CHAPTER II

LITERATURE REVIEW

1. Diabetes mellitus

1.1 Definition

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting in abnormal lipid and protein metabolisms. Hyperglycemia results from either insufficient insulin secretion or impaired insulin action on targets to maintain normal blood glucose (Alberti and Zimmet, 1998).

1.2 Classification

There are two main types of diabetes, Type 1 and Type 2, which have different etiologies. Type 1 diabetes, previously called insulin-dependent diabetes, is caused by pancreatic islet beta-cell destruction resulting in absolute insulin deficiency. An autoimmune process is the principal cause of Type 1 diabetes. In some patients, the underlying cause is unknown or idiopathic. Exogenous insulin supplementation is usually necessary to prevent the fatal complications of hyperglycemia.

Type 2 diabetes, previously called non insulin-dependent diabetes, is the most common form of diabetes in adult and is characterized by impaired insulin secretion or insulin action, but not significant loss of the pancreatic beta-cells. This results in relative insulin deficiency and hyperglycemia. Patients affected with Type 2 diabetes do not require insulin early in the course but may need insulin in the advanced stage. Specific causes are mostly unknown but evidence suggests the interaction between genetic predisposition and environmental factors, especially food consumption.

2. Diabetic neuropathy

2.1 Prevalence and significance

The long-term exposure of organs to hyperglycemia leads to chronic complications, for instance, kidney (nephropathy), retina (retinopathy), large blood vessels (arteriosclerosis) and peripheral nerve (neuropathy). Diabetic neuropathy is frequently observed in patients especially with long-term or poorly controlled diabetes (Boulton and Malik, 1998).

The progression of diabetic neuropathy has been shown to be highly related to glycemic control (The Diabetes Control and Complications Trial Research Group, 1995). Abnormal sensory functions are frequently found in the distal parts of bilateral extremities (distal symmetrical), especially toes and feet prior to fingers and hands. Reduced pain perception (hypoalgesia) particularly in the feet can lead to repeated trauma and ulceration which is aggravated by the impairment of vascular supply and immune functions required for normal wound healing. Severe foot ulcer can result in amputation and renders patients disabled.

2.2 Clinical manifestation

Diabetic neuropathy is a heterogenous condition, affecting different parts of the nervous system with diverse clinical manifestations. They may be focal or diffuse (Boulton et al., 2005). Patients with focal diabetic neuropathy (mononeuropathy) typically present with sensory symptoms although motor loss and abnormal deep tendon reflexes can be present. Although any cranial or peripheral nerve can be involved, the most common sites include the oculomotor,median,radial,and lateral popliteal nerves. Entrapment syndrome which is more common in diabetic patients, is thought to be the main cause of mononeuropathy .

Distal symmetrical sensory polyneuropathy is the most common neurologic syndrome seen in diabetes. This process involves all somatic nerves but has a strong predilection for distal sensory nerves of the feet and hands. Small unmyelinated sensory fibers transmitting pain and temperature are generally preferentially affected.

Large myelinated fibers which carry touch, vibratory, and proprioceptive sensations can be affected later in the course. With early diabetic neuropathy, most patients are asymptomatic with subtle abnormalities which can be only detected by neurological examination.

Patients typically report numbness and tingling of the distal extremities, often in the classic "glove-stocking" distribution. Quantitative sensory testing is used to assess each sensory modality, for example, vibration perception threshold, thermal perception threshold and pain perception threshold. Therefore, quantitative sensory testing can test all sensory modalities and provide a more complete picture of neuropathy relative to other tests. However, due to the fact that the values depend on a patient's interpretation of the stimuli tested, this sensory testing is less objective and produces higher variation (Valensi et al., 1993). In spite of this drawback, several studies have demonstrated that quantitative sensory tests, particularly vibration perception threshold, are useful for evaluating sensory function in diabetes (Boulton et al., 1986; Young et al., 1994; Dyck et al., 2000). Some clinical studies have shown that diabetic patients had impaired vibration and temperature perception indicating involvement of large myelinated and small unmyelinated nerve fibres, respectively (Redmond et al., 1992; Hotta et al. 1996; Laudadio and Sima, 1998).

The prominent pathological features of diabetic neuropathy are segmental demyelination and axonal degeneration which correlate well with the physiological abnormalities. NCV abnormalities can be found in the absence of clinical deficits. The slowing of NCV suggests demyelination as the primary defect followed by reduced amplitude indicating axonal degeneration in the more advanced stage. Two studies have shown decreased fibre density and axonal size in sural nerve biopsies from diabetic patients with established neuropathy (Sima et al., 1988b; Malik, 1997). It is known that potential treatments successful in improving nerve conduction in pre-clinical studies so far have failed to produce substantial effects on NCV in clinical trials although the changes were statistically significant. It is still unknown whether this is because nerve damage responsible for NCV slowing is difficult to recover, or treatments were not effective or NCV is not suitable as an end-point test.

