



Effect of Systemic Treatment by Nanoparticles on The inferior Alveolar Nerve Regeneration and Brain after Crush Injury in Rabbit.



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AXONAL regeneration of the nerve is the important target to functional recovery after the accident and peripheral nerve injury. Zinc oxide have many properties like antimicrobial activity, osteogenesis and angiogenesis, and have cytotoxic in different tissue in high dose. The effects on the nerve tissues may enhance functional recovery after peripheral nerve injury. The aim of the study was to evaluate the therapeutic effects of systemic ZnO NPs following inferior alveolar nerve (IAN) crush injury. Twelve rabbits were divided two groups six rabbits in the every group treated and control, all the animal were have the injury in left side of the face following the nerve crush injuries of the IAN, the treated group receive daily intramuscularly injections of 20 mg/kg 1 ZnO NPs. Fourteen days after induction of nerve injuries, ZnO NPs was injected over the mental foramen for the evaluation of neuronal survival. At the end of the 2 week period, histologic examination of IAN samples were performed. The microscopic evaluation showed the ability of ZnO NP to enhance the nerve regeneration in the peripheral nerve and the high dose effect to the brain tissue.

Keywords: Nerve regeneration, ZnO NPs, Cytotoxic of NPs.

Introduction

The Peripheral nerves are friable tissue and easily to be traumatized and damaged. The injury of the peripheral nerve is one of the common causes of degeneration of axons and their myelin sheaths and may become a life threaten factor on the patients [1]. It may inhibit the communication between the brain and the muscles [2]. Although, the peripheral nervous system has the ability for regeneration, it is a complicated biological process involving different cell types components of extracellular matrix that may be affected by different factors [3].

There are different methods for treatment of nerve damage such as utilizing allograft

techniques [4], cell therapy such as Schwann cell [5], stem cell, fibroblast and olfactory cells or drug therapy, and different types on nerve conduit like use of biological tubes, scaffolds with synthetic and natural materials and oriented channels, absorbable and non-absorbable synthetic and natural polymers with unique features, benefiting from new technology of nanotechnology, could improve performance for an appropriate solution to repair damaged nerve tissues [6].

Nanotechnology is known as a technology that deals with the structural differences between 1 to 1000 nanometers having the same physiochemical properties holding in larger sizes [7]. This leads to increase activities of these particles

due to their special characteristics forming nanoparticles by increase the ratio of surface to its volume allowing for more benefits than bulk chemicals [8].

Nanomaterials are highly used in repairing damaged nerve cells, spinal cord defects and peripheral ones [9]. The use of the nanomaterials in the peripheral nerve injury is used as an implantable entubulation device, e.g., nerve guidance conduit, to guide the regeneration of nerve tissue [10], chitosan tube was used as an autologous nerve graft for repairing 10-mm gap in rat sciatic nerve [11],

Nanoparticles as Iron oxide leads to prolong biological activity respect to the non-conjugated factors and stabilization of the growth factors [12] and gold nanoparticles (GNPs) are of interest as a method for improving radiotherapy [13].

Many researches have introduced toxic effects of the zinc oxide (ZnO) in such as nanoparticles (NPs) [14]. The result of nanostructural ZnO cytotoxicity have the important role in the treatment of human diseases. Many researches have shown the cytotoxicity of ZnO nanostructures, and others have not showed cell toxic effect because of changes doses of ZnO NPs [15]. ZnO nanostructures like nanowires (NWs) (16), nanorods (NRs) [17] and nanoflowers (NFs) (18) to name only a few, have been used alone for promoting the adhesion, growth, and promising antibacterial properties [19]. ZnO nanostructures, featuring antimicrobial activity, osteogenesis and angiogenesis, have been also combined with additive manufacturing technologies [20].

The aim of the study was to find out the cytotoxic of the ZnO NPs to the body and the ability to enhance the nerve regeneration.

