4. DISCUSSION

Lipomas are the most common benign form of soft tissue tumors.[131] Although lipomas are rarely life-threatening; lipomas growing in internal organs or critical sites near nerves might be considered malignant by location due to the compression of these vital structures.[3] Usually most lipomas are left without intervention because they do not pose a real threat. However, rapidly growing or painful lipomas can be treated with a variety of procedures including surgery. Nonetheless, deep lipomas have a greater tendency to recur because their complete surgical removal is not always possible.[72] Therefore, a variety of other procedures emerged to aid in the elimination of lipomas in critical sites where surgery is not feasible.

Several studies have shown that injection lipolysis using PC/DC has significant potential to treat localized collections of adipose tissue[86] and lipomas.[94] The reported side effects of injection of PC/DC formulations include swelling, bruising, and sensitivity to touch in areas with the lipo-dissolution treatments.[132] Despite this, the use of injection lipolysis using PC and DC is considered by many clinicians as a safer alternative for those patients who cannot tolerate invasive surgical procedures in the removal of fat deposits[90, 92] Nevertheless, up till now the subcutaneous injection of PC/DC is considered as an unofficially acknowleged modality[88] due to the lack of consistent studies concerning its side effects on the tissues at the injection site. Therefore, this study aimed to investigate the effect of repeated local PC/DC (Lipostabil®) injection, which is commonly used in injectable lipolysis, on different structures including neural tissues in the injection area to assess the safety of such preparation if used for lipolysis of lipomas near major nerves where surgical intervention is not considered an option. This was achieved by monitoring the histophathological changes that resulted from injection of PC/DC in rats near the femoral nerve.

4.1. Effect of PC/DC on adipose tissue

In this work, percutaneous injection of PC/DC combination (Lipostabil ®) in the groin of the right side of the rat near the femoral nerve for 4 consecutive days resulted in significant lipolysis in the inguinal fat compared to the control group receiving only saline injections. This was associated by the formation of fat cysts as well as fat necrosis. Such results are consistent with previous studies where local injection of different doses of PC/DC for 30 days in the abdominal cavity of rats or human volunteers resulted in dose-dependent reduction in cell membrane integrity of adipocytes and increased fat cyst formation. The histologic alterations induced by 30 days injects of PC/DC also included fibroplasia, band-like fibrosis in the region of the cutaneous muscle and partial muscle loss. The highest dose caused widespread fat necrosis, panniculitis and necrotic changes of the walls of small blood vessels.[133] In another study performed by Kopera and co-workers in 2006,[113] the application of PC/DC within a lipoma of a young patient caused partial reduction of subcutaneous lipoma. However, histologic examination of biopsy from the treated lipoma showed focal fibrosis, inflammatory reaction and presence of pseudomembranous degeneration of fat tissue. Recently, Park and co-workers (2013)[134] reported fat necrosis with microcalcification and cyst formation in the subcutaneous fat following PC/DC injection in the abdominal area in treated human subjects.

It is presumed that the formation of fat cysts and fat necrosis following PC/DC injection can be the result of local irritation and intense inflammatory reaction induced by PC/DC injection combined with the lipolytic effect of the formula. In support of this notion, in the current study the group treated with Lipostabil® injection showed significant intense infiltration of leukocytes at the injection site compared to the control group treated with saline, which is considered as a sign of local inflammation due to the injection of PC/DC. This inflammation can contribute to adipocyte death and fat necrosis. A possible scenario can be that PC/DC combination has a destabilizing effect on cell membranes causing adipocyte death. Further, macrophages migrated engulfing the disrupted cells in the affected area initiating inflammatory reaction. Interestingly, in 2005, Rose and Morgan[135] reported that skin biopsies from a patient who had undergone injection lipolysis with PC/DC showed mixed septal and lobular panniculitis. The fat lobules were infiltrated by increased numbers of lymphocytes and macrophages. The macrophages consisted of conventional forms, foam cells, and multinucleated fat-containing giant cells. The inflammation demonstrated in that study was associated with microcyst formation. These results led them to hypothesize that the reduction of subcutaneous fat likely follows inflammatory-mediated necrosis and resorption.

