GENTAMICIN INDUCED NEURODEGENRATIVE CHANGES IN AUDITORY CORTEX OF ADULT ALBINO RATS

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ABSTRACT

***Background**: Aminoglycoside-induced nephrotoxicity and ototoxicity is a major clinical problem. Aminoglycosides have good activity against many multi-drug resistant Gram negative bacilli and are therefore important for treating serious infections due to these organisms in adults and children including neonates. Gentamicin, due to it cost effectiveness has been used in most of the trials as compared to other aminoglycosides. Most studies documented the peripheral toxicities of gentamicin without any concern of central neurotoxicity. **Objective**: To observe Gentamicin induced neurodegenerative changes in auditory cortex of adult albino rats. **Methods**: It was an experimental study. Twenty rats were randomly divided in two groups; Group I (Experimental: n = 10) received intramuscular injection of Gentamicin for twenty one days and Group II (Control: n =10) received normal saline intramuscularly. Histological preparations were done on sections obtained from the area around lateral sulcus of rat cortex. The photomicrographs of the relevant stained sections were taken with the aid of a light microscope. **Results**: Light microscopic examination of the auditory cortex sections obtained from gentamicin injected rats (Group I) revealed severe neurodegenerative changes as compared to the rats in (Group II). **Conclusion**: Exposure of rats to Gentamicin for three weeks showed severe neurodegenerative changes. Keywords: Aminoglycosides, Auditory Cortex, Nephrotoxicity, Ototoxicity*

ملخص: يعتبر تسمم الكلى وتسمم الاذن الناتج من الأمينوجليكوزايد من المشاكل الاكلينيكية الرئيسية. للامينوجليكوزايد خاصية جيدة في القضاء علي كثير من العصيات سلبية الجرام المقاومة لأكثر من عقار، وبالتالي فهي مهمة لعلاج الالتهابات الخطيرة الناتجة من هذه العصيات في البالغين والأطفال بما في ذلك حديثي الولادة. ويستخدم الجنتاميسين في معظم التجارب بالمقارنة مع الأمينوجليكوزايد الأخرى نظرا لقلة تكلفته. معظم الدراسات اهتمت بالتسمم الطرفي للجنتاميسين دون أي تركيز علي التسمم العصبي المركزي. **الهدف**: ملاحظة التلف الذي يسببه الجنتاميسين للأعصاب في القشرة السمعية للجرذان البالغة. **منهج الدراسة**: دراسة تجريبية، حيث تم تقسيم عشرون فأر عشوائيا الي مجموعتين، المجموعة الأولى (التجريبية وعددها = 10) وتم حقنها في العضل بالجنتاميسين لمدة واحد وعشرون يوما. والمجموعة الثانية (للمقارنة وعددها = 10) وحقنت بمحلول ملح بالعضل. وقد أخذت عينات نسيجية من المنطقة المحيطة بالتلم الخارجي للقشرة المخية للفئران. تم التصوير المجهري من الأقسام ذات الصلة بمساعدة المجهر. **النتائج**: كشف الفحص المجهري لنسيج القشرة السمعية التي تم الحصول عليها من الفئران التي حقنت بالجنتاميسين (المجموعة الأولى) كشف عن تلف شديد للأعصاب بالمقارنة مع الفئران في (المجموعة الثانية). **الاستنتاج**: تعرض الفئران للجنتاميسين لمدة ثلاثة أسابيع أدي الي تلف شديد للأعصاب

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INTRODUCTION

Gentamicin is a member of aminoglycoside family of antibiotics produced by micrmonospora pupurea. It can be used in different type of infections, including gram positive and gram negative bacteria. In addition, it has significant effect against pseudomonas. Gentamicin works by inhibiting protein synthesis. It binds very strongly to ribosome's (30 S) sub unit and interferes with Protein synthesis. Gentamicin enters the cell by binding to negatively charged phospholipids and enters the cytosol via electron transport linked system and thus need oxygen and ATP to enter cytosol and be effective. Therefore Gentamicin antibiotics are effective only in aerobic bacteria. The structure of Gentamicin is consistent with the aminoglycoside structural activity relationship (SAR), except with a few minor changes. Specifically ring one have methylated amine and one axial hydroxyl group. The methylation of amine will retain its activity and will lower the susceptibility to transferase, which are the enzyme that probably causes inactivation.

Clinically, Gentamicin is used for some urinary tract infection, burns some pneumonia and bone and joint infection caused by gram negative bacteria. The most common clinical application (either alone or as part of combination therapy) of the Gentamicin is in the treatment of serious infections caused by aerobic gram-negative bacilli. Gentamicin has also been used for the treatment of selected staphylococcal and enterococcal infections. Gentamicin is the usual all-purpose agent of choice[11]. Gentamicin is more active against serratia marcescens. Gentamicin has been shown to destabilize the outer membrane of Pseudomonas aeruginosa and form holes in the cell wall, independent of its action on ribosomes (Kadurangamuwa et al, 1993). This action of the aminoglycosides may be the most important.