2.3 Etiologies

The mechanisms underlying the development of diabetic neuropathy are not fully understood. So far, there are several hypotheses proposed which can be categorized into metabolic and vascular causes. Hypotheses in the metabolic category have been used to correlate hyperglycemia and its associated metabolic abnormalities with the progression of diabetic neuropathy, for instance, increasing activity of polyol or sorbitol pathway (Gabbay et al.,1966;Gillon et al.,1983;Tomlinson et al.,1993) and oxidative stress (Baynes et al.,1991;Young et al., 1992;Garg et al.,1996). For the vascular category, endothelial and autonomic nervous system dysfunctions have been proposed (Tesfaye et al., 1994). Moreover, impaired neurotrophic support is also believed to play a role in the development of diabetic neuropathy (Fernyhough and Tomlinson, 1999).

3. Animal models of diabetes

In order to investigate the underlying mechanisms of diabetic complications, animal models of diabetes are essential due to several limitations of human subjects, especially, the uncertain onset and duration of diabetes in human. There are two principal types of animal model which are currently used, chemical-induced diabetes and genetic-associated diabetes. The chemical-induced diabetic animal is more widely used and accepted as a model for Type 1 diabetes due to the presence of the following features: hyperglycaemia, hypoinsulinemia and weight loss. The streptozotocin (STZ)-diabetic rat is the most commonly used of this type. With intravenous or intraperitoneal injection, STZ causes beta-cell destruction, characterized by reduction in the size of islets of Langerhans, resulting in hyperglycaemia 2 days after injection (Rakieten et al., 1963). STZ diabetic rat is the most characterized animal model of diabetes. Other animal models for Type 1 and Type 2 diabetes are less frequently used and, therefore, less information regarding diabetes-associated abnormalities is available.

3.1 Similarities and differences between animal models of diabetes and human diabetes

As previously mentioned, animal models have been extensively used in the studies of diabetic neuropathy. Several disparities between animals and humans have been demonstrated raising doubts on the appropriateness of animal models of diabetic neuropathy. Below are important differences between human and experimental diabetes.

1. Biochemical changes observed in human and experimental diabetic neuropathy are not exactly the same. Levels of sorbitol and fructose accumulation generated by flux through the polyol pathway in response to hyperglycaemia per unit weight of nerve from STZ-diabetic rats are higher than in human diabetic nerve.Therefore, using diabetic rat model might result in overestimation of the influence of the polyol pathway. (Hounsom and Tomlinson, 1997).

2. In the STZ-diabetic rats, reduction of axonal size and myelin thickness was found as early as 4 weeks (Jakobsen, 1976a; Jakobsen 1976b). Decrease in axonal size and myelin thickness was also observed at 4 months (Bestetti et al., 1981a) and 12 months of diabetes (Bestetti et al., 1981b; Zemp et al., 1981). Some studies have also found ultrastructural abnormalities of Schwann cell (Bestetti et al., 1981a; Bestetti et al., 1981b) and relatively less in axon (Zemp et al., 1981) in diabetic nerves. However, demyelination and axonal loss can be found in animals with longer duration of diabetes (Sima et al., 1990). In human diabetes, demyelination and axonal loss are prominent features which are less observed in diabetic rodents. However, reduced axonal size is the common finding in both diabetic patients and animals.

3. Regarding electrophysiological abnormalities, reduction in motor NCV is observed early in the course of STZ-induced diabetes, whereas sensory NCV is firstly affected in human diabetic neuropathy. However, both motor and sensory NCV deficits develop later in both diabetic animals and patients.

In spite of these differences, animal models of diabetic neuropathy are still widely used in research due to limitations of studies in human and similarity in most parameters between animal and human diabetes. STZ-diabetic rat is preferentially used probably because of low cost to produce and maintain the condition. However, the above problems must be kept in mind when data from animal studies are applied to clinical situation.

4. MAPKs and diabetic neuropathy

Several drugs or molecules have been tested for the treatment of diabetic neuropathy, such as aldose reductase inhibitors (Pfeifer et al., 1997; Hotta et al., 2001), neurotrophins [nerve growth factor (NGF)(Apfel et al.,2000),brain-derived neurotrophic factor (BDNF) (Wellmer et al.,2001)] and some anti- oxidants especially alpha-lipoic acid (Ziegler et al.,1995). However, the results of those treatments did not show satisfactory clinical improvement. Therefore, future extensive studies in the underlying mechanisms of diabetic neuropathy are still needed to provide the effective therapy.