Several studies showed that PC/DC formulae induce lipolysis.[90, 92] Studies reported that PC can accelerate lipolysis of the cell membrane by increasing sensitivity to insulin[108] as well as other lipolytic hormones such as epinephrine.[109] Additionally, PC has apoptotic effect on adipocytes.[110] Physiologically, however, DC (the emulsifier), not PC, could be the major active component for fat lysis. However, until now, the actions of PC and DC could not be directly compared, because PC needs to be emulsified with DC.[112] Few studies reported that that DC alone can induce cell membrane lysis and fat necrosis.[110, 133] These effects are usually associated with inflammation in the injected tissue.[113] In 2009, Gupta and coworkers [91]demonstrated that DC solution was almost as effective as the PC/DC formulation, at clinical concentrations, in reducing the viability of mature adipocytes over time. Also, injection of DC alone subcutaneously in the abdominal area in patients at concentrations of 2.5 and 1% caused mild, localized heat, erythema, swelling, and intense pain along with the formation of nodules at the injection site. Histological evaluation of the biopsies from tissue at the injection site revealed necrosis of adipose tissue with adipocyte lysis, fat dissolution, acute inflammatory reaction, and intense phagocytosis of fat cells by macrophages.[136] Therefore, it is suggested that the action and side effects of PC/DC formulations are mainly due to DC rather than PC.

Recently, Hübner and his colleagues (2014)[137] performed a preliminary *ex-vivo* study to identify the pathophysiologic mechanisms of fat tissue depletion by PC/DC combination. In their study they compared the effect of incubation of Lipostabil® to saline with human adipose tissue for different time intervals (1, 3, 5, and 7 h). Histopathological analysis of these samples revealed marked damage of adipocyte cell membranes and disruption of normal cell architecture after PC/DC application. Immunohistochemical analysis showed positive results for tumor necrosis factor–alpha (TNF-α, marker for necrosis) rather than caspase-3 (marker for apoptosis) in samples treated with either PC/DC or DC, implicating that Lipostabil® and DC induce pathways of cell necrosis involving TNF-α. The results of that experiment also indicate that

Lipostabil® affects fat tissue in the way of chemical-toxic destruction rather than via a physiologically induced programmed cell death(apoptosis).

The debate that PC/DC causes necrosis or apoptosis is the focus of interest of many studies. Few studies showed that PC can induce adipocytes apoptosis.[110] Alternatively, others showed that PC/DC combination caused fat cell necrosis rather than apoptosis.[115, 137] DC is a natural emulsifier and a secondary bile acid that results from the metabolism of a primary bile acid called cholic acid by the intestinal bacteria.[95] It is worth noting that bile acid homeostasis is tightly regulated and their tissue concentrations are restricted. Therefore, under pathophysiological conditions that impair their biliary secretion, hepatocytes are exposed to elevated concentrations of bile acids which trigger cell death. Accordingly, several newly synthesized bile acid derivatives are synthesized and used to induce cell death in various conditions such as cancer.[138] However, it was reported that fat-laden hepatocytes favor a necrotic rather than an apoptotic cell death when exposed to low concentrations of bile acids. This necrotic pathway is induced via increasing reactive oxygen species production and inducing mitochondrial membrane-permeability transition pore opening.[139] Therefore, such cytotoxic effects for bile acids such as DC can be considered as part of their mechanism in inducing fat lipolysis.

