RAPID ADMINISTRATION OF LIFE SAVING DRUGS USING AUTOINJECTORS – DEVELOPMENT OF AMIKACIN AS ANTI BACTERIAL AUTOINJECTOR

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ABSTRACT

The morbidity and mortality in a number of emergency situations like accidents in civil and military persons can be reduced by immediate administration of drugs. Autoinjector with an antibacterial and an analgesic drug will be very useful for the immediate administration in such situations. These autoinjectors will contain replaceable drug cartridges and are convenient to use by the health care workers with simple instruction. The drugs will act very fast when delivered by the autoinjectors and intramuscular injection is expected to be equal to intravenous injection due to the forced delivery with a spray effect of the drugs. Amikacin drug cartridge was developed for the autoinjector and its tolerability was studied in rats by intraperitoneal injection (63 mg). Rats were given either 3 injections on 3 consecutive days or 7 injections on 7 consecutive days by the autoinjector and compared with manual injection. Blood was withdrawn on the 4th day (3 doses) or the 8th day (7 doses), and haematological and biochemical parameters viz., WBC, RBC, PCV, Hb, ALT, AST, GGT, LDH, glucose, urea and creatinine were studied. Vital organs viz., lung, liver, spleen, testis, kidney and heart were removed, weighed and preserved for histological studies. All the parameters studied were within the limits and did not show any significant difference when compared with the control. This study showed that intraperitoneal injection of amikacin by the autoinjector designed for intramuscular injection was well tolerated by the rats.

Key word: Autoinjector, antibacterial, analgesic, amikacin, intramuscular, emergency.

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INTRODUCTION

A number of emergency and critical situations occur in which the individual requires first aid measures. When a qualified medical person is not available, the emergency drug has to be administered by self or by a trained individual to reduce the morbidity and mortality. Nerve agent exposure in a war scenario and other organophosphorus pesticide poisoning require immediate administration of atropine sulphate and pralidoxime chloride to reduce the mortality (Jain *et al*, 2011). These drugs can be administered by a self administrable device, the autoinjectors (Vijayaraghavan *et al*, 2007; Vijayaraghavan *et al*, 2012). Compared with manual intramuscular injection the autoinjector enhances drug absorption rate. Healthy volunteers were administered atropine and pralidoxime chloride through the autoinjectors and it was observed that the absorption of the drug was faster by the autoinjector (Friedl *et al*, 1989). Similarly for other emergency and critical situations like seizures, anaphylaxis, migraine, etc., also require immediate administration of the recommended drugs, preferably by the autoinjectors in which the individual *per se* can administer the drug or by his companion. Other than convenience these autoinjectors are well suited for emergency and mass casualty management. In comparison to manual intramuscular injection the autoinjector can deliver the drugs by deep intramuscular injection with a spray effect, increasing the area, thereby faster drug absorption. The needle is not visible in these autoinjectors and the injection will be painless, convenient and well suited for mass casualty situations.

In seizures, anticonvulsants are required to reduce the severity and prevent brain damage. Midazolam, a water-soluble benzodiazepine agonist is preferred as an anticonvulsant, since it is absorbed faster following intramuscular administration. The plasma pharmacokinetics of midazolam administered by an autoinjector was compared with conventional intramuscular administration in pigs, and it was observed that higher plasma concentration of midazolam was detected following autoinjector administration, compared with the intramuscular injection (Levy *et al*, 2004). Acute repetitive seizures can sometimes progress to status epilepticus. The approved treatment is diazepam rectal gel as it can be administered by non-medical personnel. Rectal administration may be difficult, inconvenient and objectionable and hence diazepam autoinjector has been developed for intramuscular injection. The diazepam absorption is faster and can be safely and reliably administered using the autoinjector (Lamson *et al*, 2011). Severe allergy leading to anaphylaxis is common due to food, exercise, hymophtera venom, and latex. Epinephrine is the treatment of choice for anaphylaxis and it has to be administered promptly. Delay in administration of epinephrine is a known risk factor for food allergy related mortality. Epinephrine autoinjector has been developed for food allergy (Sicherer *et al*, 2007; Kijakovic *et al*, 2009).

Migraine is characterised by throbbing headache. Sumatriptan is one of the preferred drugs for migraine and an autoinjector has been developed as an alternative to sumatriptan injection. Sumatriptan taken subcutaneously

using an autoinjector at home is an effective and well tolerated acute treatment for migraine. In a study, preventive oral treatment with sumatriptan (100 mg three times a day for 7 days) did not produce a significant reduction in the number or severity of cluster headache attacks, but sumatriptan (6 mg) given by autoinjector showed better results (Monsted *et al*, 1995). Autoinjectors of interferon-beta-1a for multiple sclerosis, peginterferon-alfa-2a for Hepatitis C and vasoactive intestinal peptide and pentolamine for erectile dysfunction are also available (Shah *et al*, 2007; Phillips *et al*, 2011; Varunok *et al*, 2011).