Ototoxicity is the most important adverse effects clinically, and had dominated attempts to rationalize aminoglycoside dosing[2]. A well-known factor i.e. hearing loss occurred as a result of degeneration of the hair cells of the cochlea, beginning at the basal coil and progressing towards the apex. High frequency hearing loss was followed by loss of lower frequency. According to Takada and Schacht, (1982) disequilibrium and ataxia were main symptoms of vestibulotoxicity. Both acute (reversible) and chronic (irreversible) ototoxicity had been observed. Ganesan et al, (1983) described that chronic toxicity was related to aminoglycoside-phosphoinositol binding, leading to altered membrane structure and permeability. According to Kahlmeter and Dahlager[10] gentamicin toxicity was the most common single known cause of bilateral vestibulopathy. In pathologic studies, severe aminoglycoside toxicity was associated with death of inner ear hair cells[13]. Oei et al described doses that were not enough to kill hair cells but damaged their motion sensitive hairs (sterocilia), making them unable to respond to motion, at least for some months[13]. Guan et al[8] described that the mechanism of toxicity was through reduction of mitochondrial protein synthesis and the interference with mitochondrial function would be expected to cause cellular disruption through reduction in ATP production. Gentamicin is equally vestibulotoxic and ototoxic (Schacht, 1993).

Neuromuscular blockade is also commonly reported side effect. Patients with neuromuscular blockade may present clinically numbness, twitching of muscles and seizures. Said et al[14] had studied that aminoglycoside antibiotic blocked the transmurally elicited twitches of ileum in a concentration manner. According to

Santos et al[15] gentamicin enhances neuromuscular impairment and death of botulinum toxin-exposed mice. Aminoglycosides potentiate neuromuscular weakness caused by botulinum toxin.

Ototoxicity, nephrotoxicity and neuromuscular blockade are well known side effect but there are few studies that elaborated neurotoxic effect of Gentamicin. Aminoglycoside antibiotics are known to cross blood brain barrier and whole nervous system is exposed to these drugs. Evidence is available suggesting central neurotoxicity of aminoglycosides[6]. Exact mechanism of action on central nervous system is still unclear. Based on the literature we planned this study to observe the neurodegenerative effects of Gentamicin on adult albino rats.

MATERIAL AND METHODS

It was an Experimental study. Twenty adult albino rats weighing 130±20 grams were obtained from Central Animal House, J. N Medical College, AMU, Aligarh. They were divided into two groups. Group I: Experimental (n = 10) received an injection of 135 mg/kg body weight of Gentamicin intramuscularly for twenty one days (Gentamicin WHO food Additives series 34, www.inchem.org\documents). Group II: Controls (n = 10) received normal saline in same volume by intramuscular route for twenty one days.The rats were kept in plastic cages in a room 12:12 light/dark photoperiod, temperature of 20-30C and relative humidity of 50-60%. Ethical approval was sought and received from the Department of Anatomy, JN Medical College, AMU, Aligarh, UP, India.

The rats were decapitated on 22nd day of the experiment. The skin as well as soft tissues surrounding the cranium was removed. Sections were obtained from the area around lateral sulcus of rat cortex. The area around the lateral sulcus of rat cerebral cortex is the site of primary auditory area. Sections of auditory cortex to were obtained from both sides of cerebrum to observe neurodegenerative changes. After obtaining samples from the auditory area of cerebral cortex (control and experimental), tissue samples are weighed on digital weighing pan (error of weighing pan is calculated about 0.022 grams). Accurate weight of the samples has been calculated by deducting standard error from estimated weight.

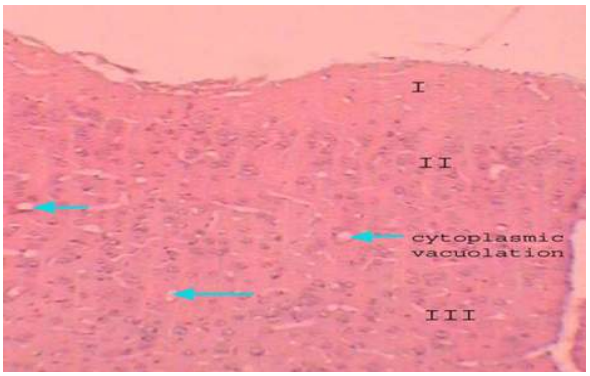


Fig.1 Photomicrograph of the auditory cortex of adult albino rat (Experimental animal) showing edema and cytoplasmic vacuolation in II (external granular) and III (pyramidal) layers (Depicted by Arrow). H&E X100.