Several lines of evidence demonstrate that PC up-regulated the expression of the apoptosis regulator protein "Bax" in 3T3-L1 pre-adipocytes cells (cell line from mice which has a fibroblast-like morphology, but, under appropriate conditions, cells differentiate into an adipocyte-like phenotype). On the other hand, PC has little effect on the expression of Bcl-2 (B-cell lymphoma 2 protein), which is a regulator protein that regulates apoptosis, by either inducing pro-apoptotic pathways or inhibiting anti-apoptotic pathways. Thus, PC increases the Bax/Bcl-2 ratio. Additionally, PC activates caspase-9 and caspase-8 as well as caspase-3, which are proteases involved in apoptosis. However, DC or PC/DC combinations were not found to show such apoptotic changes. The DC or PC/DC combination resulted in cell membrane lysis and disruption. Therefore, protein(Bax) was not detected in Western blot analysis when differentiated 3T3-L1 cells were treated with DC or PC/DC.[110] Light and electron microscopy showed fat cell necrosis in lipomas treated with PC/DC. Low levels of active caspase-3 indicated fat cell necrosis rather than apoptosis.[115] Early destruction of fat cells may suggest the involvement of detergent or osmotic mechanisms in the process. But clearly the mechanism couldn't be truly identified.

Examination of lipomas from patients injected with PC/DC at 4 and 48 h after PC/DC injection demonstrated neutrophil infiltration along with the partially destroyed fat cells. At day 10 the inflammatory process was accompanied by infiltration of T-lymphocytes. After 60 days formation of macrophages with foam cells was visible.[140] These studies demonstrated acute inflammation following injection of PC/DC combinations. This inflammation was persistent and lead to development of chronic inflammation, which finally can result in fibrosis. Such observations can suggest that caution should be taken when dealing with PC/DC for lipo-dissolution because it can lead to inflammation and scarring.

In the present work, the control group receiving saline injection showed minor inflammatory response probably due to repeated trauma resulting from repeated percutaneous injection. Also, these repeated injections can be responsible for the minor

local hemorrhage observed in this group. Alternatively, the current work also demonstrated severe significant infiltration of leukocytes in the injection area of Lipostabil ®. This inflammation was associated with deposition of collagen fibers as an early sign of fibrosis. These results are in agreement with few recent experimental and clinical studies. In 2012, Noh and Heo[130] used the bilateral inguinal fat pads of rats as an experimental model to demonstrate the lipolytic effect of PC/DC formula. In that study the bilateral inguinal fat pads of rats were elevated with the deep inferior epigastric vessel as the sole vascular pedicle. Normal saline was injected on one side as a control group and a PC/DC compound was injected on the other side, and after 4 days, the rats were euthanized for microscopic tissue examination. In the inguinal fat pad rat model, the control group and the experimental group were different significantly in the amount of normal fat tissue, inflammation, necrosis, and fibrosis. The results of microscopic examination of stained fat tissue of the group injected by PC/DC showed a histiocytic and giant cell reaction seen with fat necrosis. Neutrophil infiltration was also seen, indicating an inflammatory response suggesting more cautious approach in dealing with PC/DC formulae.

Clinically, in 2013, Reeds and co-workers [141] reported that in injection of PC/DC in one side below the umbilicus in women with body mass index above 30 caused significant reduction in the thickness of the anterior subcutaneous abdominal fat. However, the adipose tissue showed rapid increases in macrophage infiltration suggesting that PC/DC injections can effectively reduce abdominal fat volume and thickness by inducing adipocyte necrosis. Similarly, Park et al. (2013)[134] showed that injection of PC/DC formula in the abdominal area caused substitution of fat by fibrosis and marked inflammatory infiltration with micro-abscess formation in the dermis. It is worth noting that the local injection of Lipostabil® also induces changes in the cytokine production in the injection. Following injection lipolysis with Lipostabil® the pro-inflammatory cytokines TNF-α and interleukin-6 (IL-6) were elevated. This was associated with the increase of the anti-inflammatory cytokine, IL-10 mRNA levels in treated fat tissue compared to non-treated fat as a feedback mechanism along with acute panniculitis within the treated fat tissue.[142] The changes in the level of cytokine at the site of injection demonstrate an ongoing strong inflammatory reaction.