There are several emergency and critical situations in which the individual may be injured severely and the medical attention may get delayed. For instance in the Military Service life (Army, Navy, Air force and Paramilitary Staff) during training and operation, low intensity conflicts, road accidents and also natural disasters such a situation may occur in which the individual may be with great pain and there may be chances of infection. In such conditions autoinjectors with an antibacterial drug and an analgesic drug will be very much useful for field administration by an authorised person (Vijayaraghavan, 2012). The purpose of this study is to develop amikacin cartridge for the reusable autoinjector and evaluate its injection capability and tolerability in rats.

MATERIALS AND METHODS

Animals : Randomly bred Wistar male rats (120 - 200 g), bred and maintained at Biomedical Research Unit and Laboratory Animal Centre (BRULAC) of Saveetha University were used for the study. The animals were kept in polypropylene cages (3 per cage) with sterilised and dry paddy husk as a bedding material. The animals were fed with commercial laboratory animal feed (Tanuvas, Chennai) and RO water *ad libitum*. The care and maintenance of the animals were as per the approved guidelines of the 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India)'. This study has the approval of Institutional Animal Ethical Committee (SU/SMC/RD/010/2012; Dt. 21 February 2012).

Chemicals and drugs : The amikacin drug cartridge was developed with the collaboration of M/s Neon Laboratories (Mumbai). The cartridge contains 250 mg/mL of amikacin as amikacin sulphate with stabilisers. The drug volume is 2.3 to 2.4 mL and upon ejection by the autoinjector, 2.1 to 2.2 mL will be delivered. The other QAQC parameters were as per the nerve gas antidote drug cartridges (Vijayaraghavan et al, 2012). The other chemicals were either Indian Pharmacopoeial grade or analytical grade purchased from standard companies. Autoinjectors produced by M/s Sigma Engineering (Hyderabad), a technology by Defence Research and Development Organisation (DRDO) and has an option to select full dose or partial dose delivery was used.

Autoinjector usage : The tolerance of the amikacin autoinjector was studied by injecting the drug intraperitoneally in male rats. For this the drug cartridge was diluted 1:4. From the cartridge 1.75 mL of the drug solution was withdrawn under a laminar flow and 1.75 mL

sterile saline was injected back to make the concentration to 63 mg/mL. The drug cartridge was loaded in the autoinjector with the plastic clip restrictor (Fig 1). This will allow only partial volume of the drug (about half) to be injected and restricting the ejected needle length to half. The rats were held firmly on the surgical table with its back on the table. The autoinjector was unlocked and positioned gently on the lower abdomen vertically (Fig 2). The trigger button was pressed and held on to the abdomen for 10 sec. The autoinjector was then removed gently. The drug cartridge was weighed before and after injection to estimate the quantity of the drug injected. For the manual injection the drug solution removed from the cartridge was diluted 1:4 with normal saline (63 mg/mL) and 1 mL was injected intraperitoneally for each rat.

Experimental groups: The first experiment was carried out by delivering the drug through the autoinjector for 3 consecutive days. The second experiment was carried out by delivering the drug through autoinjector for 7 consecutive days. The following were the groups of this study. For each group 6 animals were used.

Group A : Control,

Group B : Amikacin injection, 63 mg/mL per rat for 3 days by autoinjector,

Group C : Amikacin injection, 63 mg/mL per rat for 3 days by manual injection,

Group D : Amikacin injection, 63 mg/mL per rat for 7 days by autoinjector,

Group E : Amikacin injection, 63 mg/mL per rat for 7 days by manual injection.

Sample collection: The animals were weighed daily, and general behaviour, food intake and water intake were recorded. Twenty four hours after the last injection (4^{th} or the 8^{th} day) blood was withdrawn from the orbital sinus of the rats after anaesthetising the animals with chloroform. Blood was collected in two tubes with anticoagulant (Na_2 EDTA) and without anticoagulant. After the collection of the blood the animals were sacrificed with over dose of chloroform. Vital organs viz., liver, heart, spleen, kidney, lung and testis were excised, blotted free of blood, weighed and preserved in formalin solution.

Haematological Parameters: The whole blood was used for the estimation of haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC) and red blood cell count (RBC). Beckman Coulter Cell Counter (UK) was used for the analysis.

Biochemical Parameters: Serum was separated from the blood, and aspartate aminotransferase (AST), alanine amonitransferase (ALT), gama glutamyl transferase (GGT), lactic dehydrogenase (LDH), glucose, creatinine and urea were estimated. For the analyses Roche Modular EVO 9000 autoanalyser (USA) was used.

Organ to body weight ratio: The organ to body weight index (OBWI) was calculated as a ratio of organ weight divided by body weight and

multiplied by 100. The tissues were fixed in formalin solution and preserved for histological studies.