The slides with mounted section from Group I and Group II were dried in an incubator at 45^0C for 4 hour for the proper attachment of the sections on the slides. The mounted sections were stained by Haematoxylin & Eosin[3] and Thionin stain. The photomicrographs of the relevant stained sections were taken with the aid of a light microscope.

RESULTS

Auditory cortex of adult albino rat (Experimental) animal showed oedema, cytoplasmic vacoulation in II (External granular) and III (pyramidal) layers [Fig.1].

Photomicrograph of the auditory cortex of adult albino rat (Experimental) animal showing neuronal (Pyramidal and Stellate) cells with cytoplasmic swelling and ill-defined cytoplasmic margins due to Gentamicin toxicity [Fig.2].

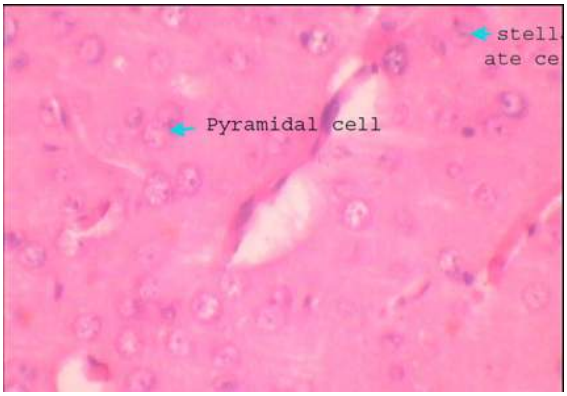


Fig.2 Photomicrograph of auditory cortex of adult albino rat (Experimental) showing neuronal cells with cytoplasmic swelling and ill-defined cytoplasmic margins due to gentamicin toxicity

Photomicrograph of the auditory cortex of adult albino rat (Experimental) animal shows signs of mild degeneration i.e., cytoplasm swelling and eccentric nucleus in II (External granular) and III (Pyramidal cell l) layers [Fig.3].

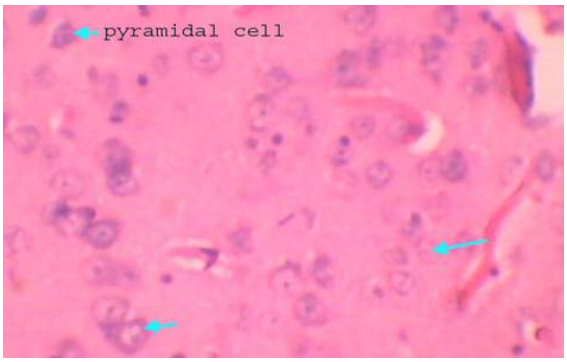


Fig. 3 Photomicrograph of the auditory cortex of adult albino rat (Experimental) cytoplasmic swelling and eccentric nucleus with mild degenerative changes (depicting by arrow). H&E X400

Generalized dispersion of Nissl substance with swollen cells is present in Photomicrograph of the auditory cortex of adult albino rat (Experimental animal) [Fig.6]

Photomicrograph of the auditory cortex of adult albino rat (Experimental animal) shows cytoplasm vacuolation, mild degree of degenerative change and lymphocytic infiltration [Fig.4].

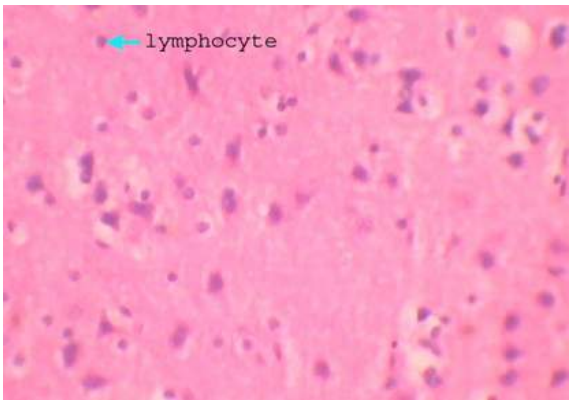


Fig. 4 Photomicograph of the auditory cortex of Albino rat (Experimental) showing cytoplasmic vacuolation, mild degree of degenerative change and lymphocytic infiltration. H&E X 400

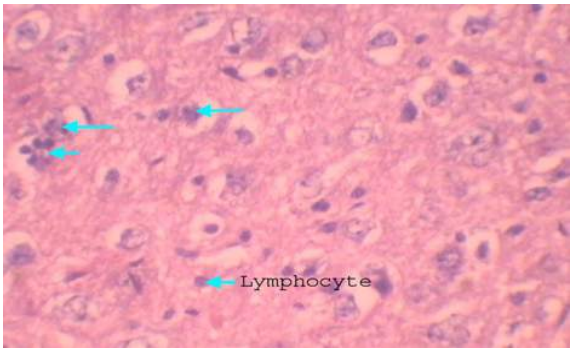


Fig. 5 Photomicrograph of the auditory cortex of adult albino rat (Experimental group) showing nuclear (irregular margins, chromatin clumping and karyorrhexis) and cytoplasmic (vacoulation) degenerative changes. H&E X400.

