が10匹である。

電気生理学的・組織学的検討：

既に報告したのと同様の方法を用いた[6)]。電気生理学的検討は再灌流6日後に、坐骨・脛骨神経の伝導検査を坐骨結節・膝・踝レベルで電気刺激、足底筋導出で検討した。また足底から内側足底筋に直角に貫通させた皮下針電極から、単位時間当たりの脱神経電位発射数を記録した。組織学的検討は再灌流7日後に、坐骨結節から踝レベルまでの坐骨・脛骨神経を採取し、エポン包埋用に大腿上部、大腿下部、腓腹上部、腓腹下部での各レベルをglutaraldehydeにて固定し、その他の大腿中部、膝関節、腓腹中部の各レベルは免疫組

Key Words：ischemic neuropathy（虚血性ニューロパチー），reperfusion injury（再灌流傷害），FK506（FK506），rat peripheral nerve（ラット末梢神経障害），macrophage（マクロファージ）

* The effect of FK506 (tacrolimus) on reperfusion injury in rat nerve

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表1．虚血前および虚血4時間・再灌流6日後の坐骨・脛骨神経の電気生理学的検討

	FKラット ($n=11$)	コントロール ($n=10$)	p value (t test)
虚血前			
CMAP amplitude* (mV)	2.72±0.85	2.51±0.53	0.5143
虚血・再灌流後			
CMAP amplitude* (mV)	0.88±1.10	0.63±0.48	0.5118
脱神経電位発射頻度 (#/s)	43.1±21.5	78.3±29.7	0.0055

*ankle-stimulated

繊染色用にformalin固定した。免疫組織染色にはED-1、ED-2、8OHdG、BrdUの各抗体を用いた。

結　果

電気生理学的検討：

坐骨・脛骨神経の伝導検査で、4時間虚血および再灌流6日後、FK投与群、コントロール群ともに虚血前に比し、虚血・再灌流後の足首刺激時CMAP amplitudeが有意の低下を示した（FK投与群：虚血前2.72±0.85、虚血・再灌流後0.88±1.10mV、$p=0.0017$、コントロール群：虚血前2.51±0.53、虚血・再灌流後0.63±0.48mV、$p<0.0001$）。しかし、FK投与群とコントロール群間では虚血前および虚血・再灌流後ともに有意差はなかった（表1）。4時間の虚血および6日間の再灌流後、足底筋での脱神経電位発射数については、FK506投与群ではコントロール群に比し有意に少なかった（表1）。

形態学的検討：

形態学的には、4時間の虚血および7日間の再灌流後の坐骨・脛骨神経で、FK投与群ではコントロールに比し軸索変性を示す有髄神経線維が減少したが（図1）、FK投与後も全例である程度の軸索変性を認めた（図1）。定量的に軸索変性を示す有髄神経線維の頻度が、FK投与群に有意に少ないことが確認された（FK投与群66.5±10.6%、コントロール群46.7±15.0%、$p=0.0026$）。

免疫組織化学的には、4時間虚血および48時間再灌流後に、大腿下部レベルの脛骨神経で神経束内のED2陽性マクロファージの減少が見られ（図2）、これらのマクロファージは

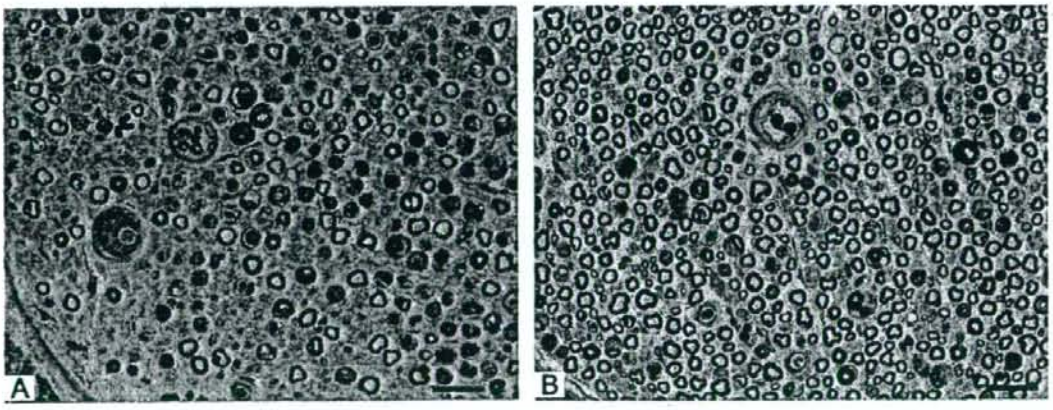

図1：4時間虚血・7日間再灌流後のラット脛骨神経（腓腹中部レベル）の横断像。
コントロールラット（A）では有髄神経線維に広範な軸索変性像がみられるが、FK投与ラット（B）では軸索変性を示す有髄神経線維の数が著明に減少している。Methylene Blue染色。Bars=30μm。

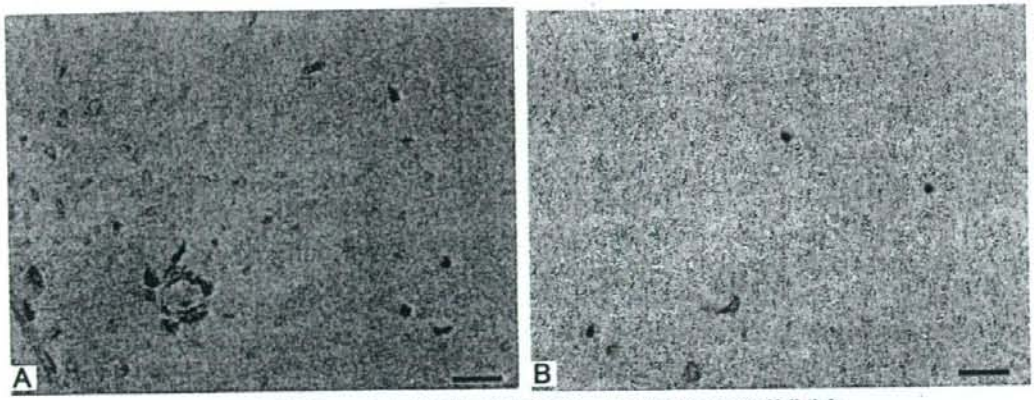

図2：4時間虚血・48時間再灌流後のラット坐骨神経（大腿下部レベル）横断面のED2抗体染色。
コントロールラット（A）ではED2陽性のマクロファージが多数認められるが、FK投与ラット（B）ではED2陽性マクロファージ数が減少している。Bars=30μm。

再灌流7日後に著明な食作用を示すマクロファージとは形態が異なり小型で紡錘形を示した。

考　察

FK506は本来、calcineurin inhibitorの作用を持つ免疫抑制剤として開発され、プログラフ（prograf®）は肝・腎・骨髄移植後に頻繁に使われている[1),2)]。さらに、FK506は実験的虚血での中枢・末梢神経における神経細胞保護作用、心筋・肝・腎などでの虚血・再灌流傷害に対する効果なども報告された。しかし、その作用機序については未だ明らかではない。

末梢神経の虚血・再灌流傷害においても急性炎症反応が起こり、その発症機序は他の組織と同様と思われる[7),8)]。本研究では、FK506は虚血4時間・再灌流48時間後の神経束内マクロファージ出現を抑制し、再灌流6日後に脱神経電位発射頻度が有意に減少し、7日後には軸索変性を示す有髄神経線維数が有意に減少した。この抑制機序については、FK506の免疫抑制作用とは別に、再灌流傷害時の炎症反応の抑制が考えられる。FK506がどの様に急性炎症を抑制するかは明らかでないが、血管内皮細胞での接着因子・ケモカインの発現抑制、好中球・単球からの炎症性サイトカイン産生抑制、抗酸化作用などが挙げられている[9),10)]。この様な微小循環での種々の細胞保護作用の集積的結果として、虚血・再灌流時の細胞保護作用が出現すると思われる。

FK506の免疫抑制剤としての作用機序研究が、T細胞の活性化機構を解明させ免疫学における基礎研究の面でも大いに貢献したように、FK506の虚血・再灌流傷害に対する細胞保護作用の機序解明が、再灌流傷害の発症機序をさらに明らかにすることが期待される。この点からは、免疫系でのFK binding protein (FKBP) やcalcineurinに代わるFK506の新たなターゲット蛋白質の存在も考えられる。

ま と め

本研究では、FK506が神経束内マクロファージ出現および軸索変性の重症度を抑制することが電気生理学的・病理学的に証明された。FK506は、ラット末梢神経の虚血・再灌流傷害による急性炎症反応を抑制し、神経細胞保護作用を示した。このFK506投与により、再灌流傷害発症機序の更なる解明が期待される。

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糖尿病神経障害の発症頻度と臨床診断におけるアキレス腱反射の意義
—東北地方15,000人の実態調査—