Statistical analysis: All the parameters were analysed using one way analysis of variance and compared with control using Dunnett's test. A probability of 0.05 and less was taken as statistically significant. The analysis and plotting of graphs were carried out using SigmaPlot 12 (Systat Software Inc., USA).

RESULTS

In one rat full dose of 500 mg of amikacin was injected intraperitoneally through the autoinjector. The animal died within 1 hour. In two rats partial dose of 250 mg was injected intraperitoneally. The delivery of the drug was perfect and the animals did not react. The rats survived after the first dose and a second dose of 250 mg was administered intraperitoneally through the autoinjector the next day. Both the rats died within 4 hours. Hence the cartridge was diluted 1:4 (63 mg/mL) and 3 injections were given on consecutive days. As the animals responded very well a second experiment was carried out by administering the drug for 7 days consecutively. Based on the amount of solution injected and the weight of the animal the injected dose was calculated. Four autoinjectors were used in this study and the dose injected per day for the rats was 546.0 ± 15.5, 515.3 ± 23.6, 496.6 ± 39.7 and 494.2 ± 26.1 (mean ± SE) mg/kg respectively. The dose administered was not significant from each other. Using the restrictor the effective needle length was 15.54 ± 0.09 mm. Without the restrictor the effective needle length was 26.42 ± 0.09 mm.

The food intake, water intake, general behaviour and body weight did not show any significant difference among the groups. Figure 3 shows the OBWI of 3 day and 7 day treatment. There was no significant difference between the control group, the groups of autoinjector and the groups of manual injection. Upon opening the abdomen no sign of bleeding or injury was observed, due to the autoinjector or the manual injection. Figure 4 shows the level of Hb, PCV, RBC count and WBC count. No significant difference was observed between all the groups. The serum parameters viz., glucose, urea and creatinine did not show any significant difference compared to control, except the glucose level was significantly higher in 7 day manual injection (Figure 5). Similarly the enzymes AST, ALT, GGTP and LDH also did not show any significant difference from the control (Figure 6).

DISCUSSION

Aminoglycosides are potent bactericidal antibiotics that act by binding to 30s ribosomal subunits. They are particularly active against aerobic, gram-negative bacteria and act synergistically against certain gram-positive organisms. Amikacin is a safer antibiotic and effective against gentamycin resistant organisms (Cunha 1988). Amikacin sulphate is water soluble and stable, and hence used for preparing the drug cartridge. Since the adult intramuscular dose is 500 mg the cartridges were made with the same dose and diluted for the animal usage. The front septum

of the cartridge is made of silicon (~30 shore hardness) and withdrawing the liquid from the cartridge and injecting saline using a 22 gauge needle was effective, as there was no leak while ejecting the liquid from the cartridge by the autoinjector. The autoinjector with the provision for two doses also functioned satisfactorily. The full dose is expected to deliver a volume of 2.1 to 2.2 mL and the new design of a restrictor in the autoinjector delivered a partial volume of 1.2 to 1.3 mL. The four autoinjectors used in this study also showed same quantity of drug delivery.

In the present study none of the haematological and biochemical parameters showed any significant difference compared with the control, manual injection and through autoinjector, showing that the delivery of the drugs through the autoinjector was well tolerated by the animals. Amikacin is known to cause ototoxicity and nephrotoxicity upon long term usage (Gonzalez and Spencer, 1998). The serum urea and creatine levels showed no significant difference, showing that the dose and duration used in this study did not cause nephrotoxicity. The liver enzymes also did not show any significant difference from the control showing that the injection with the autoinjector which delivers the drug with a force did not cause any tissue damage. All the vital organs studied did not exhibit any gross abnormality as the OBWI did not show any significant difference.

The reported LD_{50} of amikacin is 3.5 g/kg by intraperitoneal injection in rat (Material Safety Data Sheet). Since the human dose is close to the LD_{50} of rat the full dose of 500 mg and the partial dose of 300 mg (2 doses) could not be tolerated by the rats. But, drug like atropine sulphate was tolerated by pigs when the human dose was administered by autoinjectors (Nyberg *et al*, 1995). The injectable needle length was within the range and recommended for the autoinjector devices (Song *et al*, 2005; Schwirt and Seeger, 2010).

This study shows that intraperitoneal injection of amikacin by the autoinjector designed for intramuscular injection was well tolerated by the rats. The reusable autoinjector with dual dose provision also functioned very effectively. It is also possible to decrease the drug concentration by dilution with normal saline inside the cartridge aseptically and hence the autoinjectors can be used for children and animals including pet animals.

Figure 1 : Autoinjectors with drug cartridges.

Left = Autoinjector with the restrictor for the partial delivery of the drug with partially ejected needle.

Right = Autoinjector with fully ejected needle.

Figure 2 : Intraperitoneal administration of Amikacin in rat by autoinjector with restrictor for the partial delivery of the drug.

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