4.2. Effect of Lipostabil ® on skeletal muscles

In the present work, Lipostabil® injection caused marked interstitial inflammation invading the muscles causing myofiber necrosis, myophagocytosis, and regeneration and deposition of collagen fibers as early signs of fibrosis. It is worth noting that PC/DC formulation can induce the lysis of various cell types including adipocytes, normal human fibroblasts, endothelial cells, and skeletal muscle cells in a nonspecific manner.[91] Despite the results of Gupta and co-workers in 2009, it is also possible to assume that the effect of PC/DC formula on skeletal muscles can be the result of skeletal muscle ischemia secondary to impaired endothelial function rather than the direct detergent effect of DC. In support of this assumption earlier studies showed that the sarcolemma of rabbit skeletal muscles is resistant to the solubilizing effect of DC.[143] Alternatively, other studies showed that vascular endothelium plays an important role in the matching of oxygen supply to demand during supply limitation in skeletal muscle. DC can destroy vascular endothelium leading to loss of normal *in-vivo* and *in-vitro* endothelium-

dependent dilatory responses to acetylcholine, but endothelium-independent vascular smooth muscle responses remain intact. This leads to increased vascular resistance and decreased oxygen extraction by skeletal muscles causing ischemia.[144] Also, DC almost totally inactivates the isoenzyme lactate dehydrogenase-5 (LDH-5) present in the liver and striated muscles, whereas it does not affect LDH-1 present in the heart muscles.[145] Lactate dehydrogenase catalyzes the conversion of pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply.[146] Therefore, it is suggested that DC might also affect the level of energy production in the skeletal muscles under the condition of ischemia due to its effect on endothelial cells leading to degeneration of the skeletal muscles in the injection area.

The link between inflammation and skeletal muscle degeneration cannot be ignored. Indeed, skeletal muscle degeneration comprises early microvascular changes and inflammatory cell infiltration.[147] It is presumed that the endothelial damage induced by PC/DC along with the ability of DC to cause energy deprivation in the skeletal muscle due to LDH-5 inhibition can be the triggering factor that would initiate the skeletal muscle damage. The skeletal muscle injury can lead to recruitment of leukocytes to the injured area to clear the damaged tissue to start muscle regeneration. These inflammatory cells will subsequently produce pro-inflammatory cytokines that can aggregate skeletal muscle damage. In support of this view, studies show that regenerative capacity of injured skeletal muscle depends on the presence of myogenic cells, called satellite cells and ability of these cells to proliferate and then differentiate to either fuse with existing fibers or with other myogenic cells to generate new fibers.[148, 149] The regulatory mechanisms that influence skeletal muscle regeneration resemble those that occur during embryonic muscle development. However, the microenvironments in which myogenesis occurs varies dramatically between embryonic myogenesis and muscle regeneration. This is because the immune cells are relatively scarce in developing skeletal muscle. Alternatively, these inflammatory cells can be present in regenerative muscle at high concentrations.[149] The activation of these inflammatory cells and their ability to releasing numerous soluble molecules, especially cytokines can affect the process of skeletal muscles regeneration. Previous studies showed that systemic administration of pro-inflammatory cytokines results in muscle catabolism in experimental animals.[150] Alternatively, genetic blockade of pro-inflammatory cytokine signaling such as deletion of TNF-α receptors [151] or pharmacologic blockade of pro-inflammatory cytokine signaling via anti-interleukin anti-bodies [152] attenuates muscle wasting. Moreover, cell culture studies demonstrated that pro-inflammatory cytokines can also cause atrophic changes in cultured myotubes where TNF-α plus interferon gamma reduced myosin expression.[150] Indeed, the injured skeletal muscles cells can produce pro-inflammatory cytokines that recruit neutrophils to the injured area.[153] The infiltration of these inflammatory cells will cause the production of more pro-inflammatory cytokines that causes muscle necrosis [154] and the vicious cycle continues leading to more damage to the skeletal muscles at the site of injection.