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要約：糖尿病末梢神経障害の実態と臨床診断におけるアキレス腱反射の有用性を明らかにするために，東北6県448医療機関と共同で入院・通院中の糖尿病患者を対象とした調査を行った．調査に当たっては独自に作成した「糖尿病患者調査票」を用い，自覚症状とアキレス腱反射検査は必須，振動覚検査は適宜実施とした．2003年2～9月の期間に，総計14,744症例(年齢：64.2±11.9歳，罹病期間：9.7±7.7年，HbA_{1c}：7.4±2.5%，いずれも平均±標準偏差)のデータを得た．自覚症状，アキレス腱反射の低下あるいは消失，振動覚低下の発現率はそれぞれ18.8%，40.3%，52.0%であった．糖尿病神経障害の発現頻度は，主治医の総合的判断では27.6%，「簡易診断基準」を適用した場合では35.8%であった．アキレス腱反射の低下・消失は自覚症状に比べて糖尿病発症早期から発現し，また，その発現率は自他覚所見「なし」群に比べて「あり」群において，有意に高率であった．アキレス腱反射は外来で手軽にできる簡便な検査であるが，糖尿病神経障害診断における有用性が示唆された．

Key words：① 糖尿病神経障害　② 実態調査　③ 糖尿病神経障害簡易診断基準　④ アキレス腱反射　⑤ 振動覚

〔糖尿病 50(11)：799～806，2007〕

はじめに

糖尿病性の神経障害，網膜症，腎症は3大糖尿病合併症といわれる．日本においては，このなかで糖尿病網膜症と腎症はそれぞれ中途失明原因の第1位，血液透析原疾患の第1位であることから医療従事者や患者および社会的な関心が高い．一方，糖尿病神経障害は初期には診断の困難さや患者の訴えの少なさなどから，網膜症や腎症と比較すると医師の関心は低い傾向にある．しかし糖尿病神経障害は体性神経障害や自律神経障害の程度によっては患者のquality of life (QOL)が著しく損なわれるばかりでなく，重症の足壊疽や無痛性心筋梗塞など，患者の生命予後に重大な

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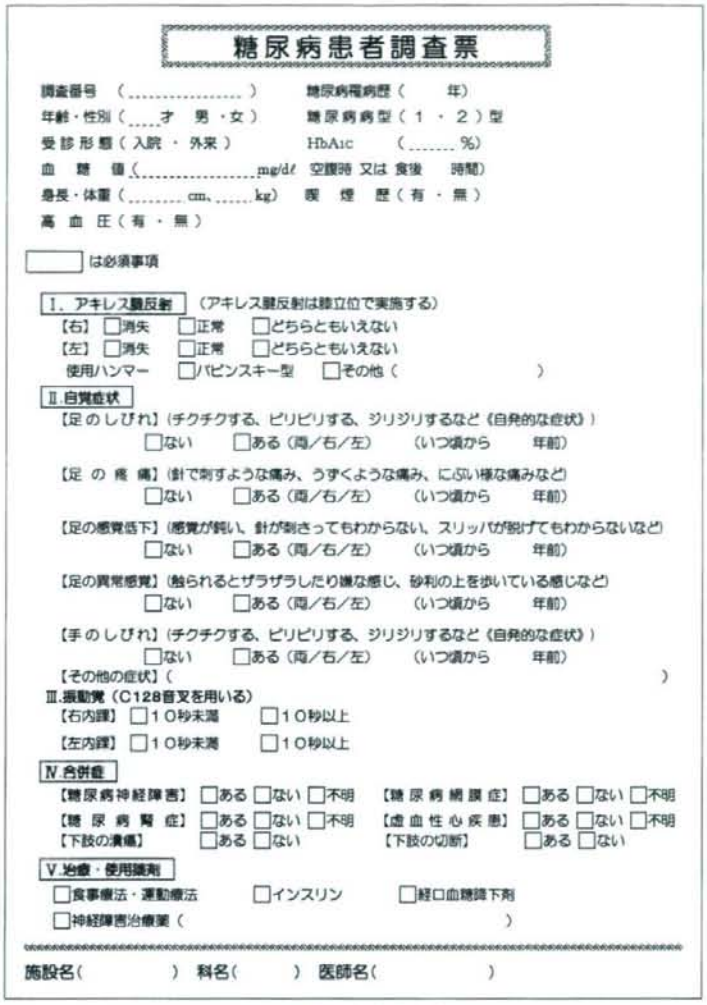

糖尿病患者調査票

調査番号（　　）　糖尿病罹病歴（　　年）
年齢・性別（　　才　男・女）　糖尿病病型（1・2）型
受診形態（入院・外来）　HbA1c　（　　%）
血糖値（　　mg/dl　空腹時 又は 食後　　時間）
身長・体重（　　cm、　　kg）　喫煙歴（有・無）
高血圧（有・無）

□は必須事項

Ⅰ．アキレス腱反射（アキレス腱反射は膝立位で実施する）
【右】□消失　□正常　□どちらともいえない
【左】□消失　□正常　□どちらともいえない
使用ハンマー　□バビンスキー型　□その他（　　）

Ⅱ.自覚症状
【足のしびれ】（チクチクする、ピリピリする、ジリジリするなど《自発的な症状》）
□ない　□ある（両／右／左）　（いつ頃から　　年前）
【足の疼痛】（針で刺すような痛み、うずくような痛み、にぶい様な痛みなど）
□ない　□ある（両／右／左）　（いつ頃から　　年前）
【足の感覚低下】（感覚が鈍い、針が刺さってもわからない、スリッパが脱げてもわからないなど）
□ない　□ある（両／右／左）　（いつ頃から　　年前）
【足の異常感覚】（触られるとザラザラしたり嫌な感じ、砂利の上を歩いている感じなど）
□ない　□ある（両／右／左）　（いつ頃から　　年前）
【手のしびれ】（チクチクする、ピリピリする、ジリジリするなど《自発的な症状》）
□ない　□ある（両／右／左）　（いつ頃から　　年前）
【その他の症状】（　　）

Ⅲ.振動覚（C128音叉を用いる）
【右内踝】□10秒未満　□10秒以上
【左内踝】□10秒未満　□10秒以上

Ⅳ.合併症
【糖尿病神経障害】□ある □ない □不明　【糖尿病網膜症】□ある □ない □不明
【糖尿病腎症】□ある □ない □不明　【虚血性心疾患】□ある □ない □不明
【下肢の潰瘍】□ある □ない　【下肢の切断】□ある □ない

Ⅴ.治療・使用薬剤
□食事療法・運動療法　□インスリン　□経口血糖降下剤
□神経障害治療薬（　　）

施設名（　　）　科名（　　）　医師名（　　）

Fig. 1　糖尿病患者調査票

影響を及ぼすことから軽視することのできない慢性合併症である．

東北糖尿病合併症フォーラムプロジェクト会では1998年から東北地方における糖尿病神経障害を中心とした糖尿病合併症の実態調査を実施してきた．1998年に行った調査では，約33,000人の糖尿病患者を対象に自覚症状を中心としたアンケート調査を行い，主治医の総合的判断によって糖尿病神経障害を診断した．この調査における糖尿病神経障害の頻度は27%，糖尿病網膜症は24%，糖尿病腎症は20%であり，神経障害は3大合併症のなかで最も頻度が高いことを報告した[1]．

糖尿病神経障害の診断基準としてはPartanenら[2]やDyckら[3]の基準が知られているが，いずれの診断基準も神経伝導速度をはじめ種々の電気生理学的検査が必要である．しかし，これらの検査には専用の機器や熟練した手技が必要であるため，ベッドサイドの臨床診断には適さない．最近，糖尿病性神経障害を考える会ではベッドサイドで使用できる「糖尿病性多発神経障害の簡易診断基準」（以下「簡易診断基準」と略す）を作成した[4]．この診断基準は下肢の自覚症状，アキレス腱反射の低下あるいは消失，内踝振動覚低下の3項目のうち2項目を満たせば糖尿病神経障害と診断できるという簡便さにもかかわらず，感度と特異度が高いものである[5]．

そこで，前回（1998年）から5年ぶりの今回の糖尿病神経障害の大規模実態調査では，調査項目に下肢の自覚症状に加えてアキレス腱反射と振動覚検査を追加

し，「簡易診断基準」を用いて糖尿病神経障害を診断した．本稿では今回の約15,000人の糖尿病患者を対象にした実態調査結果を報告し，また，「簡易診断基準」の項目のうち特にアキレス腱反射に着目して，糖尿病神経障害の臨床診断におけるアキレス腱反射の意義と有用性について考察した．

対象と方法

東北6県の448の病院・診療所のご協力を得て入院・通院中の糖尿病患者を対象とした患者実態調査を行った．

調査票をFig. 1に示す．調査票は年齢，性，糖尿病罹病期間などの患者背景とⅠ．アキレス腱反射，Ⅱ．自覚症状，Ⅲ．振動覚，Ⅳ．合併症，Ⅴ．治療・使用薬剤の5項目で構成されている．調査については主治医の日常診療への負担も考慮し，アキレス腱反射検査を必須項目としたものの，振動覚検査については適宜実施することとした．