Materials and Methods

Twelve male adult rabbits were used in the experiment where they were divided into two main groups. First group was injured and kept without treatment while the other group was treated with ZnONPs after induction of nerve injury. The animals were kept in good condition cages and good food and environment before and after the operation. Inferior alveolar nerve injury model rabbit were anesthetized with an intramuscular injection of ketamine (60 mg/kg 1) and xylazine (6 mg/kg1) combination and were then immobilized on the operation table in a sideward position. The buccal aspect of the face was shaved and disinfected with povidone iodine. A 1cm vertical incision, starting five mm below the lateral canthus of the eye, was performed. The masseter muscle was bluntly dissected immediately below the parotid's salivary duct with sharp pointed micro forceps. A self-retaining retractor was used to allow unimpeded access to the underlying bone. The Inferior alveolar nerve (IAN) injury was induced at the level 1cm rostral to the mandibular foramen where the main trunk of IAN divides into two large branches. The IAN was clamped for 30 s with forceps to induce crush injury.

The left IAN was injured while the right side was not operated and the right IAN remained intact. The masseter muscle was sutured in a layered fashion with 2.0 Vicryl suture (Ethicon, Minhang, China) and the buccal skin was sutured with 2.0 silk suture (Dogsan, Trabzon, Turkey). Throughout the procedure, a thermal blanket placed under the rabbits were kept at 37°C and rectal temperature measurements were employed to control body temperature. Animals were subsequently maintained on a standard

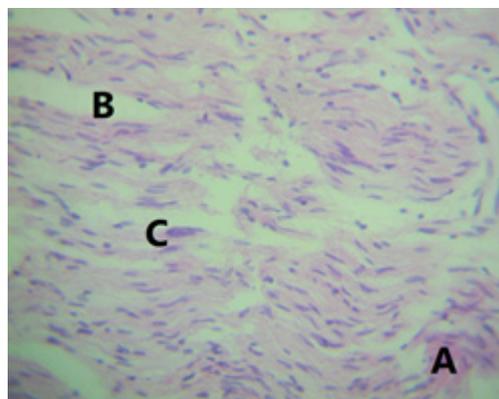


Fig.1. Perineurium with WBC infiltration (A), Newly formed blood vessels (B), Macrophages (c) (H&E X40)

diet of laboratory food and water. Systemic ZnO administration 20 mg/kg the ZnONPs were injection intramuscularly daily 14 days after induction of the IAN injury.

Results

The control group in 7 days, the nerve trunk was formed by number of many nerve fibers , each one was formed by axon surrounded by a thin layer of myelin produced by Schwann cells with presence of its nuclei at the periphery of neurolemma of Schwann cells and the Nodes of Ranvier. Most of the axon were still loss of the continuity. Each nerve bundle had many blood vessels of small size, individual macrophages were demonstrated in the perineurium and epineurium. (Fig 1)

The treated group in 7 days, the nerve was formed by nerve bundles and each bundle was containing many individual nerve fibers, each nerve fiber was present as wavy appearance, it was formed by axon surrounded by myelin sheath.

The Schwann cells which had nuclei surrounding the myelin , also presence of Nodes of Ranvier along the nerve fibers, the endoneurium as delicate loose connective tissue was investing the nerve fibers. The perineurium more fibrous tissue investing each bundle of nerve fibers and the outer layer of connective tissue was coating the whole nerve trunk which was the epineurium had blood vessels, fat tissue and certain number of WBC (Fig 2) .

The control group in 14 days the cross section of the nerve trunk revealed that the nerve fibers in each nerve bundle was formed by axons and thin layer of myelin around each one.

The layer of myelin was surrounded by nuclei of Schwann cells which was detected , in each bundle blood vessels were seen with congestion of blood in the perineurium and these are also recognized in the epineurium associated with the presence of macrophages and adipose tissue (Fig 3).

The treated group in 14 days the nerve was densely crowded with nerve fibers with presence of cellular hyperplasia of Schwann cells along the course of newly formed sprouts of nerve fibers. The fibroblasts were present in the endoneurium and perineurium. The nerve bundle of nerve trunk was containing great cavities along the course of nerve fibers which was lining by cuboidal epithelial cells. The periphery of nerve trunk was containing blood vessels , adipose tissue and macrophages. these fibers were seen with many Schwann cells present on the newly formed axons, so even the Node of Ranvier could be detected, new blood capillaries were detected in the perineurium and epineurium with presence of macrophages and other WBC (Fig. 4).