The regeneration of injured skeletal muscle depends on the severity of the injury, the inflammatory response and on the balance of the processes of development of fibrotic scar tissue, with partial loss of function in one hand and the remodeling of the scar tissue in the other. The formation of fibrotic scar tissue seems to be dependent, at least in part, on local transforming growth factor-beta 1 (TGF-β1) expression.[155] The TGF-β1 is

proteolytically derived from the carboxyl terminus of a 390 amino acid precursor molecule called pre-pro-TGF-β1. Studies demonstrated that the pro piece of pre-pro-TGF-β1 forms an inactive, latent complex with TGF-β1. These latent forms are important in the regulation of TGF-β1 activity.[156] The latent TGF-β1 is a component of extracellular matrix microfibrils.[157] Notably, mast cell chymase and leukocyte elastase efficiently released matrix-bound latent TGF-β1complexes.[158] It is worth noting that over expression and activation of TGF-β1 leads to developed muscle weakness and atrophy manifested as endomyosial fibrosis and smaller myofibers.[159] Other studies also showed that treating muscles with TGF-β1 results in a dramatic accumulation of type I collagen, substantial fiber atrophy, and a marked decrease in force production.[160] The role of DC in the development of fibrosis at the injection site cannot be overruled. Previous studies showed that increased serum level of bile acids in cholestasis can cause hepatic fibrogenesis due to their ability to stimulate the release of TGF-β1from Kupffer cells.[161] Therefore, it is presumed that the extensive infiltration of inflammatory cells in the muscle fibers at the site of inject along with the DC present in the Lipostabil® injection can be responsible, at least partially, for the activation of TGF-β1 at the site of injection with the subsequent initiation of the fibrotic cascade at the injection site.

Another factor that can contribute to degeneration of the skeletal muscle and the increased TGF-β1 and fibrosis is the neural damage. It has been previously demonstrated that progressive fibrosis of skeletal muscles after peripheral nerve injury is mostly produced by the enhanced formation of immatured collagen fibers in tissues, rather than the decrease in the amount of structural muscle proteins.[162] Studies showed that denervation alters the metabolism of the extracellular matrix in skeletal muscle, with the subsequent increase in TGF-β1 and the development of muscle fibrosis.[163] In the sciatic nerve injury model in rats in which denervated gastrocnemius was isolated for analysis a rapid increase of TGF-β1 expression for reported. This increase in TGF-β1 was associated with increased expression of collagen I. The area of the gastrocnemius muscle fiber decreased gradually following denervation along with increased interstitial fibrosis. Notably, these pathological changes were partially prevented by local injection of TGF-β1 antibodies, which caused the reduction of collagen 1 expression as well.[164] Therefore, the neural damage presented in the current study due to PC/DC injection can also participate in the development of the skeletal muscle injury following the injection.

4.3. Effect of Lipostabil ® on neural tissues

The effects of PC/DC formulae on nerves are crucial in determining the possibility of utilizing injection lipolysis in spinal lipomas being critical areas. The current findings demonstrated that injection of Lipostabil® percutaneously in the groin of the right side of the rat near the femoral nerve for 4 consecutive days resulted in significant intense infiltration of inflammatory cells entrapping the nerve bundles. Moreover, endoneural mast cells were evident in the neural tissue via light and electron microscopic imaging. The electron microscopic imaging of the nerve bundle also revealed intra-neural fibroblasts and intra-neural collagen fibers abutting the nerve fiber with marked myelin degeneration. This was associated with intra-neural thick walled blood vessels that were surrounded by fibroblast. Such results can be due to the severe local inflammation induced by PC/DC formula as well as the fibrotic and intense lipolytic effects of the PC/DC formula that can cause disintegration of the nerve myelin sheath. On