自覚症状については主治医が調査票に基づき下肢症状を中心に問診を行った．アキレス腱反射はバビンスキー型打腱器を用いて膝立位で判定した．安静状態での検査によって足関節の動きが誘発されない場合には，背筋および肘関節を伸展する反射増強法をとって叩打し，それでも反射運動がみられないものを“消失”，足関節底屈運動が生じたものを“どちらともいえない”すなわち“低下”と判定した．内踝振動覚はC-128音叉を用い，振動感知が音叉叩打から10秒未満の場合を“低下”と判定した．本研究は，参加者が実地でこれら二検査の手技統一を計ったうえで開始された．

神経障害を含めた種々の糖尿病合併症の診断については，主治医が症状や検査結果から総合的に判断しその有無を記入した．すなわち，神経障害については自覚症状，アキレス腱反射，振動覚を参考に，網膜症は眼底所見で単純性網膜症以上を，腎症は微量アルブミン尿，顕性蛋白尿などを参考に，それぞれ臨床的に診断された．虚血性心疾患は心筋梗塞の既往歴や心電図の所見によって診断された．

さらに，糖尿病神経障害に関しては，自覚症状，アキレス腱反射および内踝振動覚の3項目全ての結果がそろった患者については，主治医による総合的判定とは別に「簡易診断基準」を用いて研究会事務局が診断した．

データの表記について糖尿病罹病期間，HbA1cなど，定量値は平均値±標準偏差で表示した．アキレス腱反射異常の発現率の統計解析はノンパラメトリック技法(χ^2-検定)にて行った．

Table 1 Clinical patient profiles

		n(14,744)	(%)
Gender	Male	7,836	(53.1)
	Female	6,776	(46.0)
	Unknown	132	(0.9)
Age(y)	-39	463	(3.1)
	40-49	1,091	(7.4)
	50-59	2,957	(20.1)
	60-69	4,562	(30.9)
	70-79	4,238	(28.7)
	80-	1,030	(7.0)
	Unknown	403	(2.7)
	(Mean ±SD)	64.2±11.9	
In/outpatient	Inpatient	1,132	(7.7)
	Outpatient	12,294	(83.4)
	Unknown	1,318	(8.9)
Body mass index	<25.0	8,271	(56.1)
	≧25.0	5,256	(35.6)
	Unknown	1,217	(8.3)
	(Mean ±SD)	24.4±4.2	
Diabetes mellitus duration (y)	-4.9	3,999	(27.1)
	5-9.9	3,815	(25.9)
	10-14.9	2,768	(18.8)
	15-	3,332	(22.6)
	Unknown	830	(5.6)
	(Mean ±SD)	9.7±7.7	
Diabetes mellitus type	Type 1	371	(2.5)
	Type 2	13,926	(94.5)
	Unknown	447	(3.0)
HbA1c (%)	-4.9	195	(1.3)
	5.0-5.9	2,545	(17.3)
	6.0-6.9	4,717	(32.0)
	7.0-7.9	3,395	(23.0)
	8.0-8.9	1,659	(11.3)
	9.0-9.9	830	(5.6)
	10.0-	1,143	(7.8)
	Unknown	260	(1.8)
	(Mean ±SD)	7.4±2.5	
Blood glucose (mg/dl)	Fasting(Mean ±SD)	143.6±54.4	
	Nonfasting(Mean ±SD)	186.6±72.8	
History of smoking	Yes	4,826	(32.7)
	No	9,442	(64.0)
	Unknown	476	(3.2)
Treatment	Diet and exercise therapy	7,159	(48.6)
	Oral hypoglycemic agent	8,357	(56.7)
	Insulin	3,573	(24.2)
	Medication for neuropathy	890	(6.0)

Table 2 Frequency of subjective symptoms, absence or decrease of Achilles tendon reflex, and decreased threshold of vibration perception.

		n(14,744)	(%)
Neuropathic symptoms:			
Numbness in feet	+ (Bilateral)	1,961	(13.3)
	+ (Unilateral)	874	(5.9)
	+ (Bilateral or Unilateral*)	350	(2.4)
	−	11,482	(77.9)
	Unknown	77	(0.5)
Pain in feet	+ (Bilateral)	667	(4.5)
	+ (Unilateral)	461	(3.1)
	+ (Bilateral or Unilateral*)	164	(1.1)
	−	13,312	(90.3)
	Unknown	140	(0.9)
Hypesthesia of feet	+ (Bilateral)	861	(5.8)
	+ (Unilateral)	310	(2.1)
	+ (Bilateral or Unilateral*)	243	(1.6)
	−	13,200	(89.5)
	Unknown	130	(0.9)
Paresthesia of feet	+ (Bilateral)	976	(6.6)
	+ (Unilateral)	271	(1.8)
	+ (Bilateral or Unilateral*)	270	(1.8)
	−	13,091	(88.8)
	Unknown	136	(0.9)
Numbness in hands	+ (Bilateral)	755	(5.1)
	+ (Unilateral)	708	(4.8)
	+ (Bilateral or Unilateral*)	191	(1.3)
	−	12,875	(87.3)
	Unknown	215	(1.5)
		n(14,700)	(%)
Symptoms in bilateral feet	+	2,767	(18.8)
	−	11,933	(81.2)
		n(14,614)	(%)
Achilles tendon reflex	− (Bilateral)	5,889	(40.3)
	− (Unilateral)	1,040	(7.1)
	+	7,685	(52.6)
		(6,017)	(%)
Vibration perception of feet	<10s (Bilaterel)	3,127	(52.0)
	<10s (Uilaterel)	554	(9.2)
	≧10s	2,336	(38.8)

*Unspecified + : Yes − : No

結 果

調査期間は2003年2～9月の8カ月間で，総計14,744症例のデータを得た．

1. 患者背景

患者背景をTable 1に示した．男性7,836人(53.1%)，女性6,776人(46.0%)，平均年齢64.2歳，最も多い年齢層は60歳台(30.9%)，次いで70歳台(28.7%)の順であった．大半(83.4%)が外来患者で，35.6%が肥満(BMI≧25)であった．平均糖尿病罹病期間は9.7年で，50%以上が10年未満であった．糖尿病の分類では95%が2型であった．HbA1cは平均7.4%で，HbA1c 7.0未満の患者は全体の約半数を占め

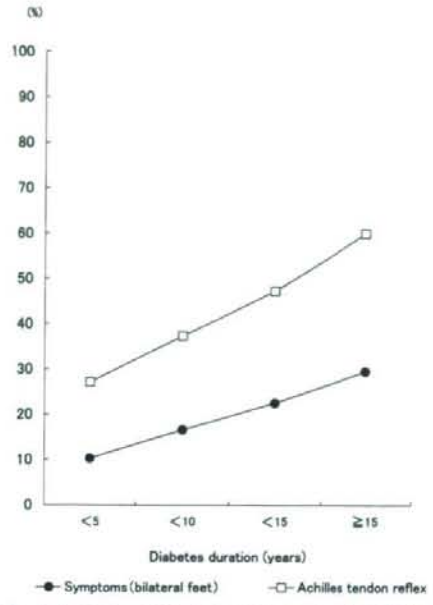

Fig. 2 Frequency of subjective symptoms* and absence or decrease of Achilles tendon reflex related to diabetes duration.
(*numbness, pain, hypesthesia, or paresthesia)

た．糖尿病治療法は食事療法・運動療法が 48.6%，経口血糖降下薬療法 56.7%，インスリン療法 24.2%（経口血糖降下薬併用例も含まれる）であった．また，アルドース還元酵素阻害剤（エパルレスタット）などの神経障害治療薬が 6.0% の患者に使用されていた（Table 1）．

2. 自覚症状，検査所見および糖尿病合併症の頻度

自覚症状およびアキレス腱反射，振動覚検査の結果を Table 2 に示す．両側下肢の "しびれ"，"疼痛"，"感覚低下"，"異常感覚" の出現率は 4.5～13.3% であった．両手の "しびれ" の出現率は 5.1% であった．全患者の 18.8% がこれらの，何らかの両側の下肢症状を訴えていた．これら両側下肢の何らかの自覚症状の発現率は罹病期間と共に上昇した（Fig. 2）．

アキレス腱反射検査では 40.3% の患者に両側の低下あるいは消失を，振動覚閾値検査では 52.0% の患者に両側の低下（10 秒未満）を認めた（Table 2）．なお，アキレス腱反射異常の内訳は「両側消失」が 29.3%，「両側の低下」が 11.0% であった．

主治医の総合的判断による糖尿病神経障害の頻度は，27.6% であった．また，その他の合併症の頻度は網膜症 20.3%，腎症 18.8%，虚血性心疾患 10.5% であった．一方，自覚症状，アキレス腱反射，振動覚の全てが測定されていた症例に（n＝5,148）「簡易診断基準」を適用した時の糖尿病神経障害の頻度は 35.8% であった（Table 3）．簡易診断基準によって診断された糖尿病神経障害のうちアキレス腱反射の低下・消失と振動覚低下のみで診断されたもの，すなわち自覚症状のない無症候性糖尿病神経障害は 53.5%（全患者の 19.2%）であった．