Brain in 14 days , The meninges were present surrounding the brain tissue and the certain areas were lost these meninges from the brain cortex, the cerebral blood vessels were congested with blood, the layers of brain cortex , molecular, granular and other layers of brain cortex such as the pyramidal layers and multiform layer of neurons were present and no abnormalities in its conformation and archetecture , except pericellular vacuoles or white zone was detected around the neurons and glial cells (Fig 5).

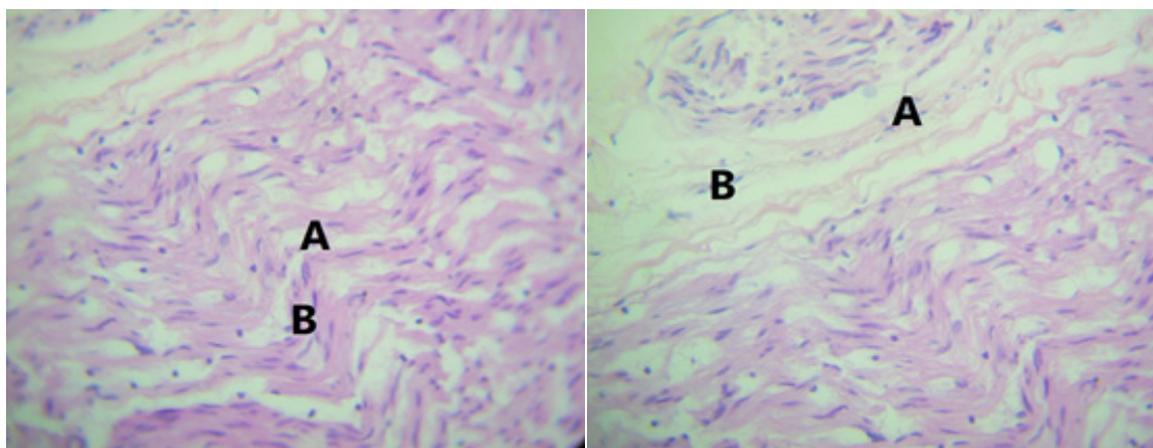


Fig. 2. Left - nerve trunk 7 days , wavy appearance (A) Schwann cells (B). Right Perineurium (A), Macrophages (B). (H&E X40)

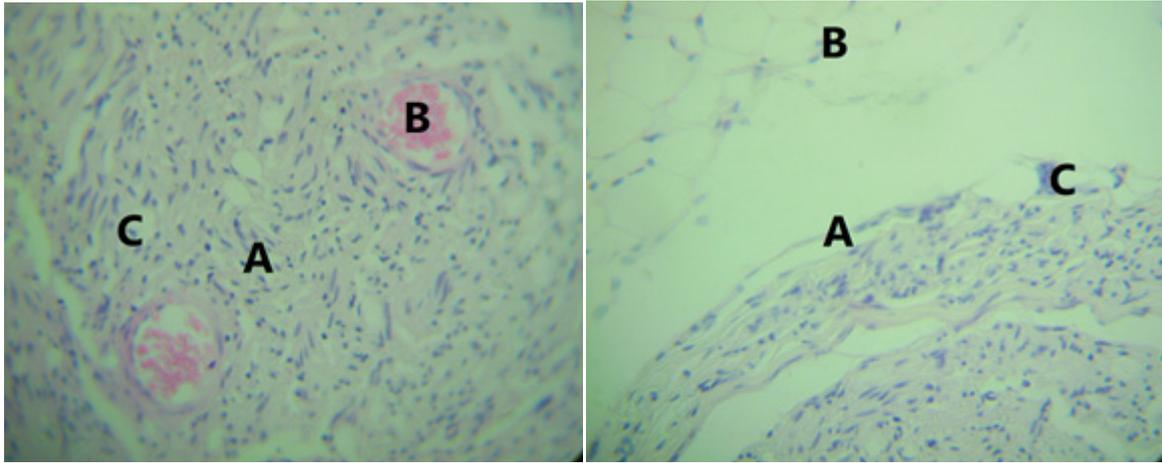


Fig. 3. Left -Nerve bundle (A), Blood vessels with blood (B) , Schwann cells (C). Right - Epineurium (A) , Adipose tissue (B) , Macrophages (C) (H&E X40).