the other hand, repeated local injection with saline resulted in limited local inflammation with no detectable neural damage. Interestingly, Uygur and coworkers (2008) demonstrated that single intrafascicular injection of 0.1 mL Lipostabil® (250 mg/5 mL) with a 30-gauge needle into the left posterior tibial nerve caused no neural damage and the nerve retained its normal architecture. Under electron microscopy, there was no demyelination, cellular infiltrate, inflammatory, or vascular reaction seen at any point along the nerve. However, the study of Uygur and coworkers (2008)[118] used only a single injection of PC/DC not repeated injections as in the real settings. Also, the neural damage was assessed on day 21 following the injection, which could be a suitable period for tissue repair and regeneration.

The effect of PC/DC in neural tissues was not studied in depth before. Studies used DC experimentally to breakdown the blood brain barrier. The breakdown of the blood brain barrier can subject the brain tissues to noxious substances causing neural damage.[165] Nonetheless, the direct effect of DC on the neural tissues, to our knowledge, was not studied.

Notably, the physiological and pharmacological effects of bile acids on nerves have been previously shown in the colon. *In-vitro* studies using isolated rabbit colon showed that bile can inhibit or stimulate the colon motoricity and the nerves conductivity, depending on its concentration. At low concentrations the bile stimulated the spontaneous colon motoricity and the conductivity of sympathetic and parasympathetic nerves. Alternatively, at higher concentrations it has inhibitory effect on colon motoricity and suppressed the nerves conductivity.[166] *In-vivo*, the direct application of DC in rat colon induced mild, transient colonic inflammation within 3 days that resolved within 3 weeks. However, exaggerated visceromotor response, referred pain to mechanical stimulation, increased spinal Fos expression and increased colonic afferent and dorsal horn neuron activity were observed indicating chronic dorsal horn hyperexcitability and visceral hyperalgesia.[167] The increased c-Fos expression at spinal cord level in the study of Traub and his colleagues (2008) may suggest neural damage at the site of application of DC. In support of this notion studies showed that chronic constriction injury of the sciatic nerve in rats evokes c-Fos expression at spinal cord level.[168] Remarkably, the reported side effects of injection of PC/DC formulations include increased sensitivity to pain in areas of lipo-dissolution treatments, [132, 136] which could be the result of neural injury at the site of injection. Also, inflammation is believed to sensitize the nerve to all incoming stimuli.[169, 170] In such a state, even minor mechanical stimulation of the nerve can evoke severe exaggerated pain.

This work took place over 4 days, a true repair process couldn't be noted, nonetheless the presence of intra-neural fibroblasts abutting the nerve fibers along with intra-neural collagen deposition was found signifying an early sign of intra-neural fibrosis and nerve damage. The intra-neural blood vessels also demonstrated wall thickening. Earlier studies showed that inter-fascicular (between the nerve bundles) scar tissue can constrict nerve fibers and thereby interfere with their function and regenerative capacity.[171] Indeed, the major outcome of such intra-neural fibrosis would be a conduction disorder.[172] The present finding of damage to intra-neural blood vessel can further aggravate the neural damage. The endo-neural capillaries form a kind of blood neural barrier that helps in optimizing endo-neural environment. The damage to such

vessels can lead to deprivation of the nerve fibers from oxygen and nutritional supplies. Additionally, the break of blood neural barrier may induce a miniature closed compartment syndrome by increasing the permeability, thereby contributing to increased endo-neural fluid pressure and development of an intra-fascicular edema with the subsequent alteration in nerve function and integrity .[173] The vascular and endothelial damage in the neural blood supply is usually associated with peri-neural and intra-neural fibrosis. Accordingly, many patients with chronic mechanical back pain demonstrate vascular, fibrotic, and inflammatory components to the problem.[174]

The presence of endo-neural mast cells can participate in the development of neural inflammation and intra-neural fibrosis in the present work as well as the reported increased pain sensation and itching following PC/DC injection in other studies. Several lines of evidence can support such assumptions. Mast cells degranulation can result in pruritus [175] and urticaria.[176] It can be suggested that mast cell degranulation may be responsible for local itching and flare that is manifested clinically after local injection by PC/DC. However, such an assumption warrants further research to delineate the effect of PC/DC combination on mast cell membrane stability and degranulation.