Table 3 Frequency of diabetic neuropathy

Diagnosis based on	Surveyed in	
	1998**	2003
Physician's general judgment	26.8% (n＝32,995)	27.6% (n＝14,744)
Simplified diagnostic criteria for diabetic polyneuropathy*	—	35.8% (n＝5,148)

*Proposed and recommended by the Japanese Study Group on Diabetic Neuropathy (Ref. 4)
**Ref. (1)

3. アキレス腱反射と自覚症状および他の合併症との関連

アキレス腱反射低下・消失は両側下肢の自覚症状に比較し，糖尿病の診断早期から高頻度に出現する傾向にあり，自覚症状と同様に糖尿病の罹病期間が長くなるほど異常の頻度は上昇した（Fig. 2）．

アキレス腱反射低下・消失と自他覚所見の有無との関係については，アキレス腱反射低下・消失の発現率が足や手の自覚症状「なし」群に比べて「あり」群において，また，振動覚低下「なし」群に比べて「あり」群において有意に高率であった（Fig. 3A）．さらにアキレス腱反射低下・消失と糖尿病合併症との関係については，アキレス健反射低下・消失の発現率は，神経障害，網膜症，腎症，高血圧，虚血性心疾患のいずれについても「なし」群よりも「あり」群のほうが有意に高率であった（Fig. 3B）．

考　察

東北糖尿病合併症フォーラムプロジェクト会は東北地区において 1998 年より糖尿病神経障害を中心とした糖尿病合併症の実態調査を実施している．1998 年には約 33,000 名の糖尿病患者を対象に自覚症状についてのアンケート調査を行いその結果は既に報告したが[1]，糖尿病神経障害を客観的に診断するには，神経伝導速度などの電気生理学的検査が必要である．しかし専用の機器と熟練した操作が必要であり，日常診療には向いていない．そこで今回の 15,000 人の調査では，主治医の総合判断に加えて，簡便で客観性もある，糖尿病性神経障害を考える会が提唱した「簡易診断基準」を用いて糖尿病神経障害を診断した．「簡易診断基準」は下肢の自覚症状，アキレス腱反射の低下あ

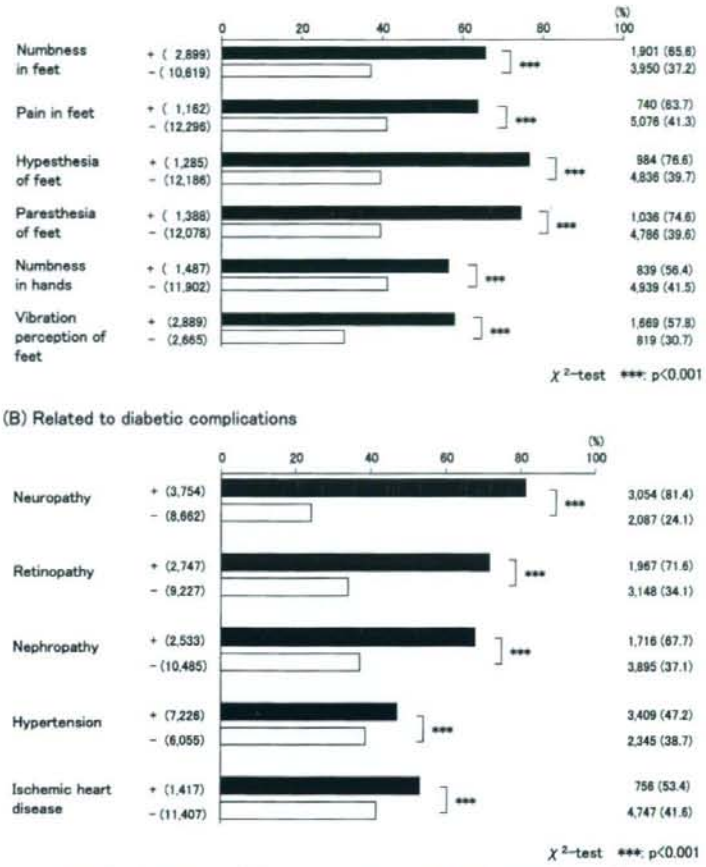

Fig. 3 Frequency of absence or decrease of Achilles tendon reflex

るいは消失，内踝振動覚低下の3項目のうち2項目を満たせば糖尿病性末梢神経障害と診断するもので，ベットサイドで簡単に診断できる割には，感度と特異性に優れていると報告されている[5]．

調査結果に基づき東北地方の平均的な糖尿病患者像をまとめると，年齢は60代が中心で，糖尿病罹病期間は10年未満が過半数であった．多くは経口血糖降下薬で治療され，約50%の患者はHbA1c 7.0%未満であり，主治医の総合的判断による糖尿病神経障害の頻度は27.6%であった．この結果は1998年の調査と同程度(26.8%)であり，前回と同様に，糖尿病神経障害は合併症のなかで最も高頻度であることが改めて示唆された．また，1998年[1]と2003年の調査において患者背景はほぼ同等であったことから，主治医の総合的判断に大きなぶれがなかったことも判明した．

今回の調査において，自覚症状，アキレス腱反射および振動覚の3項目全てが調査され，「簡易診断基準」の適用が可能であった症例が5,148例含まれていた．そこで，これらの症例に「簡易診断基準」を適用したところ糖尿病神経障害の頻度は8%上昇し35.8%となった．このうち自覚症状のない無自覚性糖尿病神経障害は約半数(53.5%)であった．高知県中核医療施設を受診中の糖尿病患者を対象とした実態調査においても，「簡易診断基準」を用いた糖尿病神経障害の頻度は本調査と同程度の37.8%と報告されている[6]．糖尿病神経障害の診断法として自覚症状に加えて，アキレス腱反射と振動覚の検査を導入した「簡易診断基準」の有用性が改めて示唆されたと思われる．

簡易診断基準においては，振動覚低下の目安として「10秒以下の感知時間」を参考記載されている．一方，高齢者では感知時間がより低下する方向も注意事項として記載されているので，われわれが採用した10秒未満という基準は，高齢者を含む集団の場合の診断基準本旨にのっとる方向として適切であったと考えてい

る.

一方，多忙な日常診療のなかへ多くの検査を取り入れることによる医師の時間的負担増も考慮する必要がある．現場の実状によっては，アキレス腱反射と振動覚検査のどちらかを選択せざるを得ない場合も生じよう．この点を念頭に置き今回の調査では，振動覚検査と比べてより簡便に実施できると考えられたアキレス腱反射検査に着目してさらに解析した．その結果，アキレス腱反射低下・消失の発現率は自覚症状に比べて糖尿病発症早期から高値を示し，自覚症状に先行することが示唆された．またアキレス腱反射低下・消失は，糖尿病神経障害以外の糖尿病合併症(網膜症，腎症，動脈硬化性病変)と平行して発現してくることも判明した．

アキレス腱反射は，反射弓の機能低下を反映する検査であり，検査結果は患者や検査医の主観に影響されないという利点がある．さらにアキレス腱反射は下肢末梢に及ぶ最長の末梢反射弓を有する腱反射であることから，両側遠位性に神経が障害される糖尿病神経障害の特性を考えると，理にかなった検査であるとも言える．

今回の実態調査から，糖尿病神経障害を的確に診断するためには，下肢の自覚症状の問診に加え，客観性のあるアキレス腱反射や振動覚検査が有用であった．特にアキレス腱反射は外来で手軽にできる簡便な検査であり，糖尿病神経障害の診断のために自覚症状の問診と共に日常診療での積極的な活用が望まれる．

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Abstract

Frequency of Diabetic Polyneuropathy (DPN) and Clinical Significance of Achilles Tendon Reflex in Diagnosis of DPN -Survey of 15,000 Patients in Tohoku, Japan-

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To determine the epidemiological status of diabetic polyneuropathy (DPN) and the usefulness of Achilles tendon reflex (ATR) in clinical DPN diagnosis, we clinically surveyed DPN in patients with diabetes mellitus, working with 448 clinics and hospitals in Tohoku, northeastern Japan. The survey consisted of questionnaires on subjective DPN symptoms and ATR examination and vibration perception.

Data came from 14,744 patients with diabetes mellitus aged 64.2±11.9 years [mean±SD], with a diabetes duration of 9.7±7.7 years, and HbA1c 7.4±2.5%. The frequency of DPN symptoms was 18.8%, the absence or decrease in ATR 40.3%, and the decrease in vibration perception threshold 52.0%. The frequency of DPN was 27.6% based on the general decision of the physician in charge and 35.8% based on simplified diagnostic criteria of the Japanese Study Group on Diabetic Neuropathy, consisting of subjective symptoms of DPN, a decrease in ATR, and decreased threshold of vibration perception. ATR disappeared earlier than the appearance of subjective symptoms of DPN after diabetes onset. The frequency of loss of ATR was significantly higher in patients with subjective symptoms of DPN than in those without symptoms.