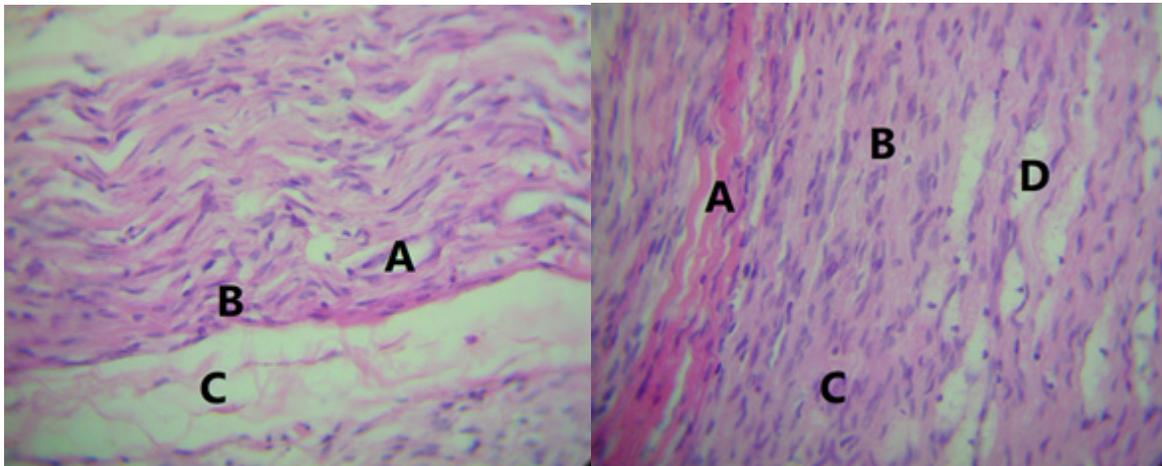


Fig. 4. Right 14 days. Nerve trunk, Newly blood vessels (A) , Schwann cell(B), Perineurium (C). Left 14 days. Collagen bundles of Perineurium (A), Spaces in between nerve fibers (B), Schwann cells (C) Newly formed blood vessels (D) (H&E X40).

Discussion

The nerve injury is a common damage to peripheral nerves, the repair of the peripheral nerve after the trauma still not clearly understands [21]. The peripheral nervous system have the ability to regenerate after severe injuries, but the regeneration is incomplete and the function is not complete recovery [22]. Many materials (nanoparticles) were used to help the peripheral nerve to regeneration faster and repair function of it [23].

The high dose of ZnO NPs can accelerate the nerve regeneration but have cytotoxic effect of author part of body like brain.

In the histological studies, the morphology of regenerated nerve fibers showed difference between treated and control groups indicating effect zinc oxide nanoparticles on the nerve regeneration. Many biomaterials have provided promising results toward improving the function of injured nervous system tissue, however, significant hurdles, such as delayed or incomplete tissue regeneration, remain toward full functional recovery of nervous system tissue [24]. Because of this need for good nervous system biomaterials, more recent approaches to design for the nervous system have incorporated nanotechnology the next generation of tissue engineering scaffolds, or more specifically, nanoscale surface feature