The role of mast cells in inflammation in well documented. Mast cells synthesize, store and release nerve growth factor [177] as well as histamine.[178] The nerve growth factor is a neurotrophin peptide, which is important for survival of nociceptive neurons during their development. It also exerts its pro-inflammatory action on mast cells, lymphocytes and neutrophils.[179] Both mast cells and nerve growth factor are involved in tissue inflammation, where nerve growth factor recruits and prims local tissue and systemic defense processes following stressful events. Also, nerve growth factor increases mast cell histamine content and intracellular tryptase activity in a dose- and time-dependent manner.[180]

The association of mast cells with different fibrotic conditions has long been observed. For instance, remarkable association between collagen increase and mast cell density in the epineurium of leprous nerves has been demonstrated. Moreover, tryptase, the most abundant protein product of mast cells, has been shown to be mitogenic for fibroblasts and to increase type I collagen production.[181] Alternatively, the neural damage due to needle stick-nerve-injury involves enhanced pain sensation along with increased intra-neural mast cells and thickening of the endoneural blood vessels wall.[182] In another model of nerve injury via sciatic nerve partial ligation, stabilization of mast cells with sodium chromoglycate reduced the recruitment of neutrophils and monocytes to the injured nerve and suppressed the development of hyperalgesia.[183] These studies demonstrated a key role for mast cells in the development of neural inflammation and the increased pain sensation following neuropathy.

In the present work, examination of the Schwann cells in the nerve bundles adjacent to the site of PC/DC injection demonstrated degenerated myelin whorls within the cytoplasm of Schwann cells. Schwann cells are cells that wrap around axons of motor and sensory neurons. Schwann cells are involved in many important aspects of peripheral nerve biology including formation of the myelin sheath, conduction of nerve impulses along axons, nerve development and regeneration, support for neurons, production of the nerve extracellular matrix as well as modulation of neuromuscular synaptic activity.[184] One of the major functions of the Schwann cells is the repair of the myelin sheath.

Therefore, many diseases that involve degeneration of Schwann cells are associated with intense neural damage.[185, 186]

4.4. Clinical implications

The identification of the histopathological effects of repeated local injection of Lipostabil® on different tissues including adipose tissue, skeletal muscles as well as neural tissues at the site of injection is important in designing new strategies for treatment of lipomas. The present observations that PC/DC injection caused intense local inflammation, myopathy and neural degeneration suggests that the off-label use of PC/DC as "fat burner" for the reduction of disturbing lipomas should be considered only on a case-to-case basis after careful consideration of possible undesired effects. The current findings can help clinician in designing better strategies for the application of injection lipolysis using PC/DC formulae in the treatment of lipomas. Given the unfavorable interaction of PC/DC combination on neural tissues in this study, the current data highlights the risk of using such combinations near nerves and the importance of the evaluation of risk/benefit ratio for the employment of injection lipolysis versus surgery in treatment of lipomas. Indeed, the present results cast a dark shadow on the use of PC/DC combination in treating lipomas and infer that the long-term safety of PC/DC for nonsurgical treatment of fat deposits is uncertain. Additionally, it can be suggested that another emulsifier or drug delivery system should be proposed to avoid the non-specific and side effects of DC.

5. SUMMARY

Spinal lipomas are benign tumors with a peak presentation between 10 and 40 years, nevertheless they have aggressive manifestations related to mass effect and secondary compressive myelopathy resulting in progressive pain, autonomic and sensorimotor deficits. Their slow growth can be tolerated being accommodated within the spinal canal all for a point where there is no space left to accommodate it, and hence compressive manifestations will then take place.