These results indicate a high frequency of DPN and the significance and usefulness of ATR, a simple and easy examination, in diagnosing DPN.

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Role of Advanced Glycation End Products in Diabetic Neuropathy

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Abstract: Diabetic neuropathy is the commonest form of peripheral neuropathy in the developed countries of the world. In diabetic patients, the presence of peripheral neuropathy increases their risks for developing foot ulceration and subsequent necrosis that necessitates lower limb amputation. Although the precise mechanisms underlying diabetic neuropathy remain unclear, there is evidence that hyperglycemia-induced formation of advanced glycation end products (AGEs) is related to diabetic neuropathy: AGE-modified peripheral nerve myelin is susceptible to phagocytosis by macrophages and contributes to segmental demyelination; modification of major axonal cytoskeletal proteins such as tubulin, neurofilament, and actin by AGEs results in axonal atrophy/degeneration and impaired axonal transport; and glycation of extracellular matrix protein laminin leads to impaired regenerative activity in diabetic neuropathy. Recently, the receptor for AGEs (RAGE) has been found to colocalize with AGEs in diabetic peripheral nerves. This suggests that, in diabetic neuropathy, AGEs and AGE/RAGE interactions induce oxidative stress, result in upregulation of nuclear factor (NF)-kappaB and various NF-kappaB-mediated proinflammatory genes, and exaggerate neurological dysfunction, including altered pain sensation. Additionally, AGE/RAGE-induced oxidative stress further accelerates formation of glycoxidation products such as Nepsilon-(carboxymethyl)lysine and pentosidine. Although new drugs that inhibit the formation of AGEs and block the AGE-RAGE interaction are being studied, no effective treatment modalities against AGE-induced nerve injury are currently available clinically. A therapeutic strategy to prevent and ameliorate diabetic neuropathy using anti-AGE agents needs to be established. In this review, the current issues involved in the role of the glycation process and the potential treatment options for diabetic neuropathy are explored.

Key Words: Diabetic neuropathy, AGEs, oxidative stress, RAGE, CML.

INTRODUCTION

Diabetic neuropathy is the commonest complication of long-term hyperglycemia and the leading cause of nontraumatic lower limb amputations in diabetic patients [1]. Although the pathogenesis of diabetic neuropathy remains enigmatic [2], there are emerging data from animal and clinical studies suggesting that hyperglycemia-induced formation of advanced glycation end products (AGEs) may play a key role in the pathogenesis of diabetic neuropathy [3], and that it is linked with other pathogenic mechanisms, such as the presence of an increased flux through the polyol pathway [4], increased oxidative stress [5], and activation of the diacylglycerol-protein kinase C (PKC) pathway [6,7]. All of these mechanisms contribute to increased oxidative stress, which in turn further increases the formation of glycoxidation products such as N-epsilon-(carboxymethyl)lysine (CML) and pentosidine. Recently, the receptor for AGEs (RAGE) has been cloned; it was found to colocalize with AGEs in the perineurium, as well as the endoneurial and epineurial vessels, of patients with diabetic neuropathy [8,9]. The formation of AGEs and their interaction with RAGE activate intracellular signaling pathways that induce transcription of proinflammatory genes and therefore cellular oxidative stress, as has been shown in peripheral nerves [8,10]. As well, hyperglycemia *per se* and subsequent activation of the polyol pathway result in increased production of intracellular dicarbonyl AGE precursors such as methylglyoxal and 3-deoxyglucosone; subsequently, these molecules modify proteins to form AGEs intracellularly and cause abnormal interactions with other matrix proteins, which eventually leads to functional and structural abnormalities in diabetic peripheral nerves. The recent development of a noninvasive method for specifically measuring tissue AGE levels showed an association between tissue AGE levels and the severity of peripheral and autonomic dysfunction, as well as the occurrence of foot ulceration in diabetic patients [11]. Thus, this review outlines the current knowledge of the interrelated biochemical mechanisms that have been hypothesized to account for AGE-mediated progressive damage and loss of diabetic nerve fibers. Furthermore, potential therapeutic approaches by which AGE formation can be inhibited or the pathogenic effects of AGE formation limited in experimental and human diabetic neuropathy are discussed.

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INTERRELATIONSHIPS BETWEEN AGES AND OTHER METABOLIC PATHWAYS

(1) AGEs and the Polyol Pathway

Increased production of fructose *via* activation of the polyol pathway enhances generation of glycated proteins. In the polyol pathway, glucose is reduced to sorbitol by the first enzyme, aldose reductase; sorbitol is then converted to fructose by the second enzyme, sorbitol dehydrogenase. Fructose is further metabolized to fructose-3-phosphate and fructose-1-phosphate by 3-phosphokinase [12] and fructokinase [13], respectively. Additionally, fructose is also converted to fructose-6-phosphate by hexokinase; fructose-6-phosphate lies within the glycolysis metabolic pathway and is converted to glucosamine-6-phosphate by glutamine:fructose-6-phosphate amidotransferase and then finally converted to uridine diphosphate *N*-acetylglucosamine in the hexosamine pathway [14] (Fig. 1). One of the reactive alpha-oxoaldehydes and a potent AGE precursor, 3-deoxyglucosone, is formed by nonoxidative rearrangement from fructose [15] and fructose-3-phosphate [16]; it rapidly reacts with protein amino groups to form AGEs such as imidazolone, pyrraline, CML, and pentosidine [17]. Methylglyoxal, another reactive alpha-oxoaldehyde, is formed by the fragmentation of glyceraldehyde-3-phosphate in anaerobic glycolysis [18,19], as well as the oxidative decomposition of polyunsaturated fatty acids [20]. Exposure of rat Schwann cells to methylglyoxal decreases intracellular reduced glutathione (GSH) content, activates p38 mitogen-activated protein kinase (MAPK), and induces apoptosis; these findings suggest that methylglyoxal has a potential role in Schwann cell injury through oxidative stress-mediated p38 MAPK activation [21]. Glyceraldehyde-derived AGEs have been found to be the most common toxic AGEs in the serum of diabetic patients, and they are formed from glyceraldehyde-3-phosphate and fructose-1-phosphate [22] (Fig. 1). Interestingly, in isolated rat Schwann cells, AGEs derived from glyceraldehyde and glycolaldehyde, but not glucose,

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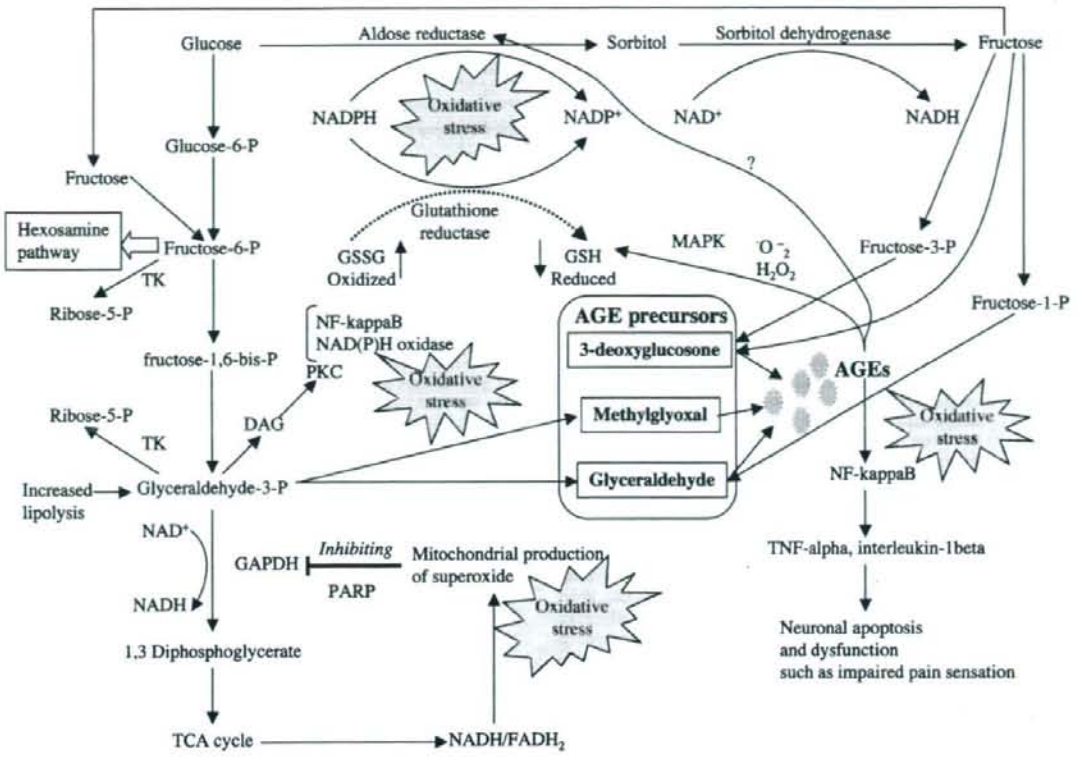

Fig. (1). Interrelationship between nonenzymatic glycation and other metabolic pathways.