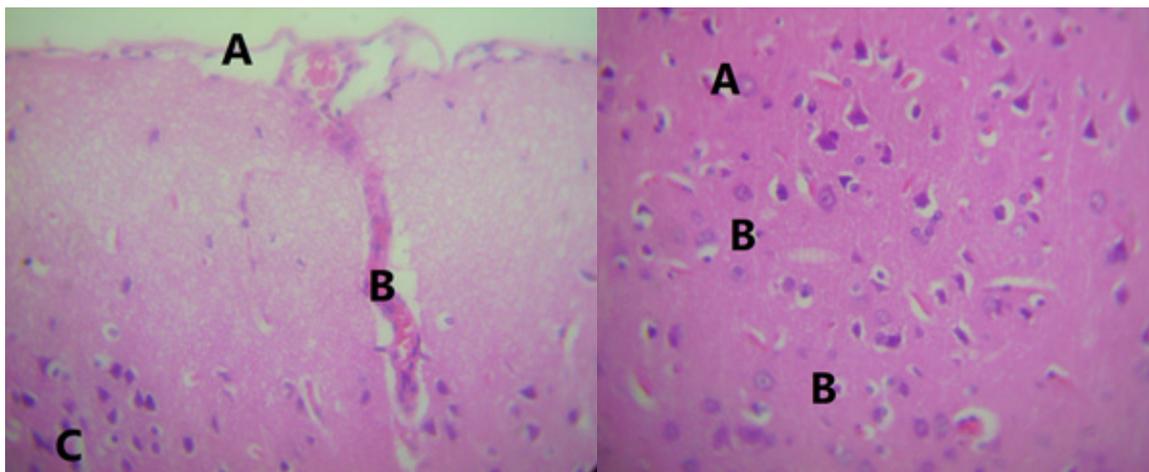


Fig.5. Left - (micro) Meninges (A), Congested cerebral blood vessels (B), Pyramidal cells (C). Right - (nano) Pericellular vacuoles around Neuron (A) around glial cells (B) (H&E X40)

dimensions which mimic natural neural tissue [25]. Compared to the control and treated groups, the nanomaterials have exhibited an ability to enhance desirable neural cell activity the complexity of neural tissue injury and the presence of inhibitory cues the absence of stimulatory cues may require multifaceted treatment approaches with customized biomaterials that nanotechnology can provide[25].

The vacuoles of the brain of the rabbit is result from the toxicity of the ZnO when it used in high dose, the toxic effects of pure zinc oxide (ZnO) in diverse forms such as nanoparticles (NPs) and nanowires [26]. The appearance and extent of nanostructural ZnO cytotoxicity play an important role in the application of ZnO NPs in the treatment. While some studies have shown severe cytotoxicity of ZnO nanostructures, other studies have not claimed remarkable cell toxic effect because of changes in cell density or amount of ZnO NPs [15].

Conclusion

- The ZnO NPs have the ability to enhance the nerve regeneration in peripheral nerve .
- The ZnO NPs have the cytotoxic effect of the brain in high dose.

Recommendation

- Using the ZnO NPs in low dose and long time
- Using another NPs in the nerve regeneration.

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Conflict of Interest

No conflict of interest

Funding statement

This research was special exertion

Ethical consideration

This study has been approved by the animal rights and ethical of Tikrit University.

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تأثير العلاج الجهازي باستخدام المادة النانوية وتأثيرها على شفاء عصب السنخي السفلي والدماع بعد تعرض العصب للسحق في الارانب

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عملية شفاء الجروح وارجاع الوظيفة للعصب من الامور المهمة جدا للعصب بعد تعرضه للاصابة. مادة الزنك او كسايدها العديد من المميزات منها القدرة على قتل البكتيريا، اعادة ترميم العظام والاعوية الدموية، كما له تأثير سمي على بعض الخلايا اذا ما تم استخدامه بالجرع العالية. كما له القدرة على اعادة ترميم الاعصاب.

الهدف من هذه الدراسة هو تقييم دور الزنك او كسايدها كمادة نانوية واستخدامها في تسريع شفاء العصب المحيطي السنخي السفلي في الارانب بعد تعرض العصب للسحق. تم استخدام اثنا عشر حيوان من الارانب وتم تقسيمها الى مجموعتين متساويتين. جميع الحيوانات تم تعريضها لسحق العصب من الجهة اليسرى من الوجه.

المجموعة الاولى تم علاجها يوميا بواسطة استخدام الزنك او كسايدها كمادة نانوية بجرعة ٢٠ مل/كغ لمدة اربعة عشر يوم الى نهاية التجربة، اما مجموعة السيطرة لم تحقن باي مادة.

كانت النتائج النسجية قدرة مادة الزنك او كسايدها على تسريع شفاء الاعصاب المحيطية والجرعة العالية كان لها التأثير الواضح على خلايا الدماغ.