Surgical treatment of spinal lipomas in symptomatic cases consists of untethering of the cord along with de-bulking of the lipomatous mass rather than total excision. De-bulking was agreed amongst most neurosurgeons because total excision necessitates more aggressive and manipulative technique; nonetheless recurrence rate is usually high due to hyperplasia of residual adipocytes and that is just to say the least about the surgical treatment when other more warning complications such as spinal injury, deprivation of the cord from blood supply through separation of the cord from the lipomatous mass, wound breakdown, CSF leakage and pseudo-meningocele takes place.

Since Pistor hypothesized in 1976 that injections of numerous medications into various levels of the skin could maximize their therapeutic effects locally; while significantly reduce their adverse effects systemically, mesotherapy including injection lipolysis using PC/DC injection was utilized in many fields. Deoxycholic acid is a natural emulsifier and a secondary bile acid that results from the metabolism of a primary bile acid called cholic acid by the intestinal bacteria. On the other hand, phosphatidylcholines are a class of phospholipids that incorporate choline. They are major component of biological membranes and can be easily obtained from a variety of readily available sources such as egg yolk or soy beans. The PC is insoluble in water, and thus requires an emulsifier, traditionally DC, to solubilize it. The PC/DC combination was approved for i.v. treatment of fat embolism in Germany and now widely used as an alternative for liposuction for reduction of subcutaneous fat. Because spinal lipomas have always represented a surgical challenge; this work aimed to evaluate the possible use of injection lipolysis as a less invasive and a safer path for treatment of lipomas near neural tissues.

In this work, 10 female Wistar rats underwent 4 successive PC/DC injections infiltrating femoral bundle on 4 consecutive days. The rats were sacrificed on the fourth day and delicate entoto excision of 1 cm of the femoral bundle was performed. The same procedure was performed on another 10 female Wistar rats as a control group being subjected to saline injection. The biopsies obtained from 6 rats in each group were subjected to light microscope study, while the other 4 underwent electron microscope study. The biopsies subjected to light microscopy assessment were the subject of semi-quantitative statistical analysis for inflammation, necrosis, fibrosis and nerve damage. Alternatively, the biopsies studied under electron microscopy were for assessing the neural ultra-structures.

Compared to the control group injected with saline, injection of 0.1 ml Lipostabil® for 4 consecutive in the groin of the right side of the female Wistar rats near

the femoral nerve caused significant intense leukocytes infiltration in the skeletal muscles causing severe inflammation and muscle damage leading to the deposition of collagen fibers as an early sign of fibrosis. These leukocytes cuffed the nerve bundle causing significant neural damage. The intense inflammation caused significant necrosis in the adipose tissue and skeletal muscles. Examination of the ultra-structures of the neural tissues in the injected area using TEM revealed the presence of intra-neural fibroblasts abutting the nerve fibers along with marked myelin degeneration. The intra-neural fibroblasts and myelin degeneration were associated with the deposition of intra-neural collagen fibers as an early sign of neural fibrosis. The local injection of Lipostabil® also affected the Schwann cells causing degeneration of myelin. Moreover, the injection of Lipostabil® affected the intra-neural blood vessels causing thickening of its walls. The sections also demonstrated endoneural inflammation.

A debate started in the past few years whether PC/DC-induced lipolysis was mediated by apoptotic or necrotic paths. The present results as well as other highlight the possibility that a process more than merely programmed cell death is involved. The PC/DC mechanism for induction of lipolysis is still not unequivocally clarified. However, this work emphasize that PC/DC combination is unsafe to be used as injection lipolysis agent for treating localized lipomas especially near neural structures. The dramatic effects of PC/DC local injection presented in this study including myelin disintegration along with intense inflammation and Schwann cell damage _not mentioning skeletal muscle damage and vasculitis found locally_ prove it unreliable in its current form to be injected near neural tissues. The use of another solvent to deliver PC is warranted in view of the side effects attributed to DC.