In the polyol pathway, the aldose reductase consumes the cofactor NADPH [117], which is also the essential cofactor for regenerating a critical intracellular antioxidant, GSH. Increased flux through the polyol pathway promotes intracellular oxidative stress. The neurotoxic intracellular AGE precursors methylglyoxal and glyceraldehyde are formed from glyceraldehyde-3-phosphate, which also activates the classical protein kinase C (PKC) pathway, since the activator of PKC, diacylglycerol (DAG), is also formed from glyceraldehyde-3-phosphate. Hyperglycemia-induced generation of superoxide inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity by activating poly(ADP-ribose) polymerase (PARP). In the unifying hypothesis, it is postulated that impaired GAPDH activity activates the polyol pathway, the PKC pathway, and the hexosamine pathway, and increases AGE formation [118]. Oxidative stress induced by AGE formation, as well as other pathways activated by hyperglycemia, enhances generation of glycoxidation products such as Nepsilon-(carboxymethyl)lysine and pentosidine. Thiamine and benfotiamine activate transketolase (TK), which stimulates the conversion of glyceraldehyde-3-phosphate and fructose-6-phosphate to ribose-5-phosphate, thereby inhibiting the hexosamine pathway, the PKC pathway, and AGE formation [119]. AGEs, advanced glycation end products; FADH, reduced flavin adenine dinucleotide; GSSG, oxidized glutathione; MAPK, mitogen-activated protein kinase, NAD(P)H, reduced nicotinamide adenine dinucleotide (phosphate); NF-kappaB, nuclear factor-kappaB; RAGE, receptor for advanced glycation end products; TCA, trichloroacetic acid; TNF, tumor necrosis factor.

increase the release of tumor necrosis factor (TNF)-alpha and interleukin-1beta, activate nuclear factor (NF)-kappaB, decrease the mitochondrial membrane potential, and induce apoptosis [23]. It has also been demonstrated that, under high glucose conditions, NF-kappaB expression is upregulated in rat Schwann cells *via* activation of the polyol pathway [24]. It has been suggested that long-term upregulation of NF-kappaB, along with PKC-beta activation linked to the polyol pathway activity, contributes to the development of peripheral nerve dysfunction, including altered pain sensation in diabetic mice [25,26]. Low TNF-alpha concentrations applied along the nerve elicit an increase in ectopic discharges of single nociceptive primary afferent fibers, whereas higher concentrations lead to reduced firing rates [27]. Thus, it is possible that, in experimental diabetic neuropathy models, along with increased flux from the polyol pathway, AGE-dependent intracellular signaling in Schwann cells leads to neurological dysfunction, including altered nociception *via* oxidative stress-induced activation of NF-kappaB and increased levels of proinflammatory cytokines such as TNF-alpha (Fig. 1).

The role of fructose as a glycator is further exemplified by the fact that diabetic patients and animals treated with aldose reductase inhibitors develop significant reductions in glycated protein levels in the aorta, lens, and erythrocytes [28-30]. Conversely, methylglyoxal has been reported to increase aldose reductase mRNA/protein expression and activity in rat aortic smooth muscle cells by inducing oxidative stress [31]. Enhanced activation of the polyol pathway by AGEs has also been demonstrated in mouse aortic smooth muscle cells and mesangial cells overexpressing the human aldose reductase gene [32,33]; this enhancement may be mediated by AGE-induced activation of the extracellular signal-regulated kinase pathway under oxidative stress [34,35]. Thus, these results suggest that increased production of fructose through activation of the polyol pathway enhances generation of glycated proteins, which in turn activate the polyol pathway in tissues that are the targets of

long-term diabetic complications. However, there is a paucity of evidence regarding the presence of such reciprocal interactions in peripheral nerves (Fig. **1**).

(2) AGEs and Oxidative Stress

"Glycoxidation" is a term used for the glycation processes that involve oxidative stress. In diabetes, major glycoxidation products, such as CML and pentosidine, are considered to be general markers of oxidative stress and protein damage; these products have been found to accumulate in human diabetic peripheral nerves [8,36-38]. Hyperglycemia-induced oxidative stress may be caused by a number of biochemical processes, including: glucose autoxidation and glycoxidation [39]; depletion of free radical scavengers and antioxidants [40,41]; the polyol pathway-dependent redox status [42]; overproduction of superoxide *via* the mitochondrial electron-transport chain [43]; mitochondrial fission [44]; altered polarization of the mitochondria [45]; activation of xanthine oxidase [46]; protein kinase C-dependent activation of reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase [47]; excessive formation of early glycation products [48] and AGEs [49]; and AGE receptor-triggering cellular oxidative stress (see below). Recent studies have demonstrated increased oxidative stress in experimental [50-54] and human diabetic neuropathy [55,56]. It has been suggested that AGEs enhance oxidative stress by reducing the level of GSH, which is the most important antioxidant in most mammalian cells [57] and, in SH-SY5Y human neuroblastoma cells, the oxidative stress in turn mediates AGE-induced cytotoxicity [58]. The AGE-induced depletion of GSH can be prevented by the radical scavengers N-acetylcysteine, alpha-lipoic acid, and 17beta-estradiol, or by the use of catalase; this indicates that superoxide and hydrogen peroxide production precedes the AGE-induced depletion of GSH [57] (Fig. **1**). Therefore, it is possible that hyperglycemia-induced oxidative stress accelerates the accumulation of AGEs, which in turn further increases the oxidative peripheral nerve injury that is associated with perturbed neuronal and Schwann cell signal transduction. In this manner, progressive fiber loss and impaired regeneration is promoted in diabetic neuropathy. Furthermore, a recent study demonstrated a concurrent increase in AGEs, neuronal nitric oxide synthase content, and oxidative stress-triggered apoptosis in pelvic ganglion neurons obtained from long-term diabetic rats. This suggests that AGEs and endogenous nitric oxide have a synergistic action in oxidative stress and irreversible nitrergic degeneration in experimental diabetic autonomic neuropathy [59].

MODIFICATION OF NEURONAL PROTEINS BY AGES AND ITS POSSIBLE IMPLICATION FOR DIABETIC NEUROPATHY

Diabetic rodent studies have shown that myelin components in both the central [60] and peripheral nerve system [61] are subject to nonenzymatic glycation. It has been suggested that AGE-modified peripheral nerve myelin is susceptible to phagocytosis by macrophages and that this stimulates macrophages to secrete protease, which may contribute to demyelination in diabetic neuropathy [62] (Fig. **2**).

Major axonal cytoskeletal proteins such as tubulin, neurofilament, and actin are also likely to undergo glycation [63-66]. These axonal proteins are central to the maintenance of axonal function and structure, and their modification by glycation may alter the structural and functional properties of the axon, thereby contributing to axonal atrophy and degeneration, as well as to slowing of axonal transport (Fig. **2**).

It has been suggested that glycation of the peripheral nerve extracellular matrix impairs peripheral nerve regeneration. The extracellular matrix protein laminin promotes the extension of neuronal processes, and glycation of a biologically active domain with laminin decreases neurite outgrowth in the murine neuroblastoma cell line [67]. More recent studies have shown similar results, in that the glycation of collagen type IV and laminin, which are major components of basal lamina, as well as of collagen type I, reduces neurite outgrowth in dorsal root ganglion neurons obtained from neonatal rats [68] and young adult mice [69] (Fig. **2**).

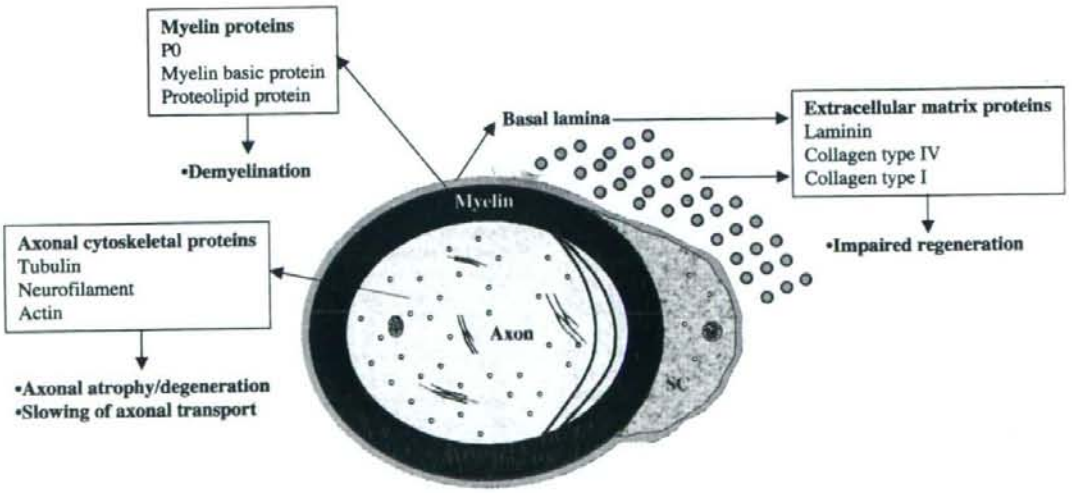

Fig. (2). Modification of neuronal proteins by AGEs and its possible implication in diabetic neuropathy.