4.1 Mitogen-activated protein kinases (MAPKs)

Mitogen-activated protein kinases (MAPKs) are serine/threonine specific kinases activated through dual phosphorylation at threonine and tyrosine residues on TXY motif (T = Threonine, Y = Tyrosine) (Robbins et al., 1993) in response to extracellular stimuli: growth factors, hormones, cytokines and cellular stresses.

MAPKs lie in protein kinase cascades co-ordinating incoming information from other signaling pathways and amplifying signals. The divergence of the pathway allows for a variety of response patterns depending on cell type and stimuli (Anderson et al., 1990; Cobb et al., 1991).So far, three main groups of members of the MAPK family are present:

1. Extracellular signal-regulated protein kinase (ERK). ERK has 5 isoforms (ERK 1-5) but only ERK1 and 2 are the most extensively studied and characterized (Gupta et al., 1996 and Horgan, 2003).
2. c-Jun NH_2-terminal protein kinase (JNK). For JNK, ten isoforms originating from three homologous genes (jnk 1-3) with molecular weights of 46 and 54 kDa due to alternative splicing have been identified (Kyriakis et al., 1994; Gupta et al., 1996).
3. p38 protein kinase. Four isoforms of p38 (α, β, γ and δ) have been found (Wang et al., 1997).

The MAPK signaling pathway is very complicated and overlap exists between each MAPK cascade. ERK is a vital mediator of a number of cellular events ranging from growth, proliferation to survival. Growth factors (Averill et al., 2001) , cytokines (Cobb et al., 1999) and oxidative stress (Stanciu et al., 2000) can activate the ERK pathway probably via the membrane receptor (Figure 1). In this cascade, MEK1 and MEK2 function as upsteam MAPKKs and Raf proteins as MAPKKKs. The cell surface receptors such as tyrosine kinase receptor and G protein-coupled receptors transmit activating signals to the Raf/MEK/ERK cascade via the small Ras-GTP. Activation of membrane-associated Ras is achieved through recruitment of SOS, a Ras-activating guanine nucleotide exchange factor. SOS stimulates Ras, allowing it to interact with Raf (Ou et al., 1995; Johnson et al., 1996) Raf as MAPKKK activates MEK 1/2 which then stimulates ERK1/2 through phosphorylation. Then, the activated ERK translocates to the nucleus and phosphorylate different transcription factors such as Elk-1 (Price et al., 1995), Signal transducer and activator of transcription 3/5(STAT3/5) (Pircher et al., 1999). Apart from the transcription factors, ERK can also phosphorylate membrane and cytoplasmic proteins for example, A-type potassium channel Kv 4.2 (Adams et al., 2002), microtububule-associated proteins and neurofilaments (Veeranna et al., 1998).

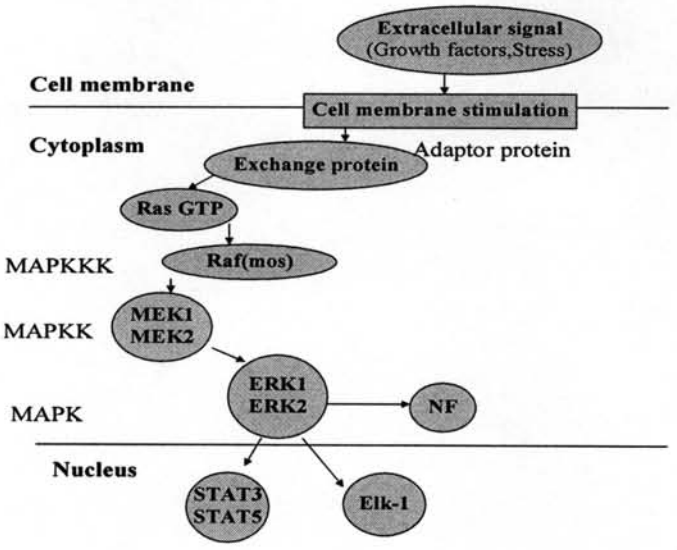

Figure 1 Pathway of MAPK ERK

4.2 Roles of MAPKs in neurons

Roles of MAPKs depend on cell type and the stimuli and can lead to cell proliferation, differentiation and survival (Marshall et al., 1999; Tran et al., 2001; Xiao et al., 2003).