Photomicrograph of the auditory cortex of adult albino rat (Experimental animal) shows signs of nuclear (irregular margins, chromatin clumping and karyorrhexis) and cytoplasmic (vacuolation) degenerative changes [Fig.5].

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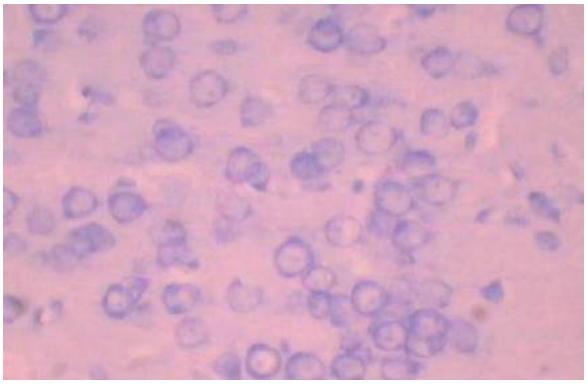


Fig. 6 Photomicrograph of the auditory cortex of an Albino rat showing cells which are swollen and their nissl substance showing generalized dispersion. Thionin stain X 400

DISCUSSION

The present study was designed to observe the neurodegenerative effects of Gentamicin on auditory cortex of adult albino rats. Findings in the literature are available showing ototoxicity, neuromuscular blockade and neurotoxicity caused by aminoglycosides.

Histological findings in present study were suggestive of degenerative changes in experimental group. Oedema and cytoplasmic vacuolation [Fig.1] were present in layer I (external granular layer) and layer II (pyramidal cell layer). Neuronal cells show cytoplasmic swelling with ill-defined cytoplasmic margins [Fig.2]. Pyramidal cells of layer II (pyramidal cell layer) show eccentric nuclei with mild degree of degenerative changes [Fig.3]. Lymphocytic infiltration was also found [Fig.5]. Cytoplasmic vacuolation [Fig.5] was also found in pyramidal cells of layer V (ganglionic layer). Irregular nuclear margins, chromatin clumping, karyorrhexis and cytoplamic vacuolation [Fig.5] were present in neuronal cells of layer V (ganglionic layer). Neuronal cell swelling with generalised dispersion of Nissl substance was found in Thionin staining [Fig.6]. Neurotoxic injury induces changes in nerve cell body which present as swelling and vacuolization[(1)]. Prominent nuclear changes with increased size, irregular outline and dispersion of chromatin are characteristic features of neurotoxic injury[(5)].

Histological findings of the present study were in conformity with neurohistological study on the effect of kanamycin on central auditory auditory pathway conducted by Faruqi *et al* which showed degenerative changes in the cochlear nucleus, inferior colliculus and auditory cortex[(5)]. Decreased staining of Nissl substance of neurons was found in the auditory cortex of kanamycin intoxicated rats. It was explained by Hotz et al that aminoglycoside therapy affects both inner ear and central auditory pathways[(9)].

Histochemical study of gentamicin intoxicated rats showed increased activity of acetylcholinesterase in cochlear nucleus[(4)]. Gentamicin might produce central toxicity by altering concentration of acetylcholine. Watanabe et al reported distinctive lesions occurred in brain stem of patients who were treated with parenteral and intrathecal gentamicin sulfate for Pseudomonas aeruginosa meningitis [(16)].

In an experimental study conducted by Wantabe *et al*, it was reported that a single intracisternal injection of 0.4 ml of 1.25 and 2.5 percent gentamicin sulfate with preservative to healthy adult rabbits produce multiple, minute, disseminated, spongy lesions with cytoplasmic vacuolation of nerve cells [(16)].

This finding is similar with cytoplasmic vacuolation found in neuronal cells of auditory cortex in present study. Convulsions, encephalopathy, confusion, hallucinations, mental depression and sometimes pleocytosis observed in

cerebrospinal fluid of humans, are clinical side effects of gentamicin on central nervous system[7].

CONCLUSION

Exposure of rats to Gentamicin for three weeks showed severe Neurodegenerative changes at microscopic level.

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