Modification of major axonal cytoskeletal proteins and myelin components by AGEs may lead to axonal degeneration and demyelination. The extracellular matrix protein laminin may be modified by AGEs and involved in impaired regeneration in models of experimental diabetic neuropathy. However, the proposed mechanisms of AGE-induced nerve injury remain largely speculative and need to be explored in future studies. SC, Schwann cell.

ROLE FOR AGE-RAGE INTERACTION IN DIABETIC NEUROPATHY

Oxidative stress is a mediator of hyperglycemia-induced cell injury and may be promoted by the activation of intracellular signaling pathways *via* an interaction between AGEs and their receptors. RAGE is a multiligand member of the immunoglobulin superfamily of cell surface molecules with a diverse repertoire of ligands [70]; it is present in various tissues, including the peripheral nervous system [71,72]. Earlier studies reported that interaction between AGEs and RAGE results in induction of endothelial cell oxidative stress and in the activation and translocation of NF-kappaB from the cytoplasm into the nucleus [10,73], and induces vascular hyperpermeability *via* an oxidant-sensitive mechanism in diabetic vascular endothelial cells [74]. A more recent study [75] revealed that dorsal root ganglion neurons express functional RAGE and respond to the RAGE ligand with similar downstream signaling, oxidative stress, and cellular injury as occurs in other diabetic complication-prone tissues. In these neurons, activation of the RAGE-mediated signaling pathway involves formation of reactive oxygen species, caspase-3 activation, and nuclear DNA degradation; all of these are prevented by antioxidant alpha-lipoic acid treatment [75]. *In vitro* studies have shown that RAGE, through regulation of NF-kappaB expression, is an important mediator for neurite outgrowth and cell survival [76,77]. Human sural nerve biopsy studies have revealed that RAGE ligands, the receptor itself, and NF-kappaB colocalize in the perineurium, as well as in the endoneurial and epineurial vessels of patients with diabetic neuropathy [8,9] and those with impaired glucose tolerance-related peripheral neuropathy [78]; these findings suggest that activation of the AGE/RAGE/NF-kappaB signaling pathway contributes to the pathogenesis of diabetic and prediabetic neuropathy. Bierhaus *et al.* [8,10] have demonstrated that diabetes-associated loss of pain sensation and upregulation of peripheral nerve NF-kappaB and NF-kappaB–dependent proinflammatory gene expression are induced by AGE-RAGE interaction and are diminished in RAGE-null mutant mice or with the use of soluble RAGE that consists of the AGE-binding domain. A new study has reported that diabetic RAGE-null mutant mice do not develop the characteristic functional and structural abnormalities of experimental diabetic neuropathy, such as nerve conduction deficits and axonal atrophy [79] (Fig. **3**).

Exposure of vascular smooth muscle cells to high glucose levels causes NF-kappaB activation and leads to oxidative stress and cellular activation *via* PKC activation[80]. In the diabetic peripheral nerve, PKC is activated in the *vasa nervorum* of diabetic rats [81] and in the vessel-rich epineurial tissue of diabetic mice [25]. Importantly, fractioned analysis of peripheral nerves has revealed that membrane PKC-alpha decreases in nerve fibers, while membrane PKC-beta increases in vascular tissues of diabetic mice overexpressing human aldose reductase, but not in vascular tissues of wild-type diabetic mice [26]. As previously described, increased polyol pathway flux contributes to generation of AGEs *via* formation of methylglyoxal and 3-deoxyglucosone, which are central precursors in the generation of an array of AGEs, including CML and pentosidine. Thus, it is possible that high glucose- and polyol pathway-mediated PKC activation and AGE accumulation, as well as AGE-RAGE interaction, occur in diabetic *vasa nervorum*. This underscores the synergistic action of the polyol pathway and the AGE/RAGE-dependent pathway in oxidative stress and NF-kappaB activation, which exaggerates neurovascular dysfunction in experimental diabetic neuropathy (Fig. **3**). Furthermore, the interaction of AGEs with RAGE may lead to perturbed vascular barrier function, which is associated with an enhanced expression of vascular cell adhesion molecule-1, monocyte chemoattractant protein 1, and E-selectin [82,83]. However, it is noteworthy that there are other AGE receptors, such as the macrophage scavenger receptor and the galectin-3 receptor, that might have similar deleterious effects to RAGE when they interact with AGEs [84]. On the other hand, intracellularly generated AGEs and AGE-modified extracellular matrix lead to the generation of reactive oxygen species and thus oxidative stress *via* nonreceptor mechanisms [85].

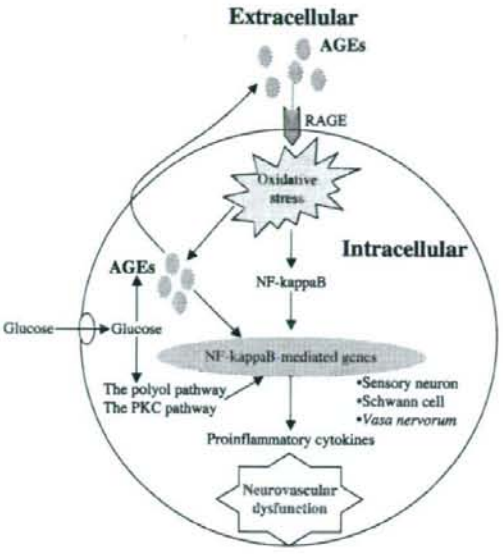

Fig. (3). Role of AGE-RAGE interaction in experimental diabetic neuropathy.

Along with increased flux through the polyol pathway and the protein kinase C pathway, AGE-RAGE-interaction induces perturbed neuronal function and structure resulting in altered pain sensation, a nerve conduction deficit, and axonal atrophy *via* upregulation of peripheral nerve nuclear factor (NF)-kappaB and NF-kappaB–dependent proinflammatory genes in diabetic animals.

AGEs, advanced glycation end products; RAGE, receptor for advanced glycation end products.

AGE ACCUMULATION IN HUMAN DIABETIC NEUROPATHY

An immunohistochemical study revealed that immunoreactivity for pyrraline, an AGE, is increased in the sclera, pia mater, cribriform plates, and connective tissues of human diabetic optic nerve[86]. Using an antiserum that specifically recognizes protein-bound CML, which is a major product of oxidative modification of glycated proteins, we [36] and other investigators [8,37] demonstrated immunolocalization of CML in perineurial basal laminae, axons, Schwann cells, and endoneurial microvessels of human peripheral nerves. The staining intensities were significantly increased in diabetic patients and were correlated with myelinated fiber loss [36]. Despite the preferential localization of CML immunoreactivity in these lesions, no direct link between the pathological changes noted in the nerve fibers and the localization of CML accumulation has been made. In this regard, perineurial cells and their basement membranes are thought to play a role in the maintenance of perifascicular diffusion barrier to the macromolecules. It is therefore possible that excessive accumulation of CML-modified adducts in the perineurium and endoneurial microvasculature alters the endoneurial microenvironment and microcirculation, thereby contributing to the development of diabetic neuropathy [36]. However, this hypothesis needs to be addressed in future studies.

A new study examined skin biopsy specimens using the immunoperoxidase technique to estimate CML and RAGE content. It was found that the perineurium of peripheral nerves and the *vasa nervorum* in the dermis were more intensely stained for CML, while stronger RAGE immunoreactivity was observed in the bundles of axons and *vasa nervorum* in type 1 diabetic patients receiving kidney transplantation alone, compared to those receiving a successful islet-after-kidney transplantation [87]. Pentosidine levels were also increased in cytoskeletal and myelin protein extracts of human sural nerves [38]. More recently, pronounced AGE immunoreactivity was detected in the axons and myelin sheaths of 90% of type 2 diabetes patients, while no AGE immunoreactivity was detected in control subjects; the intensity of the axonal AGE immunoreactivity was correlated with the severity of the structural changes that are characteristic of diabetic neuropathy, such as perineurial thickening, microvascular luminal narrowing, and axonal loss [88].