In PC12 cells and sympathetic neurons, ERK plays an anti-apoptotic role (Xia et al., 1995; Anderson et al., 1999). In contrast, ERK is associated with apoptosis of others neuron and supporting cell (Bhat et al., 1999; Blazquez and Satoh, 2000; Stanciu, 2000 and 2002). In vitro,activation of JNK leading to neuronal death is well recognized. JNK and its main transcription factor c-Jun are activated in PC12 cells and cultured cerebellar granule neurons leading to cell death (Lelkes et al., 2001; Cao et al., 2004). Similarly,evidence suggests that p38 activation plays a role in neuronal apoptosis. Spinal cord injury has been report to cause p38 and caspase-3 activation while SB 203580,an inhibitor of p38 , significantly reduced the number of apoptotic cells in the injured spinal cord (Wang et al.,2005). Therefore, from the above evidence, the role of ERK in neuronal cell death is still controversial and appears to be dependent on cell type, while the implication of JNK and p38 in neuronal cell death is more constantly evident.

In the peripheral nervous system (PNS), ERK is present in dorsal root ganglion (DRG) neuron, satellite cell including Schwann cell (Averill et al., 2001). ERK in the PNS is likely involved in nerve injury and regeneration because it is activated after nerve injury (Sheu et al., 2000). In addition, ERK is also stimulated by the neurotrophins to promote the axonal outgrowth in neuronal culture (Sjogreen et al., 2000).

4.3 Roles of MAPKs in diabetic neuropathy

In the study of p38 (Purves et al.,2001; Agthong and Tomlinson ,2002) , stimulation of p38 was found in sensory neurons cultured in hyperglycemic environment and those removed from diabetic animals and the inhibition of p38 corrected abnormal nerve conduction in diabetic rats .In addition , the stimulation of p38 in the ganglia may partially result from hyperosmotic stress caused by hyperactivity of polyol pathway because treatment with the polyol pathway inhibitor can decrease the p38 stimulation (Price et al.,2004). The stimulation of p38 leadind to decreased nerve conduction velocity may involve sodium channel because one study has shown that p38 can affect the function of Nav1.6 channel (Wittmack et al., 2005).

Accordind to the recent study of JNK (Middlemas et al.,2006), in sensory neurons of diabetic rats activated JNK and c-Jun were found. Hence, it is possible that the stimulation of JNK may be associated with abnormal functions of these neurons.

ERK is simultaneously activated in the spinal ganglion neurons of diabetic rats with both p38 and JNK. However, the role of ERK in diabetic neuropathy is still unknown. There is evidence showing that ERK may affect the function of potassium channel Kv4.2 (Schrader et al.,2006) including the change in expression and function of sodium channel in sensory neuron of diabetic rats (Hong and Wiley,2006). Furthermore, Purve et al., 2001 reported that after inhibiting ERK function in neurons cultured in diabetic-like condition, rate of cell apoptosis was decreased. In addition ,several stimuli increased in DM such as oxidative stress, hyperosmotic stress (Bogoyevitch et al.,1995;Guyton et al.,1996;Luo et al.,1998;Alessandrini,Kung and Bhat,1999;Stanciu et al.,2000;Purves et al.,2001) can stimulate ERK in neurons

resulting in apoptosis. Therefore, ERK might be involved in sensory neuronal dysfunction and damage in diabetes.

All three MAPKs may play a role in another neurologic abnormality observed in diabetic neuropathy-neuropathic pain because recent study has linked the activation of MAPKs with development of mechanical hyperalgesia in diabetic rats (Laurence et al., 2006).

In conclusion, present evidence indicates that MAPKs likely play a role in sensory abnormalities of diabetic neuropathy, especially p38. In contrast, evidence for ERK is still lacking. Existing evidence suggest that ERK may mediate stress-induced neuronal dysfunctions and possible apoptosis in diabetic neuropathy. However, its precise role in this complication of diabetes remains to be investigated. To elucidate this issue, inhibition of ERK must be used and various parameters of diabetic neuropathy are examined.

4.4 Inhibitors of the ERK pathway and applications in the nervous system

To study the role of ERK in several conditions, inhibition of the ERK pathway is required. Inhibitor of ERK can be achieved by pharmacological or genetic methods. Specific inhibitors are more widely used because sophisticated technology is not needed.

Subramaniam et al., 2003 found the decreased rate of neuronal death following the treatment with the ERK inhibitor, PD 98059. Moreover, u0126, another ERK inhibitor, can reduce the brain damage due to cerebral ischemia in rodents (Namura et al., 2001; Wang et al., 2004). These inhibitors can inhibit ERK by preventing MEK-induced phosphorylation on ERK which is nesessary to stimulate ERK. Purves et al., 2001 have also applied u0126 to neuronal culture in high glucose condition and found that the neuronal death was reduced. These findings indicate that these inhibitors of ERK, especially u0126, are effective against stress-induced ERK activation both in vitro and in vivo.

As a result, the aim of this study was to study the role of ERK inhibitor in diabetic neuropathy in terms of decreased nerve conduction velocity and abnormal nerve structure in diabetic rats. Results from this study may lead to development of novel treatment for diabetic patients in the future.