SERUM AND SKIN AGES RELATED TO SEVERITY OF HUMAN DIABETIC NEUROPATHY

Accumulation of AGEs in the skin, serum, and other specimens obtained from diabetic patients has been linked to the progression of microvascular complications, including diabetic neuropathy. Earlier studies using gas chromatography-mass spectrometry measured the levels of glycoxidation products such as CML and/or pentosidine in collagen obtained from skin-punch biopsies and demonstrated that the levels of these glycoxidation products were correlated with the severity of nephropathy, retinopathy, and vasculopathy in type 1 diabetic patients [89-91]. A more recent cross-sectional study involving 50 patients with type 2 diabetes measured AGE levels in skin, serum, saliva, and urine using spectrofluometry HPLC; the AGE levels in all specimens except for the urine increased as the patients' neuropathy, retinopathy, and nephropathy progressed [92]. Another recent study [93] measured serum CML levels using enzyme-linked immunosorbent assay with a monoclonal anti-CML antibody (6D12) in 94 type 1 diabetic children and adolescents with or without background retinopathy, microalbuminuria or neuropathy; CML levels were higher in patients with chronic complications than in patients without complications. Taking for granted the fact that CML formation depends on the oxidative condition, the increased CML levels found in the serum and tissue proteins of diabetic patients suggest that CML has a role as an endogenous biomarker of oxidative damage. Meerwaldt el al. [94] developed an AutoFluorescence Reader, a noninvasive method that uses fluorescence properties to specifically measure skin AGE contents. Using this apparatus, they assessed skin AGE accumulation in 24 diabetic patients with a history of foot ulceration, in 23 diabetic patients without clinical neuropathy, and in 21 control subjects; they found that the skin autofluorescence, which reflected tissue AGE accumulation, increased during early stages of diabetic neuropathy and was correlated with the severity of peripheral and autonomic nerve abnormalities, as well as with the presence of foot ulceration [11]. In addition, in a large group of type 2 diabetic patients, the same group confirmed that skin autofluorescence was higher in patients than in age-matched control subjects and was associated with the severity of diabetes-related complications [95]. They proposed that measurement of skin autofluorescence is a rapid and helpful tool that can be used in diabetes outpatient clinics to identify patients who are at risk for developing complications.

EFFFECTS OF ANTI-AGE AGENTS ON DIABETIC NEUROPATHY

Aminoguanidine is a highly reactive nucleophilic reagent that prevents the formation of AGEs by reacting with the carbonyl groups of reducing sugars, as well as alpha- and beta-dicarbonyl compounds such as methylglyoxal, glyoxal, and 3-deoxyglucosone. Aminoguanidine was the most promising agent for preventing AGE-mediated tissue damage caused by diabetes. In fact, initial rat studies confirmed the beneficial effect that aminoguanidine had on the development of retinopathy, nephropathy, and neuropathy. In particular, long-term aminoguanidine treatment improved the nerve conduction deficit and myelinated fiber pathology in diabetic rats [96]. Impaired nerve blood flow in diabetic rats was also normalized after aminoguanidine treatment, but such treatment had no effect on oxygen free radical activity [97]; this suggests that the hemodynamic changes were modulated without affecting oxidative stress. Short-term treatment with aminoguanidine ameliorated nerve conduction slowing and Na^+/K^+-ATPase defects, but not endothelial damage, as reflected by systemic thrombomodulin concentrations [98]. Treating diabetic rats with aminoguanidine improved endoneurial blood flow but not the impairment of endothelium-dependent vasodilation of epineurial vessels of the sciatic nerve [99,100]. The beneficial effects that aminoguanidine had on the structural alterations of endoneurial microvessels were also documented in long-term diabetic rats [101]. In contrast to these rat studies, in type 1 diabetic baboons who had diabetes for less than 5 years, treatment with aminoguanidine for 3 years did not affect impaired nerve conduction velocity, heart rate response, and myelinated fiber pathology. This suggests that, in the type 1 diabetic primate, the accumulation of AGEs is involved only in nerve damage that occurs after a more prolonged observation period [102].

A double-blinded, multiple-dose, placebo-controlled, randomized clinical trial of aminoguanidine in diabetic patients with overt diabetic nephropathy (ACTION) was completed in 1998; ACTION I involved 690 type 1 diabetic patients, and ACTION II involved 599 type 2 diabetic patients. These studies were designed to evaluate the safety and efficacy of aminoguanidine in slowing the rate of progression of renal disease in patients with overt diabetic nephropathy. The primary endpoint was doubling of the baseline serum creatinine. In ACTION I, patients received placebo, high-dose (100-600 mg per day) aminoguanidine, or low-dose (50-300 mg per day) aminoguanidine; the combined aminoguanidine dose group showed decreased progression of diabetic retinopathy and lower triglyceride, LDL cholesterol, and urinary protein levels, as well as a non-significant trend towards a slower doubling of serum creatinine. However, ACTION II was terminated prematurely due to safety concerns and apparent lack of efficacy. Reported side effects of aminoguanidine included gastrointestinal disturbance, liver function test abnormalities, flu-like symptoms, and a rare vasculitis [103]. Based on the outcomes and the side effects noted in these trials, there is no benefit in using aminoguanidine.

Other anti-AGE agents, including the thiazolidine derivative OPB-9195, have been investigated; OPB-9195 has been shown to prevent the progression of diabetic nephropathy in rats [104]. It has also been shown to improve motor nerve conduction slowing without affecting body weight and blood glucose levels in diabetic rats; the improvement was associated with reduced serum AGE levels and peripheral nerve expression of immunoreactive AGE and immunoreactive 8-hydroxy-2'-deoxyguanosine, which is a marker for oxidative stress-related DNA damage, as well as an increase in peripheral nerve (Na^+, K^+)-ATPase activity [105]. An alternative approach is to reduce tissue AGE accumulation by selectively cleaving the resultant AGE crosslinks. Diabetic rats were found to have increased mesenteric vascular AGE accumulation and mesenteric vascular hypertrophy; both of these were prevented by treatment with N-phenacylthiazolium bromide (PTB), which is a prototypic AGE crosslink breaker that attacks covalent carbon-carbon bands of dicarbonyl-derived crosslinks both *in vitro* and *in vivo* [106]. However, a more recent study has demonstrated that although AGE-breakers such as PTB and N-phenacyl-4,5-dimethylthiazolium cleave model crosslinks *in vitro*, they do not significantly cleave AGE crosslinks formed *in vivo* in skin collagen of diabetic rats [107]. Clinical trials of ALT-711, a novel AGE breaker, have shown favorable results with respect to blood pressure and vascular elasticity in aged persons with stiffened vascula-

ture [108], but treatment with ALT-711 for 2 weeks had no effects on motor nerve conduction deficit, C-fiber-mediated nociceptive dysfunction, or impaired pressure-induced vasodilation in diabetic mice after 8 weeks of diabetes [109]. Interestingly, benfotiamine, a lipophilic analogue of thiamine, is a transketolase activator that inhibits three of the four major biochemical pathways implicated in the "unifying hypothesis" of the pathogenesis of hyperglycemia-induced vascular damage: the hexosamine pathway, PKC activation, and AGE formation [110] (Fig. **1**). It has higher bioavailability after oral administration and normalizes cell replication, lactate production, and AGE formation in human umbilical vein cells and bovine retinal endothelial cells cultured in high glucose concentrations [111]. In diabetic rats, the efficacy of benfotiamine with respect to peripheral nerve function and AGE accumulation has been documented, with nearly normalized nerve conduction velocity and inhibition of neural imidazole-type AGE and CML formation after 6 months of benfotiamine treatment [112]. In nondiabetic and diabetic rats, benfotiamine also reduced inflammatory and neuropathic nociception [113]. Therefore, the ability of benfotiamine to inhibit three major pathways simultaneously might be clinically useful in preventing the development and progression of diabetic complications, including neuropathy [114,115]. Finally, the formation of AGEs can also be limited by inhibitors such as pyridoxamine, tenilsetam, and 2,3-diaminophenazone, but their efficacy for treating diabetic neuropathy still remains to be explored [116].

SUMMARY

Taken together, these results indicate that accumulation of AGEs and their binding to RAGE play a key role in the pathogenesis of experimental diabetic neuropathy with increased generation of reactive oxygen species, proinflammatory cytokines, and adhesion molecules, and oxidative stress associated with activation of the NF-kappaB signaling pathway. In addition to polyol pathway hyperactivity, AGE-RAGE-mediated cellular oxidative stress further enhances the accumulation of glycoxidation products such as CML and pentosidine. Although several AGEs have been identified that accumulate in the peripheral nerve of diabetic humans and animals, in diabetic patients the accumulation of AGEs is evident in undamaged axons, Schwann cells, and *vasa nervorum* [36]. Thus, a direct linkage between AGE accumulation and nerve injury that eventually leads to progressive damage and loss of unmyelinated and myelinated nerve fibers is still lacking in the human diabetic nerve. Furthermore, no agents are in current clinical use to block AGE/RAGE signaling in diabetic patients. Therefore, further studies are needed to explore the precise mechanisms that underlie AGE-induced nerve injury and to establish the optimal therapeutic strategy for AGE/RAGE signaling blockade in human diabetic neuropathy.

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ABBREVIATIONS

AGEs	=	Advanced glycation end products
PKC	=	Protein kinase C
CML	=	N-epsilon-(carboxymethyl)lysine
RAGE	=	Receptor for AGEs
GSH	=	Reduced glutathione
MAPK	=	Mitogen-activated protein kinase
TNF	=	Tumor necrosis factor
NF-kappaB	=	Nuclear factor-kappaB
NAD(P)H	=	Reduced nicotinamide adenine dinucleotide (phosphate)
ACTION	=	Aminoguanidine in overt diabetic nephropathy
PTB	=	Phenacylthiazolium bromide

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